

POPs, Birmingham  
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HYGIENE  
& TROPICAL  
MEDICINE



# **Diseases linked to PFOA exposure: Epidemiology and biomarkers of PFOA**

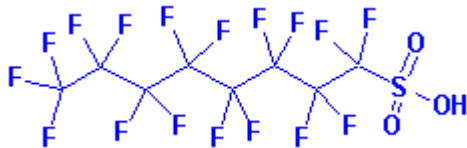
*Tony Fletcher,  
SEHR, LSHTM*

# Outline

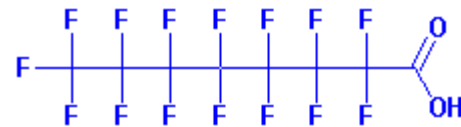
- Background PFOA exposure trends
- The C8 Study of PFOA
- Modelling exposure in the community
- Key findings: links of PFOA to disease
- Examples of the potential for PFAA biomarkers studies to give misleading results

# Background - Perfluoroalkyl acids

- Synthetic fluorinated compounds used predominantly as surfactants (both water and oil resistant)
- Many consumer and industrial applications...cleanings, coatings, greaseproofing of food containers, lubricants, fire-fighting foams, insecticides... PTFE manufacture
- C8 (PFOA and PFOS) compounds are most abundant



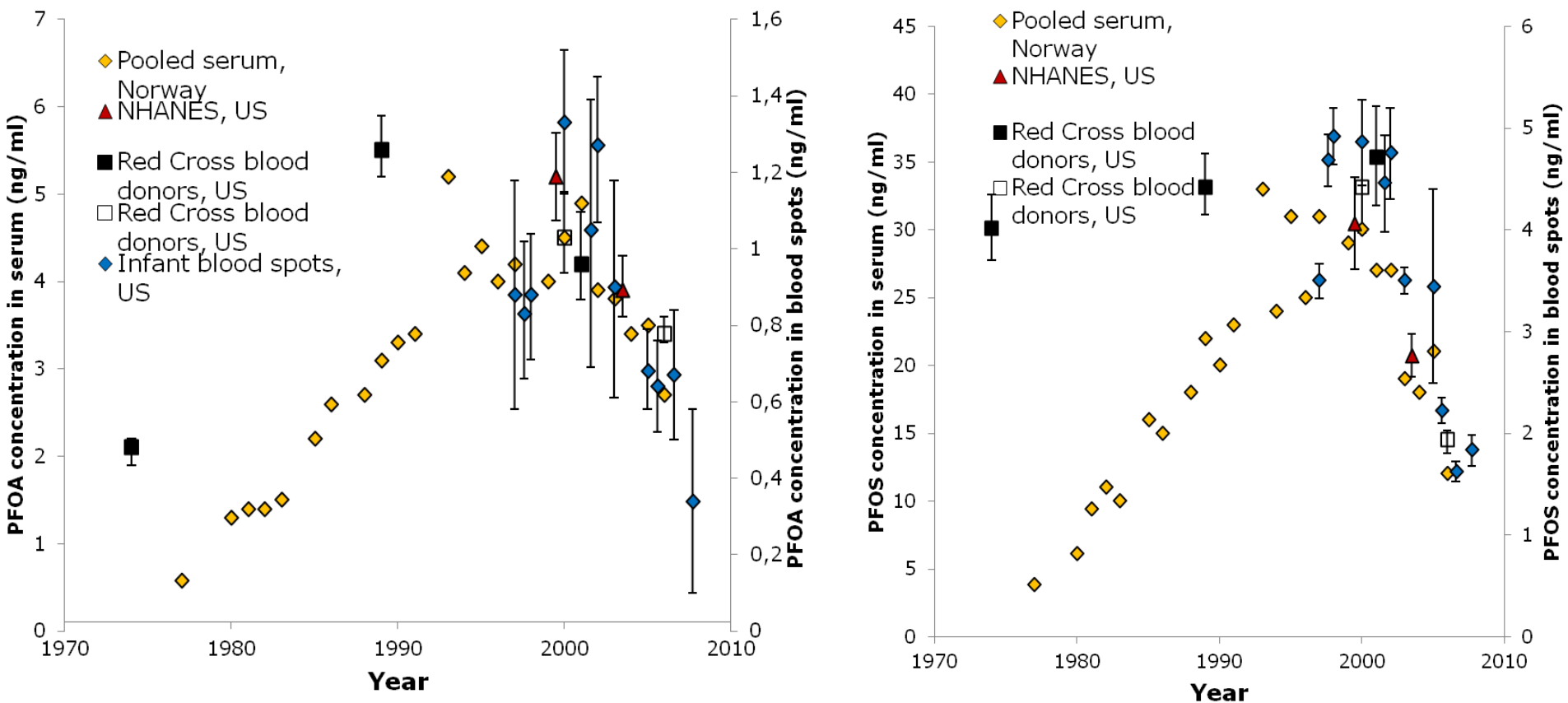
Perfluorooctane Sulphonate  
(PFOS)



Perfluorooctanoic Acid (PFOA)

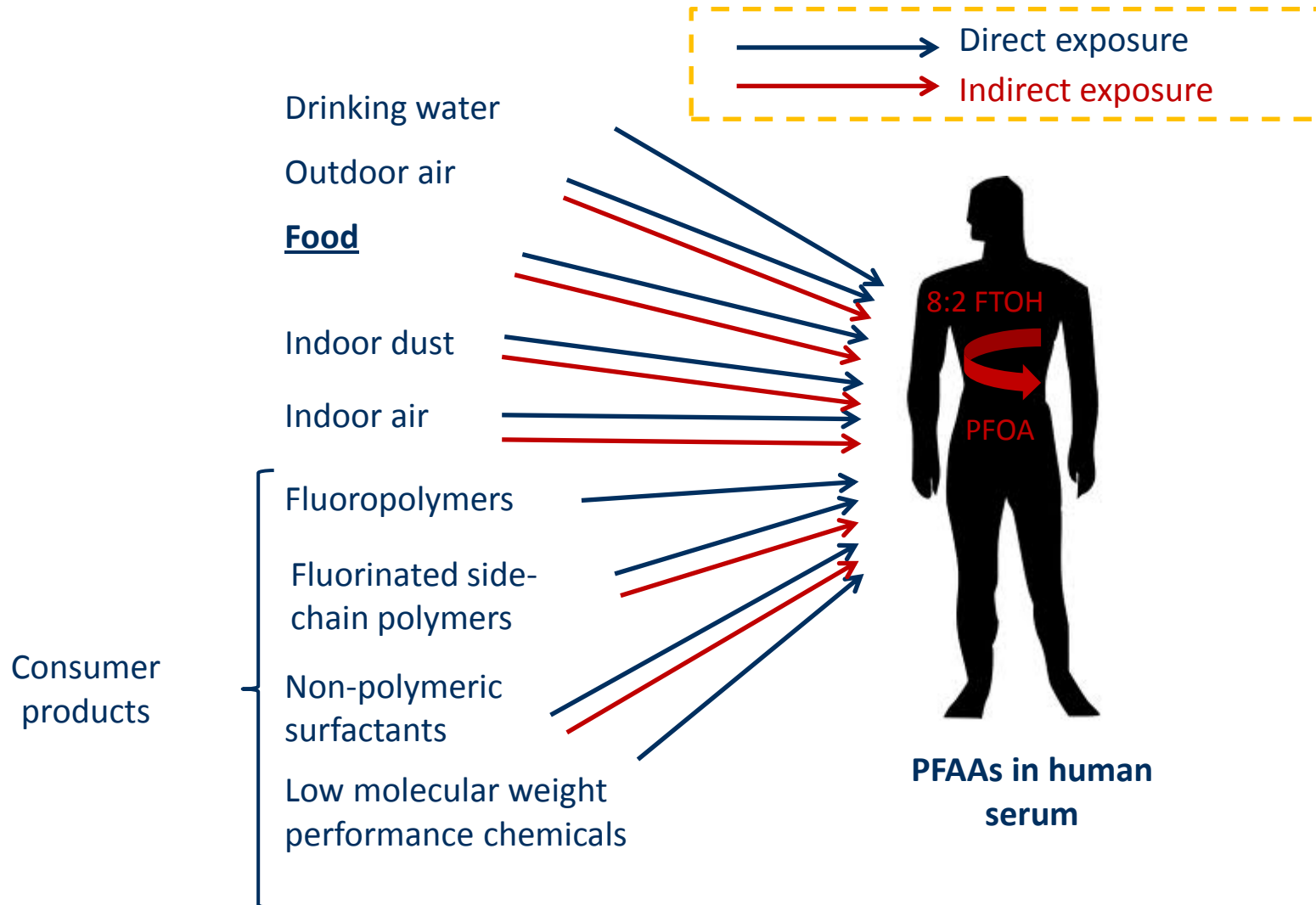
- PFOA (and PFOS) has long half life in humans (mean 2-4 years).
- Serum levels offer good stable biomarker of body burden
- PFOA serum levels have been associated with numerous health effects
- Many published studies show cross sectional associations of serum PFOA and a clinical marker or disease state

# Temporal trends for PFOA and PFOS in human serum – general populations



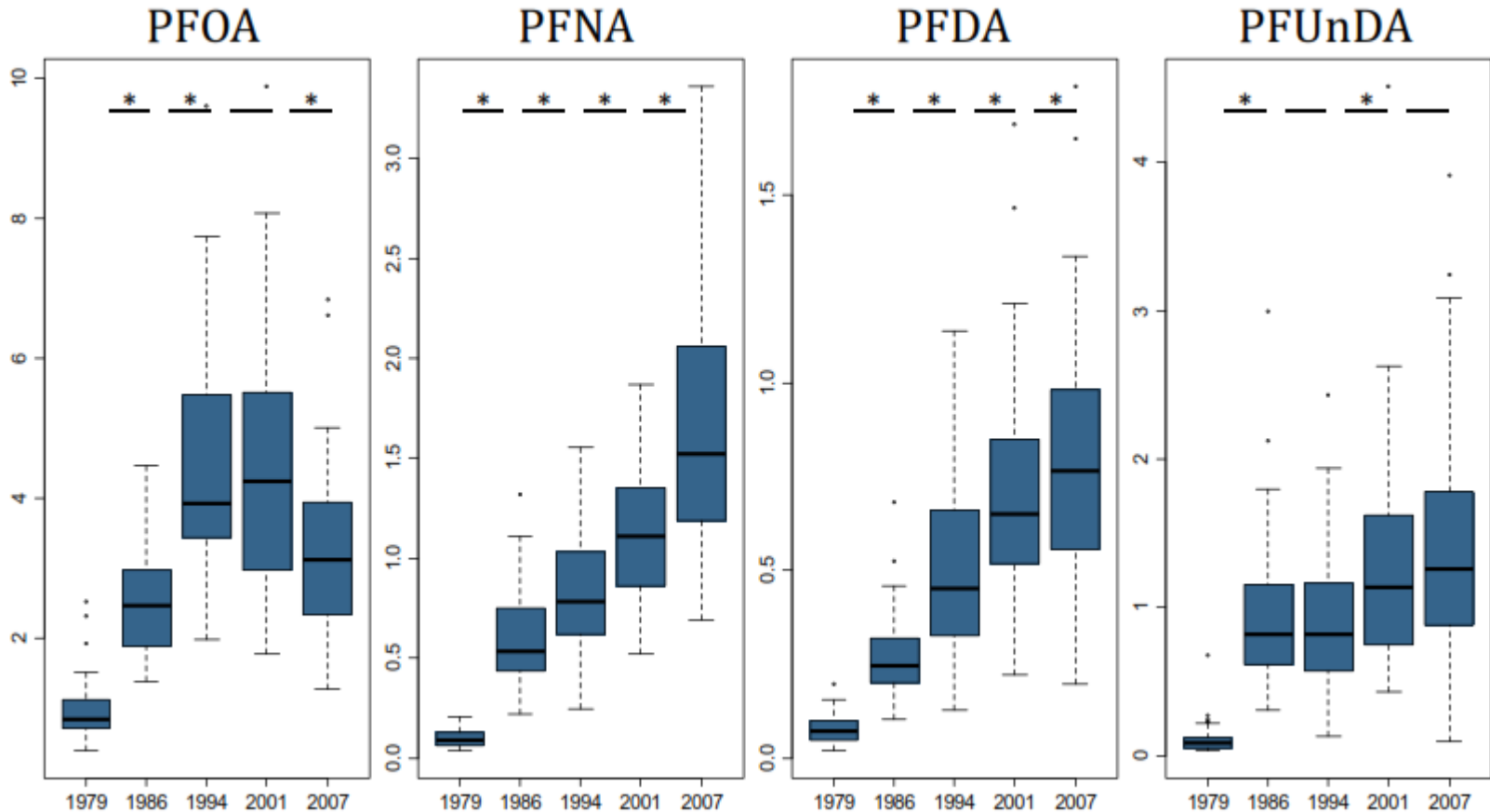
Vestergren and Cousins (2009) ES&T, 5565–5575

# Human exposure to PFAAs



# Trends in other long chain PFAAs

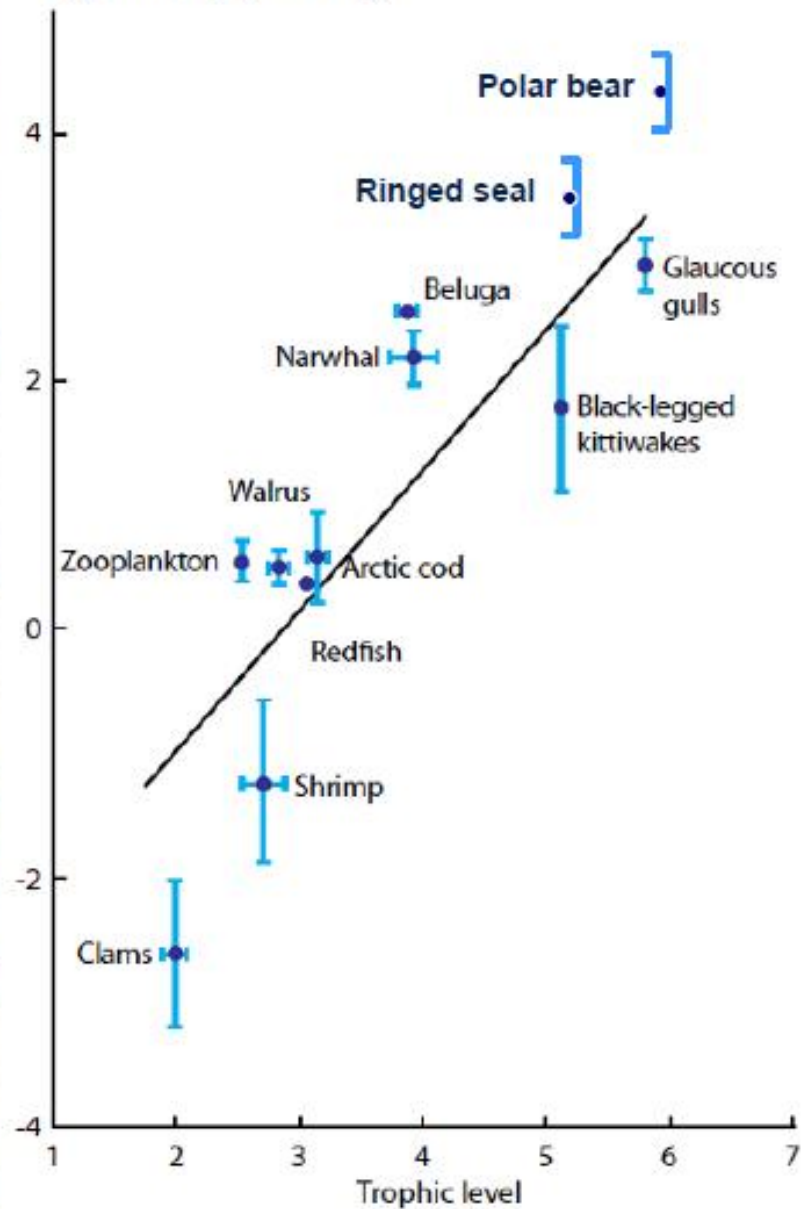
(data from Tromsø, Norway (54 males))



# Biomagnification of PFOS in Arctic food chains



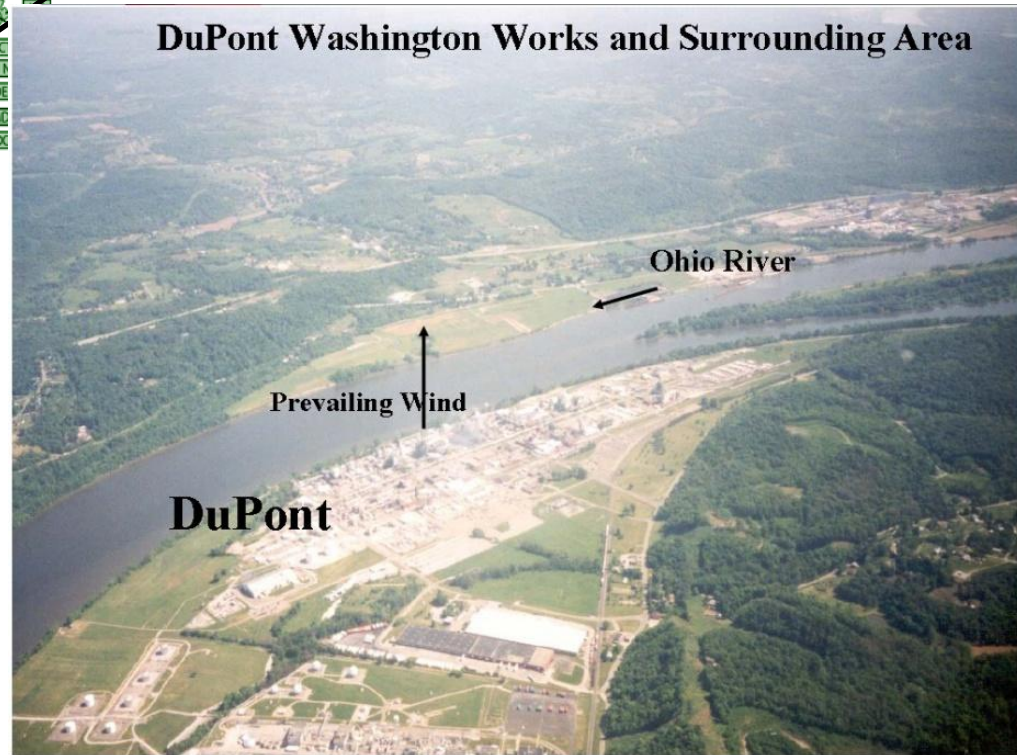
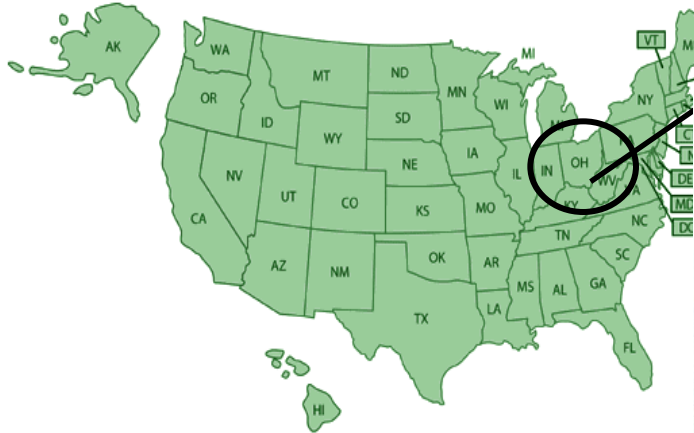
Log<sub>n</sub> [PFOS], ng/g wet weight





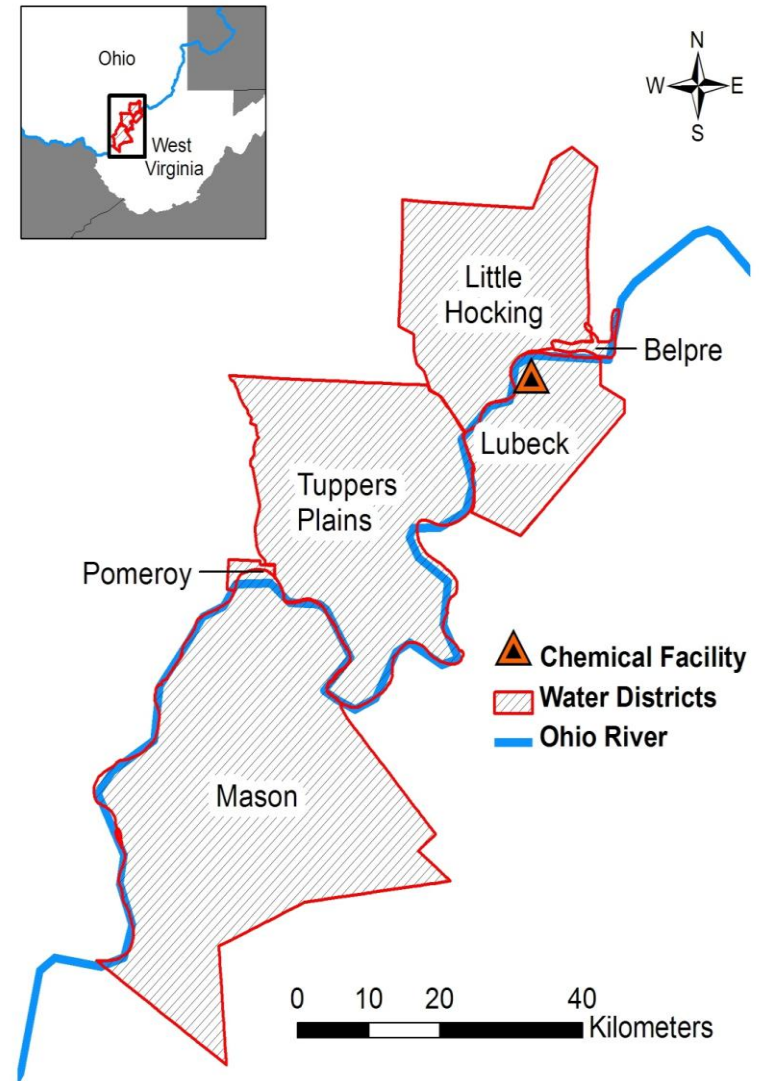
# C8 Study area

- West Virginia/Ohio



# C8 study population in Mid Ohio, exposed to PFOA or “C8” : Perfluoro-octanoic acid

- PFOA-Detected at higher levels in water supplies near Dupont Washington Works Plant due to emissions to air and river
- Led to a **class action** court case against Dupont
- “C8 Science Panel” set up & Biomonitoring survey



**C8 Health Project data included biomarkers in blood and questionnaire data for 69030 people in 2005-06.**

**10 perfluorochemicals measured.**

Highest was PFOA (mean 82.9 ng/mL), then PFOS (mean 23.3)

Table 3. Continued

Age/sex	Measure	PFHxA <sup>a</sup>	PFHxA <sup>b</sup>	PFHS	PFHpA <sup>a</sup>	PFHpA <sup>b</sup>	PFOA	PFOS	PFNA	PFDA <sup>a</sup>	PFDA <sup>b</sup>
Total population											
Female	Mean	0.8	1.4	4.3	0.6	1.2	68.8	20.7	1.5	0.5	0.8
	Median	0.5	1.0	2.7	0.3	0.8	23.6	17.6	1.3	0.3	0.7
	Geometric mean	0.5	1.1	2.8	0.4	1.0	27.9	17.0	1.3	0.4	0.7
	SD	1.0	1.1	6.2	0.8	1.2	190.6	14.1	0.8	0.4	0.4
Male	Mean	0.9	1.4	5.9	0.7	1.3	98.2	26.0	1.7	0.5	0.8
	Median	0.6	1.0	3.8	0.3	0.9	33.7	22.9	1.5	0.3	0.7
	Geometric mean	0.6	1.2	4.0	0.4	1.0	39.4	21.9	1.5	0.4	0.7
	SD	1.2	1.4	12.8	0.9	1.3	284.3	16.5	0.9	0.6	0.8
Total	Mean	0.9	1.4	5.1	0.6	1.2	82.9	23.3	1.6	0.5	0.8
	Median	0.5	1.0	3.2	0.3	0.9	28.2	20.2	1.4	0.3	0.7
	Geometric mean	0.6	1.1	3.3	0.4	1.0	32.9	19.2	1.4	0.4	0.7
	SD	1.1	1.3	10.0	0.9	1.2	240.8	15.6	0.9	0.5	0.7

# C8 Science Panel Role

- A) Plan and carry out epidemiological studies
- Cross sectional study of clinical markers of exposure and disease
  - Longitudinal linking disease to modelled exposure
  - Geographic studies of cancer and birth records

B) Report to Court on whether PFOA linked to disease:

**“Given available scientific evidence, is it more probable than not that a connection is present between C8 (PFOA) exposure and human disease?”**

# Categories of Disease considered

Based on prior epi and tox literature

**Reproductive Health** (Preg Ind Hyper, Birth Defects, Miscarriage, Low Birth Weight)

**Autoimmune Disease** (Colitis, Lupus, Crohn's Disease, Diabetes Type 1, Multiple Sclerosis, Rheumatoid Arthritis)

**Thyroid Disease**

**Cancer** (Testes, Kidney, Pancreatic, Liver... 21 sites in total)

**Cardiovascular Disease** (High Cholesterol, BP, CAD, Stroke)

**Kidney Disease**

**Liver Disease**

**Respiratory Disease** (Asthma, COPD)

**Infectious Disease** (Resp., GI, Other)

**Neurological disease** (Children, PD)

**Diabetes** (Type 2)

**Osteoarthritis**

# Categories of Disease considered

## 6 positive probable links (out of 47)

Reproductive Health (**Preeclampsia/PIH**, Defects, Miscariage, LBW)

Autoimmune Disease (**Ulcerative Colitis**, Lupus, Crohn's, MS, RA, T1 Diabetes)

**Thyroid Disease**

Cancer (**Testes, Kidney**, Pancreatic, Liver... 21 sites in total)

Cardiovascular Disease (**High Cholesterol**, High BP, Cor Art Dis, Stroke)

Kidney Disease

Liver Disease

Respiratory Disease (Asthma, COPD)

Infectious Disease (Resp., GI, Other)

Neurological disease (Children, PD)

Diabetes (Type 2)

Osteoarthritis

# Example of probable link: High Cholesterol

- Background: cholesterol positively associated with higher PFOA in 10 human studies, six statistically significant
- PFOA not lipophilic, binds with albumin
- Most prior studies cross-sectional, prohibiting causal inference
- Positive human association contradicts animal data, where association is negative

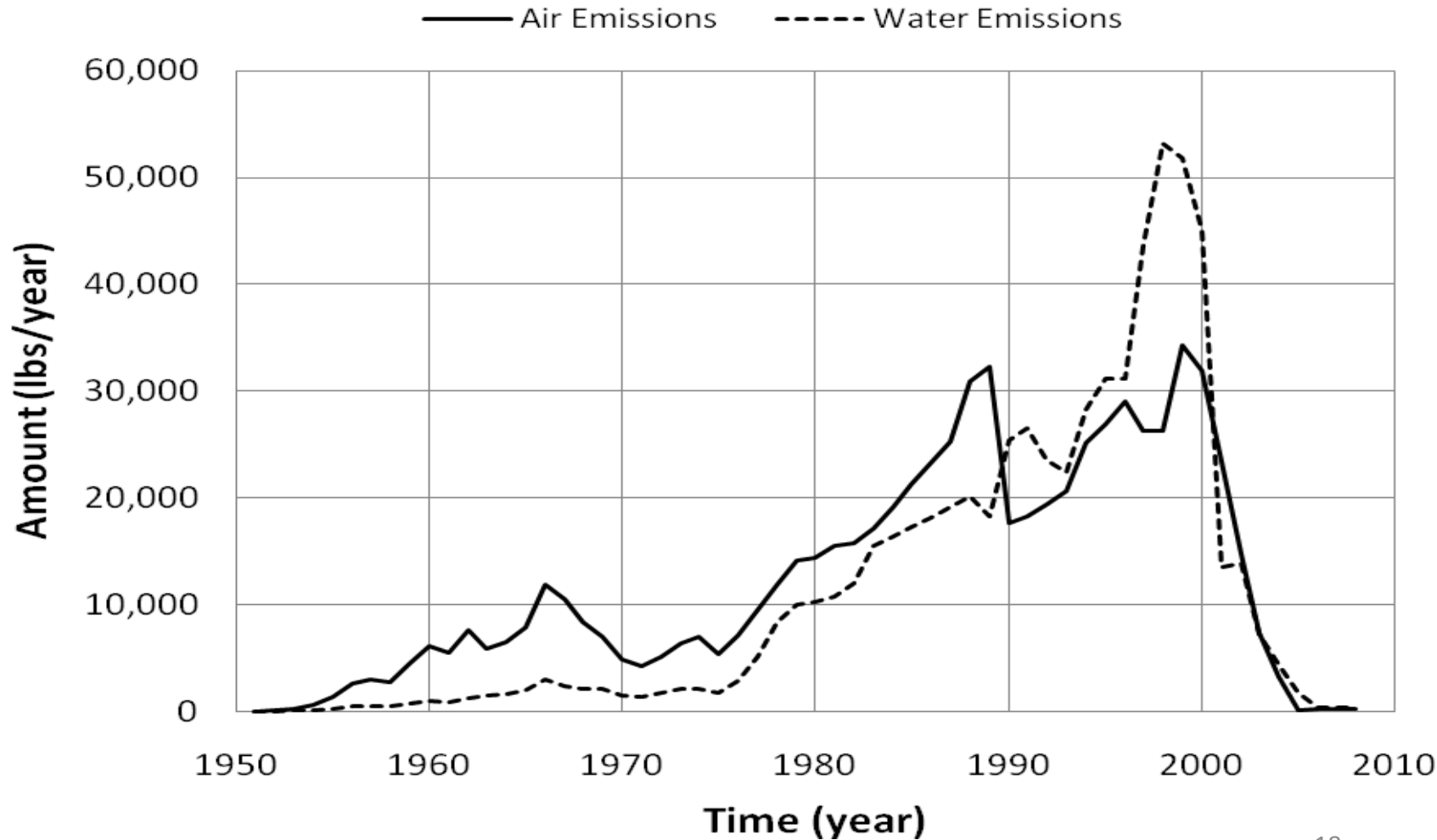
# Cholesterol Science Panel Studies in mid Ohio valley

- 1) Cross sectional study of 55,000 people not taking lipid lowering medication in the mid-Ohio valley
- 2) Cohort study of 32,000 adults
- 3) Longitudinal study of 560 adults with blood drawn in 2005 and 2010

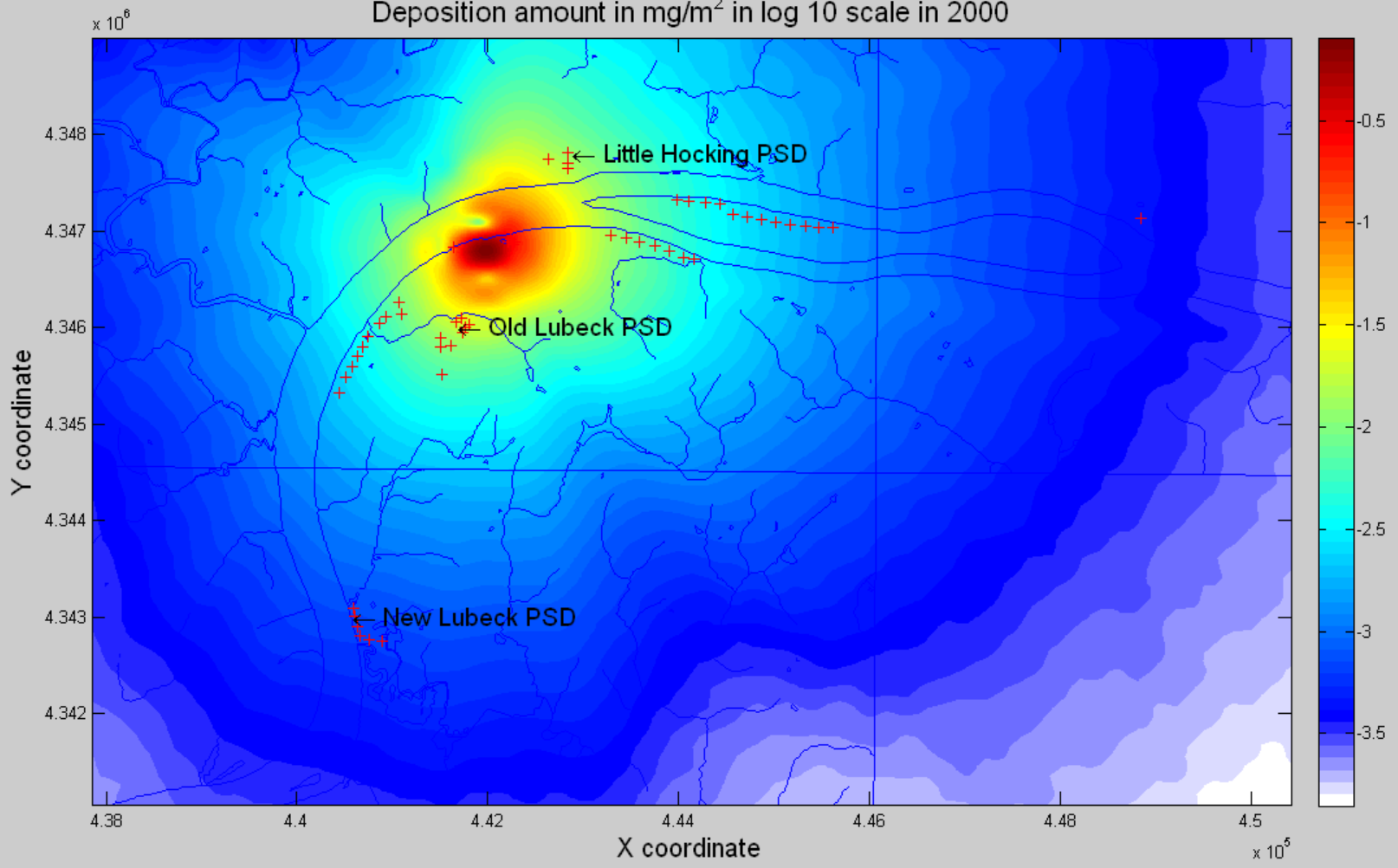


# Modelling exposure

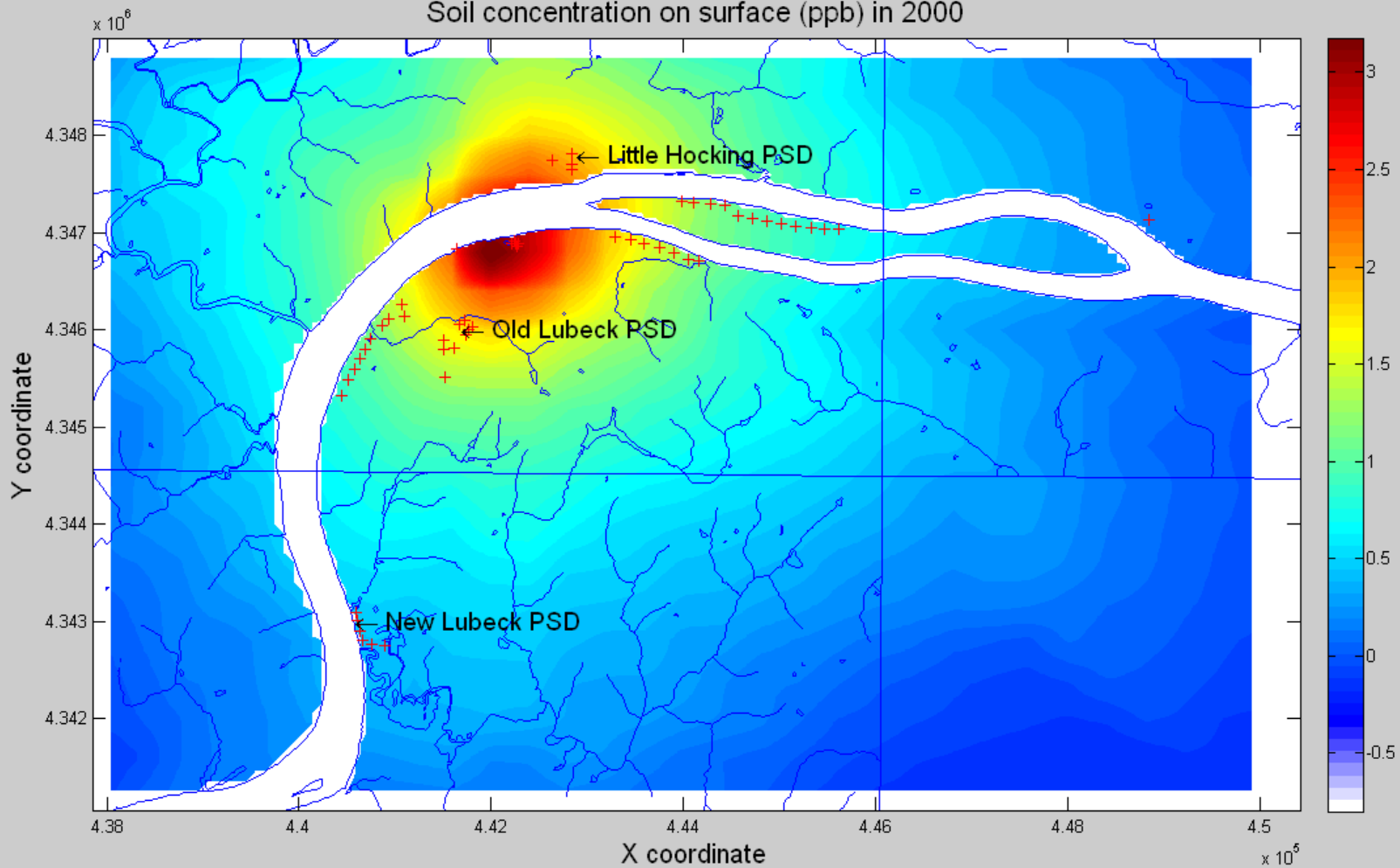
# Plant emissions to air and river, 1950-2005



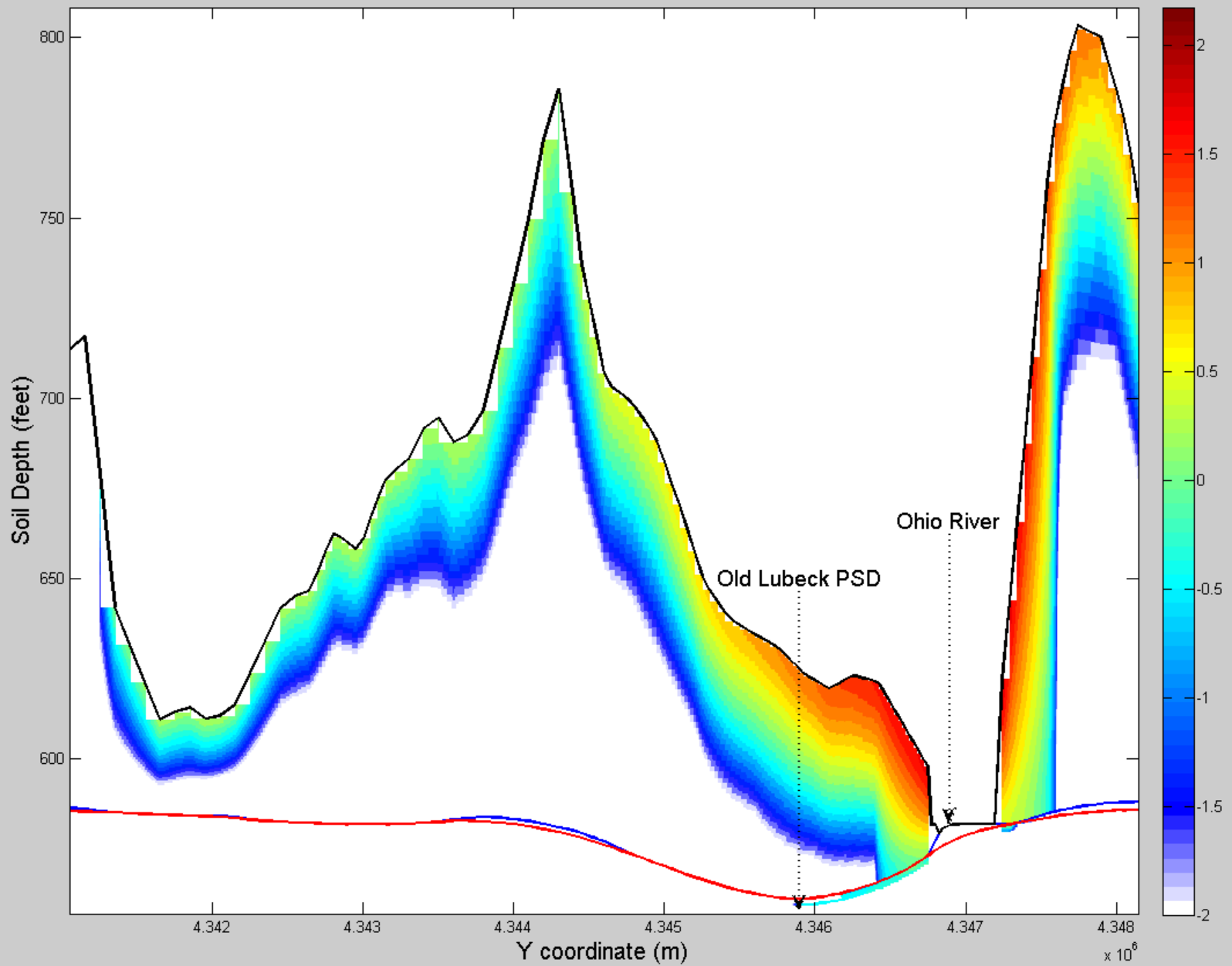
Deposition amount in  $\text{mg}/\text{m}^2$  in log 10 scale in 2000



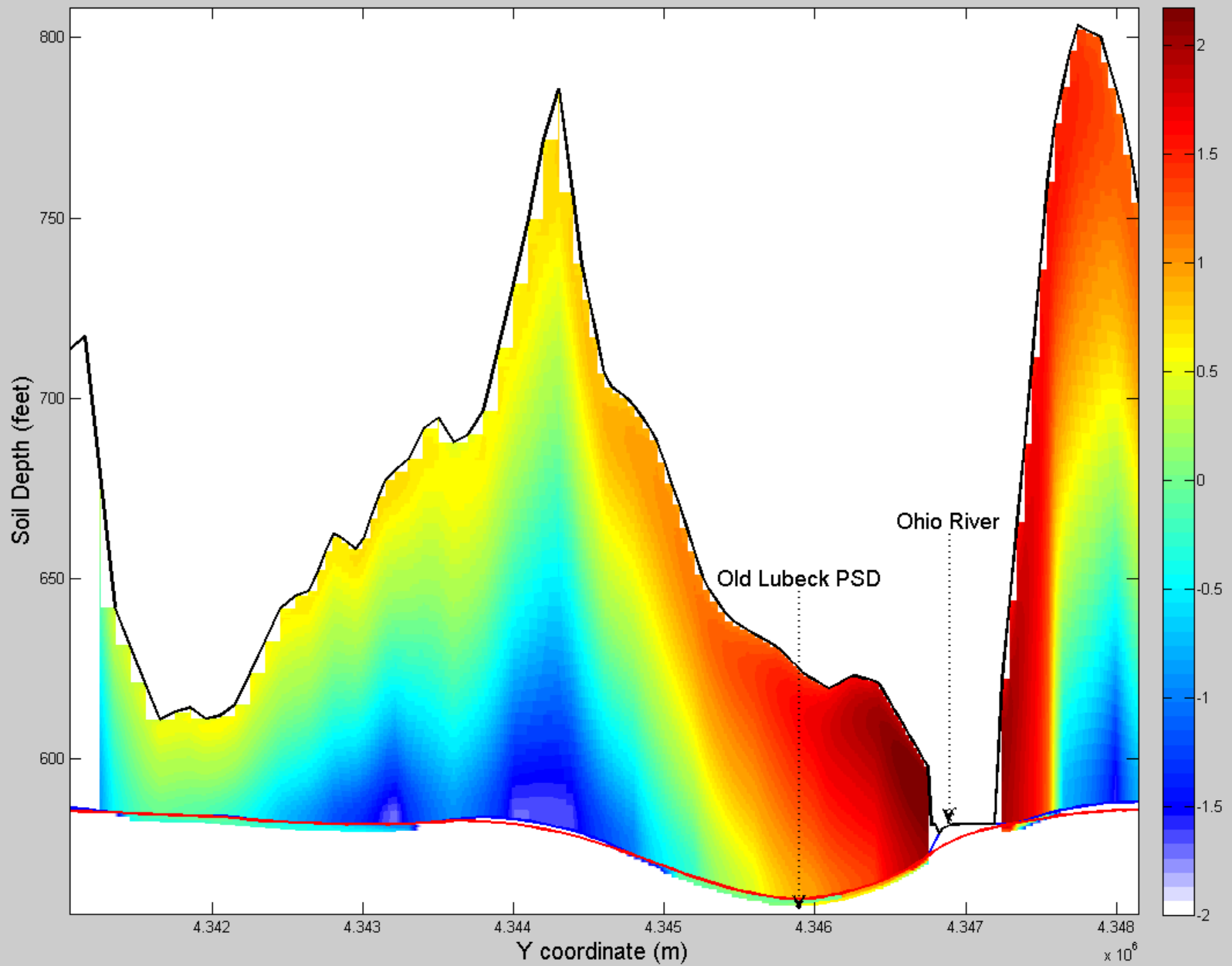
Soil concentration on surface (ppb) in 2000



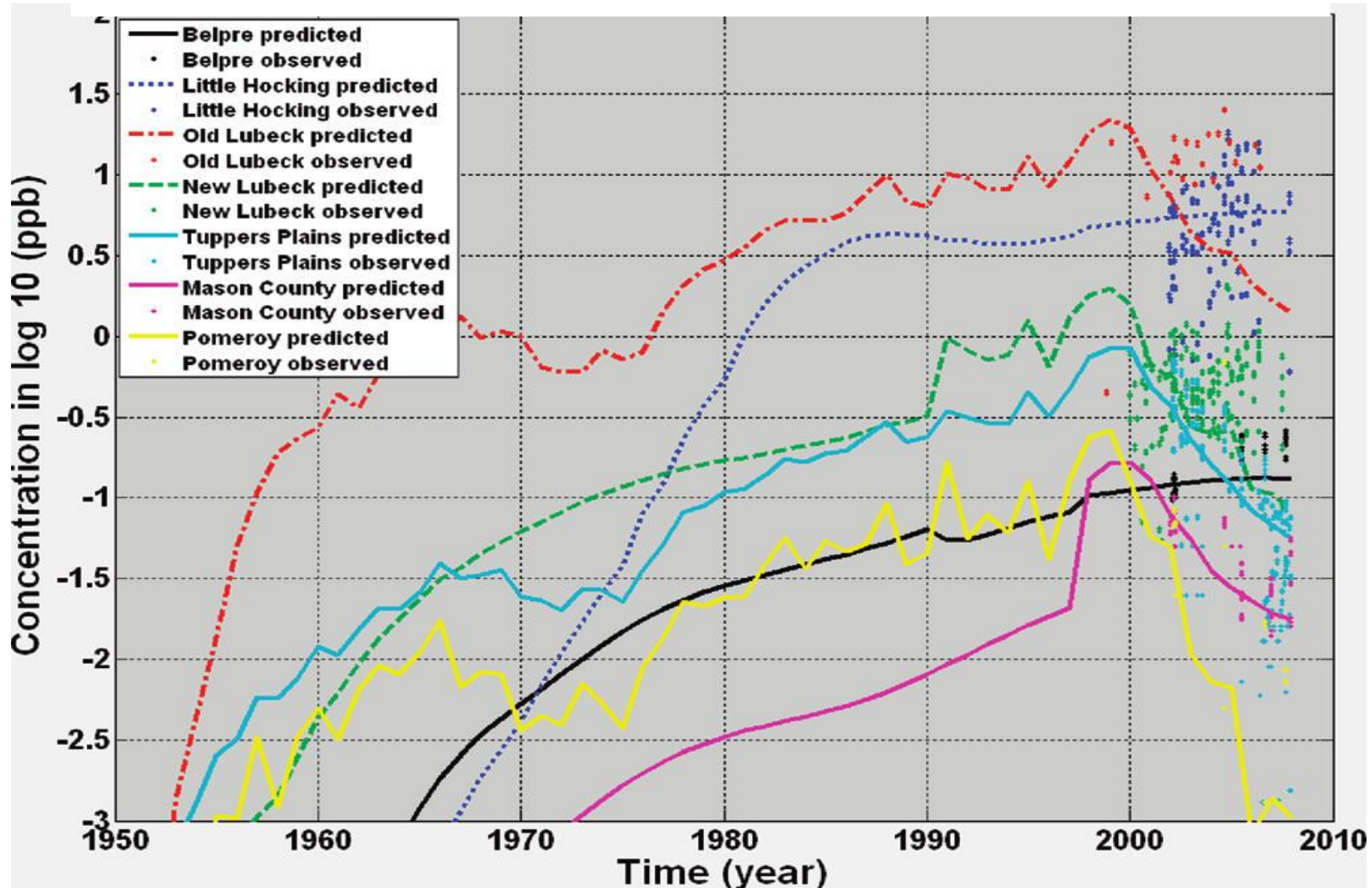
Subsurface soil concentration in lateral view (ppb) in log 10 scale in 1970



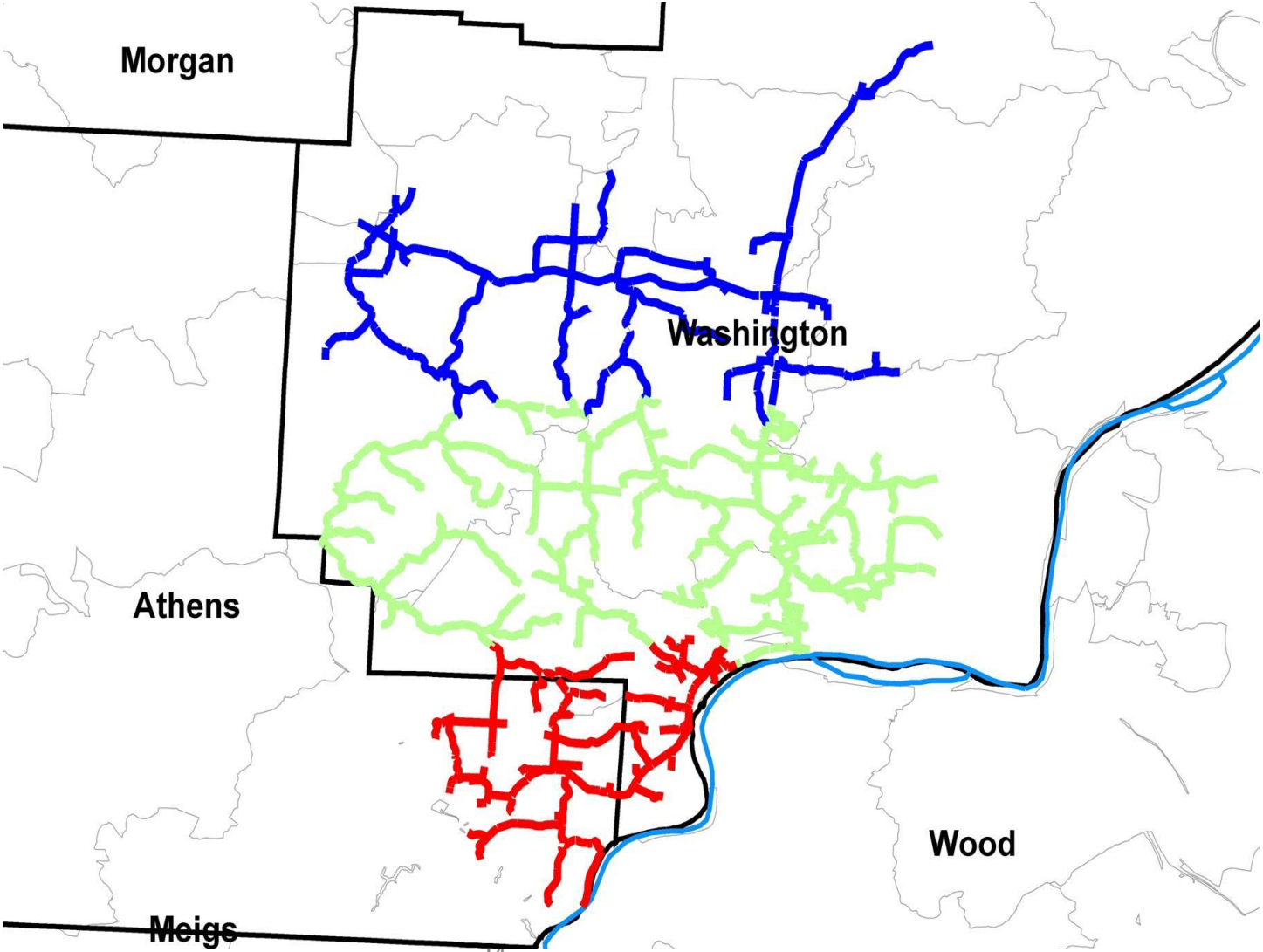
Subsurface soil concentration in lateral view (ppb) in log 10 scale in 2008



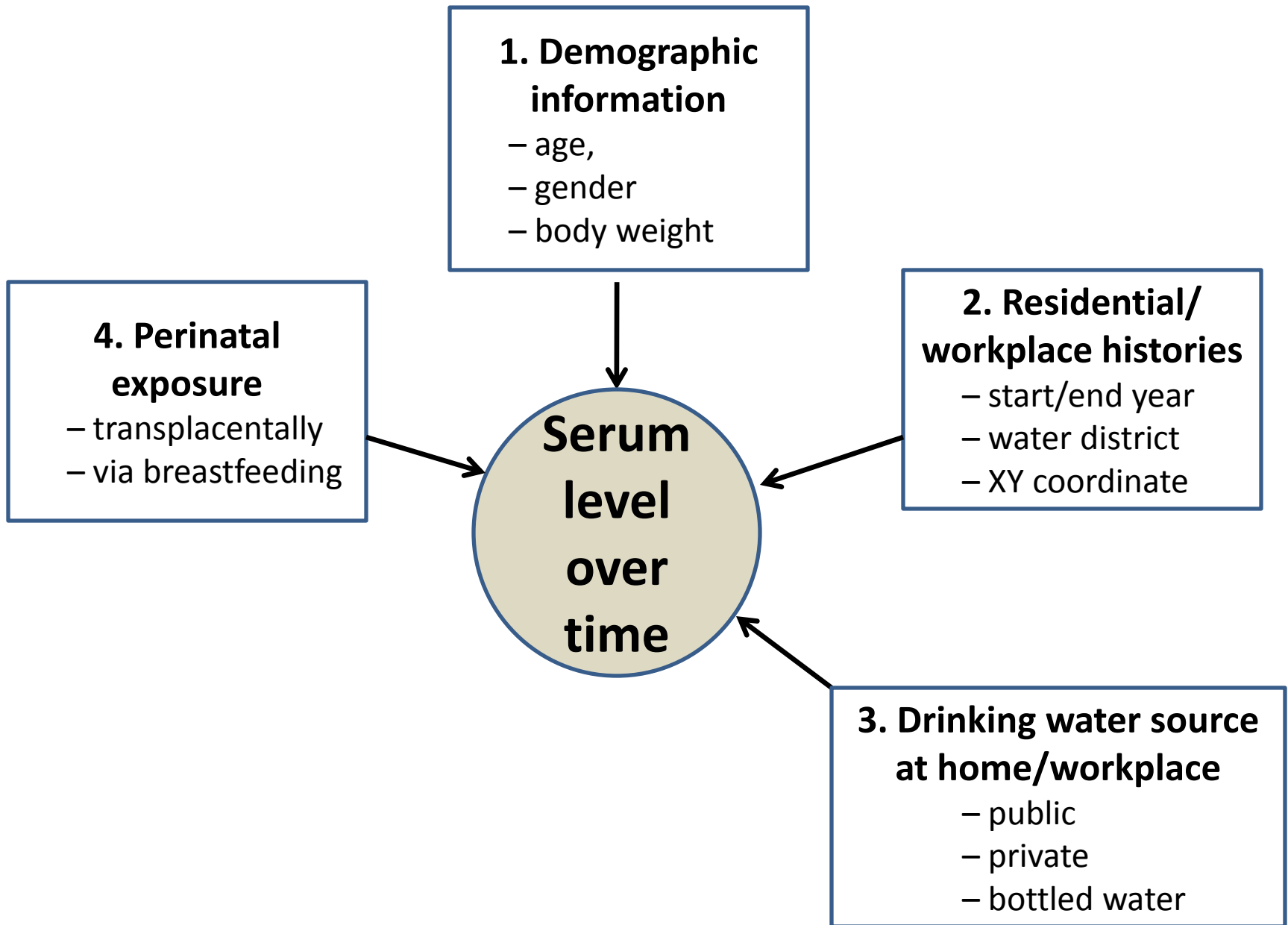
# Predicted water district well PFOA concentrations & recent observations

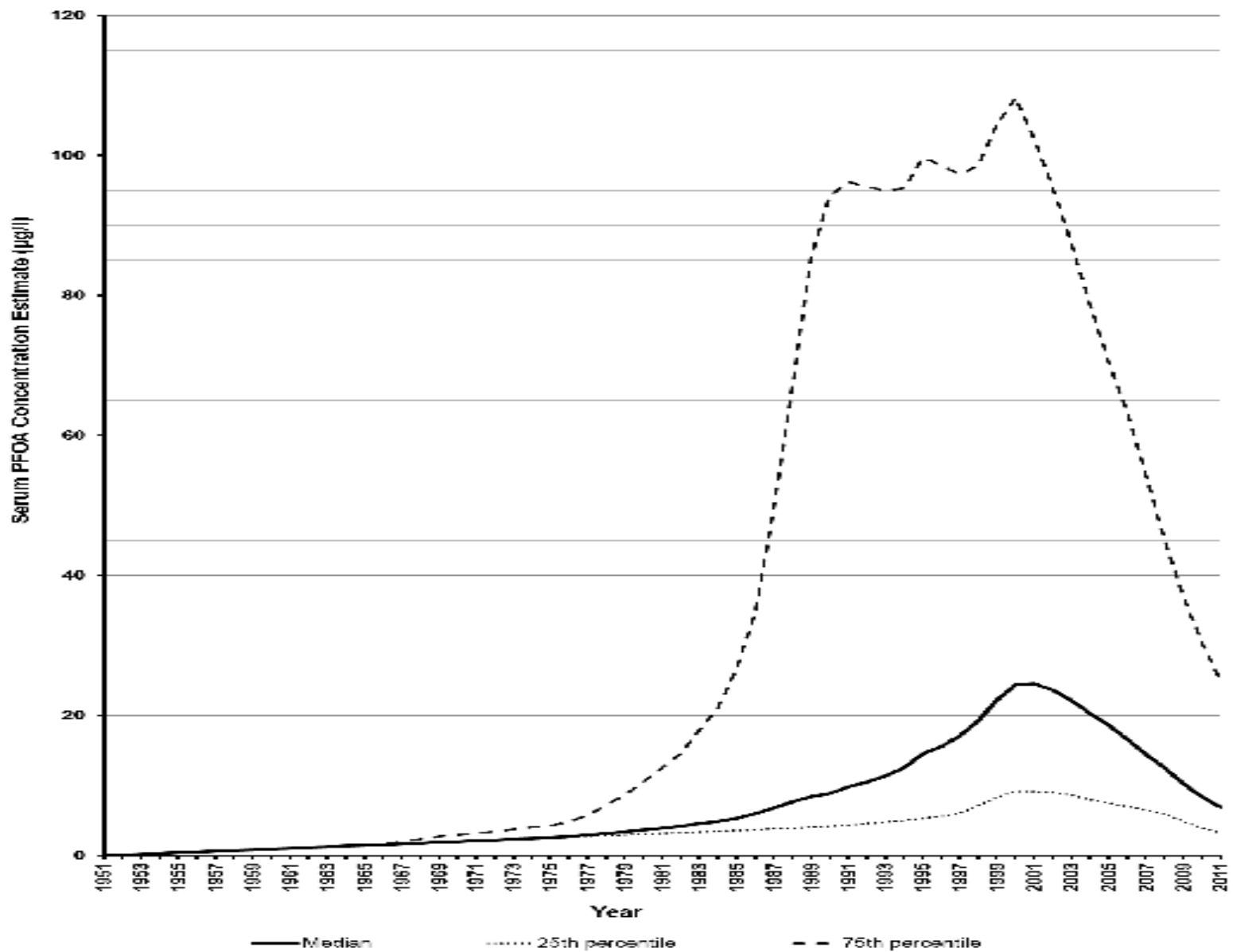


# Pipes added to district in stages

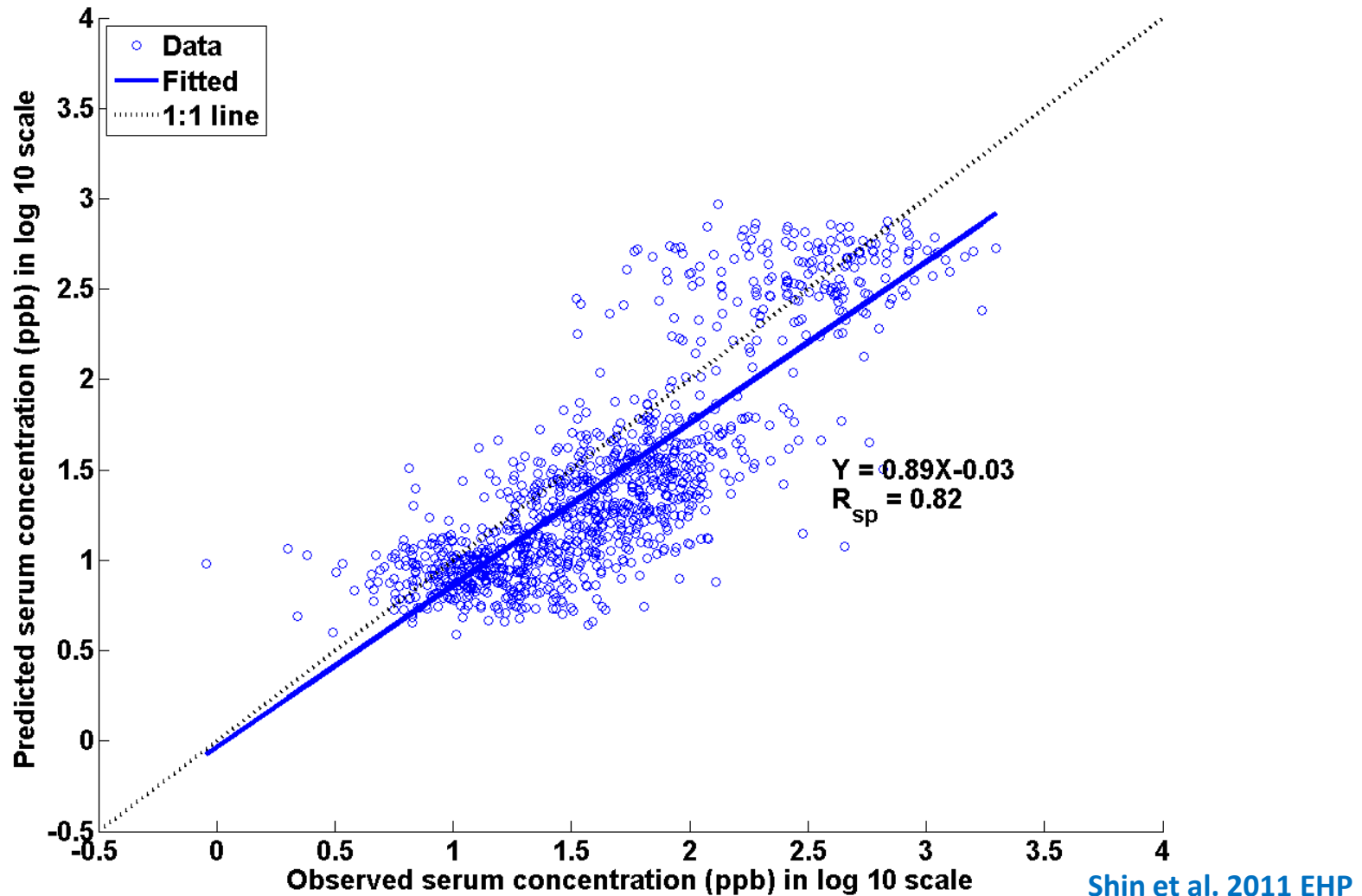








# Modelled vs measured PFOA concentrations in serum



# Science Panel Cohort study

- Study population: 28,000 community adult residents and 4,000 workers at Dupont plant, most participated in C8 Health Project 2005/2006
- Interviewed twice in 2009-2011 to collect medical and residential history
- Follow-up from 1952 or age at birth until time of interview: some prospective analyses limited to after 2005/2006 C8 Health Project
- Validation of self-reported disease via medical records

# Longitudinal study of doctor diagnosed raised cholesterol

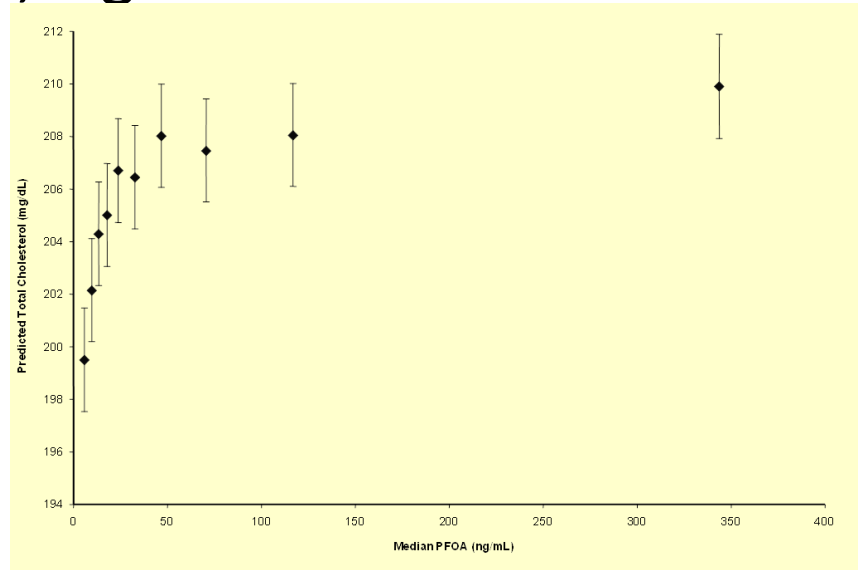
Past diagnoses “high cholesterol with prescription medication” reported and associated with modelled PFOA at year of diagnosis: 9653 cases in population of 32k

Quintile of PFOA	Odds ratio
1	1.0
2	1.07
3	1.11
4	1.05
5	1.20

$P_{\text{trend}} < 0.001$

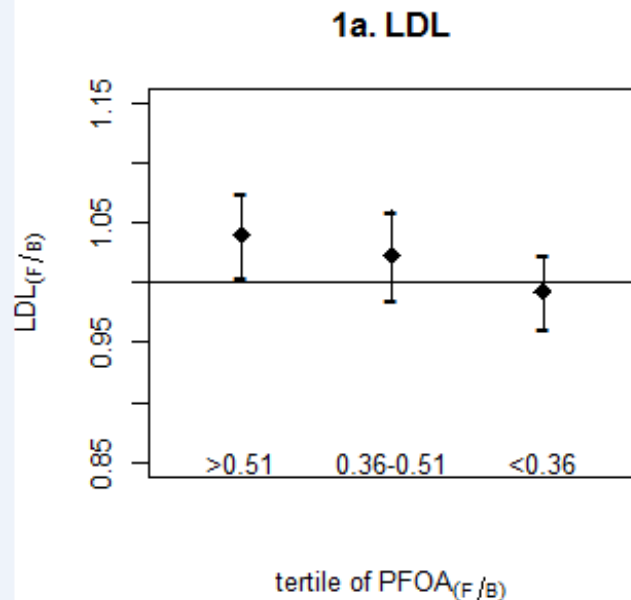
# Cross sectional data

- In cross sectional analyses, significant association between cholesterol and PFOA
- Logistic regression of high cholesterol (<240) significant trend with increasing PFOA
- LDL relationships steeper than total chol.
- Slope steeper at lower levels <40 ng/ml
- Consistent with multiple studies



# Longitudinal study of lipids

- Lipid measures repeated 4 years after first survey  
n=560
- Does LDL fall again the more the PFOA is cleared from blood ?



Response

% change per halving

(LDL) cholesterol

**-3.58 (-1.47, -5.66)**

[Fitz-Simon, Fletcher et al, Epidemiology 2013]

# **Probable link conclusion: cholesterol**

Given the evidence from a variety of different study designs, it was concluded that there is a probable link between PFOA and diagnosed high cholesterol.



# Cohort study results: cancer

- The kidney cancer relative risks RRs by quartile were 1.0, 1.2, 1.4, and 1.6, (test for trend with log cumulative exposure  $p=0.09$ ). (113 confirmed cases).
- For testicular cancer the corresponding risks were 1.0, 1.8, 2.2, 2.7 (test for trend with log cumulative exposure  $p=0.04$ ). (19 confirmed cases)

# Workers at Dupont plant: kidney cancer

## Results

- Retrospective cohort mortality of 6000 workers
- Evidence of a positive trend for kidney cancer with increasing exposure ( $p=0.0003$ ), but based on only 12 deaths
- SMR (10 years latency) - highest 2 quartiles 3.85 (1.05-9.85, 4 deaths) and 9.12 (3.07-9.85, 7 deaths)

# Probable link conclusion: cancer

- For kidney and testicular cancer, there is evidence of positive dose-response in both the cohort and geographical studies (although there is some overlap of cases between studies), and evidence for kidney cancer in the worker study (again, with some overlap).
- + animal evidence for testicular cancer
- No other cancer sites showed a consistent pattern.
- Conclusion: probable link for kidney and testicular cancer

**Some associations were inverse**

# Colon cancer

- Outcome: reported and verified colon cancer diagnosed in last 10 years prior to interview
- Analyses logistic regression by PFOA or PFOS, with adjustment for age, gender & SES. By exposure quartile or continuous in relation to logPFOA/S
- Exposure 1 Measured PFOA or PFOS in 2005/6
- Exposure 2 Modelled PFOA only predicted at same time

# Colon cancer pOR by PFOA/S

	n	PFOS OR (ci)	n	PFOA OR (ci)
Q1	59		48	
Q2	23	0.30 (0.18,0.49)	31	0.42 (0.24,0.70)
Q3	25	0.26 (0.16,0.41)	46	0.54 (0.34,0.85)
Q4	18	0.13 (0.08,0.22)	38	0.43 (0.27,0.70)
per logunit		0.26 (0.19,0.38)		0.50 (0.35,0.69)

# Colon cancer pOR for PFOA, comparing measured with modelled serum levels

	n	PFOA measured OR (ci)	n	PFOA modelled OR (ci)
Q1	48		34	
Q2	31	0.42 (0.24,0.70)	33	0.80 (0.50,1.30)
Q3	46	0.54 (0.34,0.85)	49	1.11 (0.71,1.72)
Q4	38	0.43 (0.27,0.70)	41	0.85 (0.54,1.34)
per logunit		0.50 (0.35,0.69)		0.94 (0.73,1.20)

# Colon vs non cancer: impact on biomarkers

	colon cancer	no colon cancer	95% CI for difference in means	
PFOA	67.3	90.9	7.8	39.4
PFOS	18.7	23.8	2.9	7.3



# Kidney function and PFOA

- Outcome: Glomerular filtration rate (derived from serum creatinine with age, sex, race) - Low values indicate kidney disease
- Analyses fitted regression of eGFR on PFOA, with age, gender, & other potential confounders
- Exposure 1 Measured PFOA in 2005/6
- Exposure 2 Modelled PFOA predicted at same time

# Results: Measured Serum PFAAs

C8 Health Project Participants children (n=9660)

## Associations between measured serum PFAAs and eGFR

	Change in eGFR* (95% CI)	p-value
PFOA	-0.75 (-1.41, -0.10)	0.02
PFOS	-1.10 (-1.66, -0.53)	0.0001
PFNA	-0.83 (-1.35, -0.30)	0.002
PFHxS	-0.95 (-1.57, -0.32)	0.003

\*Mean change in eGFR for an IQR shift for each PFAA; adjusted for age, sex, race, smoking, and household income

# PFOA and Kidney function

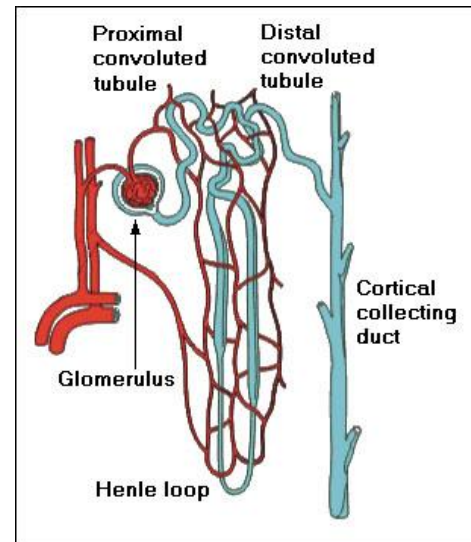
Does exposure to PFOA cause decreased GFR?

- Animal studies suggest kidney is target organ for PFOA
- *In vitro* studies show increased permeability and fluidity of cell membranes

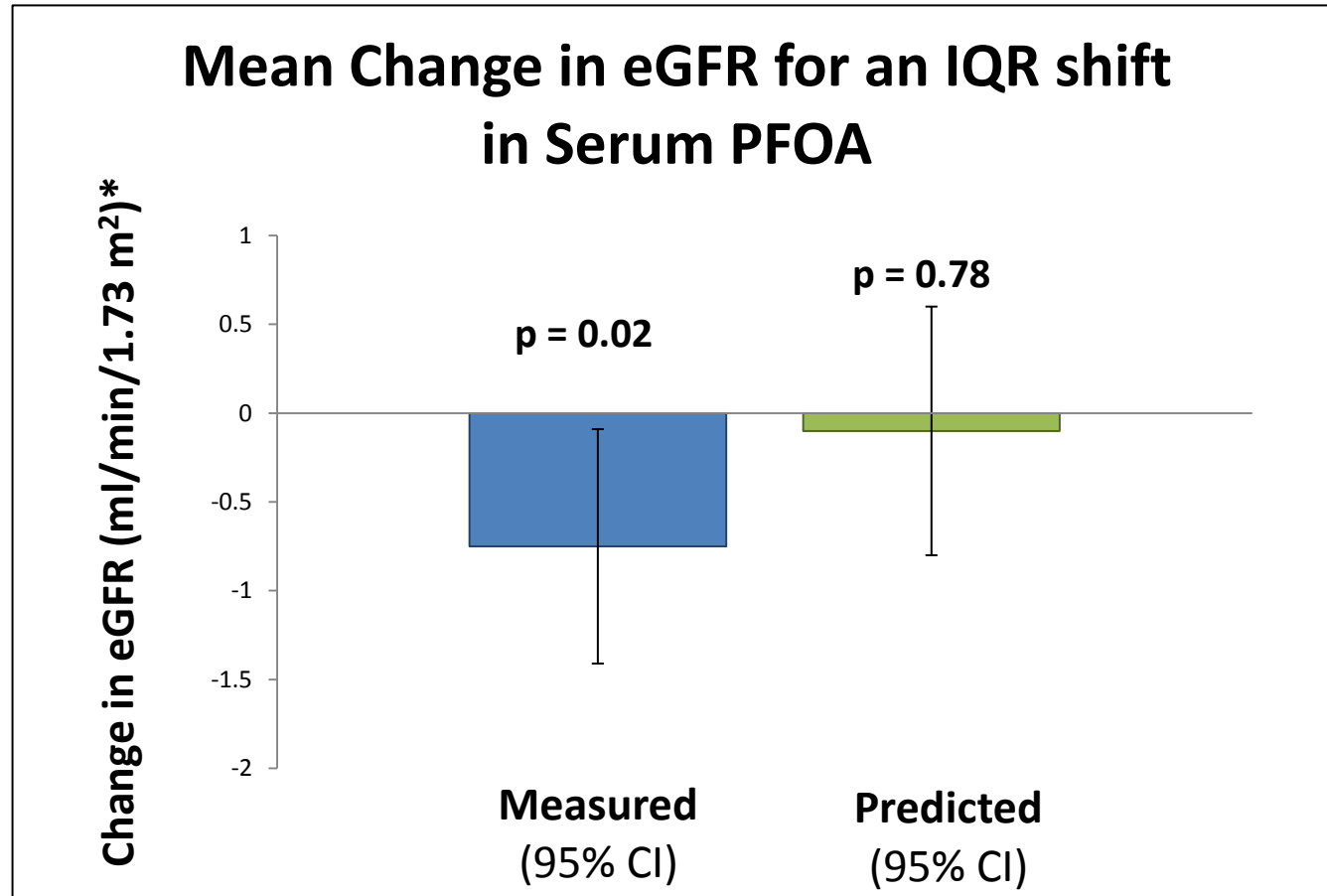
OR

Does decreased GFR cause increased serum PFOA?

- PFOA concentrates in the kidneys
- PFOA filtered out of blood in the glomerulus, but 99% reabsorbed in tubules
- Decrease rate of travel through tubules, increasing time for PFOA reabsorption



# Kidney results (for children, n=9660)

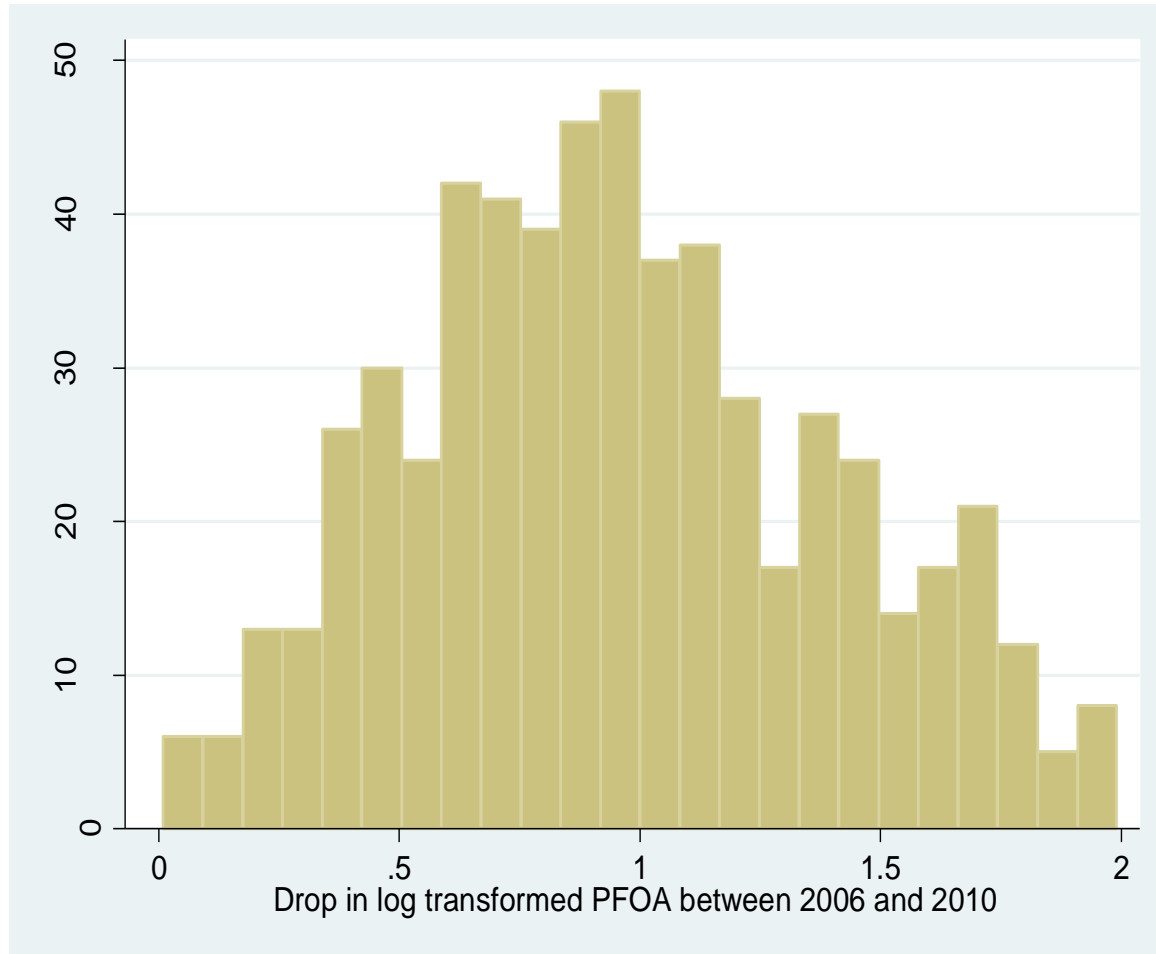


\*Adjusted for age, sex, race, smoking, and household income

# Conclusions - Kidney function

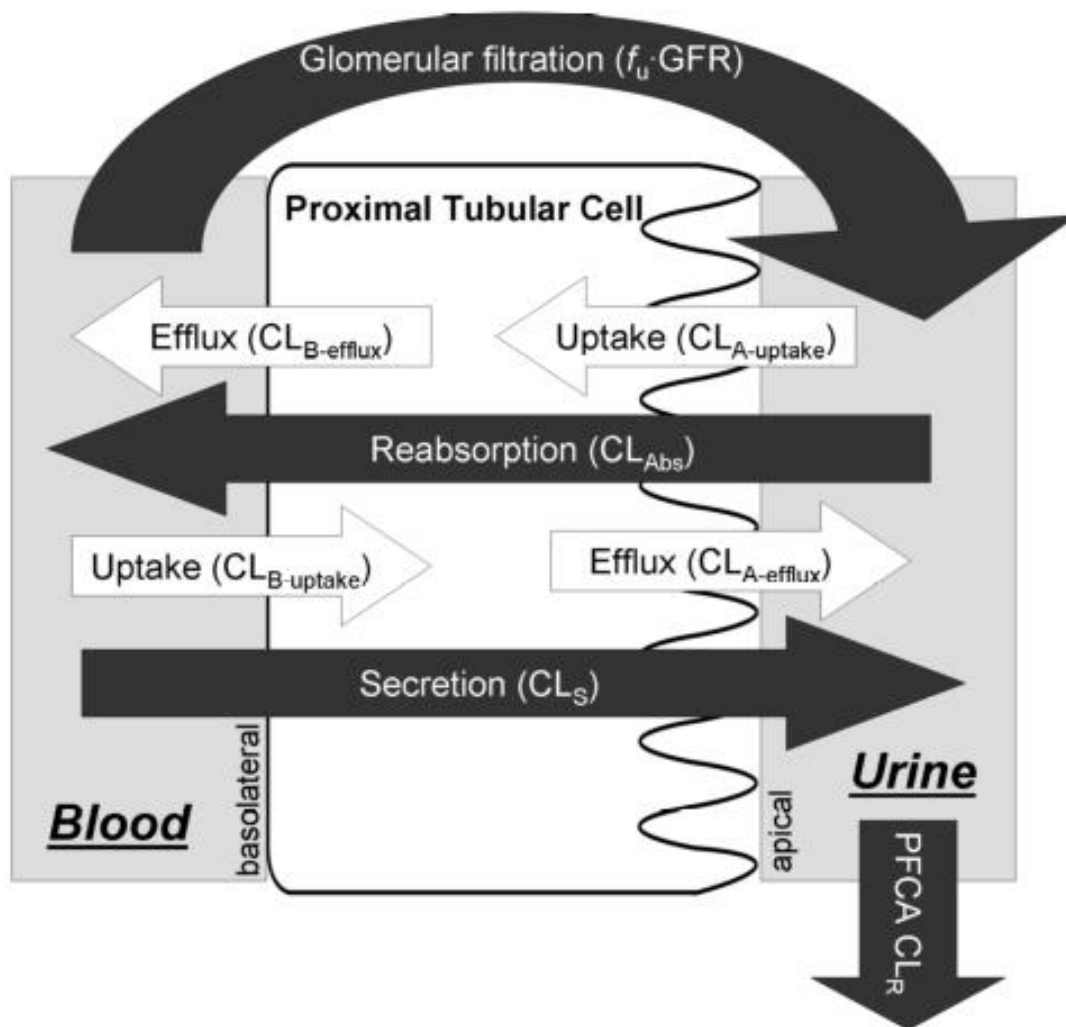
- Some, most, perhaps all, of the association between eGFR and PFOA is explained by GFR affecting PFOA excretion.
- Revealed by having a measure of intake independent of biomarker level.
- Not possible to make same comparison for other PFAAs in this study population
- Need prospective studies of exposure preceding effect

# Variability of excretion: fall in PFOA over 4 years (n=755)



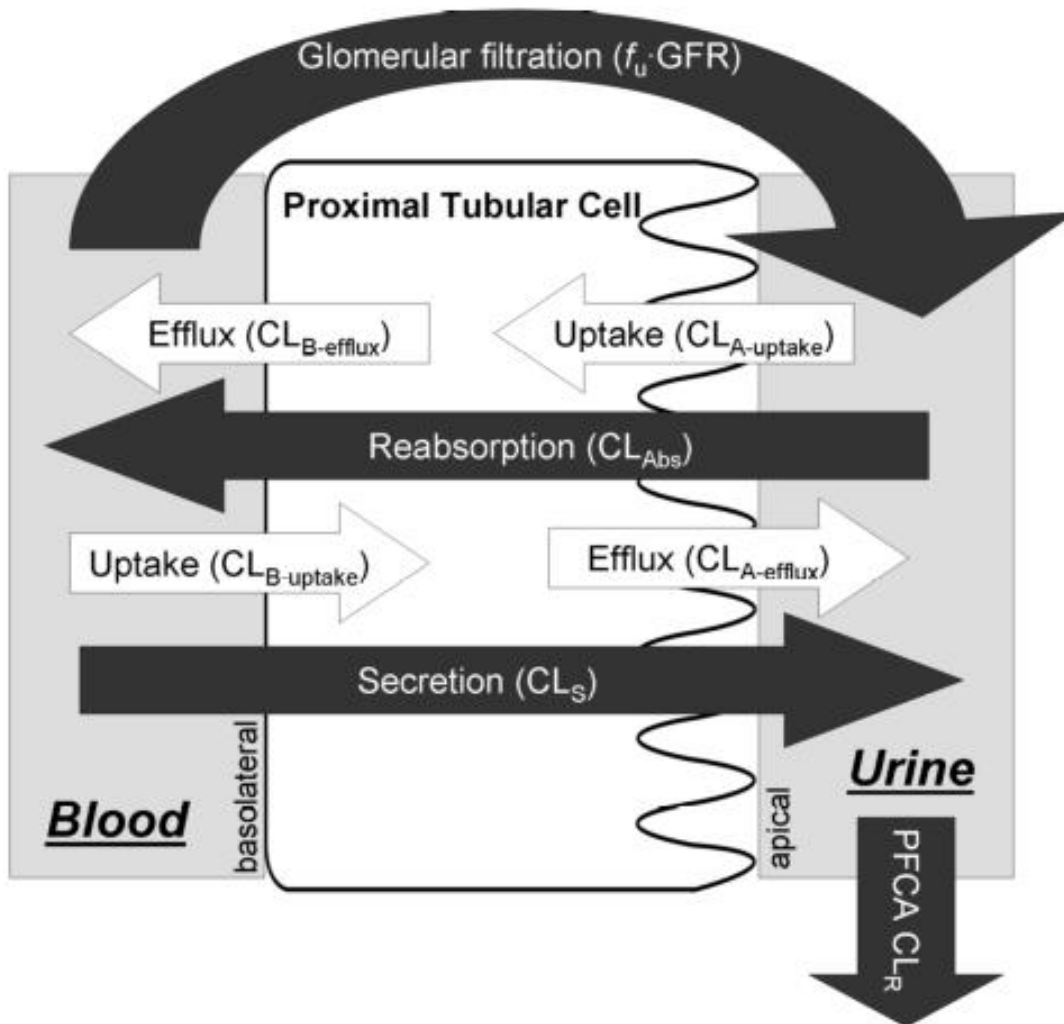
# Schematic for PFOA passage in kidney

$$\text{PFCA Renal Clearance (CL}_R\text{)} = f_u \cdot \text{GFR} + \text{CL}_S - \text{CL}_{\text{Abs}}$$



# Schematic for PFOA passage in kidney

$$\text{PFCA Renal Clearance (CL}_R) = f_u \cdot \text{GFR} + \text{CL}_S - \text{CL}_{\text{Abs}}$$



Transporter proteins involved:

Basolateral uptake

**OAT1**

**OAT2**

**OAT3**

Apical uptake

**OAT4**

**URAT1**

**OATP1A2**



# Overview of results

Significant ( $p < 0.05$ ) SNPS out of 33 SNPs

Analysis	SNP	Gene	Protein	p-value
Recessive	rs4963326	<i>SLC22A8</i>	OAT3	0.02
Additive	rs3809069	<i>SLC22A8</i>	OAT3	0.03
Dominant	rs3764043	<i>SLCO1A2/IAPP</i>	OATP1	0.02
“	rs7316461	<i>SLCO1A2/IAPP</i>	OATP1	0.04
“	rs11045994	<i>SLCO1A2/IAPP</i>	OATP1	0.03

# Conclusions

Positive findings for kidney and testes cancer, thyroid disease, preeclampsia, ulcerative colitis and high cholesterol.

Cross sectional biomarker analyses potentially vulnerable to confounding by determinants of excretion

# Thank you

Details on C8 website

[www.c8sciencepanel.org](http://www.c8sciencepanel.org)