Diseases linked to PFOA exposure:
Epidemiology and biomarkers of PFOA

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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
• 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

Drinking water
Outdoor air
Food
Indoor dust
Indoor air
Fluoropolymers
Fluorinated side-chain polymers
Non-polymeric surfactants
Low molecular weight performance chemicals

Direct exposure
Indirect exposure

PFAAs in human serum

Consumer products

8:2 FTOH
PFOA
Trends in other long chain PFAAs (data from Tromsø, Norway (54 males))

Nøst et al 2013, Nordfluor meeting
Biomagnification of PFOS in Arctic food chains

Log$_n$ [PFOS], ng/g wet weight

- Polar bear
- Ringed seal
- Beluga
- Narwhal
- Walrus
- Zooplankton
- Arctic cod
- Redfish
- Shrimp
- Black-legged kittiwakes
- Glaucous gulls
- Clams
- Redfish
- Trophic level

Nøst et al. 2013, Idstein

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)
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

[Shin et al ES&T 2011]
Deposition amount in mg/m² in log 10 scale in 2000

- Little Hocking PSD
- Old Lubeck PSD
- New Lubeck PSD
Predicted water district well PFOA concentrations & recent observations

[Shin et al ES&T 2011]
Pipes added to district in stages
1. Demographic information
   - age,
   - gender
   - body weight

2. Residential/workplace histories
   - start/end year
   - water district
   - XY coordinate

3. Drinking water source at home/workplace
   - public
   - private
   - bottled water

4. Perinatal exposure
   - transplacentally
   - via breastfeeding

[Shin et al EHP 2011]
Modelled vs measured PFOA concentrations in serum

![Graph showing the relationship between modelled and measured PFOA concentrations in serum. The graph includes data points and a fitted line with the equation Y = 0.89X - 0.03 and R² = 0.82. The data is from Shin et al. 2011 EHP.](image)
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

<table>
<thead>
<tr>
<th>Quintile of PFOA</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>1.07</td>
</tr>
<tr>
<td>3</td>
<td>1.11</td>
</tr>
<tr>
<td>4</td>
<td>1.05</td>
</tr>
<tr>
<td>5</td>
<td>1.20</td>
</tr>
</tbody>
</table>

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

[Steenland AJE 2009]
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?

<table>
<thead>
<tr>
<th>Response</th>
<th>% change per halving</th>
</tr>
</thead>
<tbody>
<tr>
<td>(LDL) cholesterol</td>
<td>-3.58 (-1.47, -5.66)</td>
</tr>
</tbody>
</table>

[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

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>PFOS OR (ci)</th>
<th></th>
<th>n</th>
<th>PFOA OR (ci)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>59</td>
<td></td>
<td>Q1</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td>23</td>
<td>0.30 (0.18, 0.49)</td>
<td>Q2</td>
<td>31</td>
<td>0.42 (0.24, 0.70)</td>
</tr>
<tr>
<td>Q3</td>
<td>25</td>
<td>0.26 (0.16, 0.41)</td>
<td>Q3</td>
<td>46</td>
<td>0.54 (0.34, 0.85)</td>
</tr>
<tr>
<td>Q4</td>
<td>18</td>
<td>0.13 (0.08, 0.22)</td>
<td>Q4</td>
<td>38</td>
<td>0.43 (0.27, 0.70)</td>
</tr>
<tr>
<td>per logunit</td>
<td>0.26 (0.19, 0.38)</td>
<td>per logunit</td>
<td>0.50 (0.35, 0.69)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Colon cancer pOR for PFOA, comparing measured with modelled serum levels

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>PFOA measured OR (ci)</th>
<th>n</th>
<th>PFOA modelled OR (ci)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>48</td>
<td></td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Q2</td>
<td>31</td>
<td>0.42 (0.24,0.70)</td>
<td>33</td>
<td>0.80 (0.50,1.30)</td>
</tr>
<tr>
<td>Q3</td>
<td>46</td>
<td>0.54 (0.34,0.85)</td>
<td>49</td>
<td>1.11 (0.71,1.72)</td>
</tr>
<tr>
<td>Q4</td>
<td>38</td>
<td>0.43 (0.27,0.70)</td>
<td>41</td>
<td>0.85 (0.54,1.34)</td>
</tr>
<tr>
<td>per logunit</td>
<td>0.50 (0.35,0.69)</td>
<td>0.94 (0.73,1.20)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Colon vs non cancer: impact on biomarkers

<table>
<thead>
<tr>
<th></th>
<th>Colon cancer</th>
<th>No colon cancer</th>
<th>95% CI for difference in means</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>67.3</td>
<td>90.9</td>
<td>7.8 39.4</td>
</tr>
<tr>
<td>PFOS</td>
<td>18.7</td>
<td>23.8</td>
<td>2.9 7.3</td>
</tr>
</tbody>
</table>
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)

<table>
<thead>
<tr>
<th>Associations between measured serum PFAAs and eGFR</th>
<th>Change in eGFR* (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>-0.75 (-1.41, -0.10)</td>
<td>0.02</td>
</tr>
<tr>
<td>PFOS</td>
<td>-1.10 (-1.66, -0.53)</td>
<td>0.0001</td>
</tr>
<tr>
<td>PFNA</td>
<td>-0.83 (-1.35, -0.30)</td>
<td>0.002</td>
</tr>
<tr>
<td>PFHxS</td>
<td>-0.95 (-1.57, -0.32)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*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)

Mean Change in eGFR for an IQR shift in Serum PFOA

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

Watkins et al EHP 2013
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

PFCA Renal Clearance ($CL_R$) = $f_u \cdot GFR + CL_S - CL_{Abs}$

[Han 2011 Chem Res Tox]
Schematic for PFOA passage in kidney

PFCA Renal Clearance ($CL_R$) = $f_u \cdot GFR + CL_S - CL_{Abs}$

Transporter proteins involved:
- **Basolateral uptake**
  - OAT1
  - OAT2
  - OAT3
- **Apical uptake**
  - OAT4
  - URAT1
  - OATP1A2

[Han 2011 Chem Res Tox]
## Overview of results

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

<table>
<thead>
<tr>
<th>Analysis</th>
<th>SNP</th>
<th>Gene</th>
<th>Protein</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recessive</td>
<td>rs4963326</td>
<td><em>SLC22A8</em></td>
<td>OAT3</td>
<td>0.02</td>
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<tr>
<td>Additive</td>
<td>rs3809069</td>
<td><em>SLC22A8</em></td>
<td>OAT3</td>
<td>0.03</td>
</tr>
<tr>
<td>Dominant</td>
<td>rs3764043</td>
<td><em>SLCO1A2/IAPP</em></td>
<td>OATP1</td>
<td>0.02</td>
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<tr>
<td>&quot;</td>
<td>rs7316461</td>
<td><em>SLCO1A2/IAPP</em></td>
<td>OATP1</td>
<td>0.04</td>
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<tr>
<td>&quot;</td>
<td>rs11045994</td>
<td><em>SLCO1A2/IAPP</em></td>
<td>OATP1</td>
<td>0.03</td>
</tr>
</tbody>
</table>
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