Breast Cancer Risk and Serum Levels of Perfluoroalkyl and Polyfluoroalkyl Substance (PFASs): A Case-Control Study Nested in the California Teachers Study

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PFASs: Historical Uses

- Large family of synthetic chemicals
- Introduced in the 1950s for their water resistant, stain resistant and lubricating properties
- Widely used in a variety of industrial applications and consumer products
PFASs – Global Contaminants

- Many are persistent, bioaccumulative and toxic global contaminants
- Widespread human exposures

PFOS/PFOA level

PFOS/PFOA pollution enlargement globally

- Wild life; white bear
  - PFOS (1.3 ~ >4000ng/g)
  - PFOA (<2.0 ~ 8.6ng/g)
  - (2004, Martin et al)

- Human serum
  - PFOS (<1.3 ~ 164ng/ml)
  - PFOA (<3 ~ 256ng/ml)
  - (2004, Kannan et al)

- Environmental water and air

- Biota
  - PFOS (<1 ~ 3680ng/g)
  - (2002, Kannan et al)

From: Takeshi Nakano, Osaka University
PFASs Production and Phase-outs

- Voluntary use reductions and regulatory restrictions began in early 2000s
- Many PFASs still in use

Figure 2: Timeline of production, commercialization and legislation of PFASs. POSF (perfluorooctane sulfonate fluoride) is the major raw material used to manufacture PFOS. Red flags represent events and actions that may have resulted in increased concentrations in the environment. Green flags represent important findings and phase-outs that may result in decreased concentrations in the environment.

From: Land et al. Environmental Evidence 2015, 4:3
Human Exposures – Biomonitoring Data

Temporal Changes in the Levels of Perfluorinated Compounds in California Women’s Serum over the Past 50 Years

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- Declines in body burden for some, but not all PFASs

PFAS: Cancer Concerns

- Tumor promotion
- Developmental toxicity
- Hepatotoxicity
- Immunotoxicity
- Genotoxicity (?)
- Endocrine disruption (thyroid, sex-steroid hormones, lipid metabolism)

Formal cancer evaluations only for PFOA:
- IARC: 2B possible human carcinogen (testicular, renal)
- US EPA: US EPA 2006 Scientific Advisory Board Review – ‘likely to be carcinogenic’
Scant and conflicting epi data for breast cancer

- **No association:**
  - C8 Science Panel Studies [Barry et al. 2013; Vieira et al. 2013]
  - Danish Birth Cohort [Bonefeld-Jorgensen et al. 2014; Ghisari et al. 2017]

- **Increased risks:**
  - Greenlandic Inuits – significantly increased risks for PFOA, PFOS, PFHxS, ΣPFSA [Bonefeld-Jorgensen et al. 2011; Ghisari et al. 2014; Wielsoe et al. 2017]
Objective

Evaluate the risk of breast cancer associated with serum levels of several PFASs among participants of a large case-control study, nested within the California Teachers Study (CTS)
Study Population

California Teachers Study (CTS):
- Statewide prospective cohort study of breast cancer
- 133,479 female professional public school employees
- Initiated in 1995-1996
- On-going follow-up: surveys, linkage to cancer and mortality data

Nested Breast Cancer Case-Control Study participants (n=1,760):
- No prior history of invasive or *in situ* breast cancer at cohort entry
- Provided blood sample & completed survey at blood draw in 2011-2015
- Continuous resident of California from cohort entry (baseline) to time of blood draw
Case/Control Definitions

Breast Cancer Cases (n=902)
- Identified by routine annual linkages of the CTS to the California Cancer Registry (CCR)
- Primary invasive breast cancer (SEER Site Code=26000, ICD-03 histology <8590)
- Details on tumor characteristics (estrogen and progesterone receptor status, Luminal A, Triple Negative)

Controls (n=858)
- Breast cancer free at blood draw
- Randomly selected and frequency-matched to cases by 5-year age group, race/ethnicity, broad geographic area
Serum Collection

- Collected October 2011 – August 2015
- 10 mL of blood collected in serum separation tubes by trained phlebotomists
- Spun in field using portable centrifuges
- Frozen and stored at -20°C for 4-6 weeks
- Shipped to Environmental Chemistry Lab (ECL) at California’s Department of Toxic Substances Control (DTSC), CalEPA
- Frozen and stored at -20°C until chemical analysis
Laboratory Assays

- PFASs concentrations (ng/mL) measured by online SPE-HPLC-MS/MS (Symbiosis Pharma, IChrom Solutions, Plainsboro, NJ, and ABSciex 4000 QTrap mass spectrometer, ABSciex, Redwood City, CA)
- Native & isotopically-labeled PFAS standards (Wellington Labs)
- Quality Control:
  - In-house spiked calf serum samples
  - NIST 1958 Standard Reference Material run in duplicate within each 20-sample batch
  - QCL, QCM, QCH
- Routine performance tests:
  - Centers for Disease Control
  - Arctic Monitoring and Assessment Programme
- 12 compounds measured
## Restricted to PFASs with DF > 95%

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Name</th>
<th>Detection Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFHpA</td>
<td>Perfluoroheptanoic acid</td>
<td>63.6</td>
</tr>
<tr>
<td>PFOA</td>
<td>Perfluorooctanoic acid</td>
<td>99.9</td>
</tr>
<tr>
<td>PFNA</td>
<td>Perfluorononanoic acid</td>
<td>99.2</td>
</tr>
<tr>
<td>PFDA</td>
<td>Perfluorodecanoic acid</td>
<td>90.1</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>Perfluoroundecanoic acid</td>
<td>96.5</td>
</tr>
<tr>
<td>PFDaDA</td>
<td>Perfluorododeconic acid</td>
<td>9.6</td>
</tr>
<tr>
<td>PFBS</td>
<td>Perfluorobutane sulfonic acid</td>
<td>18.9</td>
</tr>
<tr>
<td>PFHxS</td>
<td>Perfluorohexane sulfonic acid</td>
<td>99.9</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluoroctane sulfonic acid</td>
<td>99.6</td>
</tr>
<tr>
<td>PFOSA</td>
<td>Perfluoroctane sulfonamide</td>
<td>74.5</td>
</tr>
<tr>
<td>EtPFOSA</td>
<td>2-(N-Ethyl-perfluoroctane sulfonamido) acetic acid</td>
<td>76.4</td>
</tr>
<tr>
<td>MePFOSAA</td>
<td>2-(N-Methyl-perfluoroctane sulfonamido) acetic acid</td>
<td>96.4</td>
</tr>
</tbody>
</table>
Covariate Information

- Collected through a series of CTS mailed surveys (1995-2013) and an interview-administered survey at blood draw (2011-2015)

- Included information on:
  - age at blood draw, race/ethnicity
  - reproductive history (parity, breastfeeding, age at first full-term pregnancy, age at menarche, menopausal status, age at menopause)
  - BMI, changes in BMI
  - diet, alcohol consumption, smoking, menopausal hormone use, physical activity
  - family history of breast cancer
  - sample collection date, season
  - neighborhood socioeconomics and urbanization
Statistical Analysis

- PFASs < level of detection (LOD) were imputed = LOD/√2
- PFAS levels log_{10} transformed to symmetrize the data
- Unconditional multivariable logistic regression analyses – odds ratios
  - Separate model for each PFAS, modeled both as a continuous and categorical variable (based on tertiles in controls)
  - Summary measures: ∑PFSA=\text{sum}(PFOS, PFHxS, MeFOSAA);
    ∑PFCA=\text{sum}(PFOA, PFNA, PFUnDA)
- Covariate selection: backwards step-wise, 10% change in PFAS odds ratios
- Subset analyses (selected \textit{a priori} based on lit review):
  - Breast cancer subtypes: post versus pre/peri-menopausal; ER+ or PR+ tumors/ER- and PR- tumors; luminal A tumors; triple-negative tumors
  - Host characteristics: never/ever HT users; nulliparous/parous; categories of BMI
Results: Study Population Characteristics

Age: Median = 67 yrs; Range 41-92 yrs

- 80+ yrs: 7%
- 70-79 yrs: 34%
- 60-69 yrs: 42%
- 50-59 yrs: 13%
- 40-49 yrs: 4%

Race/Ethnicity: 89% NH-White

- NH-White: 89%
- Black: 4%
- Hispanic: 2%
- Asian/PI: 2%
- Other: 3%
Results: Characteristics of Cases & Controls

Compared to controls, cases more likely to:

- report a family history of breast cancer
- smoke tobacco
- have higher BMI
- be nulliparous
- have later age at first full term birth
- consume more red meat and pork
- have blood sampled during spring and summer
Serum PFAS Concentrations

PFCAs

<table>
<thead>
<tr>
<th>Serum concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA Cases</td>
</tr>
<tr>
<td>PFOA Controls</td>
</tr>
<tr>
<td>PFNA Cases</td>
</tr>
<tr>
<td>PFNA Controls</td>
</tr>
<tr>
<td>PFUnDA Cases</td>
</tr>
<tr>
<td>PFUnDA Controls</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Max</th>
<th>Min</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.35</td>
<td>2.48</td>
<td>0.85</td>
</tr>
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</table>

PFSAs

<table>
<thead>
<tr>
<th>Serum concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOS Cases</td>
</tr>
<tr>
<td>PFOS Controls</td>
</tr>
<tr>
<td>PFHxS Cases</td>
</tr>
<tr>
<td>PFHxS Controls</td>
</tr>
<tr>
<td>MeFOSAA Cases</td>
</tr>
<tr>
<td>MeFOSAA Controls</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Max</th>
<th>Min</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.70</td>
<td>6.95</td>
<td>1.52</td>
</tr>
</tbody>
</table>
Odds Ratios (ORs) for Breast Cancer

<table>
<thead>
<tr>
<th>Substance</th>
<th>OR</th>
<th>Upper 95% CI</th>
<th>Lower 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.82*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFNA</td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΣPFCA</td>
<td>0.76+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‡ ORs for high vs low (3rd vs 1st tertile) of serum level
* p-trend < 0.05
Odds Ratios for Breast Cancer

Odds Ratios (OR), 95% Confidence Intervals (CI)‡

‡ ORs for high vs low (3rd vs 1st tertile) of serum level
ORs for Breast Cancer, by Menopausal Status

**Post-menopausal**

$n = 1657$: 859 cases, 798 controls

<table>
<thead>
<tr>
<th>Substance</th>
<th>Upper CI</th>
<th>Lower CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.92</td>
<td>0.96</td>
<td>0.80</td>
</tr>
<tr>
<td>PFNA</td>
<td>0.92</td>
<td>0.96</td>
<td>0.81</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.80</td>
<td>0.81</td>
<td>0.80</td>
</tr>
<tr>
<td>ΣPFCA</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
</tr>
</tbody>
</table>

**Pre/Peri-menopausal**

$n = 102$: 43 cases, 59 controls

<table>
<thead>
<tr>
<th>Substance</th>
<th>Upper CI</th>
<th>Lower CI</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.56</td>
<td>0.24</td>
<td>1.87</td>
</tr>
<tr>
<td>PFNA</td>
<td>0.56</td>
<td>0.24</td>
<td>1.87</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.24</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>ΣPFCA</td>
<td>0.75</td>
<td>0.75</td>
<td>0.75</td>
</tr>
</tbody>
</table>

‡ ORs for high vs low (3rd vs 1st tertile) of serum level
ORs for Breast Cancer, by Menopausal Status

Post-menopausal
n = 1657: 859 cases, 798 controls

Pre/Peri-menopausal
n = 102: 43 cases, 59 controls

‡ ORs for high vs low (3rd vs 1st tertile) of serum level
**ORs for Breast Cancer, by ER/PR Tumor Status**

**ER+ or PR+ Tumors**  
$n = 1601$: 743 cases, 858 controls

<table>
<thead>
<tr>
<th>Serum Level</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.84 (0.79, 0.78)</td>
</tr>
<tr>
<td>PFNA</td>
<td>0.93</td>
</tr>
<tr>
<td>PFUnDA</td>
<td>0.79</td>
</tr>
<tr>
<td>ΣPFCA</td>
<td></td>
</tr>
</tbody>
</table>

**ER- and PR- Tumors**  
$n = 965$: 107 cases, 858 controls

<table>
<thead>
<tr>
<th>Serum Level</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFOA</td>
<td>0.64 (0.52*, 0.65)</td>
</tr>
<tr>
<td>PFNA</td>
<td></td>
</tr>
<tr>
<td>PFUnDA</td>
<td></td>
</tr>
<tr>
<td>ΣPFCA</td>
<td></td>
</tr>
</tbody>
</table>

‡ ORs for high vs low (3rd vs 1st tertile) of serum level  
* p-trend < 0.05
ORs for Breast Cancer, by ER/PR Tumor Status

ER+ or PR+ Tumors
n = 1601: 743 cases, 858 controls

<table>
<thead>
<tr>
<th>PFOS</th>
<th>PFHxS</th>
<th>MeFOSSA</th>
<th>ΣPFSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.92</td>
<td>0.83</td>
<td>0.84</td>
<td>0.89</td>
</tr>
</tbody>
</table>

ER- and PR- Tumors
n = 965: 107 cases, 858 controls

<table>
<thead>
<tr>
<th>PFOS</th>
<th>PFHxS</th>
<th>MeFOSSA</th>
<th>ΣPFSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.52 *</td>
<td>0.45 *</td>
<td>0.46 *</td>
<td></td>
</tr>
</tbody>
</table>

‡ ORs for high vs low (3rd vs 1st tertile) of serum level
* p-trend < 0.05
Breast Cancer ORs: Subset Analyses

Generally, null patterns of risk seen for:
- Cases with Luminal A tumors
- Cases with triple negative tumors
- Never/ever users of hormone therapy
- Nulliparous/parous women
- Women with low, medium and high BMI
Results Summary

- Overall, no evidence of breast cancer risk associated with serum PFASs measured at the time of (or shortly after) diagnosis

- Some suggestion of reduced risk associated with some PFASs, particularly for cases with non-hormonally responsive tumors

- Conclusions need to consider results in context of:
  - potential mechanisms
  - other epidemiologic findings
  - limitations of current study
Discussion – Potential Mechanisms

- Proposed mechanisms for PFAS and breast carcinogenesis*:
  1. Developmental toxicity -- mammary gland, hormonal signaling
  2. Genotoxicity – either direct or indirect
  3. Hormonal tumor promotion

- Timing of exposure matters!

PFASs Developmental Toxicity

- Early life exposures lead to permanent changes in mammary gland morphology and/or alterations in sex steroid hormone signaling pathways
- Laboratory evidence – pubertal delays & mammary gland abnormalities (in mice, dependent on dose & timing)
- Human evidence – onset of puberty (limited and mixed)
- Critical periods of exposure: prenatal & puberty
Genotoxicity of PFASs

- Little evidence of direct genotoxicity
- Some evidence of indirect genotoxicity through formation of reactive oxygen species (ROS) induced by oxidative stress
- Mammary tissue is thought to be most susceptible to genotoxic effects of carcinogens during puberty and pregnancy but exposures anytime during life could be relevant
PFAS: Tumor Progression

Progression of breast tumors are promoted by estrogens
- Promote tumor growth by increasing cellular proliferation and/or decreasing apoptosis
- Both endogenous & exogenous (e.g. menopausal HT)

Estrogenic effects of PFASs are unclear:
- Laboratory data: estrogen disruption but not well-understood
  - Don’t bind to estrogen receptors
  - Effects vary by compound, species, strains within species, by dose (not necessarily monotonic dose-response)
  - Effects may be dependent on co-exposures to estradiol (estrogenic alone, anti-estrogenic with high levels of estradiol)
- Human data – more limited, also very complex
  - Both estrogenic and anti-estrogenic effects
  - Effects may vary by host characteristics (genetic polymorphisms, nulliparity)

Chronic/later exposures are relevant
Age of Study Participants Limits Potential for Early Life Exposures

- Widespread PFASs exposures in the U.S. began in 1980s and 1990s
- Our study population: all born before 1972, 75% born before 1952
- Limits our ability to detect risks:
  - Mediated by developmental toxicity induced by prenatal & pubertal PFASs exposures
  - Mediated by genotoxic effects induced by PFAS exposures during puberty & pregnancy
- Our study is best suited to evaluate risks for hormonally-mediated tumor promotion associated with chronic, later in life PFAS exposures
PFAS & Breast Cancer: Other Epi Studies

No association:
- C8 Science Panel Studies [Barry et al. 2013; Vieira et al. 2013]
  - PFOA only (high exposures due to historical water contamination)
  - Estimated chronic lifetime PFOA exposure (modeled, not measured)
  - Overall a very large study (n=1,000+ cases of breast cancer) but few cases in heavy exposure group (n=29)
- Danish Study [Bonefeld-Jorgensen et al. 2014; Ghisari et al. 2017]
  - Only included premenopausal women
  - Case control (n=250 cases/250 controls)
  - PFAS levels in sera collected during pregnancy, 10-15 years prior to diagnosis/enrollment
  - Some suggestion of an inverse association for PFHxS and positive association for PFOSA

Increased risks:
- Greenlandic Inuits [Bonefeld-Jorgensen et al. 2011; Ghisari et al. 2014; Wielsoe et al. 2018]
  - Small case-control (n=77 cases/84 controls)
  - PFAS levels in sera collected at diagnosis/enrollment
  - Significantly increased risks for PFOA, PFOS, PFHxS, ΣPFSA
  - Risks modified by polymorphisms in CYP and COM genes
  - Risks did not differ by tumor hormone responsiveness
  - Also reported increased risks for PCBs
  - Serum levels of some, but not all PFASs very high (PFOS, PFHxS, PFNA)
Summary: Epidemiologic Evidence

- Small body of literature
- Inconsistent findings – may be due to varying:
  - exposure assessment methods
  - windows of exposure
  - exposure levels
  - host characteristics of study population (e.g., premenopausal, genetic polymorphisms, endogenous estrogen levels)
Limitations of Our Study

- Age structure precludes ability to evaluate risks associated with early life exposures (can’t assess risk associated with developmental toxicity)

- Adequacy of our biomarkers of exposure
  - Do they represent levels in breast tissue?
  - How well do they represent chronic exposures?
  - Reverse causality?

- Inability to incorporate host characteristics
  - Genetic susceptibility
  - Endogenous estrogen levels
Study Strengths

- Large well-defined study population, specifically designed to study breast cancer
- Ability to control for breast cancer confounders
- Examination of risks separately for subtypes of breast cancer and for potentially susceptible subpopulations
- Quantitative measures of PFAS exposures (serum concentrations)
Conclusions

- Our results do not support an association between breast cancer risk and serum levels of PFASs at the time of (or shortly after) diagnosis in middle-aged & older California women.

- In context of tox and epi literature, further research is warranted:
  - Other populations with potentially more relevant exposure profiles (e.g. younger women)
  - Consideration of co-exposures to estrogens – both endogenous and exogenous
  - Polymorphisms in genes that regulate estrogen biosynthesis and metabolism
  - Consideration of breast cancer subtype
  - Consideration of mediating factors on the causal pathway (e.g. timing of menarche, menopause, BMI)

- Clarification of risks and mechanisms important to inform the choice of replacement PFASs to avoid regrettable substitutions.
Acknowledgments

Funders

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- National Cancer Institute (Grants R01 CA77398)

Study Team

- CPIC: Peggy Reynolds (PI), Debbie Goldberg, David O. Nelson
- ECL: Myrto Petreas, June-Soo Park, Miaomiao Wang, Weihong Guo, Shuhash Harwani, Erika Houtz

CTS Participants

Questions or comments: susan.hurley@cpic.org