State of the Science Panel

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Outline

1. Endocrine Disrupting Chemicals (EDCs) - the basics
2. Perfluoroalkyl substances (PFAS) - in the body
3. Non-Cancer Endpoints
   - Cholesterol, pre-eclampsia and NAFLD
   - Fetal exposures and outcomes

   Dr. Ducatman

   - Thyroid
   - Neurodevelopment
   - Immune function
   - Mammary gland development

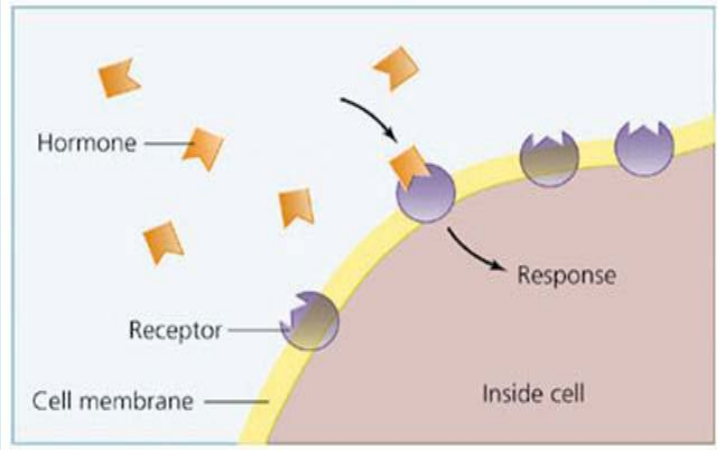
   Dr. Carignan

4. Early indications of toxicity and harm
5. Cancer Endpoints

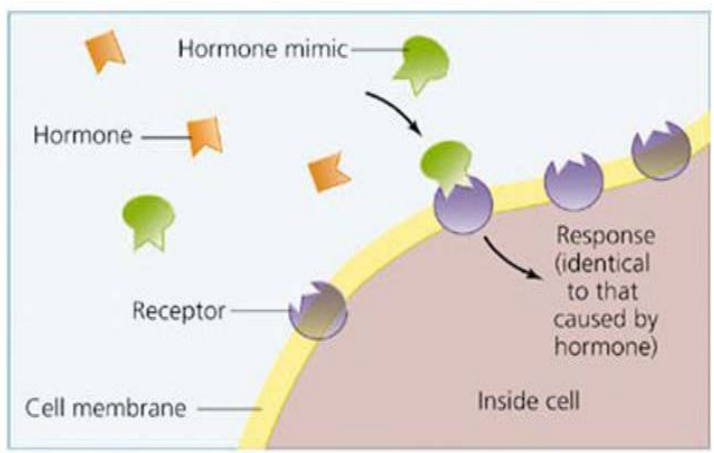
Dr. Clapp

Endocrine Disrupting Chemicals (EDCs)

The hundreds or more exogenous chemical(s), or mixtures of chemicals, that **interfere with any aspect of hormone action.**



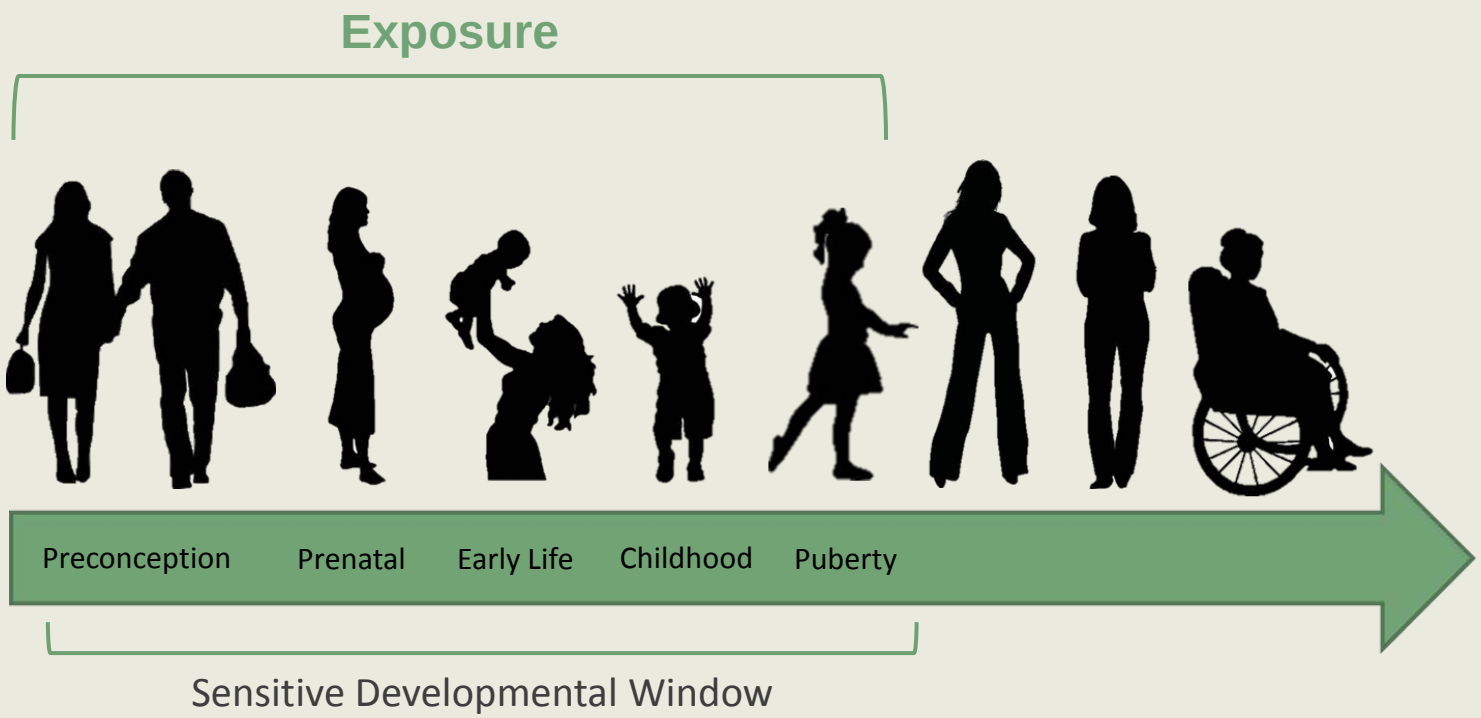
(a) Normal hormone binding



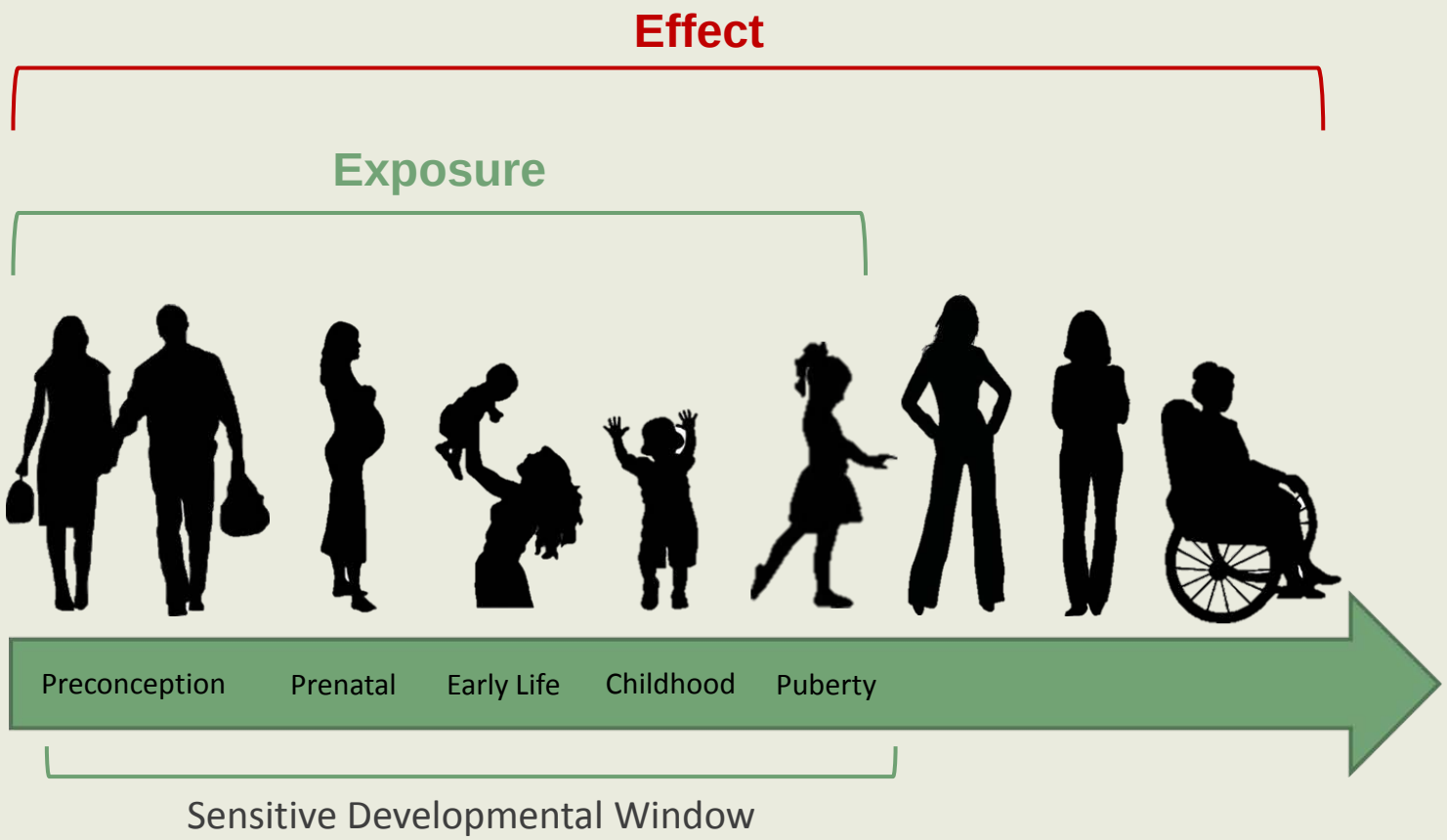
(b) Hormone mimicry

- Act at low levels in the body
- Act in sensitive time windows of development

Act in Sensitive Developmental Windows



To have Later Life Effects



DoHAD: Developmental Origins of Health and Disease

Evidence that exposure can impact

- Fertility & Reproduction
- Neurodevelopment
- Neuroendocrine system
- Obesity & diabetes
- Hormone-sensitive cancers

Gore et al. (Endocrine Reviews) 2015

EDCs are in many products

Many have not been captured by our regulatory frame work

Consumer products

PFAS (stain-proof, non-stick)

Flame retardants (foam, electronics)

Food packaging

PFAS (paper)

Phthalates (plastic)

Personal care products

Phthalates

Parabens

Triclosan

Benzophenone

Canned foods

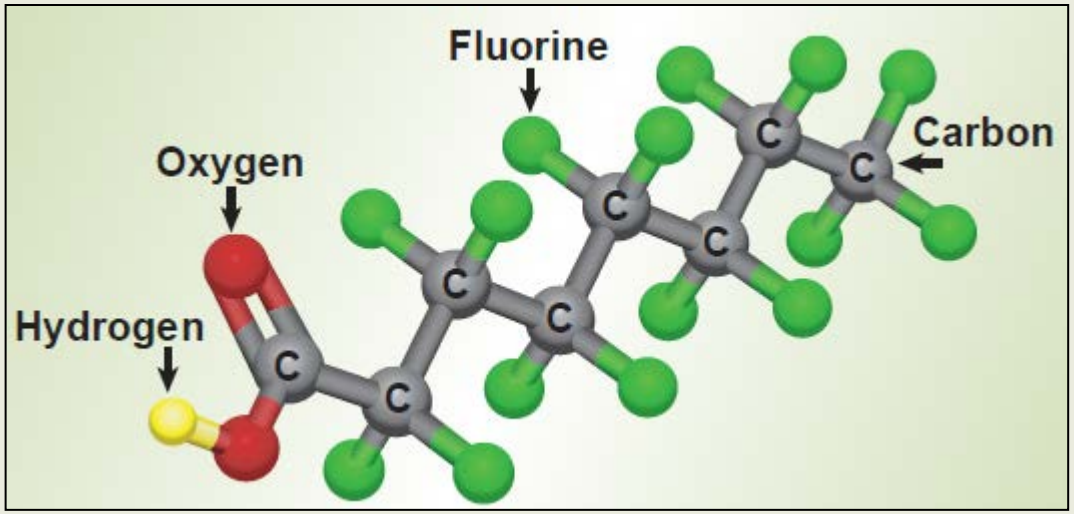
Bisphenol-A

Bisphenol-S

Bisphenol-F

Perfluoroalkyl Substances (PFAS)

Also known as Perfluorinated Chemicals (PFCs)



Hydrophilic functional group
Hydrophobic/lipophilic fluorinated tail
C-F bond is very strong – does not break down easily
Recirculates in blood stream bound to protein

PFAS used in many products

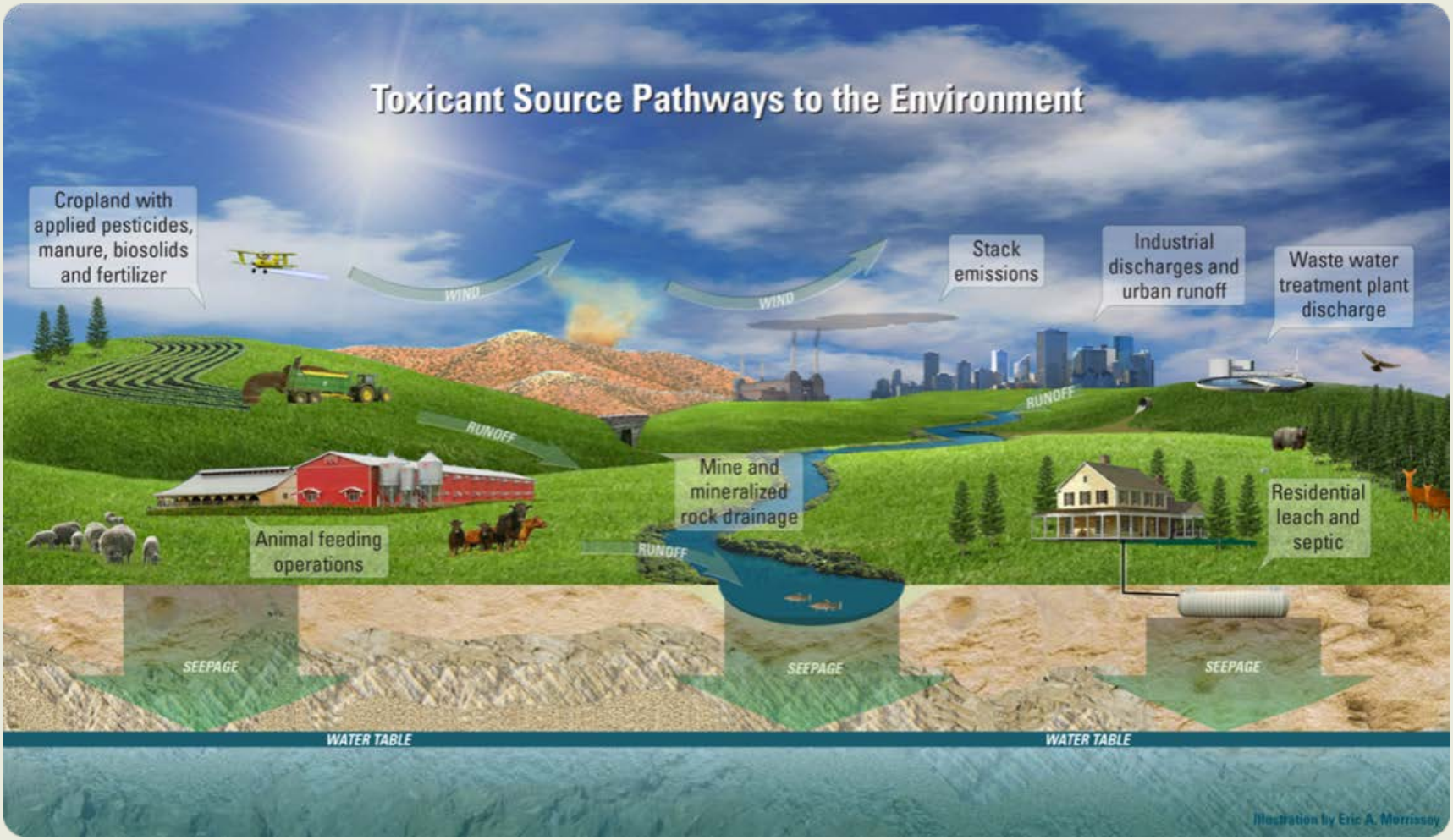
Stain and Water Proofing

Food packaging

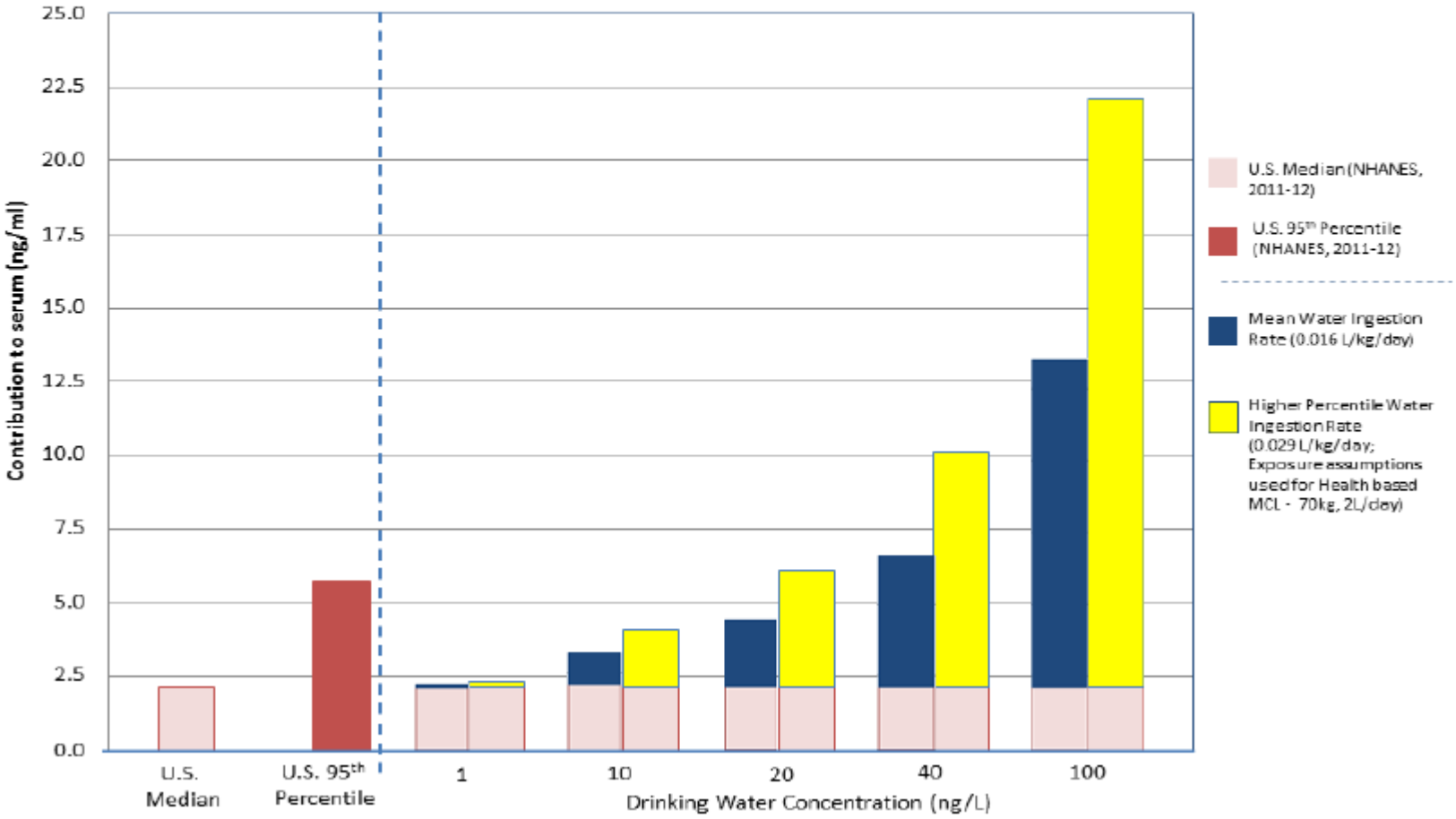
Non-stick

Fuel Fires (AFFF)

PFAS Migrate into Groundwater



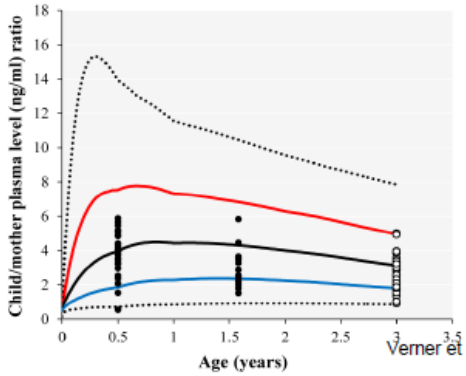
Increases in Serum Concentrations Predicted from Ongoing Exposure to PFOA in Drinking Water



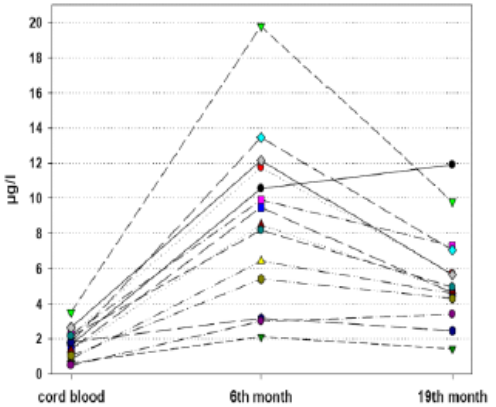
NJ DWQI, 2016

Early Life Exposure

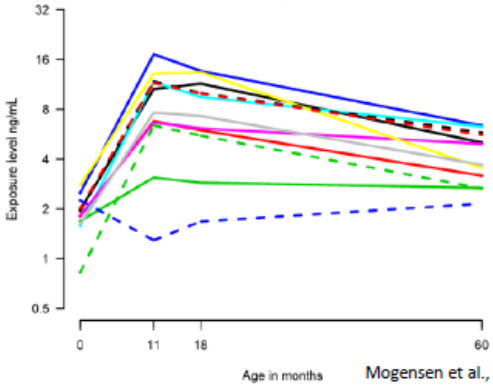
Increases in Infant PFOA Serum Levels after Birth



Verner et al., 2016



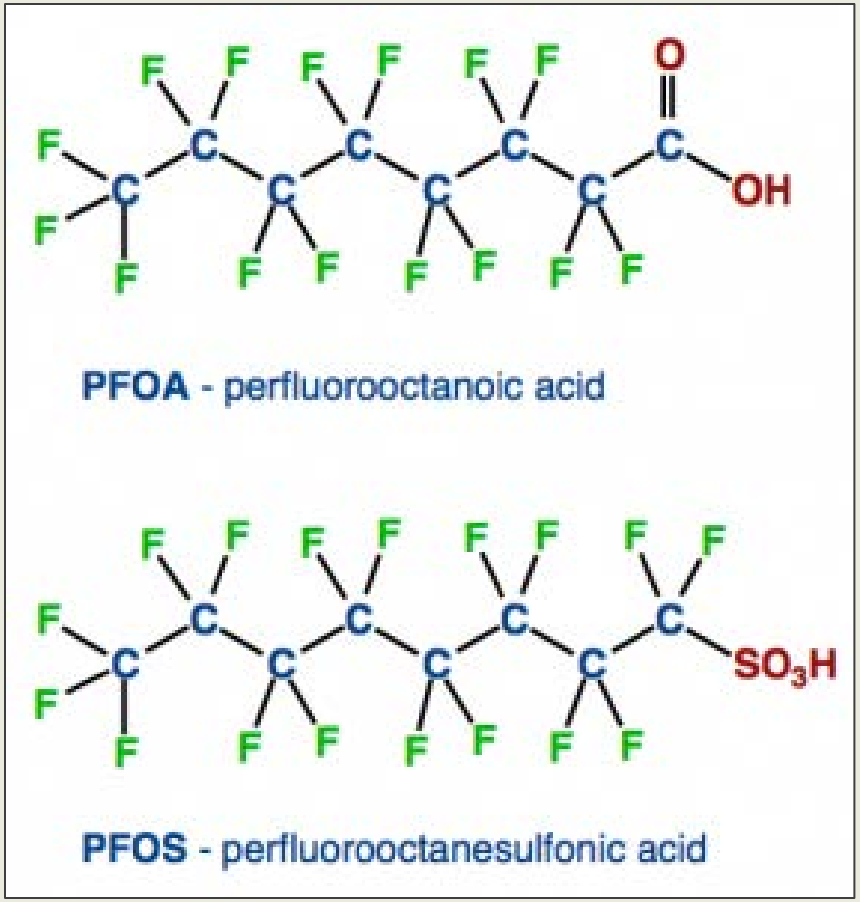
Fromme et al., 2010



Mogensen et al., 2015

NJ DWQI, 2016

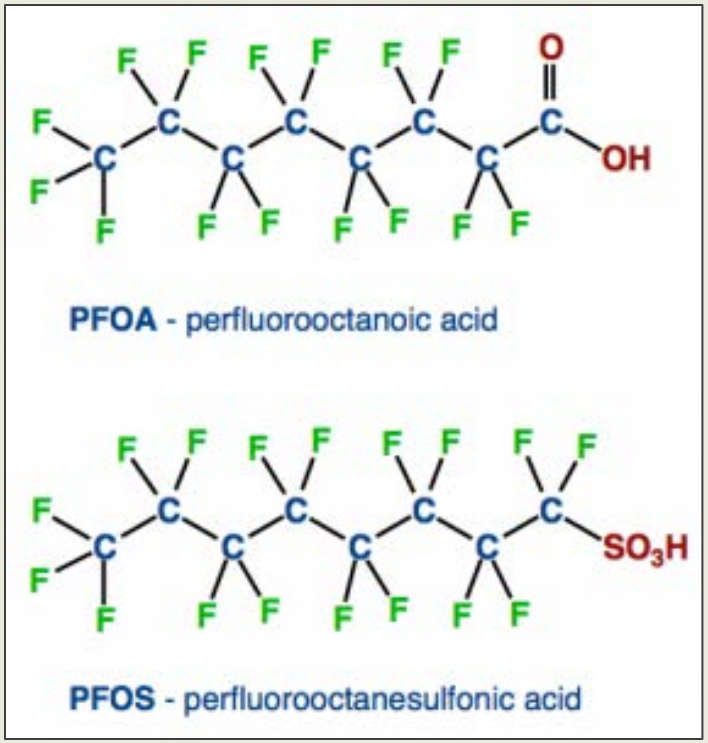
Some PFAS phased-out



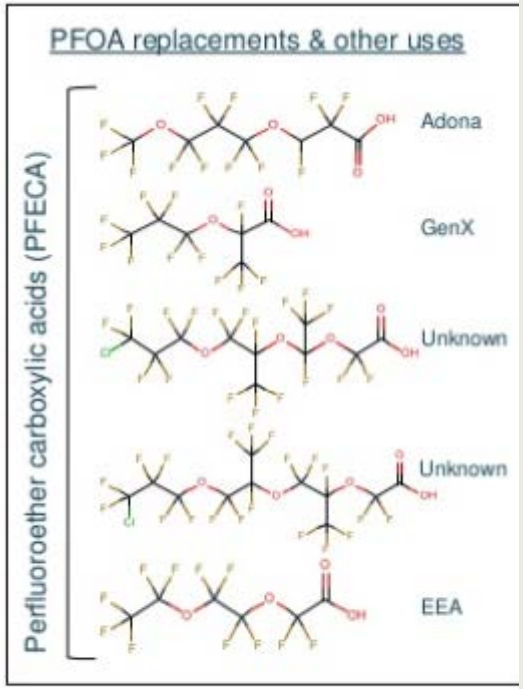
- Persistence
  - Years (in body)
  - Millenia (environment)
- EDCs with impacts on human development and reproduction
- Probable carcinogen

Replacement PFAS

'Old'
Long-Chain
PFOA, PFOS, PFHxS



'New'
Short-chain
C6, GenX



Wang et al., (2013) Environ. Int., 60, 242-248.
Wang et al., (2015) Environ. Int., 75, 172-179.

Most Health Data is on PFOA

PFC: Perfluorinated chemicals

PFAS: Perfluorinated alkyl substances

Standard PFC Serum Panel
PFOA
PFOS
PFHxS
PFUA
PFOSA
PFNA
PFDeA
Me-PFOSA-$AcOH_2$
Et-PFOSA-AcOH

PFCs in AFFF

6:2 FTS	$C_8F_{15}H_4SO^-_3$
PFOSA	$C_8F_{17}SO_3NH^-$
PFBS	$C_4F_9SO^-_3$
PFHxS	$C_6F_{13}H_4SO^-_3$
PFHpS	$C_7F_{15}SO^-_3$
PFOS	$C_8F_{17}SO^-_3$
PFDcS	$C_{10}F_{21}\ SO^-_3$
PFBA	$C_4F_9COO^-$
PFPeA	$C_5F_{11}COO^-$
PFHxA	$C_6F_{13}COO^-$
PFHpA	$C_7F_{!5}COO^-$
PFOA	$C_8F_{17}COO^-$
PFNA	$C_9F_{19}COO^-$
PFDcA	$C_{10}F_{21}COO^-$

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PFAS State of the Science- Clinical population review: liver, lipids, little ones

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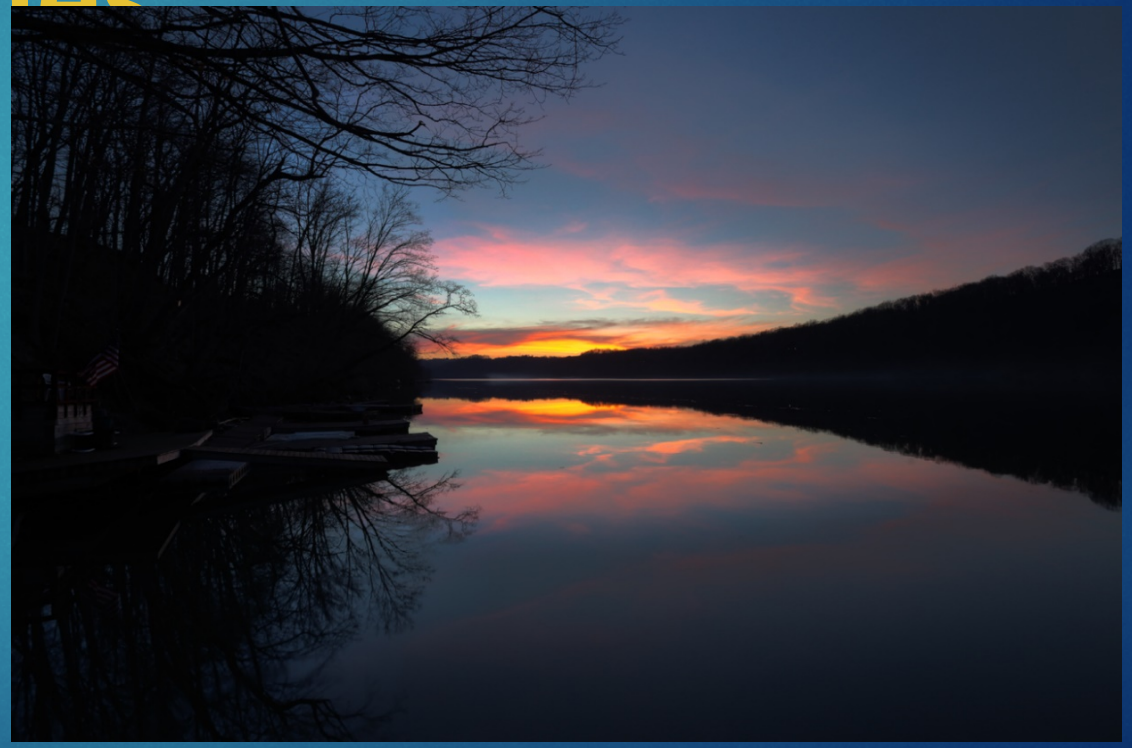


Declarations

- Principle Investigator for Creation of Public Website (2006-2010) for Public Data Communications of the “C8 Health Study” pertaining to 69,030 participants in the mid-Ohio Valley, residents of two states affected

- Future: plan to again participate with affected communities, including municipal and other government, water utilities, and representatives

Goals

Attendees will be able to provide an overview of:

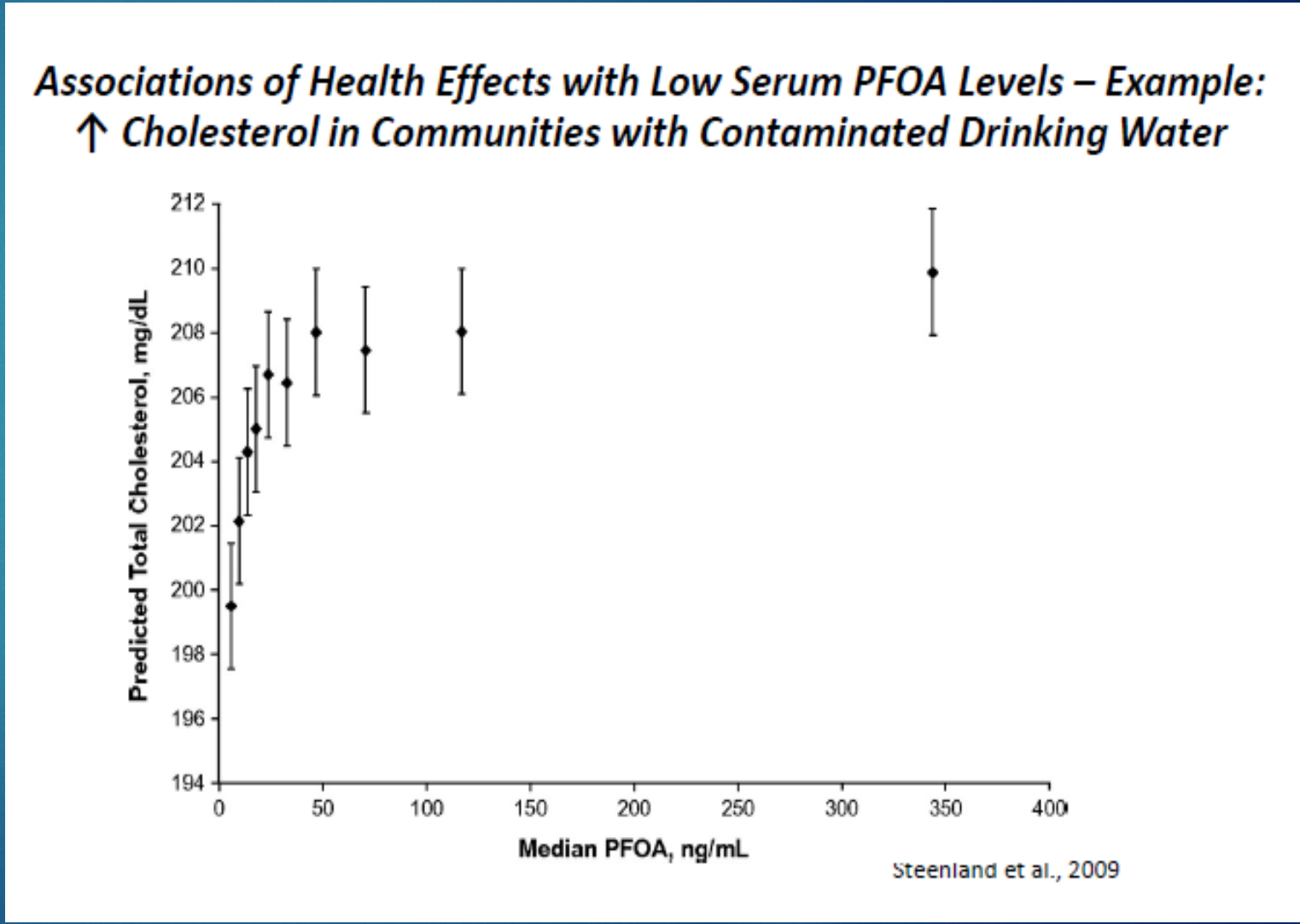
- Human Data
- How human data and animal data are related
- How different kinds of biomarker findings can be physiologically related
- Hit the high points for community attendees, leave the data on the table for scientists and journalist.
- (Lecturing as an aerobic sport?)

Lipids Dose & Risk

Nonlinear (asymptotic) dose-response curves shown is **Steenland et al** about adults Am J Epi 2009, Aug15

(Saturation mechanism?)

Physiologically active at human serum concentrations as low as we have measured them.



Start with Lipids. Multiple studies.

: "There is disagreement among studies." This fundamental misinterpretation was common until recently, is now becoming uncommon

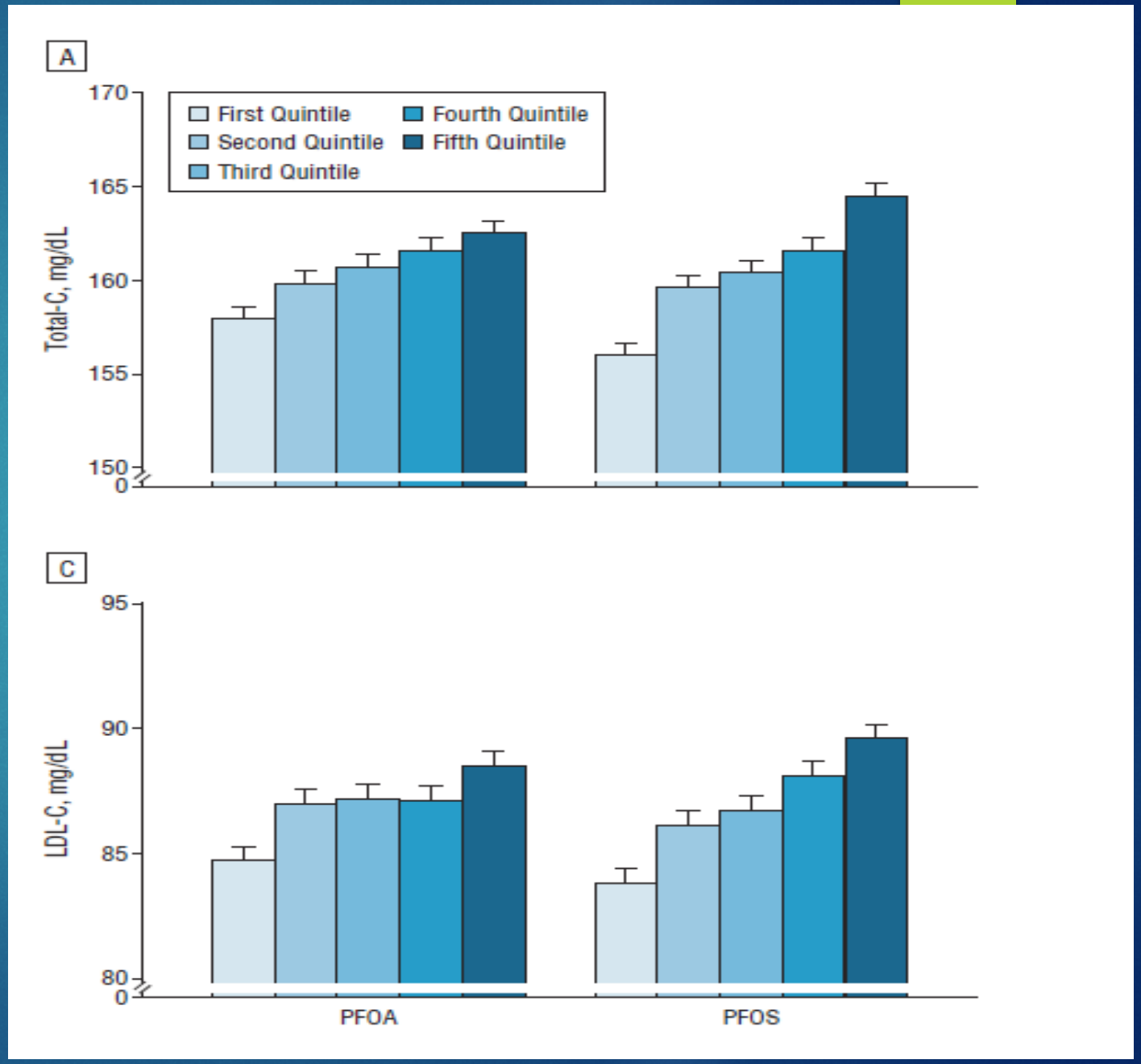
*Reality: **dozens of similar human findings** of elevated LDL or total cholesterol. To understand the similarities, Important to consider*:

- Most "long" (≥C7 or C6S) chain PFAS show the association.
- *The PFAS species present* in the environment is commonly the one with the most apparent effect. Example: if PFTA (C14) is dominant, that can be the one with the strong association (Zeng XW et al. Sci Tot Environ 2015). These are not disagreements, these are confirmations of a likely **shared mechanism**.
- Most "action" is at the low end of the curve (**asymptotic curve**), so the effect is hard to see at high doses in small populations (present, but maybe not statistically significant). This is common (in medications!), not exotic.
- A few of the chemicals may also raise the HDL or triglycerides, that is less clear.

Lipid association: also in children

GLM Covariable estimated marginal means for PFOA (left) and PFOS right for Total Cholesterol and LDL Cholesterol, in 6459 children (age <18, range 1-17, mean 11.2) from the mid-Ohio Valley

Source: Frisbee SJ et al. Arch Pediatr Adolescent Med; 164: 2010



LIPIDS in Pregnancy or following *in Utero*

Pregnancy Starling et al, In addition Skuladottir et al showed absence of confounding by diet for this

Prenatal Maisonet M et al. – Environ Int'l 2015 **Table** is mean Total and LDL-C in 199 7-year old and 15-year old children by tertile of **PFOS** exposure

Tertiles of exposure	PFOS			
	Age 7		Age 15	
	Mean	95% CL	Mean	95% CL
TC				
Lower tertile	167.6	158.1, 177.1	140.8	131.1, 150.4
Middle tertile	176.7	169.2, 184.2	159.6	149.7, 169.5
Upper tertile	179.5	169.3, 189.6	159.9	146.5, 173.3
LDL-C				
Lower tertile	86.6	78.4, 94.8	76.7	67.8, 85.7
Middle tertile	91.1	84.5, 97.8	91.8	83.0, 100.5
Upper tertile	93.9	87.1, 100.8	89.8	79.8, 99.7

Good designs refute wishful

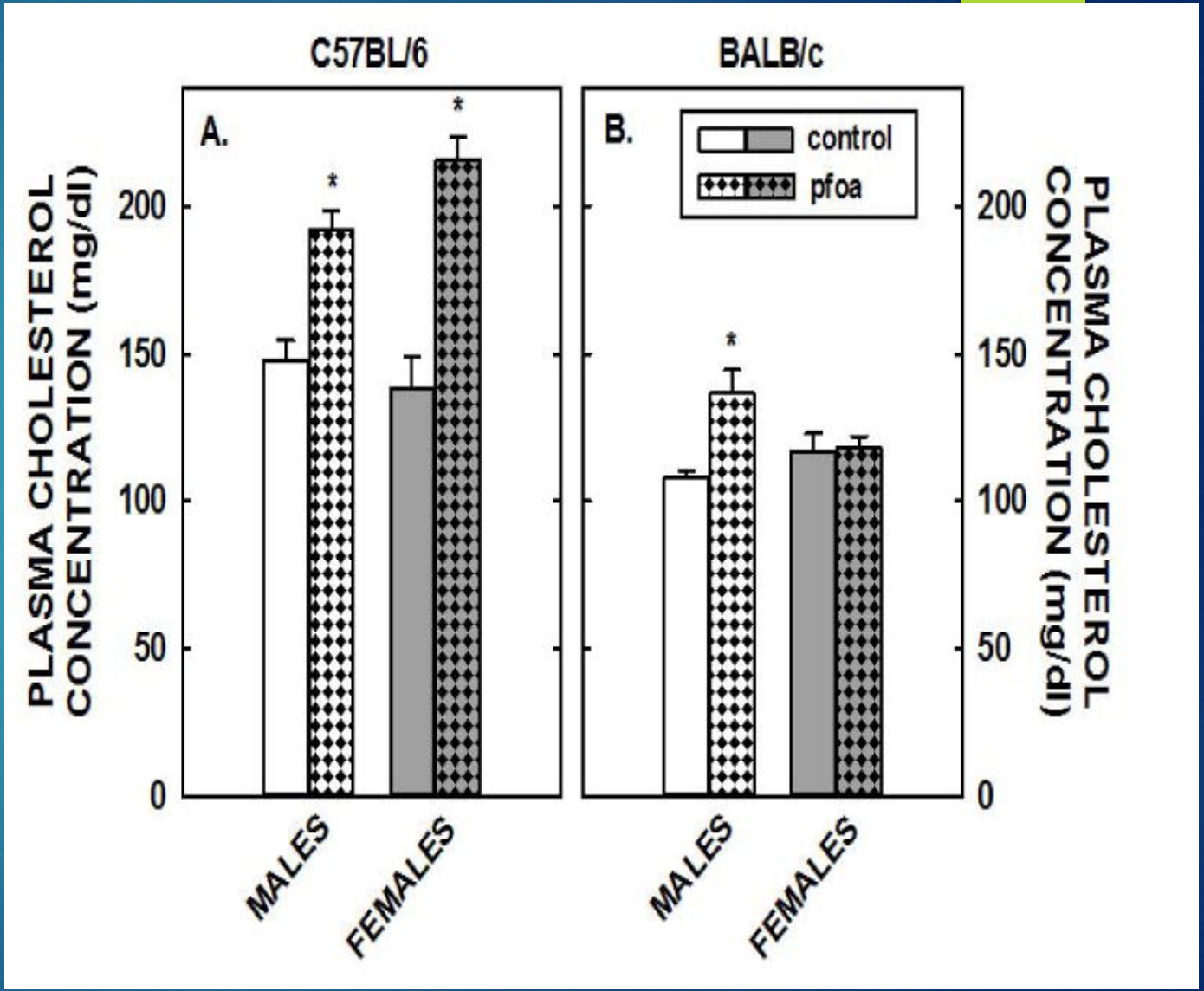
Wish 1. PPAR-a, but, humans do not have much PPAR-a response, so……

Wish 2. The cholesterol goes down in rodents, so …

Wish 3. The curve is nonlinear, so..

Good design: Rebholz SL et al. Toxicol Rep 2016 Plasma cholesterol concentrations in C57BL/6 (A) and BALB/c (B) male and female mice. Mice were weaned and fed diets containing fat plus cholesterol. Half of the mice received no dietary PFOA and half received 3.5 mg/kg diet .

Diet dependent, strain-dependent (and also not PPAR only).



By the Way

The predicted outcome of a PFAS association with lipids is an association with abnormal lipid levels, (above treatment recommendation thresholds)

In large enough populations, That is what is seen.

Implications……

Codeable diagnoses

Lipid lowering **medications** (may in turn lead to some bias and underestimation of LDL effect in adults)

However, related concern about **heart disease** is **mostly not seen**. There are a couple of **caveats** about that.

- Treatment effects (lipid lowering agents work very well to reduce death/disease!)
- **Relevant subgroup studies needed** including:

  Obese? Metabolic syndrome?

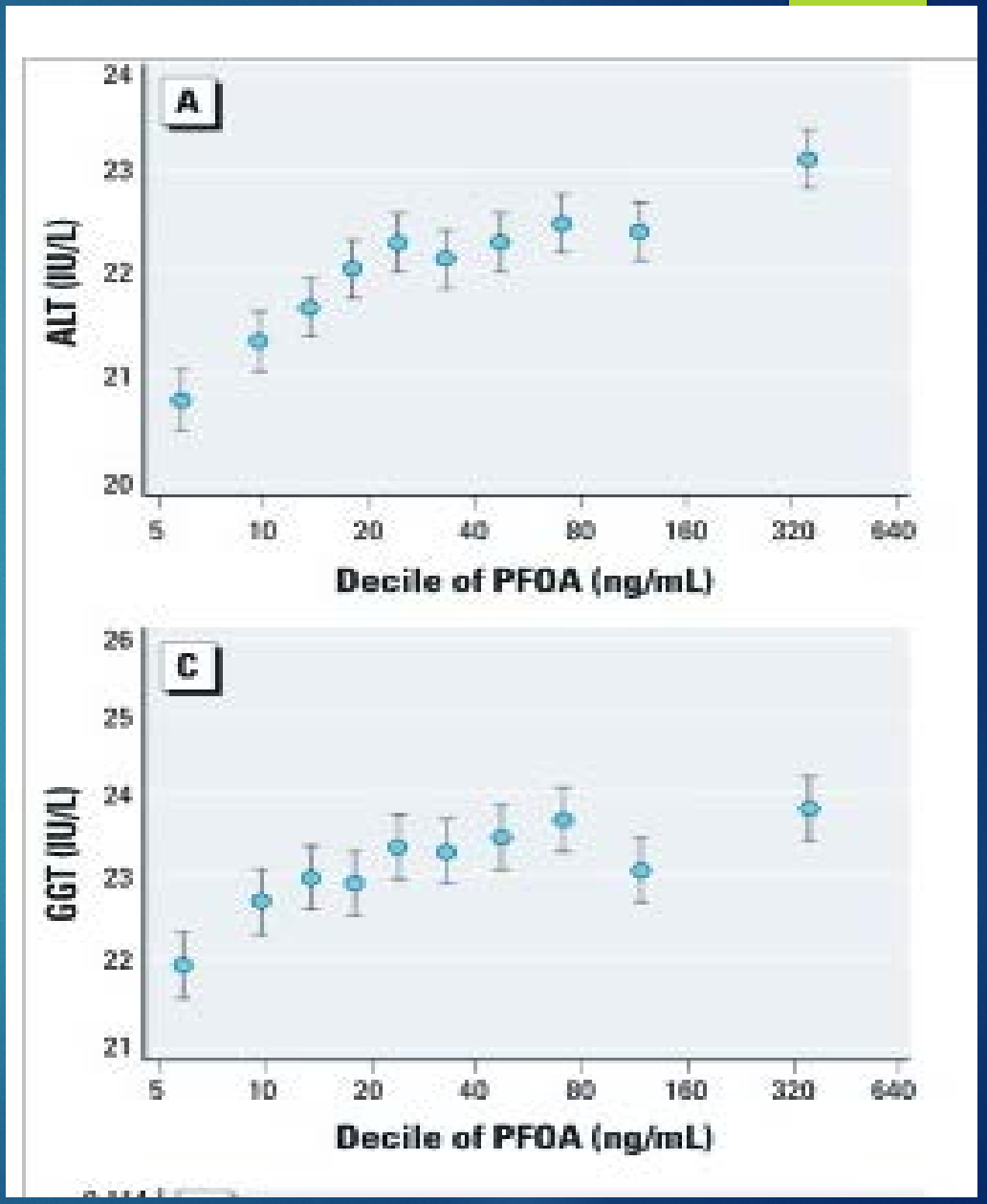
  strong NAFLD family history or predictive gene variants?

Higher **'Liver Functions'**, especially PFOA and ALT

Original human observation by **Gallo V et al, EHP** 120 (2012) C8 Health study (**Figure at right**)

Additional data showing increased observations above clinical cutoffs from Gleason JA, et al. Environ Res 136 (2015) (NHANES data) and from Darrow LA et al EHP 124 (2016) based on a modeled exposure from the C8 health study

Although both occur, I contend discussion about "higher" is more pertinent than discussion about 'abnormal' in a large population survey. Basis follows……..



"Liver functions" insensitive – studies need other approaches in addition.

Liver 'enzymes' or "functions" (such as aminotransferases) are insensitive (and not specific) to preclinical liver damage. Low yield when pre-test probability is uninformed by clinical presentation. (Why we screen for glucose or HbA1c, and lipids, but not for LFTs in absence of indications).

TABLE 1. MAJOR LFTS AND PLASMA PROTEINS

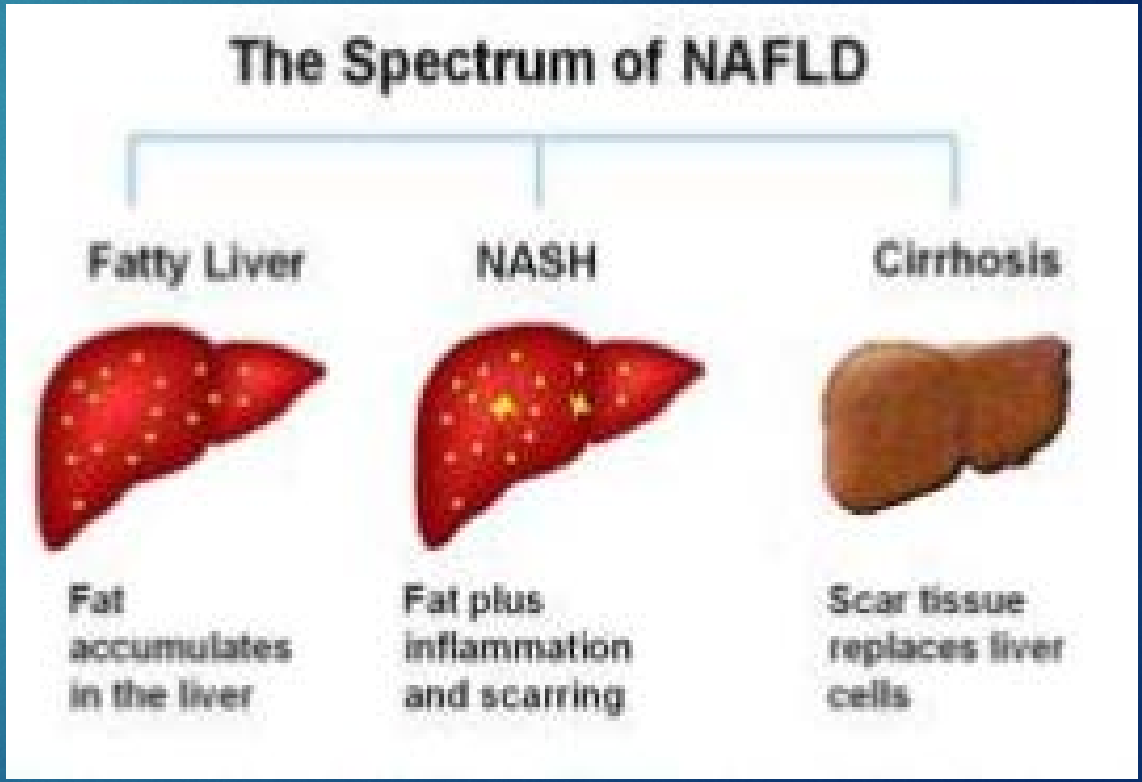
Analyte	Reference range
Bilirubin	<17μmol/L
Alkaline phosphatase (ALP)	95-320IU/L
Gamma-glutamyl transpeptidase (GGT)	5-55IU/L
Alanine aminotransferase (ALT)	5-42IU/L
Asparate aminotransferase (AST)	10-50IU/L

NB: Reference ranges differ - check those used by your own service

When clinicians think about LFTs and Lipids together, the differential includes Metabolic Syndrome and ***Non-Alcoholic Fatty Liver Disease*** – "NAFLD", "NASH"

Animals fed a western diet and PFAS exhibit both elevated cholesterol and increased liver weight, similar to humans with NAFLD

Rebholz S, et al. Toxicol Reports 2016; 3: 46-54



PPAR-a important, but not alone or even required for PFAS-induced lipid accumulation/liver damage

Several studies, including industry work.

Early: Filgo AJ et al, Toxicol Pathol 43 (2015), doi: 10.1177/0192623314558463 in context of prenatal exposures. Other studies have confirmed.

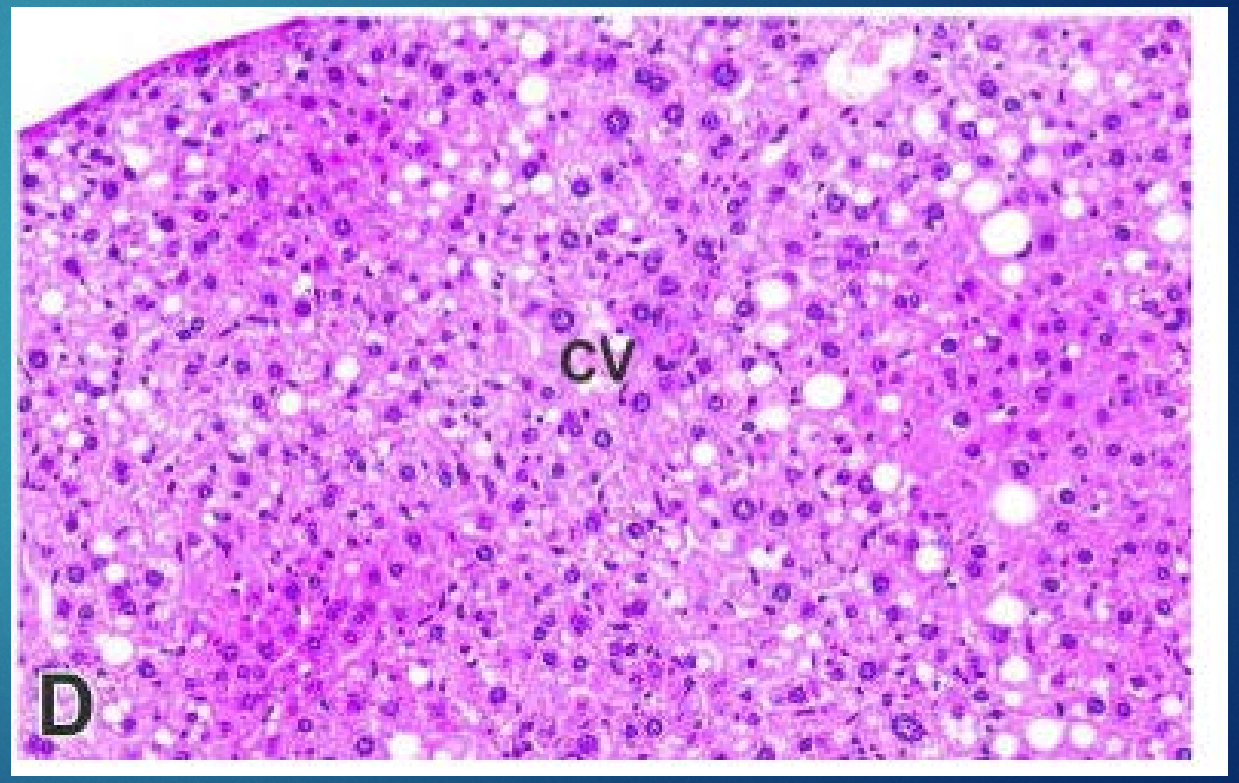
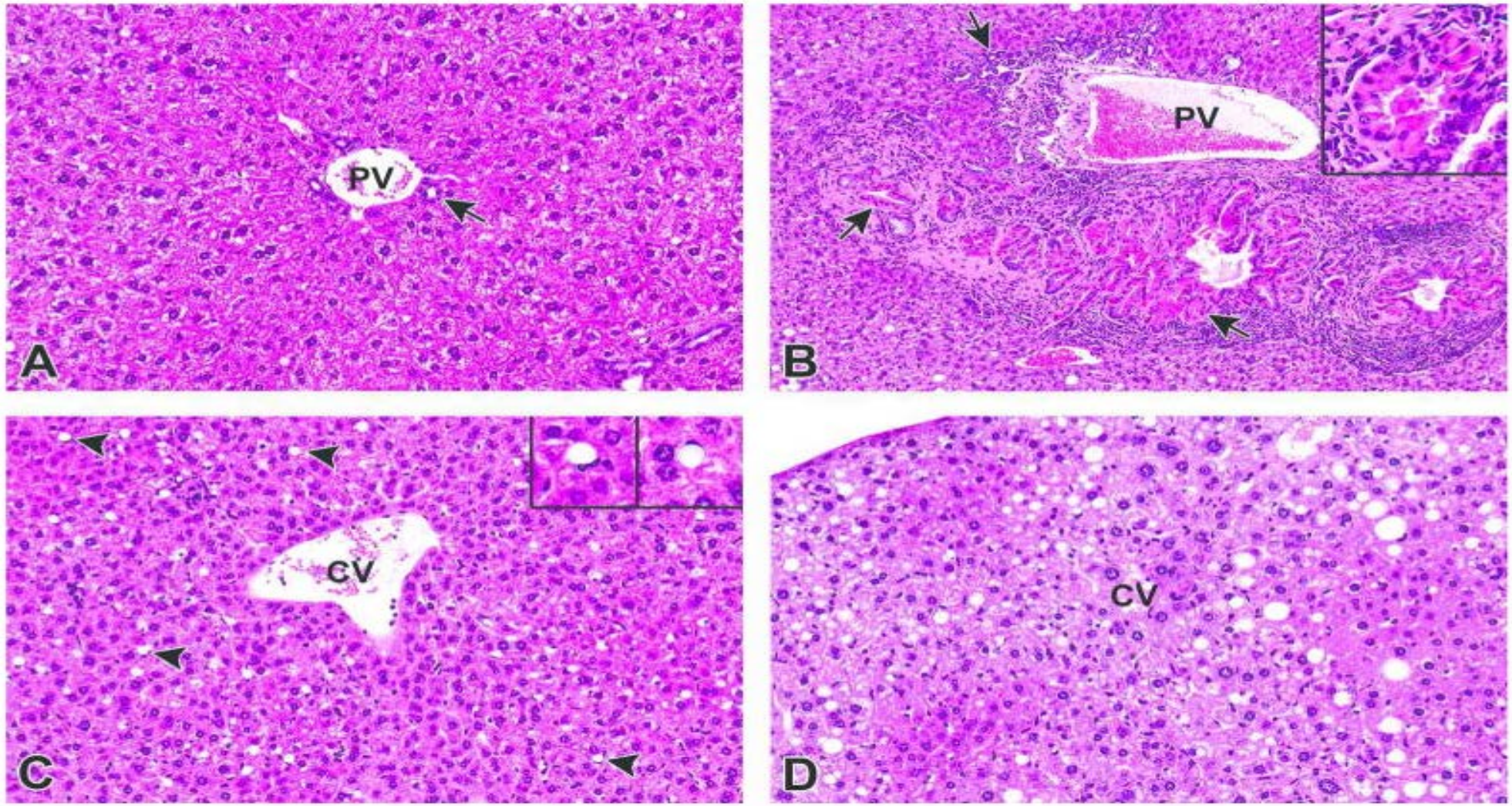


Figure is high magnification from suite of 4 (next PPT), showing the vacuolation.



Non-neoplastic hepatic lesions in PPARα-KO mice. **A**) Liver, PPARα-KO mouse, 0 mg/kg PFOA. Control liver from an untreated PPARα-KO mouse. PV = portal vein, arrow = bile duct. 40X. HE. **B**) Liver, PPARα-KO, 3.0 mg/kg PFOA. There is marked bile duct proliferation relative to the control mouse A (bile duct hyperplasia) as well as marked accumulations of homogenous, intracytoplasmic, brightly eosinophilic, globular material within biliary epithelial cells (hyaline droplet accumulation). Note the accumulation of primarily lymphocytic

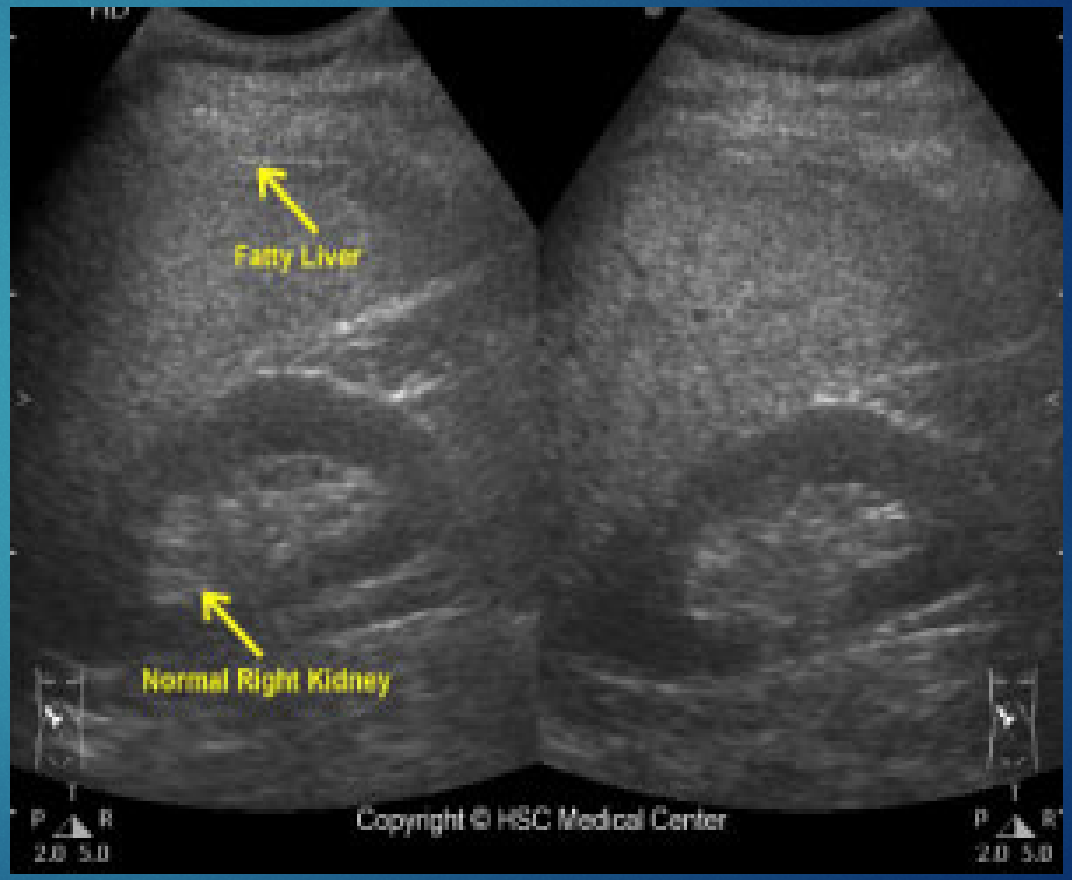
NAFLD: a challenging diagnostic problem

Simple steatosis often "benign,"but 15-25% progress. And, 80% have components of "Metabolic syndrome."

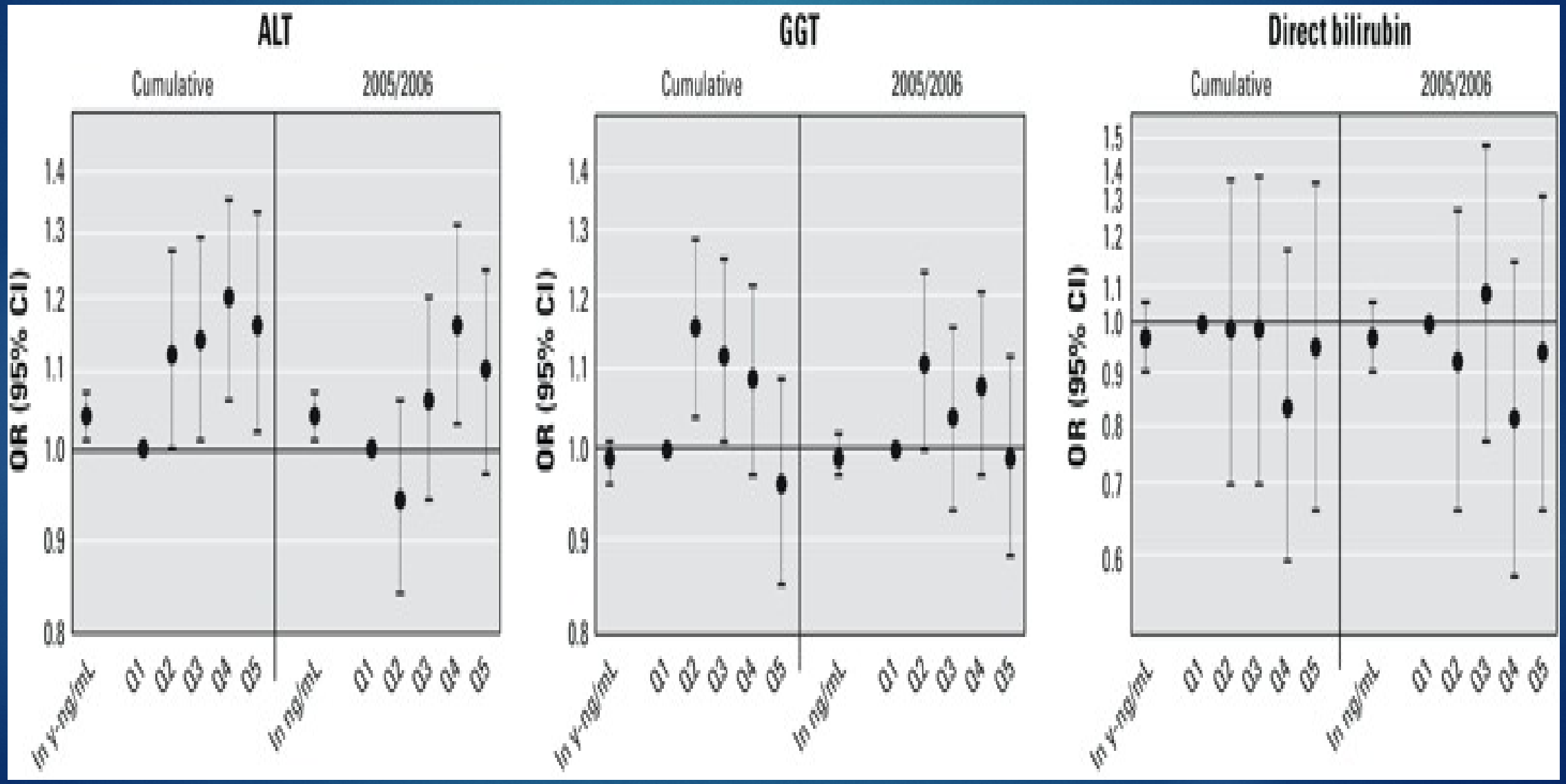
Prevalence **estimated** >> 3% all US population, > 25% of obese.

Common cause of non-infectious persistent elevated LFTs (above cut-offs), often with ALT>AST (opposite of alcohol).

However, ***Liver enzyme levels normal in a large percentage of patients with steatosis***. Detection when suspected is with ultrasound and confirmation with unenhanced CT (or biopsy depending on findings)



Odds ratios and 95% CIs for above normal ALT, GGT, and direct bilirubin per log increase and by quintile of cumulative and 2005/2006 year-specific modeled PFOA serum concentration. Darrow LA et al et al EHP 2016



PFAS and NAFLD? – in Mice (**and Men?**)

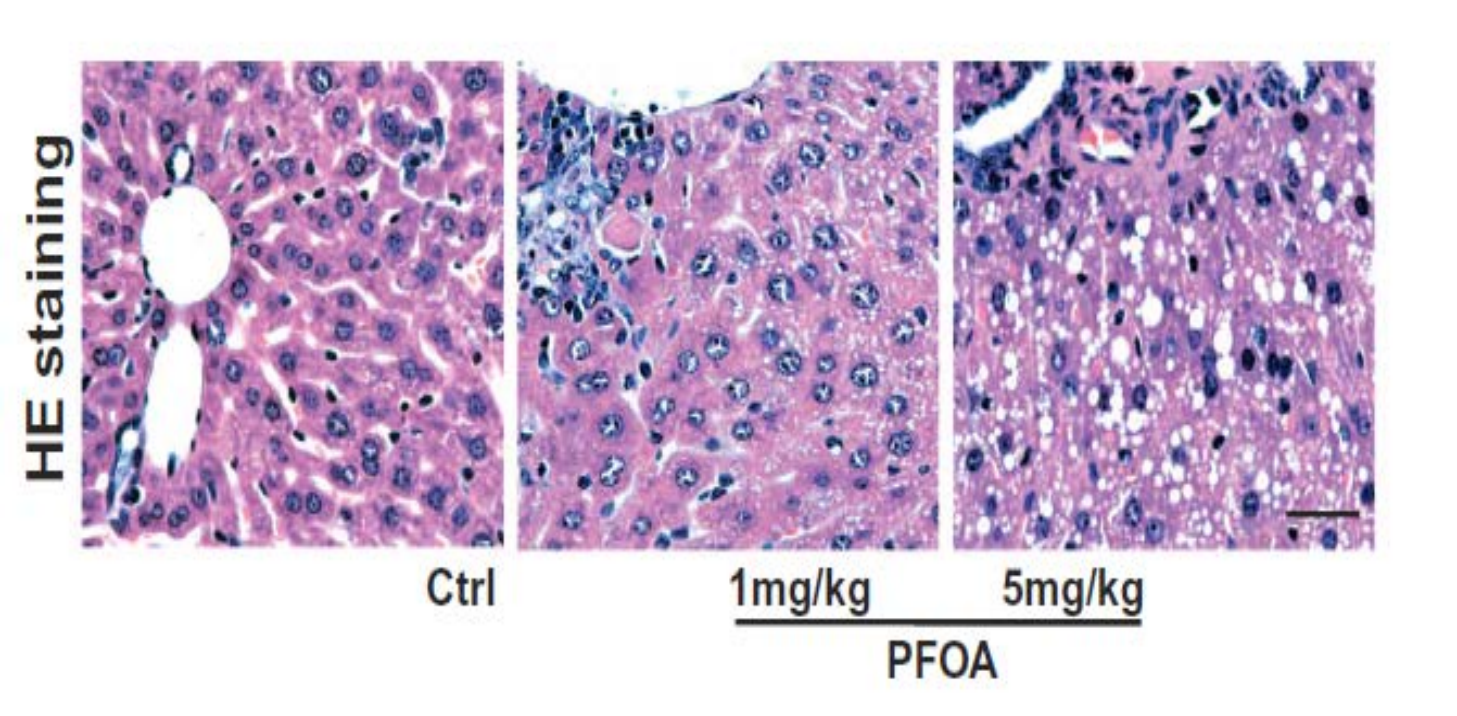
Hui Z, et al. Gene 622(2017)

PFAS stored in liver (many species)

Activate gene pathways associated with hepatocellular lipid metabolism, including down-regulation of lipid uptake-associated mRNA of apolipoprotein

Increase Liver enzymes in rodents similar to increases seen in humans

Picture shows vacuolation in hepatocyte cytoplasm and altered architecture.

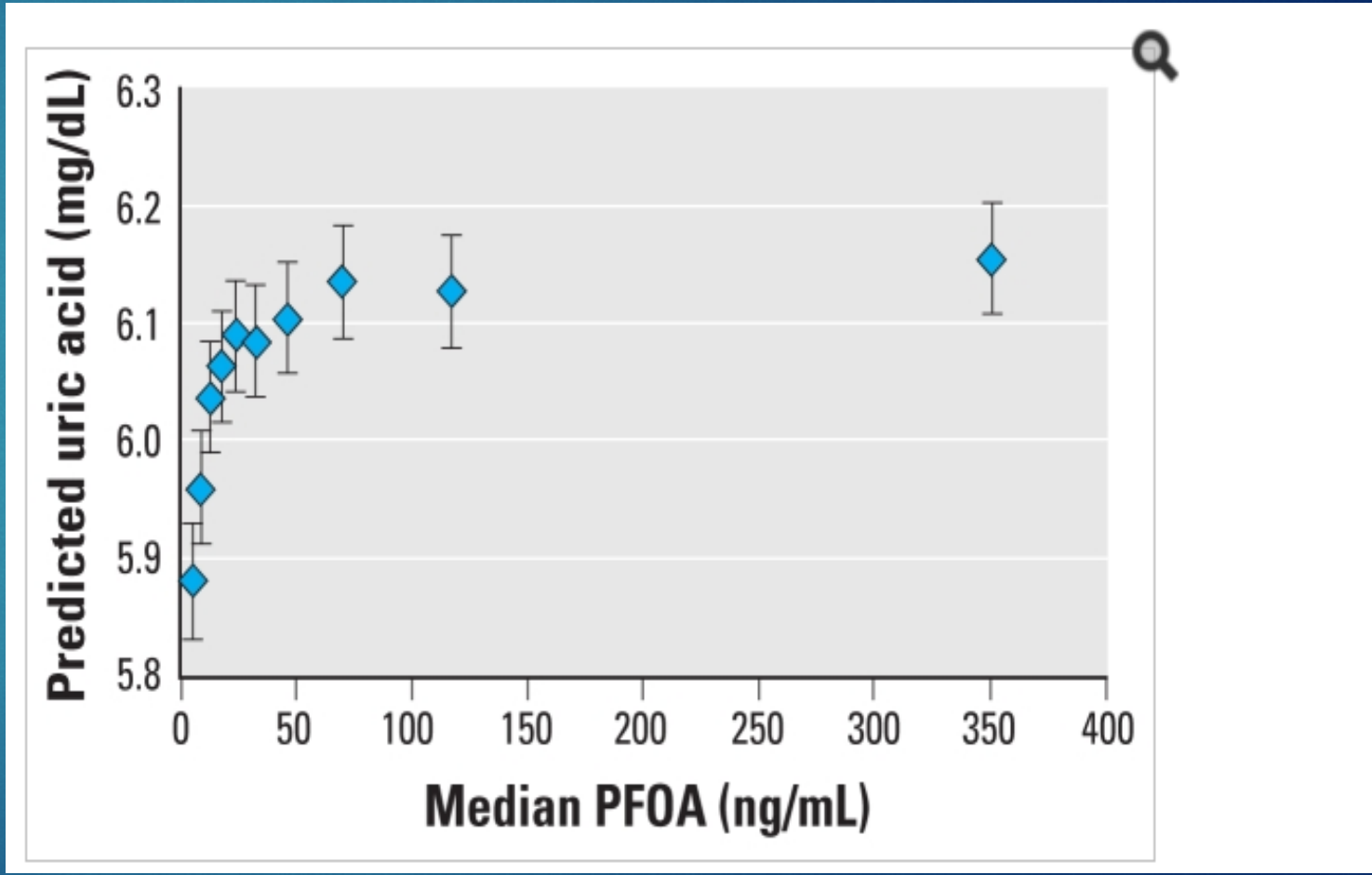


PFOA induces lipid dysmetabolism in rodent liver; involved in dysregulation of Fatty Acid trafficking.

By the Way – Uric Acid also associated with NAFLD, and with PFAS exposure

ELEVATED SERUM URIC ACID LEVELS ARE ASSOCIATED WITH NON-ALCOHOLIC FATTY LIVER DISEASE INDEPENDENTLY OF METABOLIC SYNDROME FEATURES IN THE UNITED STATES: (LIVER ULTRASOUND DATA FROM THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY)

Figure : regression model from **Steenland K** et al, **EHP** 2010 118 (2010),in nonsmoking, nondrinking C8 study with, 95% CI bars. There are multiple confirmatory studies, including other PFAS. (PFOS similar but less steep.)



So, how can we Screen for NAFLD in populations?

- **Liver functions** – easy. Good for effects, weak for diseases.
- Population **biomarker**s such as caspace-cleaved cytokeratin M30 and M65(for apoptosis) are more sensitive. There are other potential markers
- Liver ultrasound

SHOWN: Unpublished preliminary 200 random participant serums from the C8 data set, based on biomarker cutoffs by Classification and Regression Trial Analysis (CART). (A Ducatman, S Wen, J Bassler, M Cave, J Barnett)

Full model includes BMI, EGFR, EtoH, Age, Sex (SES was tested and did not interact).

Full model preliminary associations

	Beta		p	
	M30	M65	M30	M65
C8	2.1	1.46	.005	.044
C8S	0.536	0.557	.18	.15
C6S	0.889	0.966	.042	.022
C9	0.567	0.254	.031	.320

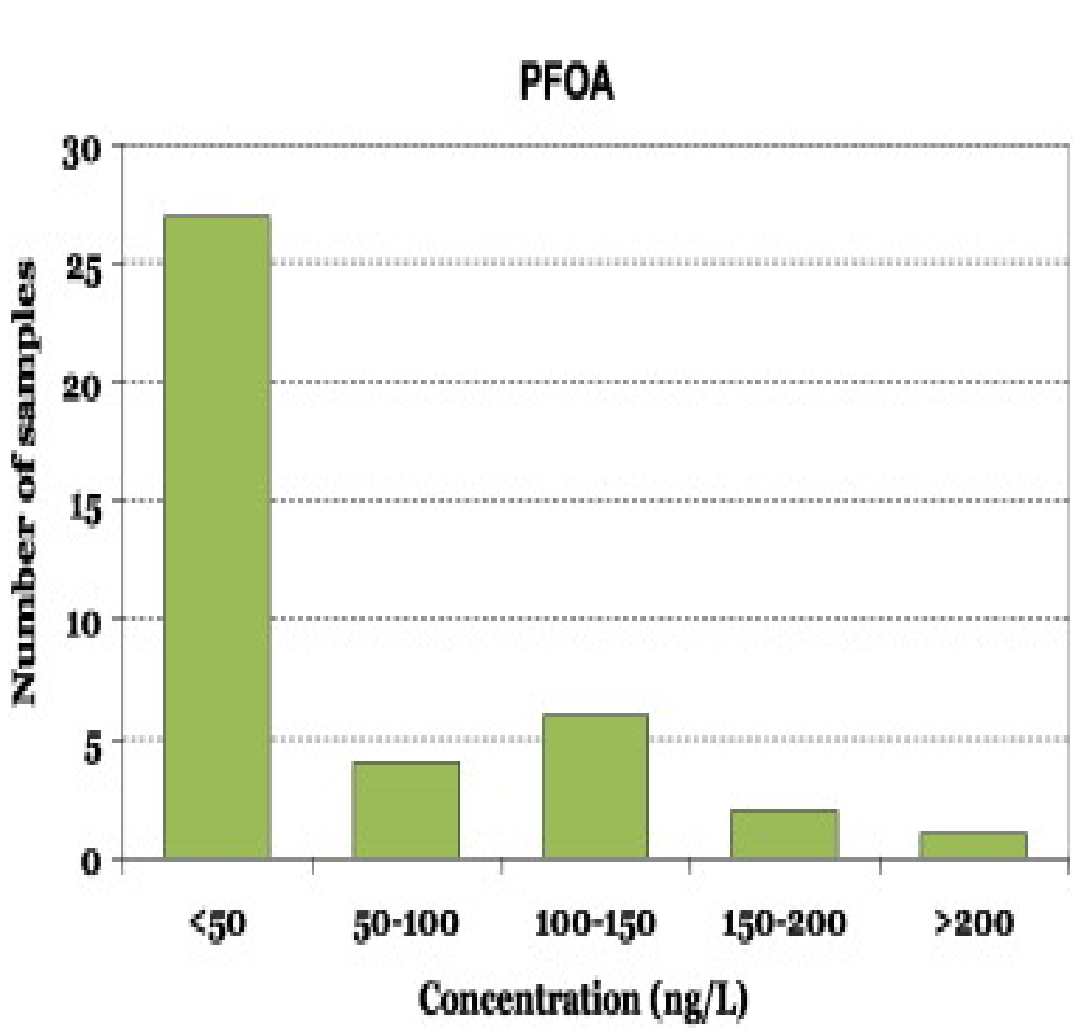
Not a pivot: Developing humans –breast milk: (PFOA in 67 nursing mothers, Spain)

Fish consumption associated with higher concentration in adults, and in colostrum

For developing humans, parity and breast feeding inverse-associated with maternal serum PFAS concentrations. This is **NOT** good news.

Breast Feeding has transfer, but, likely < less than in utero

image: Guzman et al. Sci Total Environ
http://dx.doi.org/10.1016/j.scitotenv.2015.11.059



For the PFAS, we know transplacental PFAS transfer. And PFAS in breast milk.

And, aldo about lipids in children, and about lipids in children following fetal exposure

Does It matter?? What do we really know about consequences?

Developing humans: topic of greatest importance, and hardest to study.

Many but not all studies show small correlations of PFAS with birth weight, or other similar measure. This is a consensus but small relationship. Is it important? Possibly to probably.

Small association with preeclampsia, gets stronger as geocoding methods are refined. and Monte Carlo simulations done.

Figure is from first in series of articles, Stein CR, et al, An J Epi 170(2009) ,

Table 3. Crude and Adjusted[a] Associations Between Serum Perfluorinated Compounds and Preeclampsia[b] Within 5 Years of C8 Health Project Enrollment, Mid-Ohio Valley, 2000–2006

Serum PFC	PFOA Exposure (n = 1,589)				PFOS Exposure (n = 4,566)			
	No. of Cases	Crude OR	Adjusted		No. of Cases	Crude OR	Adjusted	
			OR	95% CI			OR	95% CI
Per increase from the 25th to the 75th percentile[c]	156	1.2	1.1	0.9, 1.4	407	1.2	1.1	0.9, 1.3
<50th percentile	64	1.0	1.0		280	1.0	1.0	
≥50th percentile	92	1.5	1.3	0.9, 1.9	127	1.5	1.3	1.1, 1.7
<50th percentile	64	1.0	1.0		163	1.0	1.0	
50th–<75th percentile	52	1.8	1.5	1.0, 2.3	117	1.4	1.3	1.0, 1.7
75th–90th percentile	27	1.3	1.2	0.7, 2.1	65	1.3	1.1	0.8, 1.6
>90th percentile	13	0.9	0.9	0.5, 1.8	62	2.0	1.6	1.2, 2.3

Abbreviations: CI, confidence interval; OR, odds ratio; PFC, perfluorinated compound; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

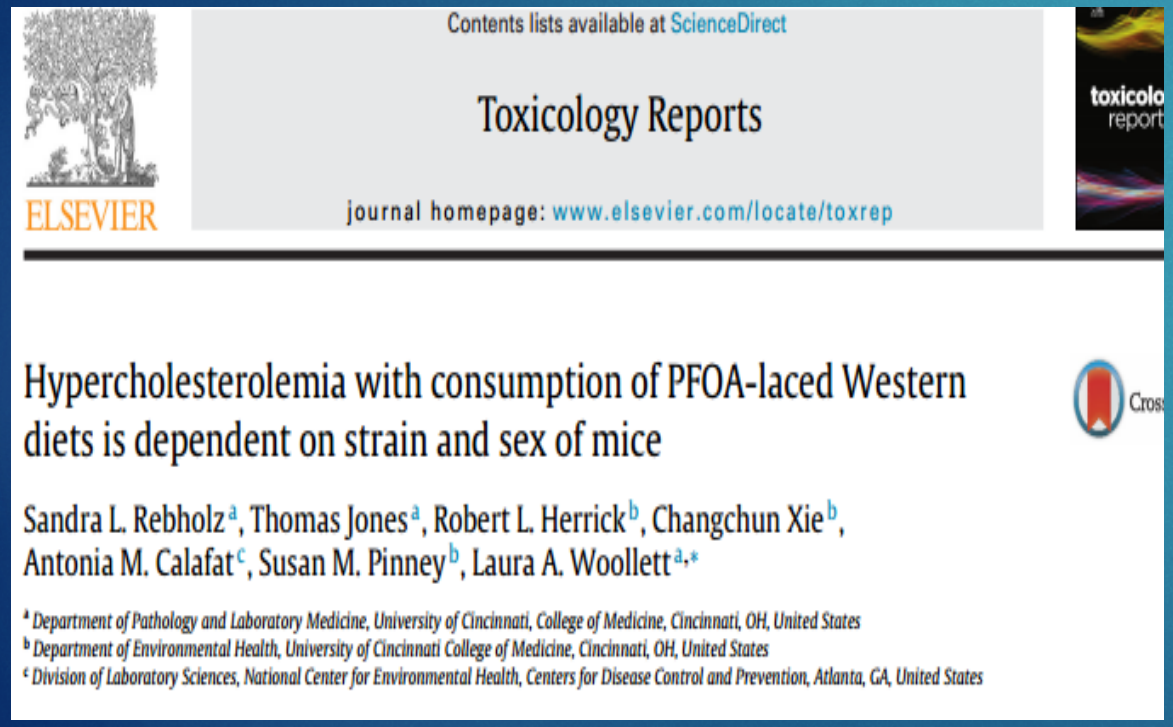
[a] Adjusted for maternal age, parity, educational level at interview, smoking status at interview, and PFOS in the analysis of PFOA and PFOA in the analysis of PFOS.

[b] Modeled by using generalized estimating equations with an exchangeable correlation structure to account for multiple pregnancies per woman.

[c] Change from the 25th to the 75th percentile corresponds to a shift from 10.3 ng/mL to 49.8 ng/mL for PFOA and from 9.0 ng/mL to 17.7 ng/mL for PFOS.

Am J Epidemiol 2009;170:837–846

Cholesterol metabolism disruption and endocrine disruption - about sterols


Contents lists available at ScienceDirect

Toxicology Reports

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

Hypercholesterolemia with consumption of PFOA-laced Western diets is dependent on strain and sex of mice

Sandra L. Rebholz[a], Thomas Jones[a], Robert L. Herrick[b], Changchun Xie[b], Antonia M. Calafat[c], Susan M. Pinney[b], Laura A. Woollett[a,*]

[a] Department of Pathology and Laboratory Medicine, University of Cincinnati, College of Medicine, Cincinnati, OH, United States
[b] Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH, United States
[c] Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, United States

- mRNA levels of genes associated with sterol input were reduced only in C57BL/6 females, the mice with the greatest increase in plasma cholesterol levels. Strain-specific PFOA-induced changes in cholesterol concentrations in mammary tissues and ovaries paralleled changes in plasma cholesterol levels…

Endocrine disruption and preeclampsia

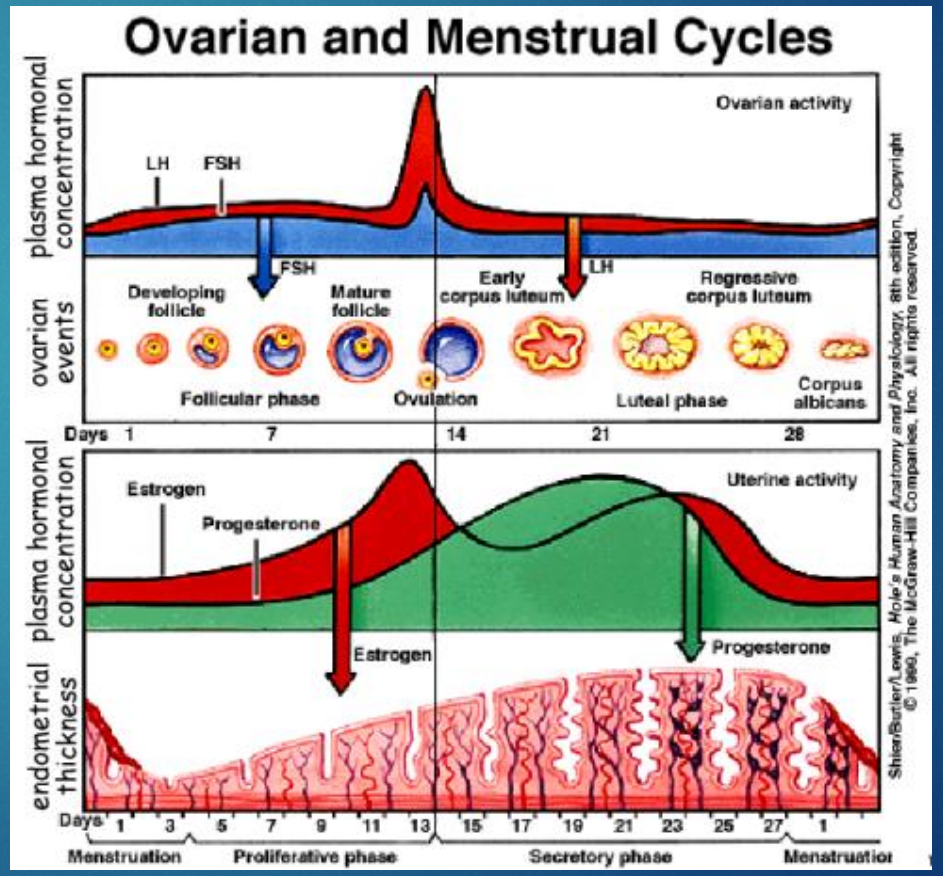
At this point, we should be worried but unsure if preeclampsia is an outcome of PFAS exposure. **We are sure that endocrine disruption occurs in preeclampsia and that sterols are affected by PFAS.**

- **Increased maternal and fetal cholesterol efflux capacity and placental CYP27A1 expression in preeclampsia**[S] J
- **Mistry HD et al J Lipid Res 58 (2017)** 10.1194/jlr.M071985. Epub 2017 Apr 10.

Female reproduction

PFOS associated with lower concentrations of ovarian hormones, E_2 (estrogen) and progesterone

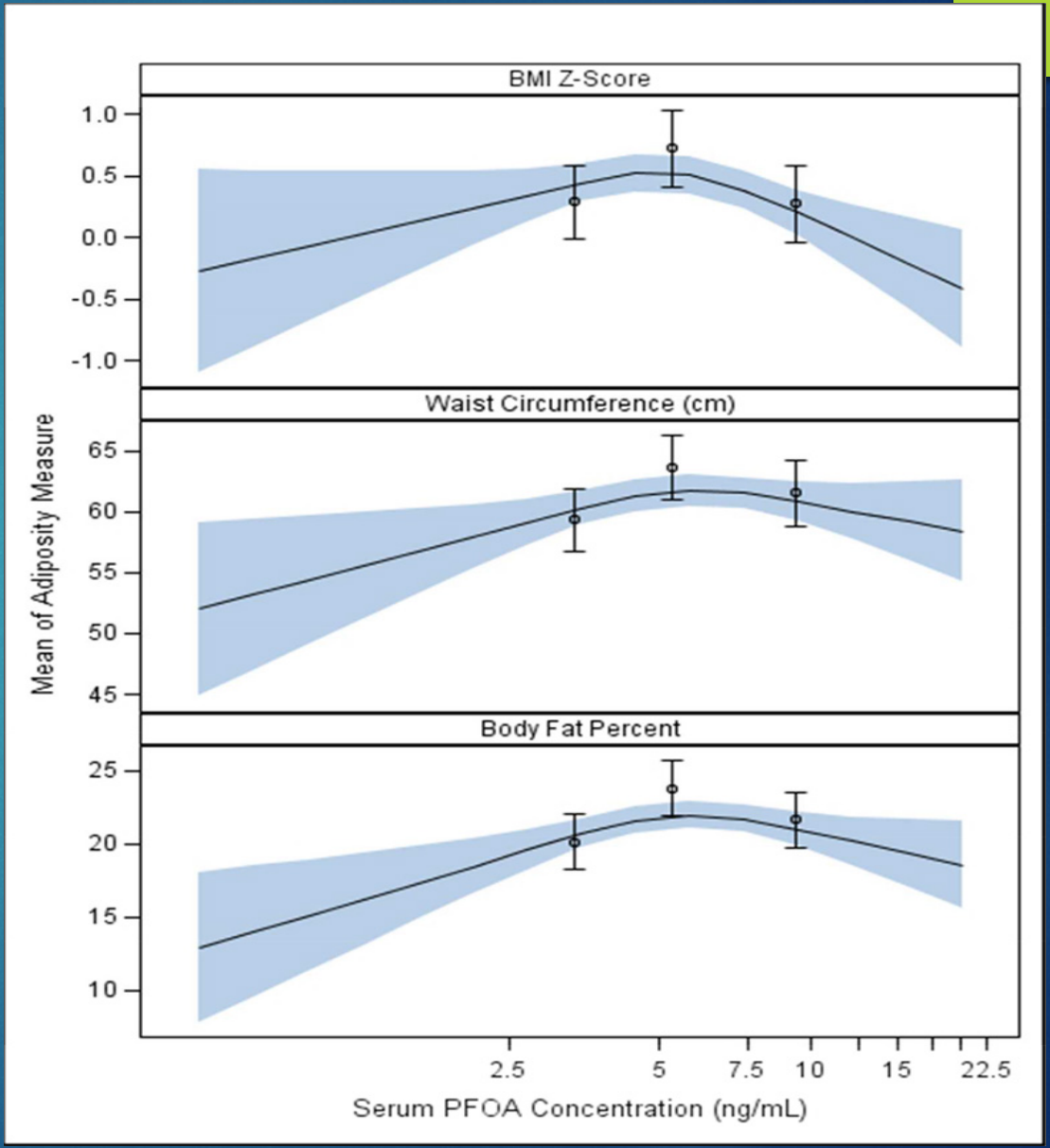
(Knox et al. 2011, Barrett et al. 2015)



NAFLD - primarily a Disease of the Obese . So what about early life exposure and adiposity?

Barry V et al Environ Res 2014 – no relationship to obesity modeled early life PFOA in serum (C8 Health Study)

But: Braun JM et al. Obesity doi: 10.1002/oby.21258. Epub 2015 Nov 11. **(see figure** from HOME study, n=204 Cinn.).



Maternal serum concentrations (2 weeks post partum) and overweight (ages 1.5 & 5)

- Faroese Island study. Unique and important – much of the PFAS exposure is from fish. Same population also part of childhood immune down-regulation studies.
- **Karlson M et al Reprod Toxicol** (doi: 10.1016/j.reprotox.2016.08.002) found a complex association across toxicants including PFAS
- Linear regression and generalised linear models assessed the associations with continuous and dichotomous BMI z-scores, respectively, at ages 18 months and/or 5 years. Maternal serum concentrations of HCB, **PFOS and PFOA** were associated with increased BMI z-scores and/or overweight risk (i.e. BMI z-score ≥ 85th WHO percentile). No clear association was found for maternal serum-PCBs, *p,p'*-DDE, PFHxS, PFNA and PFDA. In cross-sectional analyses, we observed a pattern of inverse associations between child serum-POPs and BMI z-scores at age 5, perhaps due to reverse causation that requires attention in future prospective analyses.

Increases in adiposity in girls exposed prenatally

- 1,645 pregnant women (median 9.6 weeks gestation) and their children in 2 afe groups. Median ages of measurement, 3.2 (n=1006) and 7.7 (n=876)

Mora AM et al. EHP 125 (2017) http://dx.doi.org/10.1289/EHP246.

Results are mostly borderline significant.

- **Among girls, each interquartile range increment of prenatal PFOA concentrations was associated with 0.21 kg/m2 (95% CI: –0.05, 0.48) higher body mass index, 0.76 mm (95% CI: –0.17, 1.70) higher sum of subscapular and triceps skinfold thickness, and 0.17 kg/m2 (95% CI: –0.02, 0.36) higher DXA total fat mass index in mid childhood. Similar associations were observed for PFOS, PFHxS, and PFNA. We observed null associations for boys and early-childhood adiposity measures.**

Consensus? Maybe, at least preponderance of evidence but also messy.

Among 359 girls (age 9),

%BF was higher [1.4%; 95% confidence interval (95% CI): 0.3 to 2.5] for each one unit (ng/mL) higher PFOA among girls with mothers in the middle education group, but lower (−0.6%; 95% CI: −1.12 to −0.04) for the corresponding comparison among girls with mothers with the highest education. %BF was lower (−0.2%; 95% CI: −0.3 to −0.1) for each one unit higher PFOS among girls with the most educated mothers.

Hartman TJ et al, Childhood Obesity 13 (2017)

Table 2. Regression Coefficients (β) for the Relationship between Perfluoroalkyl Substances and Measures of Body Fatness Overall[a] and by Educational Status (Low, Medium, High)

	DXA-total body fat (%) ($N = 319$)			DXA-trunk fat (%) ($N = 319$)			BMI (kg/m^2) ($N = 312$)			WC (cm) ($N = 319$)		
Analyte	β	95% CI	p	β	95% CI	p	β	95% CI	p	β	95% CI	p
PFOS (ng/mL)												
Overall[b]	−0.07	−0.16 to 0.02	0.12	−0.06	−0.12 to −0.01	**0.02**	−0.04	−0.07 to 0.00	**0.03**	−0.12	−0.20 to −0.04	**0.005**
Low education	0.02	−0.17 to 0.22	0.81	−0.05	−0.17 to 0.06	0.37	−0.01	−0.08 to 0.06	0.76	−0.03	−0.21 to 0.16	0.78
Medium	0.09	−0.08 to 0.26	0.30	0.07	−0.03 to 0.18	0.17	0.02	−0.04 to 0.08	0.60	0.04	−0.12 to 0.20	0.62
High	−0.19	−0.31 to −0.07	**0.003**	−0.13	−0.21 to −0.06	**<0.001**	−0.07	−0.11 to −0.03	**0.001**	−0.23	−0.35 to 0.12	**<0.0001**
P-interactions			**0.02**			**0.006**			0.06			**0.01**
PFOA (ng/mL)												
Overall[b]	−0.30	−0.76 to 0.16	0.20	−0.27	−0.55 to 0.00	0.05	−0.16	−0.32 to 0.00	0.05	−0.54	−0.97 to −0.11	**0.01**
Low education	−1.03	−2.35 to 0.29	0.13	−0.51	−1.31 to 0.28	0.21	−0.63	−1.10 to −0.16	**0.009**	−1.29	−2.52 to −0.07	**0.04**
Medium	1.41	0.28 to 2.54	**0.01**	0.35	−0.33 to 1.04	0.31	0.41	0.02 to 0.81	**0.04**	1.16	0.11 to 2.21	**0.03**
High	−0.58	−1.12 to −0.04	**0.03**	−0.38	−0.70 to −0.05	**0.02**	−0.22	−0.41 to −0.03	**0.02**	−0.82	−1.32 to −0.32	**0.001**
P-interactions			**0.005**			0.14			**0.003**			**0.002**

Also: Adults exposed to PFOA prenatally? Halldorsson TI et al. EHP 120 (2012) + BMI Outcomes in Women (only).

Associations[a] between in utero exposure to PFOA and the offspring BMI and waist circumference at 20 years of age for females (n = 345) and males (n = 320).

PFOA in quartiles [median (range)]b		ΔBMI [mean (95% CI)]		ΔWaist circumference [mean (95% CI)]	
		Crude	Adjusted[c]	Crude	Adjusted[c]
Females					
1	2.3 (0.1–2.8)	Referent	Referent	Referent	Referent
2	3.2 (2.8–3.7)	0.2 (–0.7, 1.2)	0.4 (–0.6, 1.3)	1.0 (–1.7, 3.7)	1.4 (–1.4, 4.2)
3	4.2 (3.7–4.8)	0.8 (–0.2, 1.8)	0.9 (–0.1, 1.9)	0.9 (–1.9, 3.6)	1.2 (–1.7, 4.0)
4	5.8 (4.8–19.8)	1.6 (0.6, 2.5)	1.6 (0.6, 2.6)	4.2 (1.5, 6.9)	4.3 (1.4, 7.3)
p-Value for trendd		0.0007	0.001	0.005	0.006
Malese					
1	2.4 (1.2–2.8)	Referent	Referent	Referent	Referent
2	3.3 (2.8–3.7)	0.5 (–0.4, 1.4)	0.6 (–0.3, 1.5)	1.3 (–1.7, 4.3)	1.3 (–1.5, 4.1)
3	4.2 (3.7–4.8)	0.3 (–0.7, 1.2)	0.2 (–0.7, 1.1)	1.0 (–2.0, 4.0)	1.0 (–1.9, 3.8)
4	5.8 (4.8–16.6)	0.4 (–0.5, 1.3)	0.6 (–0.3, 1.5)	0.7 (–2.2, 3.6)	1.3 (–1.6, 4.1)
p-Value for trendd		0.47	0.30	0.72	0.48

Not quite done

Other examples of childhood development?

- TTP and sperm morphology?
- **immune downregulation** asthma/allergy/infection/vaccine/lung function
- **thyroid/endocrine**
- neurospsychologic measures including ADHD/behavior, communication
- uric acid

No associations found:

- preconception exposure &incident pregnancy loss
- prenatal exposure & testosterone in girls at age 15

Even a Large Ham can be cured.

Finish Line: Don’t Forget

Health Professionals and Communities , what can inhabitants, patients, doctors, and communities do?

3:30 PM tomorrow, about what patients and clinicians can do together.

Preview: versatile clinician applying PFAS treatment

Appreciation for presentation support: Heather Henderson, MA.

Outline

1. Endocrine Disrupting Chemicals (EDCs) - the basics
2. Perfluoroalkyl substances (PFAS) - in the body
3. Non-Cancer Endpoints
   - Cholesterol, pre-eclampsia and NAFLD
   - Fetal exposures and outcomes

   Dr. Ducatman

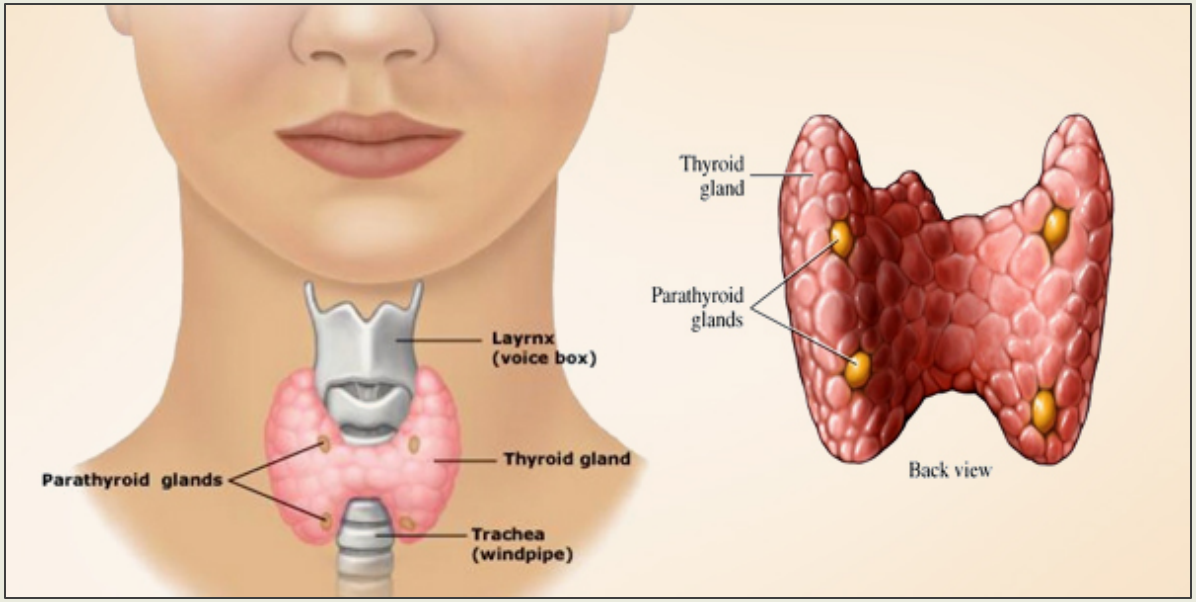
   - Thyroid
   - Neurodevelopment
   - Immune function
   - Mammary gland development

   Dr. Carignan

4. Early indications of toxicity and harm
5. Cancer Endpoints

Dr. Clapp

The Thyroid



Regulates…

- Metabolism & lipid homeostasis
- Respiratory, cardiovascular, nervous, & reproductive systems (Choksi, 2003)
- Growth/neurodevelopment (de Escobar, 2004)

Slide from Emma Preston

Thyroid Function

Complex system of feedback loops

Hypothalamus:

- Thyrotropin releasing hormone (TRH)

Anterior Pituitary:

- Thyroid stimulating hormone (TSH)

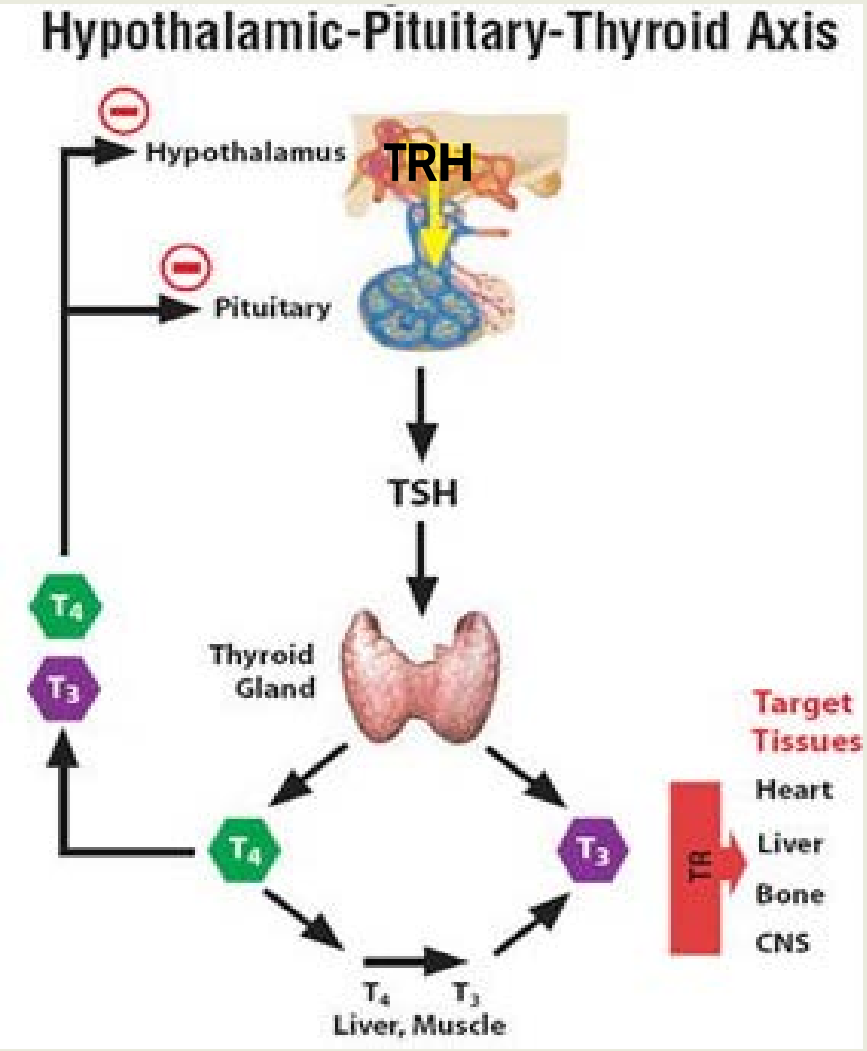
Thyroid gland:

- Thyroxine (T_4)
- Triiodothyronine (T_3)

  **Iodine*

- Free & protein bound
  - Thyroxine-binding globulin (TBG), transthyretin (TTR), albumin

TSH inversely related to T_4 & T_3



Slide from Emma Preston

Clinical Disease

<u>Hypothyroidism:</u> ↓ T_4 ↑ TSH

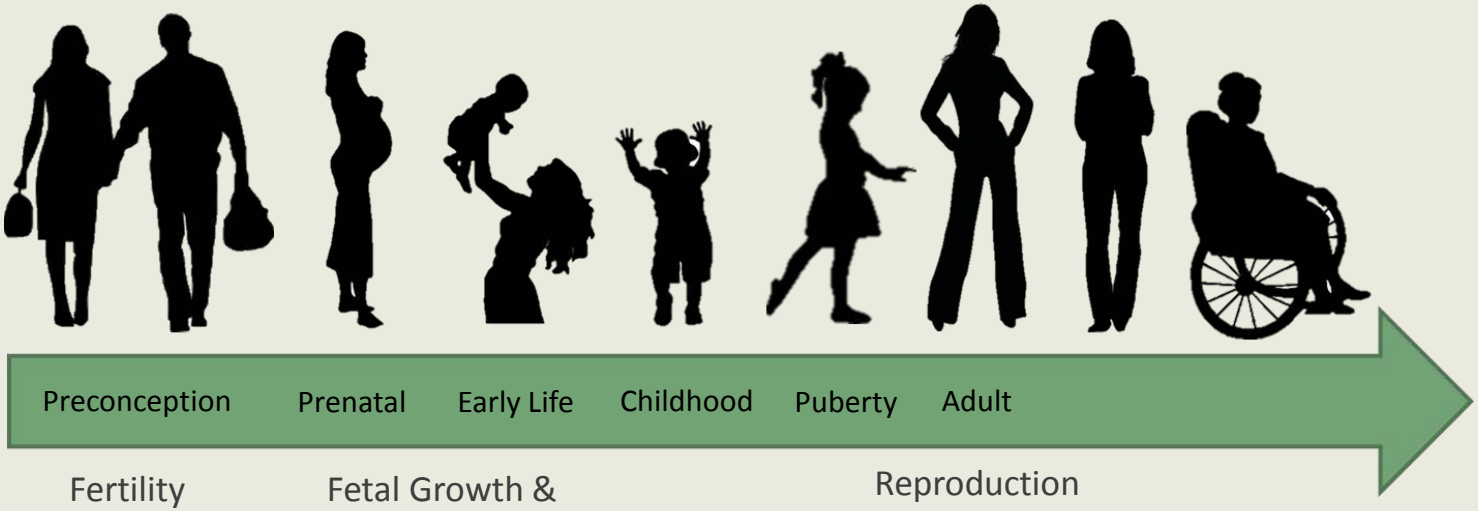
- Fatigue, weight gain, depression, etc.
- >3 million US cases/year

<u>Hyperthyroidism:</u> ↑ T_4 ↓ TSH

- Weight loss, anxiety, tachycardia, etc.

Slide from Emma Preston

Subclinical Thyroid Dysfunction



Fertility

Fetal Growth &
Neurodevelopment

Reproduction
Cognition
Cardiac

Slide from Emma Preston

de Escobar, 2004; Spyridoula, 2016

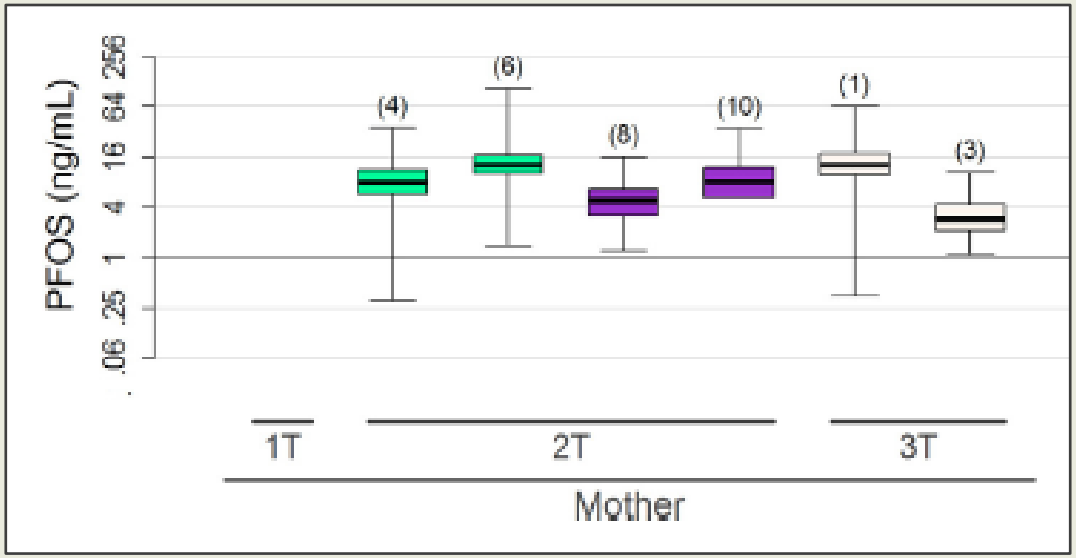
Review of Human Studies

- Systematic review (PRISMA Statement)
- Chose studies if investigated associations of TSH, T_3, T_4 or thyroid dysfunctions with PFHxS, PFOA, PFOS and/or PFNA
- Pregnant women and children up to 19 years old

Ballesteros et al. *Environ Int* (2016)

Review of Human Studies

Positive association of PFOS and TSH

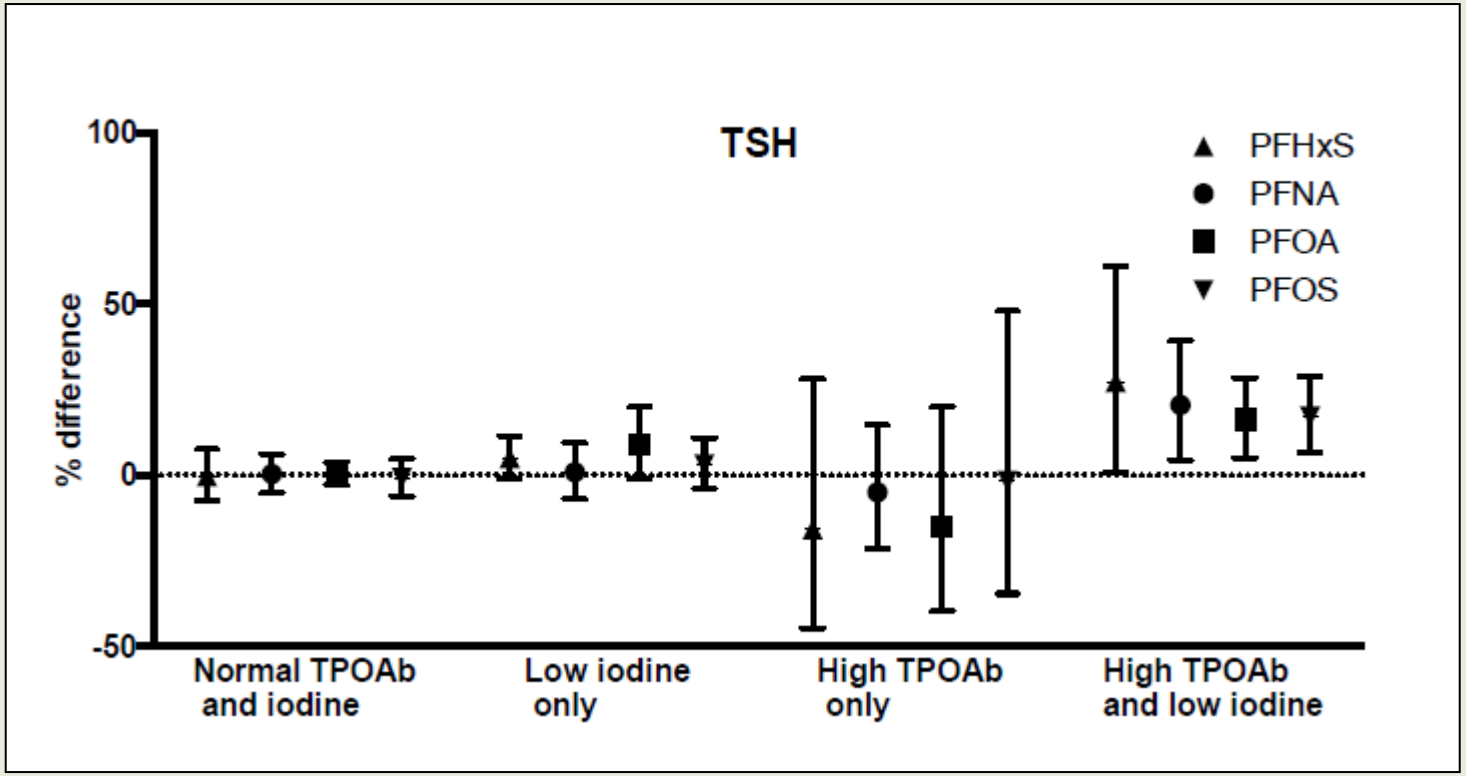


Ballesteros et al. *Environ Int* (2016)

Subpopulation

Stronger effect in subgroup with low iodine status and high TPOAb

(TPOAb is a marker of autoimmune hypothyroidism)



Webster et al *Environ Health Perpect* (2015)

Implications

- Thyroid hormone is especially important for brain maturation and development
- Thus thyroid disruption is a potential mediator for neurodevelopmental toxicity

Neurodevelopment

- Higher serum PFAS concentrations were associated with parent-reported behavioral problems:
  - Hyperactivity, peer relationship, and conduct problems
  - Internalizing and externalizing problems
  - Autism screening composite scores
- Related to post-natal, but not prenatal, exposure
- Adverse effects in girls and null or positive effects in boys

Oulhote et al. *Environ Int* (2016)

Impacts on Immune Function

National Toxicology Program (2016):

"Presumed immune hazard to humans"

- PFOA and PFOS: Antibody response suppression in animals and humans
- PFOA: Reduced infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence in humans
- PFOS: Suppresses disease resistance and natural killer cell activity

National Toxicology Program Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid (PFOA) or Perfluorooctane Sulfonate (PFOS) (September 2016)

Reduced Infectious Disease Resistance

PFAS in Children (Norway)

Children whose blood had higher PFAS levels:

produced fewer antibodies to rubella vaccination at 3 years of age &

had increased frequencies of the common cold and gastroenteritis

Granum et al. *J Immunotoxicol* (2013)

Antibody Response Suppression

Children with higher blood levels of PFAS produce fewer antibodies after vaccination for diphtheria and tetanus (DTaP)

Morgensen et al. 2015

VACCINATION USED AS A MODEL OF IMMUNE FUNCTION

Extrapolation suggests drinking water standard closer to 1 ppt

Grandjean and Clapp *New Solutions* (2015)

Delayed Mammary Gland Development

- Occurs at **much lower doses** than most other developmental effects.
  - Most sensitive endpoint with data for dose-response modeling.
- Conclusions of detailed evaluation: **adverse** and **relevant to humans**.
- Reported in **nine separate studies** from perinatal (fetal or neonatal) exposure to mice.
  - Reported in dams and female offspring, in two strains of mice, and from gestational and/or lactational exposure.
  - Not found in one study with problematic issues.
  - **Structural changes that persist until adulthood**.
- Effects differ with lifestage (perinatal v. peripubertal exposure).
- Insufficient toxicology data to make conclusions about effects on lactational function.
  - Possibly relevant – several humans studies associated PFOA with decreased duration of breastfeeding.

NJ DWQI, 2016

ATSDR Physician Fact Sheet

Questions Patients May Ask	Key Patient Messages	Key Message Supporting Facts
		1-888-SAFEFOOD (1-888-723-3366).
Could my health problems be caused by PFAS exposure? **(Based on the health problems the patient has, there are two possible responses to this question.)** **(a)** If the patient's health problem is in the list below, it may potentially be associated with PFAS exposure, based on limited evidence from human studies. The potential health effects include: - Thyroid function (potential to affect T_4 and TSH levels) - High cholesterol - Ulcerative colitis - Testicular cancer - Kidney cancer - Pregnancy-induced hypertension - Elevated liver enzymes - High uric acid **(b)** If the patient's health problem is not in the bulleted list above, then there is no current evidence that it is related to PFAS exposure. (However, research is ongoing and not all health outcomes have been adequately studied.)	**(a)** Although the evidence is not conclusive, your health problem could potentially be associated with exposure to PFAS. However, health effects can be caused by many different factors, and there is no way to know if PFAS exposure has caused your health problem or made it worse. **(b)** Based on what we know at this time, there is no reason to think your health problem is associated with exposure to PFAS.	For supporting facts on the listed health effects in this question **(a)**, see **"How can PFAS potentially affect human health."** The information on potential illnesses and health effects will be briefly reviewed for each of these illnesses or health effects. This information can be found in this fact sheet on page 3 and 4. If your patient presents with health concerns that might be associated with PFAS exposure, it is appropriate to discuss the patient's concerns and perform a thorough health and exposure history and also a physical exam relative to any symptoms reported.

https://www.atsdr.cdc.gov/pfc/docs/pfas_clinician_fact_sheet_508.pdf

C8 Health Study Medical Monitoring

You can see which screening protocols are recommended for you in the following table:

SCREENING BY AGE	
< 15 years	– High cholesterol – Thyroid disease (at parents' discretion) – Testicular cancer (exam not part of Program, but done as regular care)
15-18 years	– High cholesterol – Thyroid disease (at parents' discretion) – Ulcerative colitis – Testicular cancer
18-19 years	– High cholesterol – Thyroid disease – Ulcerative colitis – Testicular cancer
20 or older years	– High cholesterol – Thyroid disease – Ulcerative colitis – Testicular cancer – Kidney cancer
Pregnant Females	– Blood pressure & urine protein should be measured at each prenatal visit – these tests are part of standard prenatal care and may not be reimbursed by the Program. **Pregnant women may receive blood pressure monitoring devices provided by the Program.**

http://www.c-8medicalmonitoringprogram.com/docs/med_panel_education_doc.pdf

Routine Physical

- Cholesterol
- Thyroid
- Iodine sufficiency
- Vitamin D sufficiency
- Kidney function
- Reproductive cancers

Outline

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   Dr. Ducatman

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   - Mammary gland development

   Dr. Carignan

4. Early indications of toxicity and harm
5. Cancer Endpoints

Dr. Clapp

PFAS: Emerging knowledge about cancer risks

Richard W. Clapp, D.Sc., MPH
B.U. School of Public Health
U.Mass.- Lowell

Outline

- Early indications of toxicity
- Indications of harm in exposed workers
- Class action lawsuit and C8 Health Study
- Emerging insights about risks
  - See Grandjean P and Clapp R. Perfluorinated Alkyl Substances: Emerging Insights Into Health Risks. *New Solutions:A Journal of Environmental and Occupational Health Policy*. Vol. 25(2):147-163, 2015.

Early toxicology

- Unpublished monkey study reported immunotoxic effects of PFAS in 1978
  - Goldenthal, et al., 1978
- Animal toxicity studies published in 1980
  - Griffith and Long, JAIHA, 1980
- Carcinogenicity of PFOA in rodents reported in studies published in 1980s and 1990s
  - Cook, et al. TAP, 1992 reported Leydig cell tumors

Early studies of workers

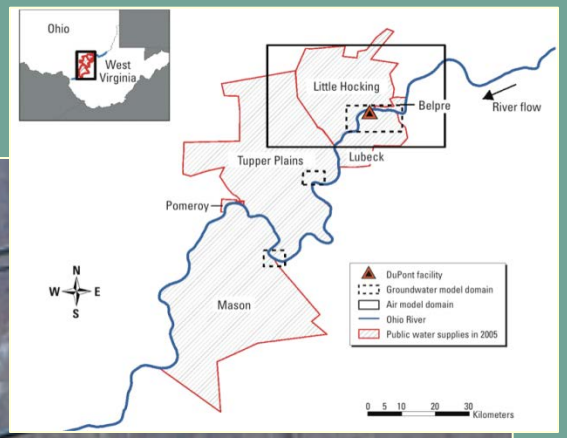
- 3M PFOA worker mortality study (1993) reported 3-fold excess prostate cancer with more than 10 years employment
- Subsequent 3M PFOS worker mortality study (2003) reported excess bladder cancer with high exposure jobs
- Internal DuPont cancer registry showed excess kidney cancer

More recent PFAS worker studies

- DuPont Parkersburg, WV workers study (2008) reported slight excess kidney cancer mortality [SMR=152 (95% CI: 78-265)]
- 3M PFOA workers follow-up study (2009) reported increased prostate cancer with moderate to high exposure, plus suggestive increase in cerebrovascular disease deaths.

C8 Health Study

DuPont Washington Works Facility near Parkersburg, West Virginia



C8 Health Panel Studies

- Class action lawsuit settlement in 2005 established epidemiologic panel and collected blood and exposure histories on ~70,000 residents and workers near Parkersburg, WVA
- Large number of community-based and occupational studies published beginning in 2009 (see c8sciencepanel.org)

C8 Health Panel (cont.)

- “Probable links” found to high cholesterol, thyroid disease, pre-eclampsia, ulcerative colitis, kidney and testicular cancer.
- Plaintiffs in 2005 agreement can now bring claims for these outcomes in Court. First three cancer cases resulted in a verdicts for plaintiffs. Settlement announced in 2017.

WVa/OH community risks of cancer

(95% confidence interval)

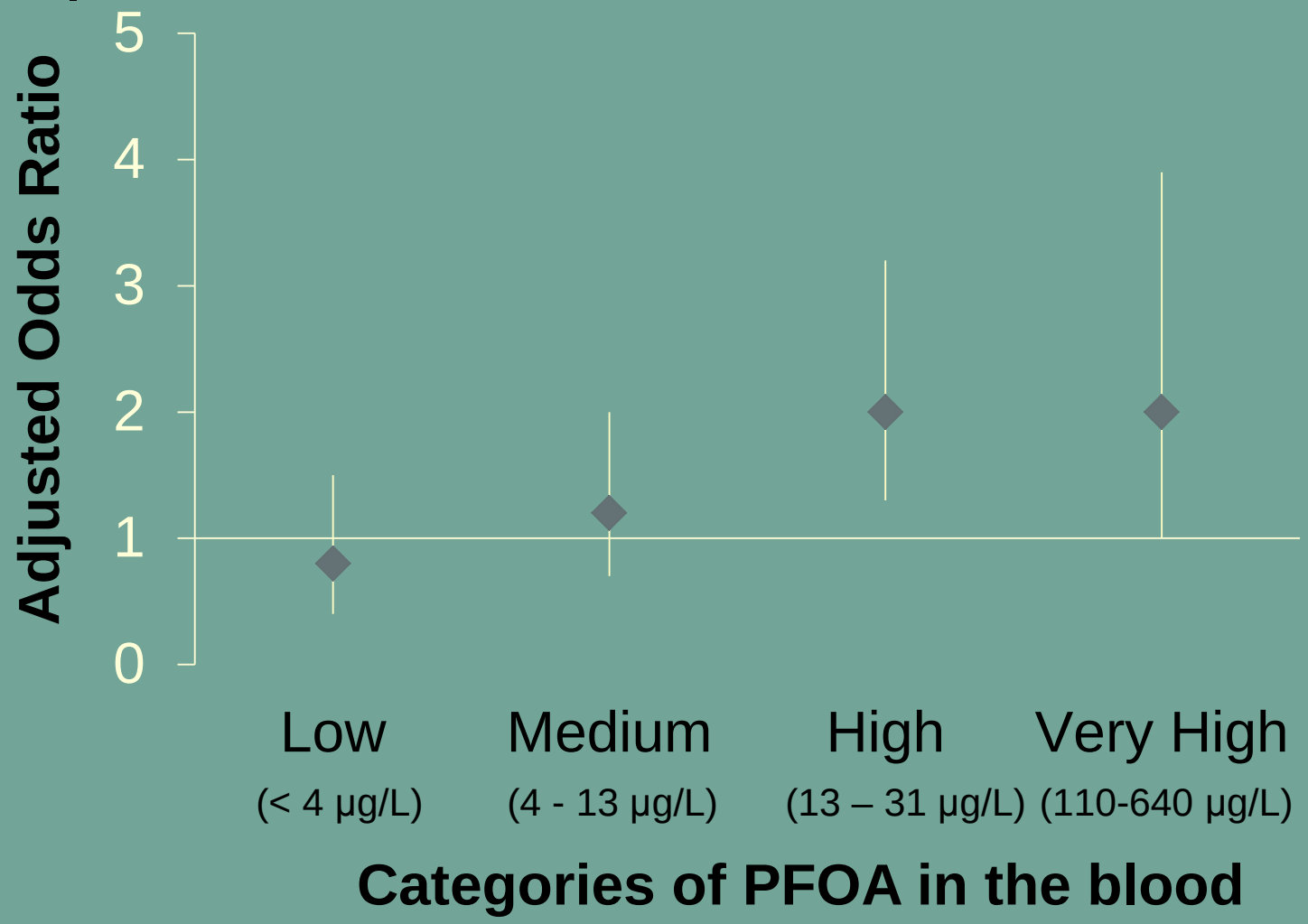
Cancer	Low	Medium	High	Very High
Brain	1.5 (0.8, 2.7)	1.8 (1.1, 3.2)	0.6 (0.2, 1.6)	—
Female breast	0.9 (0.7, 1.2)	1.1 (0.8, 1.5)	0.7 (0.5, 1.0)	1.4 (0.9, 2.3)
Kidney	0.8 (0.4, 1.5)	1.2 (0.7, 2.0)	2.0 (1.3, 3.2)	2.0 (1.0, 3.9)
Non-Hodgkin lymphoma	1.0 (0.6, 1.6)	1.5 (1.0, 2.2)	1.1 (0.7, 1.9)	1.8 (1.0, 3.4)
Ovary	0.5 (0.2, 1.4)	1.4 (0.7, 2.7)	1.4 (0.7, 2.9)	2.1 (0.8, 5.5)
Prostate	1.1 (0.8, 1.5)	0.8 (0.6, 1.0)	0.8 (0.5, 1.1)	1.5 (0.9, 2.5)
Testis	0.2 (0.0, 1.6)	0.6 (0.2, 2.2)	0.3 (0.0, 2.7)	2.8 (0.8, 9.2)

Adapted from Vieira et al. 2013

Wide confidence intervals are because of small numbers of cases

Slide from Dr. Carignan

Increasing risks of kidney cancer



Slide from Dr. Carignan

Adapted from Vieira et al. 2013

IARC Possible Carcinogen (2B)

Based on *limited evidence* in human and animal studies.

Testicular cancer
- 2 human studies
- 2 rat studies

Kidney cancer
- 4 human studies

Liver cancer
- 2 rat studies
- 2 studies of rainbow trout

Pancreatic cancer
- 1 rat study, male only

IARC Monographs, 2016 Slide from Dr. Carignan

Conclusions

- TSCA (1976) did not require testing of PFASs already in commerce.
- Toxicity was demonstrated in experimental animals in late 1970s and 1980s, and carcinogenicity in 1990s.
- Early epidemiologic indications, including carcinogenicity, not followed up until 2000s.
- PFOA now considered ”possible” carcinogen; PFOS ”possible.”