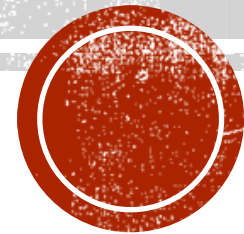# PFAS-RELATED HEALTH EFFECTS: LESSONS FROM THE FAROESE STUDIES AND RECENT FINDINGS ABOUT THE MICROBIOME

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Outline

1. **The Faroese birth cohorts**

2. **Important finings from these cohorts**

   A. PFAS exposure pathways

   B. Potential Health effects of PFAS

3. **Lifetime exposure to PFAS in relation to the Microbiome**

   A. Methods

   B. Findings

FAROE ISLANDS

© Encyclopædia Britannica, Inc.

Faroe Islands have a homogeneous population with high consumption of marine mammals

- Homogeneous: SES, genetics
- High participation rate in prospective studies
- Total population - 48,000
- In deep assessment of exposures

Cohort 1 n=1022

Cohort 2 n=182

Cohort 3 n=656

Cohort 4 n=148

Cohort 5 n=475

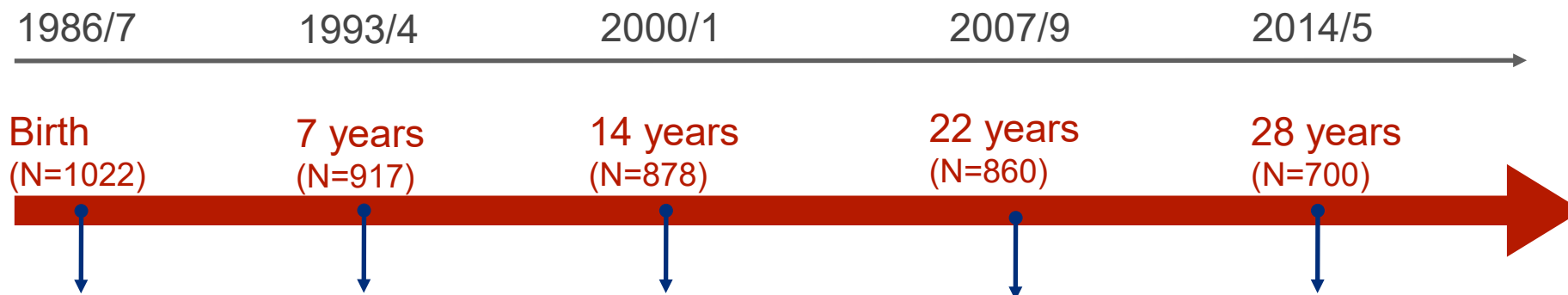1986-1987   1994-1995   1998-2000   2000-2001   2007-2009

4

DESIGN AND DATA COLLECTION

1986/7	1993/4	2000/1	2007/9	2014/5
Birth (N=1022)	7 years (N=917)	14 years (N=878)	22 years (N=860)	28 years (N=700)

Cord whole blood*:
PFAS:
PFOS, PFOA, PFHxS, PFDA, PFNA, Other POPs

*Maternal serum concentrations estimated using cord blood:maternal serum ratio (Eryasa et al. Environ Int 2019)

Offspring serum PFAS: PFOS, PFOA, PFHxS, PFDA, PFNA

Multiple health endpoints assessment:
- **Anthropometric measures**
- **blood pressure**
- **2h-oral glucose tolerance test (OGTT)**
- **Neurodevelopment**
- **Immune system**

Obstetric history from birth medical records, interview-based questionnaires on parental and offspring lifestyle factors, offsprings' physical examination by clinician and biological samples collected in all follow-up visits.
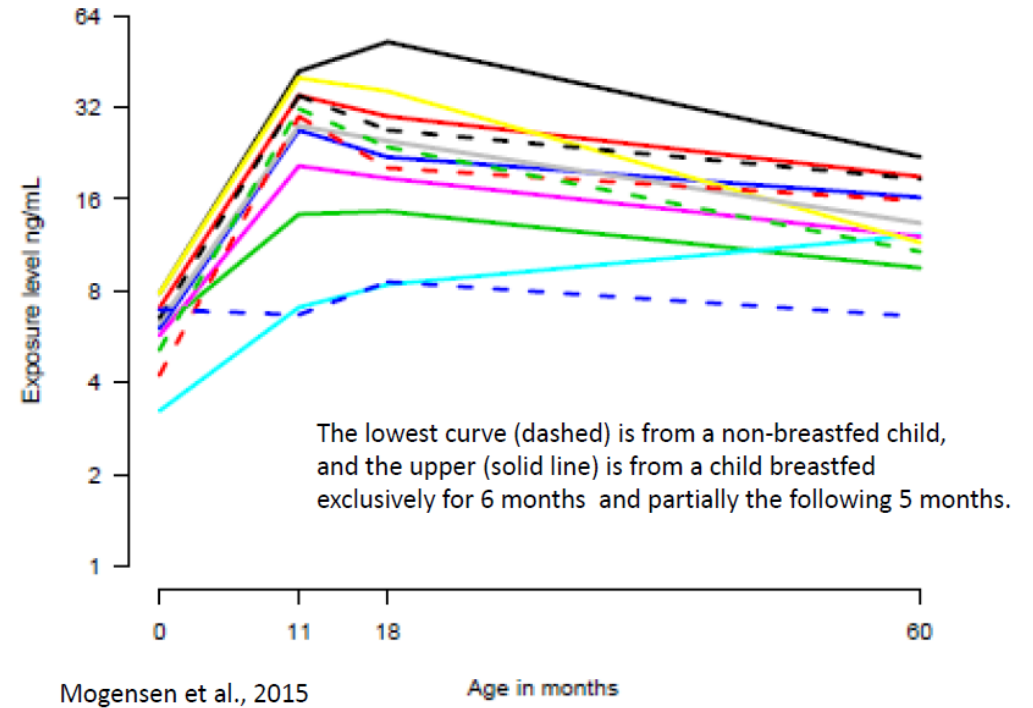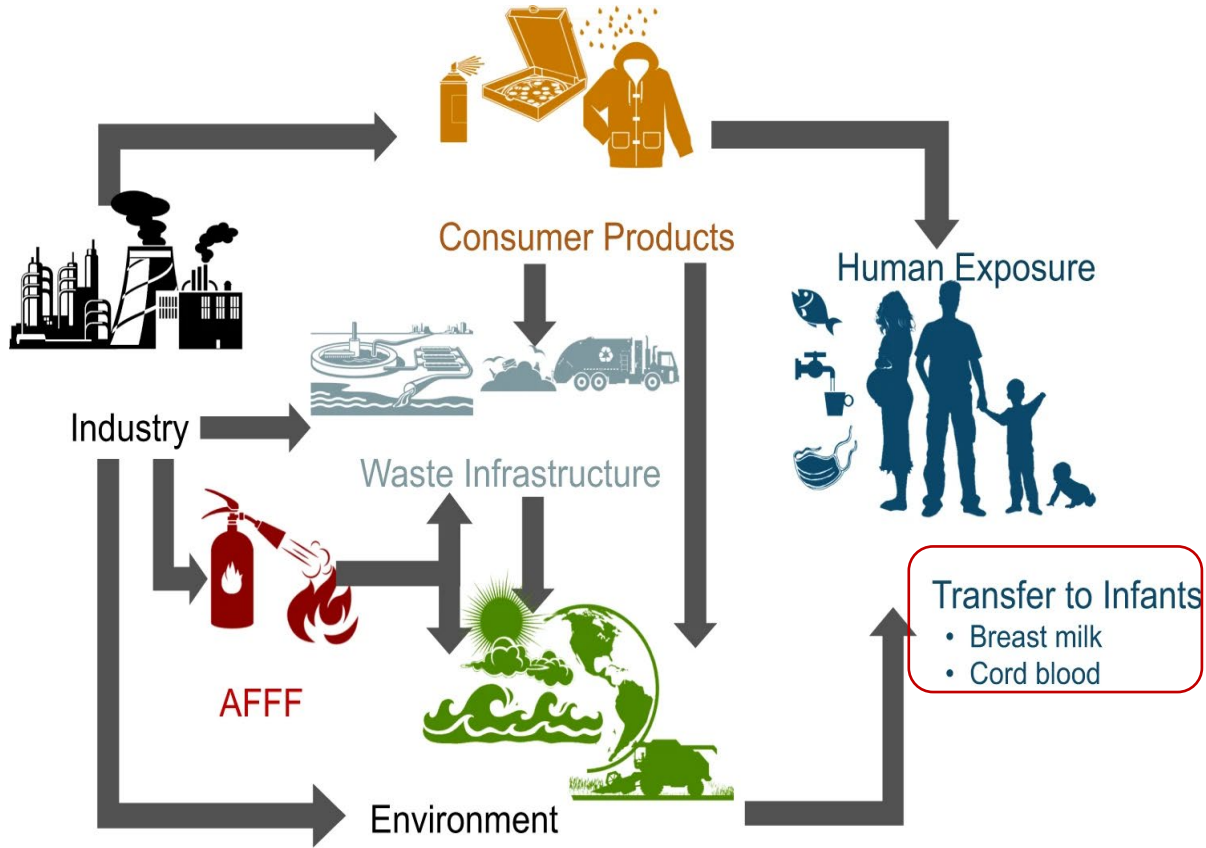
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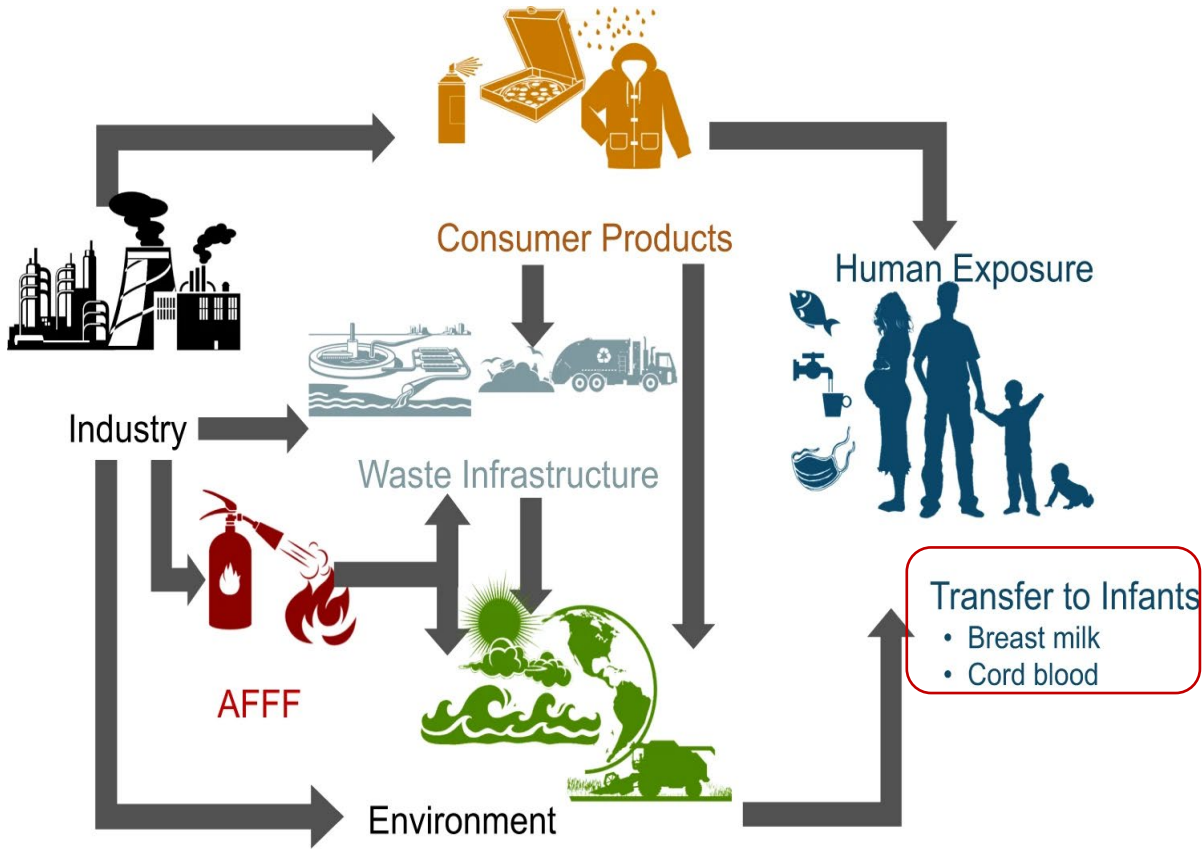
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PFAS EXPOSURE PATHWAYS FOR DIFFERENT HUMAN POPULATIONS

The lowest curve (dashed) is from a non-breastfed child, and the upper (solid line) is from a child breastfed exclusively for 6 months and partially the following 5 months.

Mogensen et al., 2015

Sunderland et al. JESEE 2019

PFAS EXPOSURE PATHWAYS FOR DIFFERENT HUMAN POPULATIONS

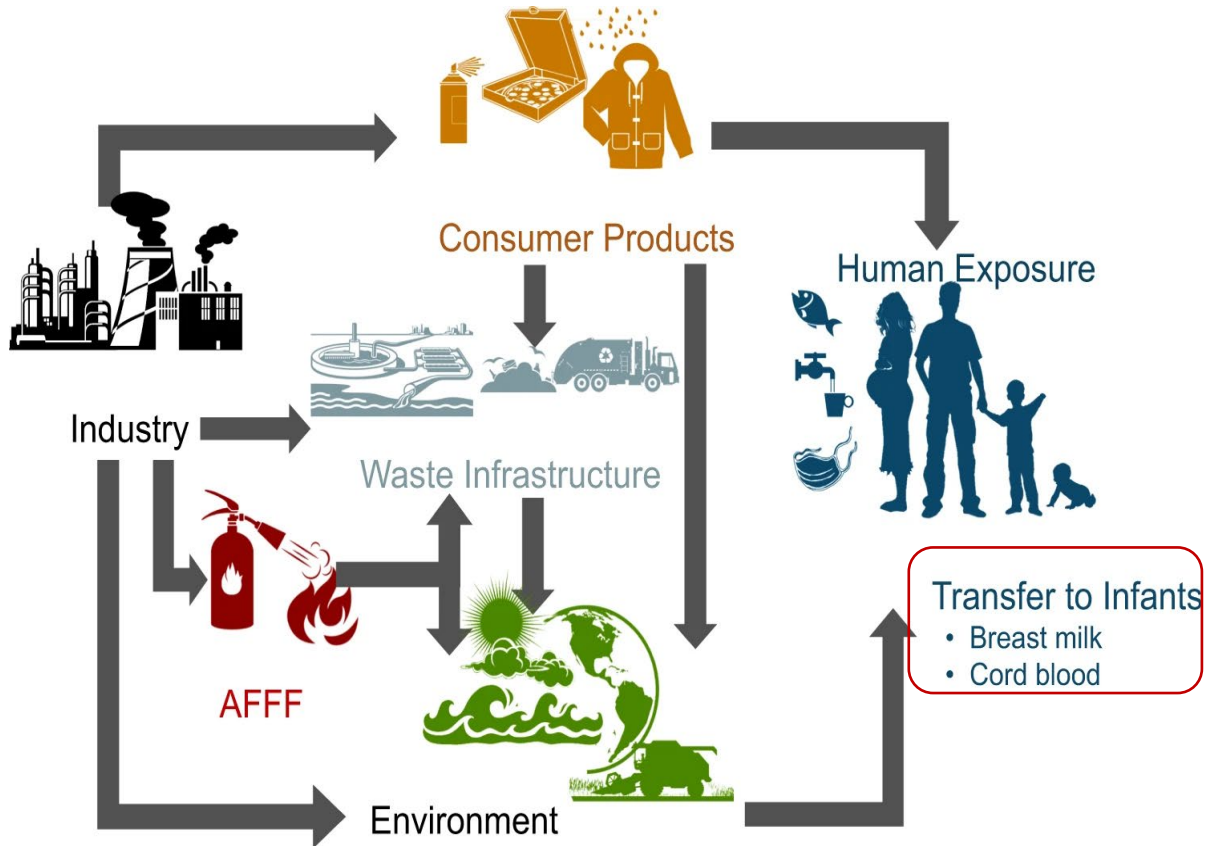Sunderland et al. JESEE 2019
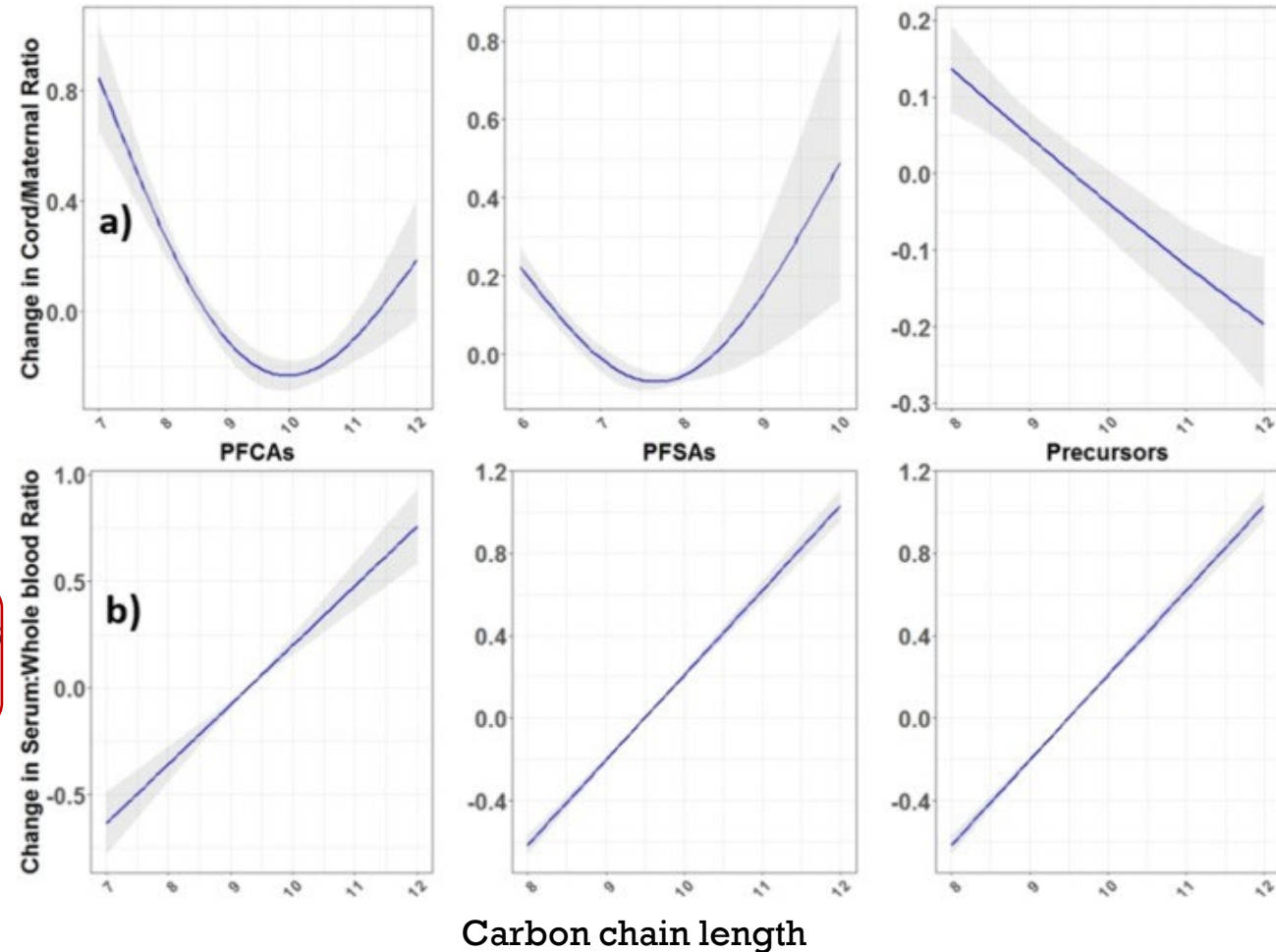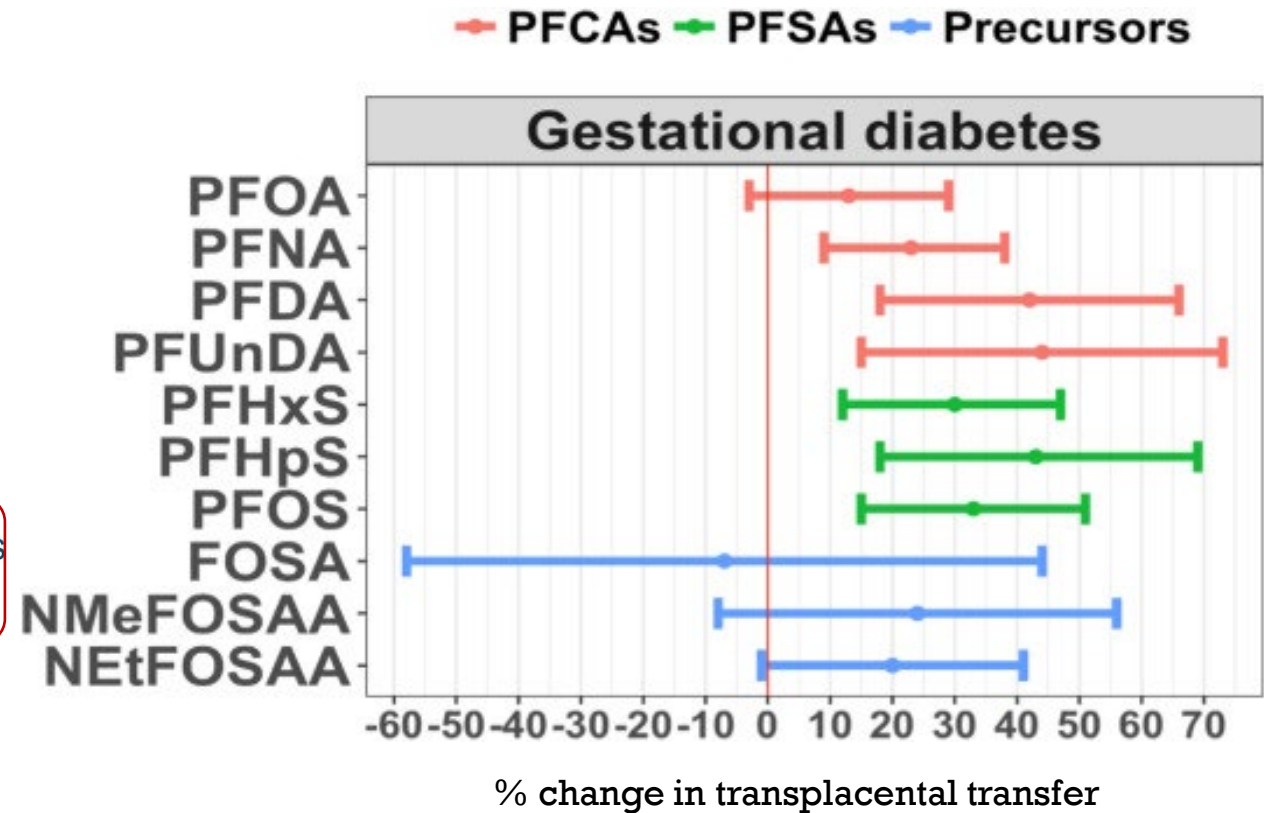
Eryasa et al. Env. Int 2019

PFAS EXPOSURE PATHWAYS FOR DIFFERENT HUMAN POPULATIONS

Sunderland et al. JESEE 2019

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- DuPont's West Virginia Washington Works Plant in southwest Parkersburg released C8 into the air and Ohio River from the 1950s until the early 2000s.
- C8 (PFOA) reached drinking water supplies by entering the groundwater and was detected in six water districts near the DuPont plant in 2002.

- **After a class action:**
  1. DuPont paid $70 million in damages upfront
  2. Paid for a state-of-the-art cleanup of the Parkersburg area's water supplies
  3. Agreed to fund an independent panel of scientists – the C8 Science Panel – to study the links between C8 and various diseases between 2005 and 2013 (n~70,000 residents).
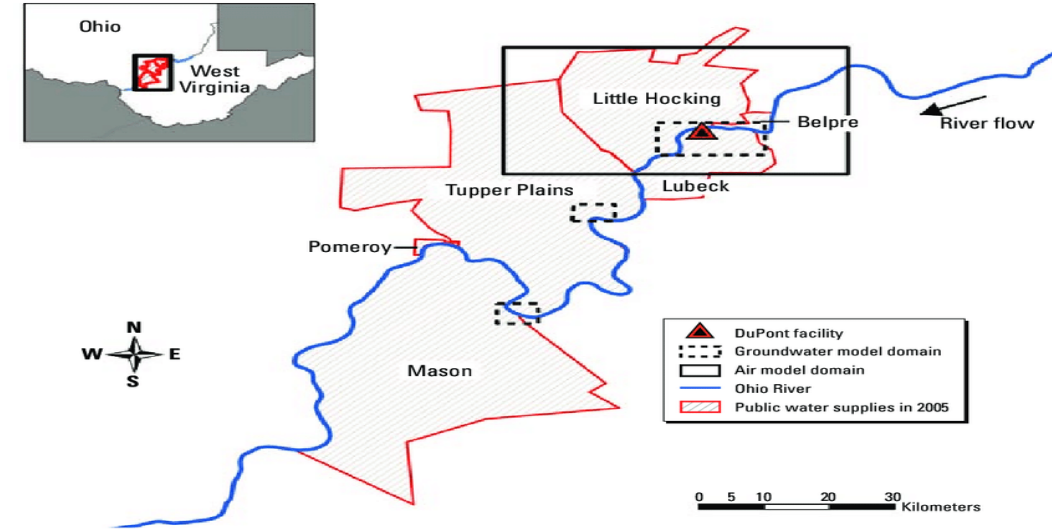
Findings:
- **High cholesterol (dyslipidemia)**
- Thyroid disease
- Pregnancy induced hypertension
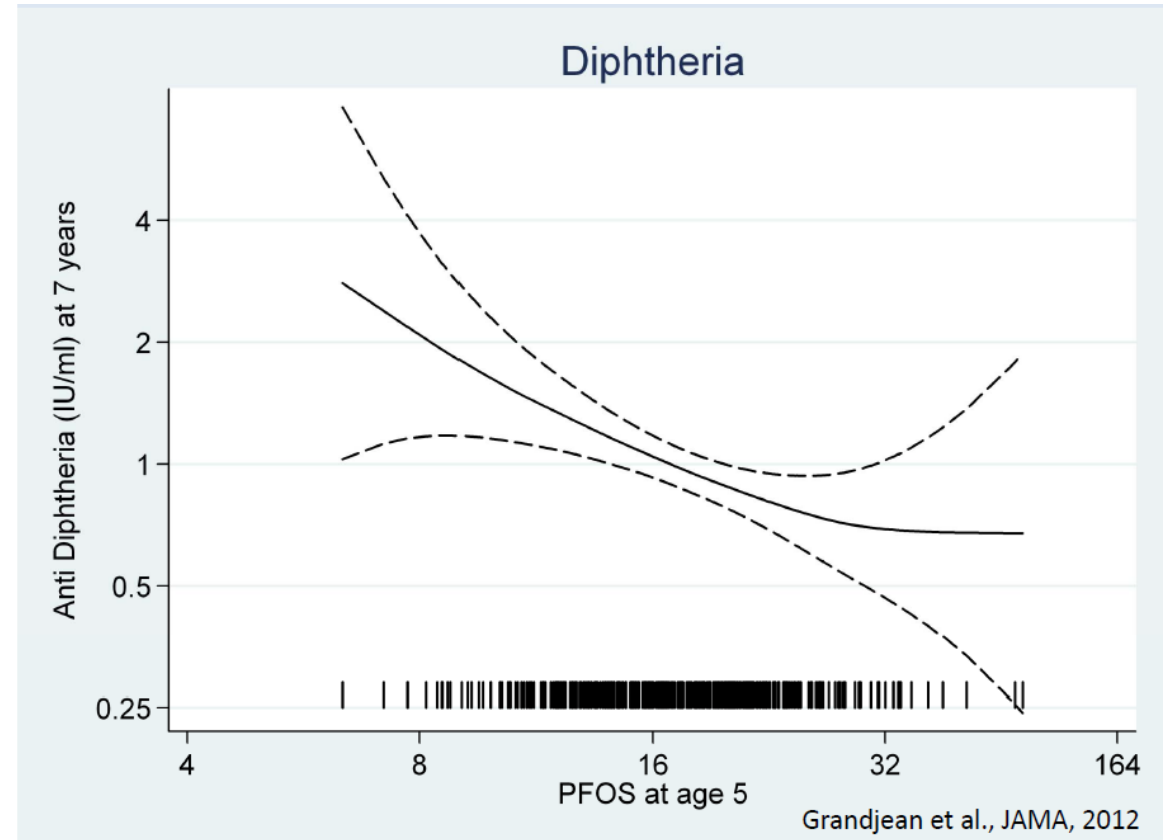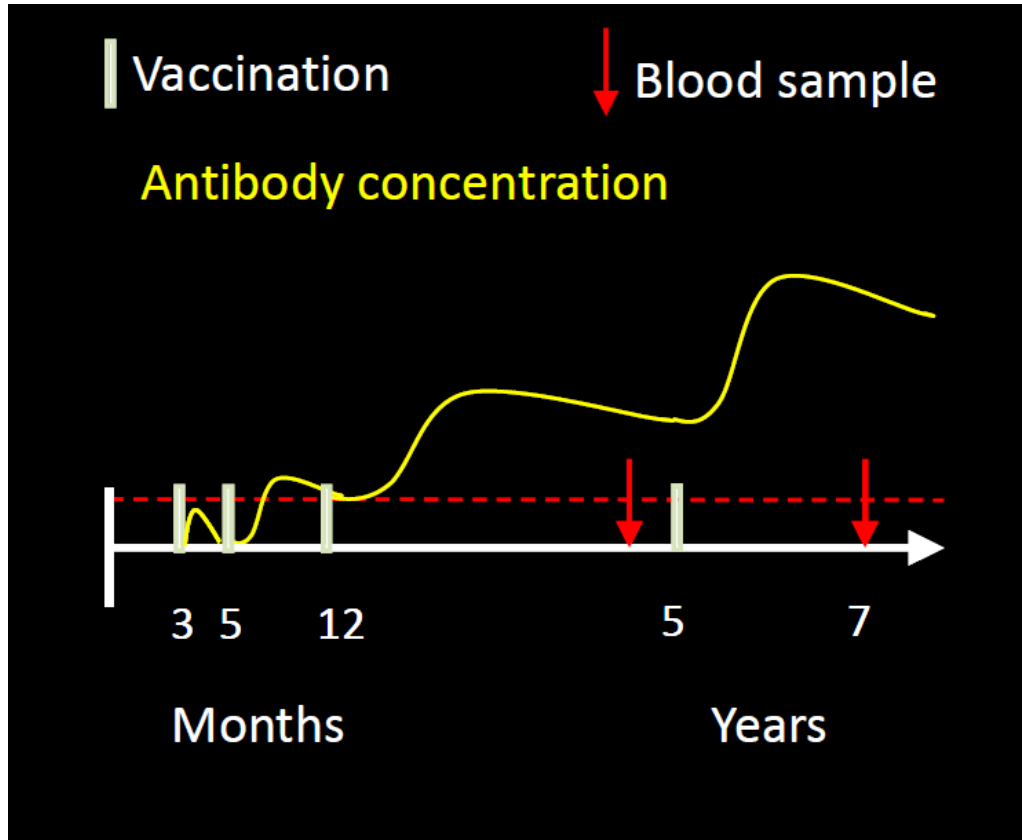- **Ulcerative colitis**
- Kidney and Testicular cancer

Some challenges:
- Most studies were based on modeled levels, not measured ones.

PFAS-RELATED HEALTH EFFECTS
Immunosuppressive effects

Courtesy of Dr. Grandjean

PFAS-RELATED HEALTH EFFECTS
Immunosuppressive effects

Since then, evidence emerged about

- Rubella antibodies
- Increased risk of common infections (cold and gastroenteritis)
- Higher frequency of fever and its symptoms

- Cytokines levels and white blood cell counts
- Hypersensitivity and autoimmunity

National Toxicology Program
U.S. Department of Health and Human Services

NTP Monograph

Immunotoxicity Associated with Exposure to Perfluorooctanoic Acid or Perfluorooctane Sulfonate
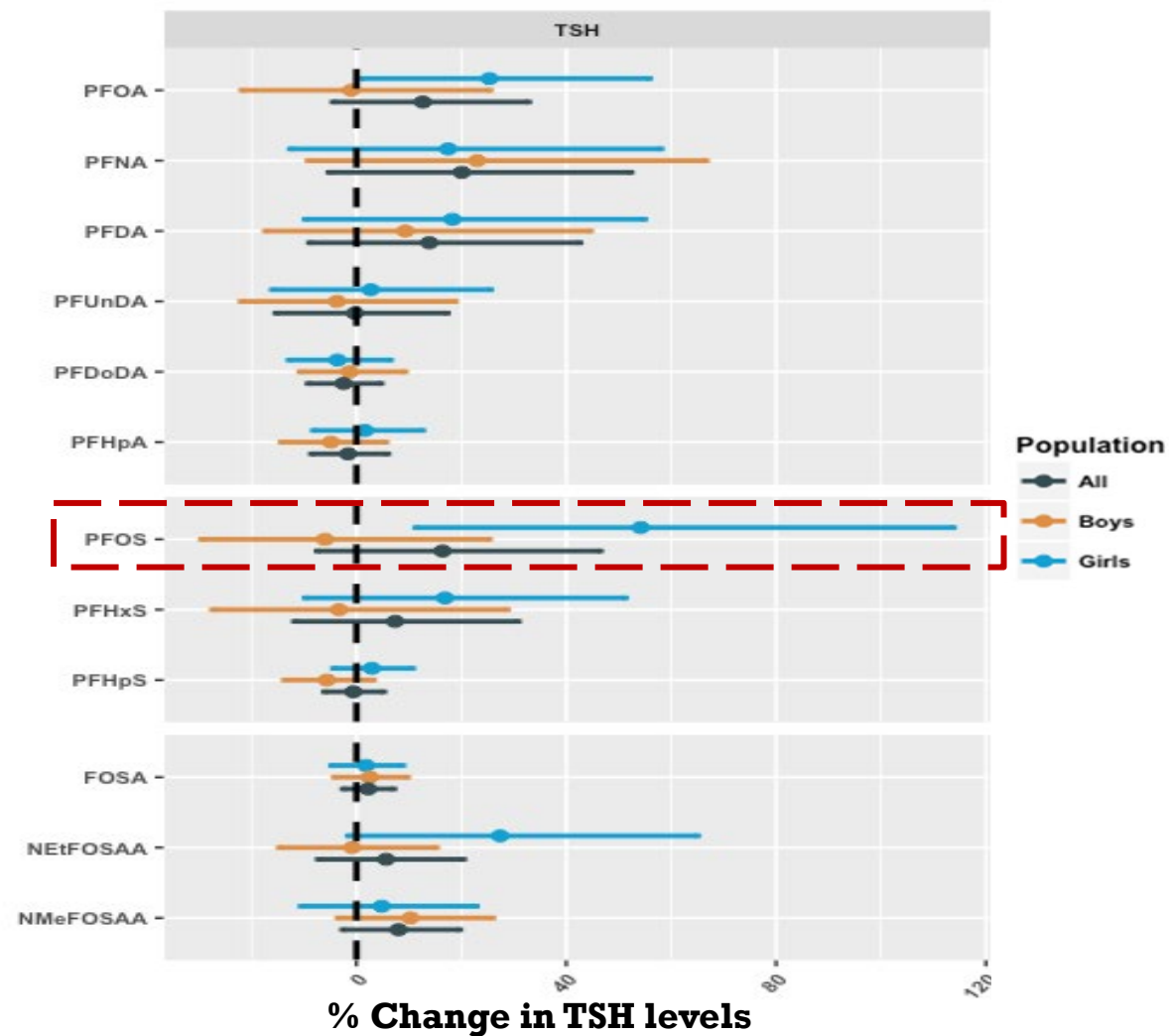
2016

| Category of Immune Response	Immune Outcomes	Confidence Ratings in the Body of Evidence		Level of Evidence in the Body of Evidence		Hazard Conclusion
		Human	Animal	Human	Animal	
Immunosuppression	Antibody response	Moderate	High	Moderate	High	Presumed to be an Immune Hazard to Humans

Liew, Goudarzi & Oulhote 2018

Xiao et al. JCEM 2019

- Most studies showed decreased birth weight for PFOA

- Less consistent results for PFOS,

- Not enough studies for other PFAS

% Change in birth outcomes z-scores

Xiao et al. JCEM 2019
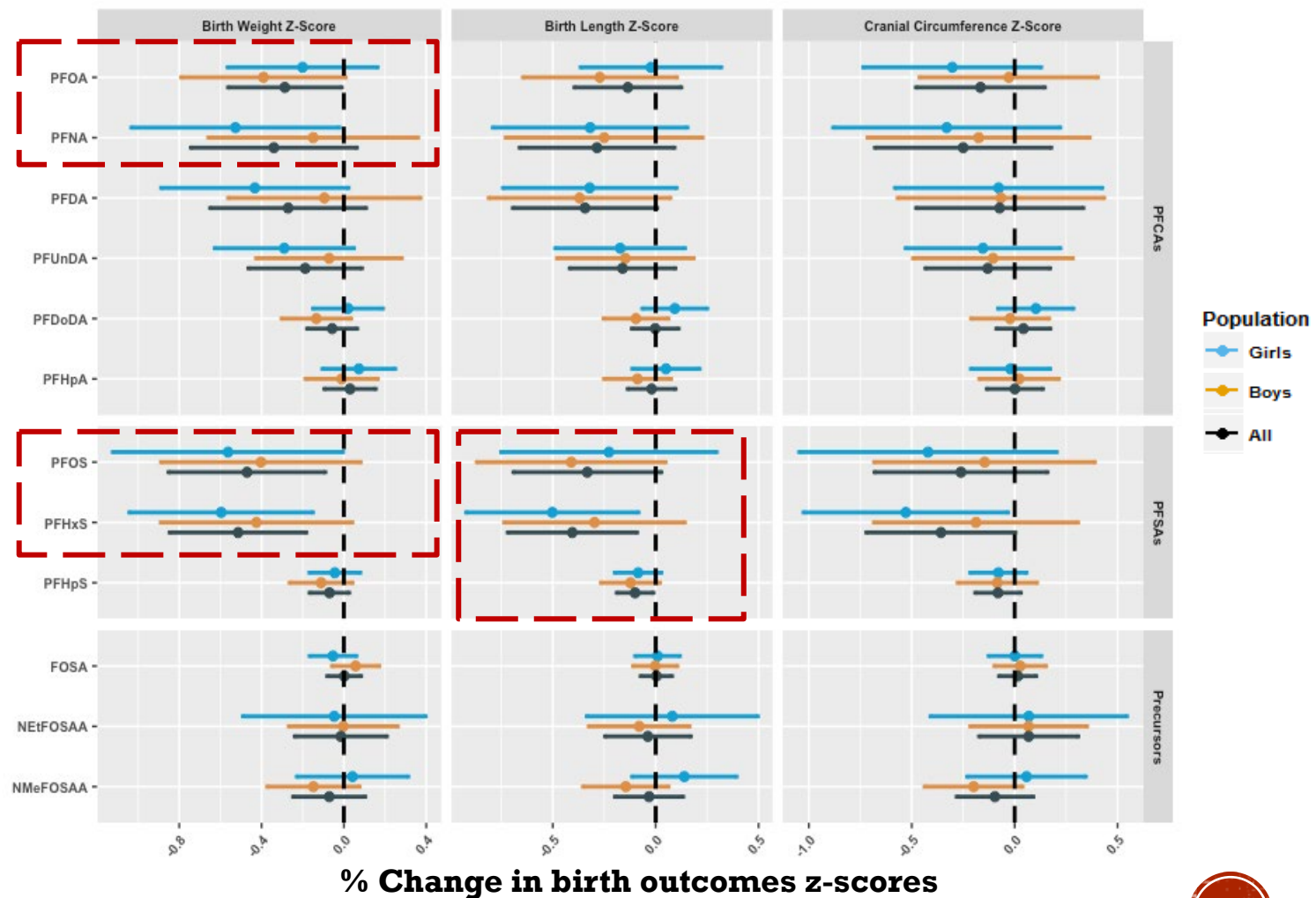
PFAS-RELATED HEALTH EFFECTS
Markers of Type 2 Diabetes

Estimates from Multiple Informant GEE models: N=653; 5 PFAS measures/subject
Geometric mean % change in outcome per doubling of PFOS
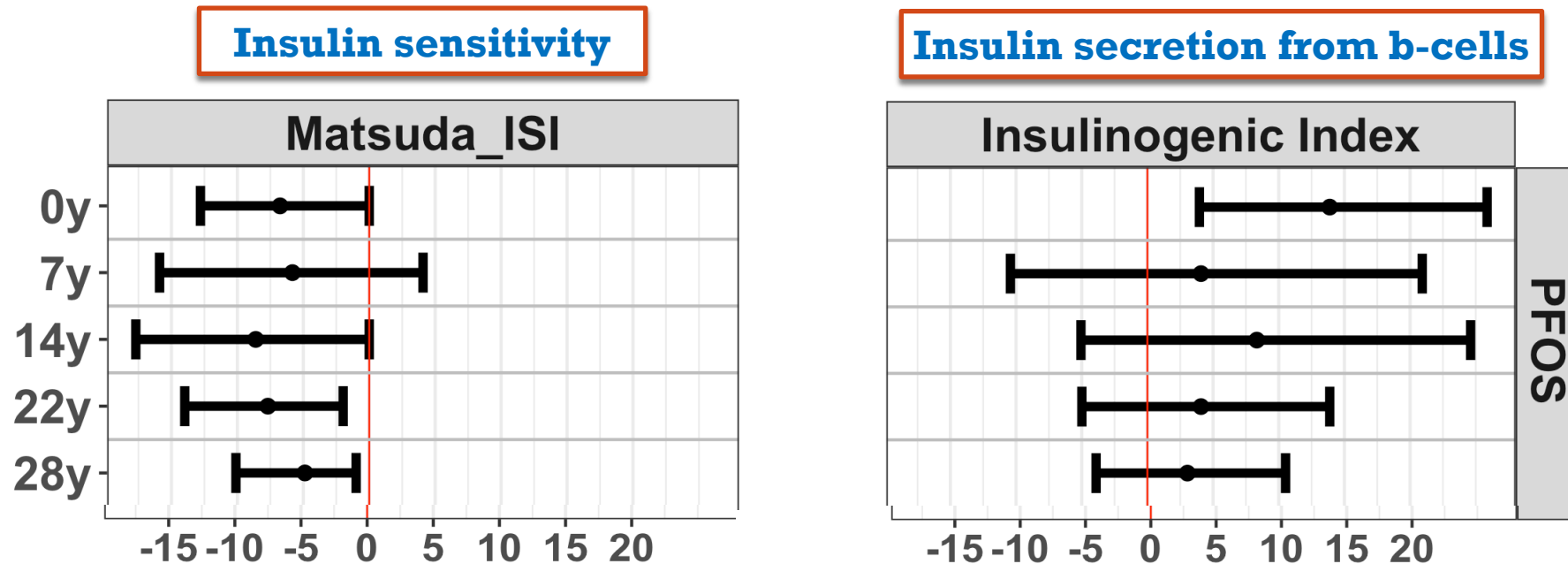
Estimates are adjusted for age*PFAS, sex, maternal prepregnancy BMI, gestational diabetes, parity, smoking in pregnancy, participant's smoking and alcohol consumption in adult examinations and (time-varying) exact age at visits and fish/whale intakes.
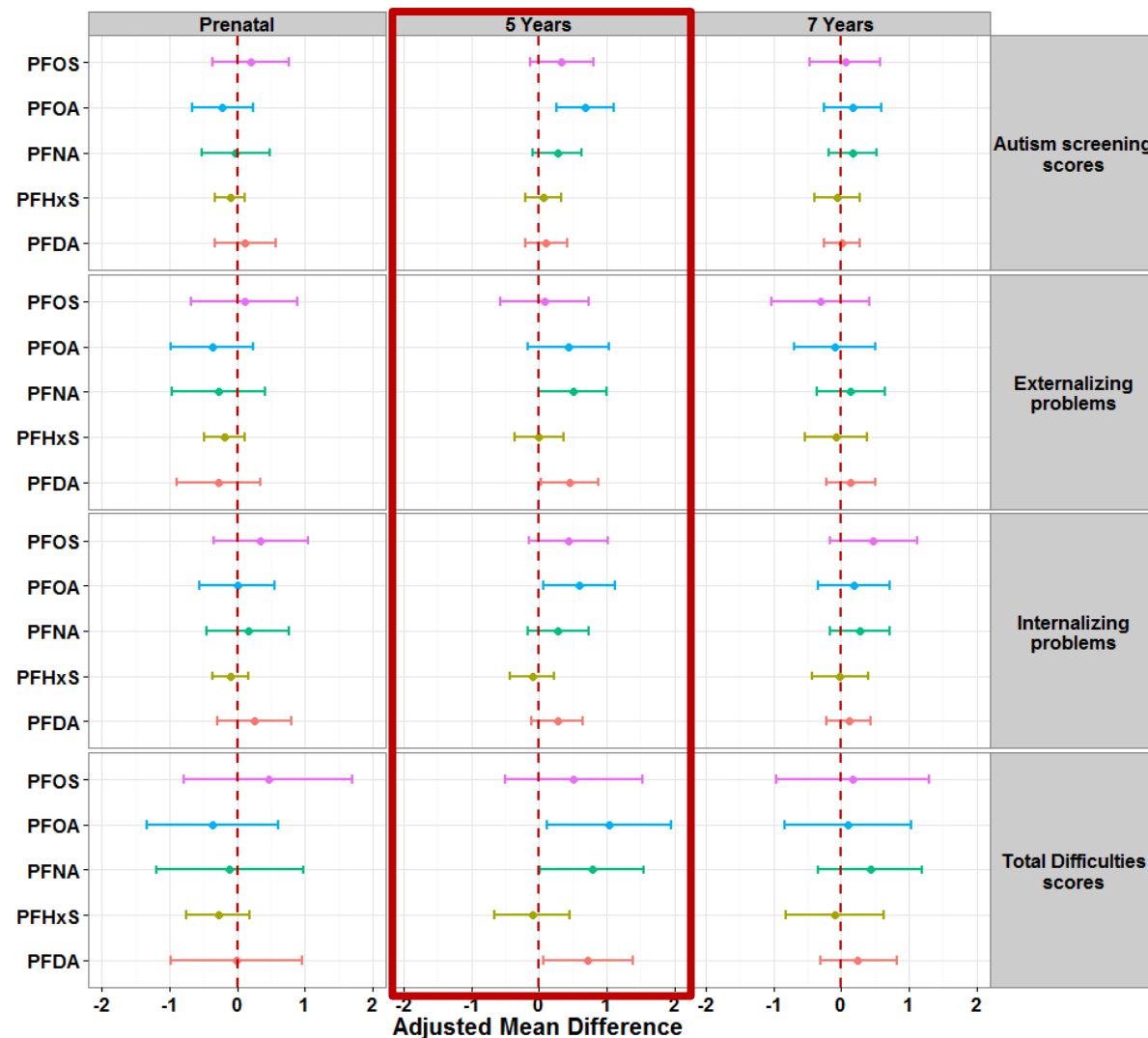
Valvi et al. in Review

PFAS-RELATED HEALTH EFFECTS
Neurodevelopment

- Inconsistent findings in regard to child behavioral and cognitive functions

- There appears that effects may be sex-specific

Oulhote et al. 2017

PFAS-RELATED HEALTH EFFECTS
Neurodevelopment

- Inconsistent findings in regard to child behavioral and cognitive functions

- There appears that effects may be sex-specific

- Further studies should take into account cumulative effects of PFAS and PFAS with other chemicals
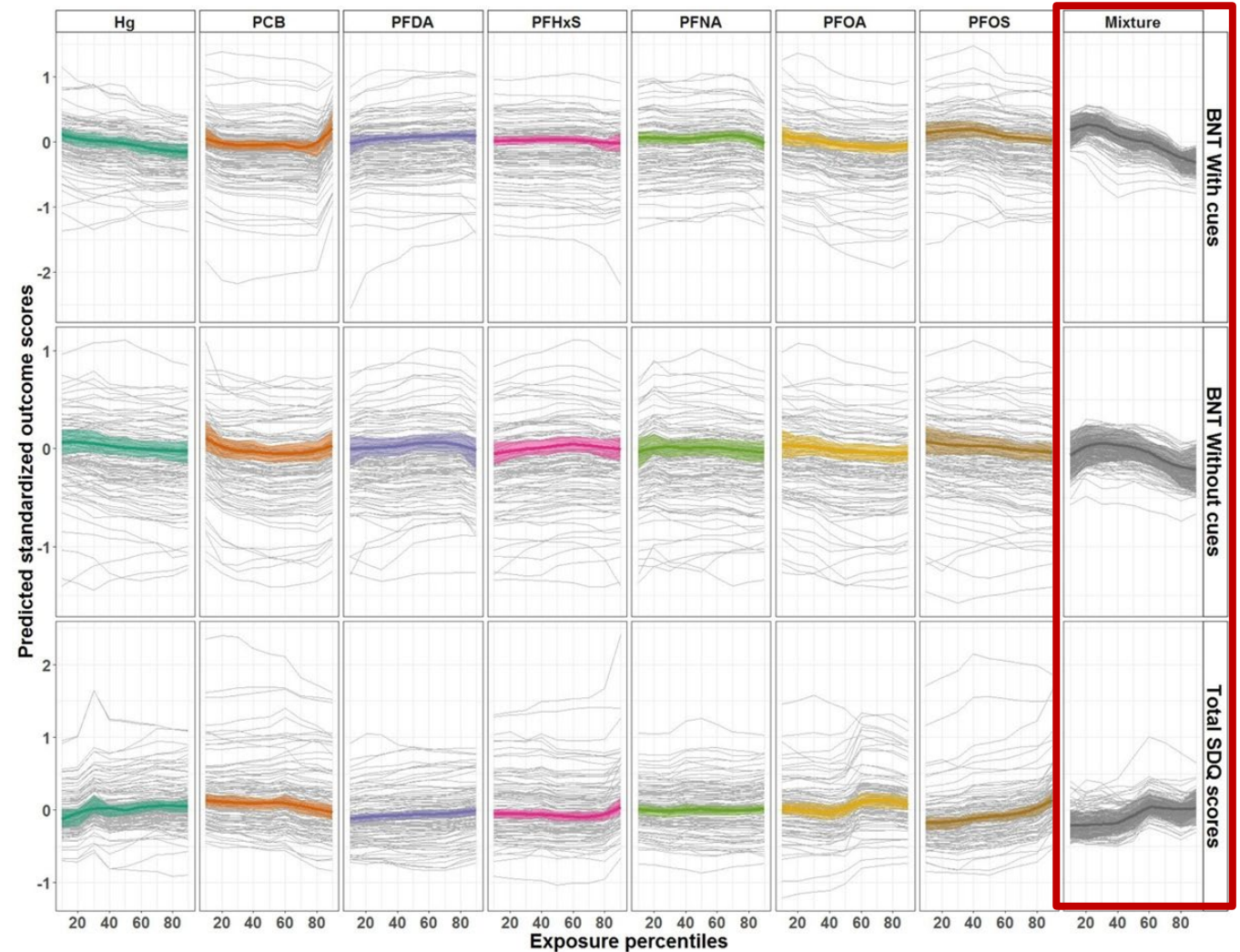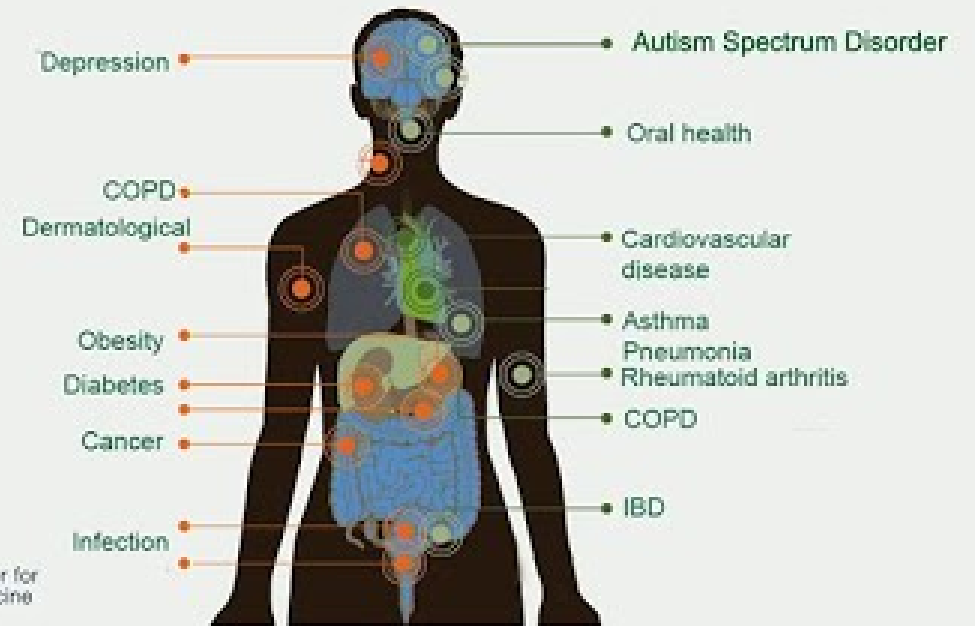

Oulhote et al. 2019

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MICROBIOME SIGNATURES AS A POTENTIAL MECHANISM

- Many of the POPs have antimicrobial properties,

- Chemicals can alter the composition of the gut microbiome,

- These alterations have been tentatively linked to the development of several diseases, including asthma, obesity, and the inflammatory bowel diseases (IBD)
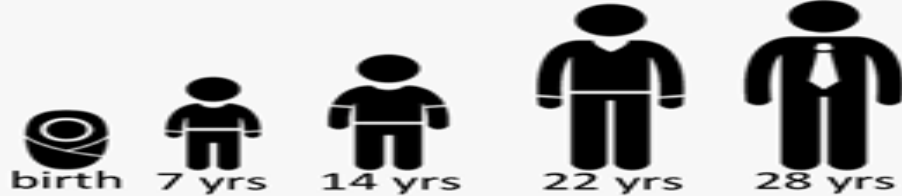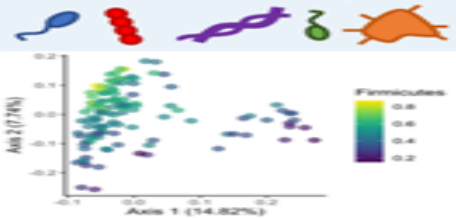

Microbiome Perturbation Associates with Disease

Depression • — Autism Spectrum Disorder

• Oral health

COPD •
Dermatological • — Cardiovascular disease

• Asthma
Obesity • Pneumonia
Diabetes • • Rheumatoid arthritis
• COPD
Cancer •

• IBD
Infection •

UCSF The Benioff Center for Microbiome Medicine

METHODOLOGY: MATERIAL

Dr. Kelsey Thompson
HSPH & Broad Institute

METHODOLOGY: STATISTICAL METHODS

- Pre-Processing:
  - ✓ Final read count per sample averaged 70,089,019 (±15,719,889) → 1 sample removed (< 1.5 M reads) We used the marker gene-based approach from MetaPhlAn 2 to assign taxonomy to each sample.

  - ✓ Functional profiling was performed using HUMAnN 2 to provide taxon-specific profiles of UniRef gene families, enzymes, and MetaCyc pathways.

- Development of a robust multi-methods approach to isolate effects
  - ✓ Univariable permutational multivariate analysis of variance (PERMANOVAs) on Bray-Curtis dissimilarities.

  - ✓ Generate maximum predicted concentrations of each contaminant in each individual, the age of Max concentration, the slope of fastest change in relation to beta diversity.

  - ✓ For feature testing: we used MaAsLin 2 for parametric feature-wise multivariable testing and HAllA for nonparametric, blockwise hierarchical testing.

  - ✓ Quantile G-computation to estimate the difference from the average pathways and taxonomic distances expected when increasing all pollutant levels by one quantile, simultaneously, conditional on covariates
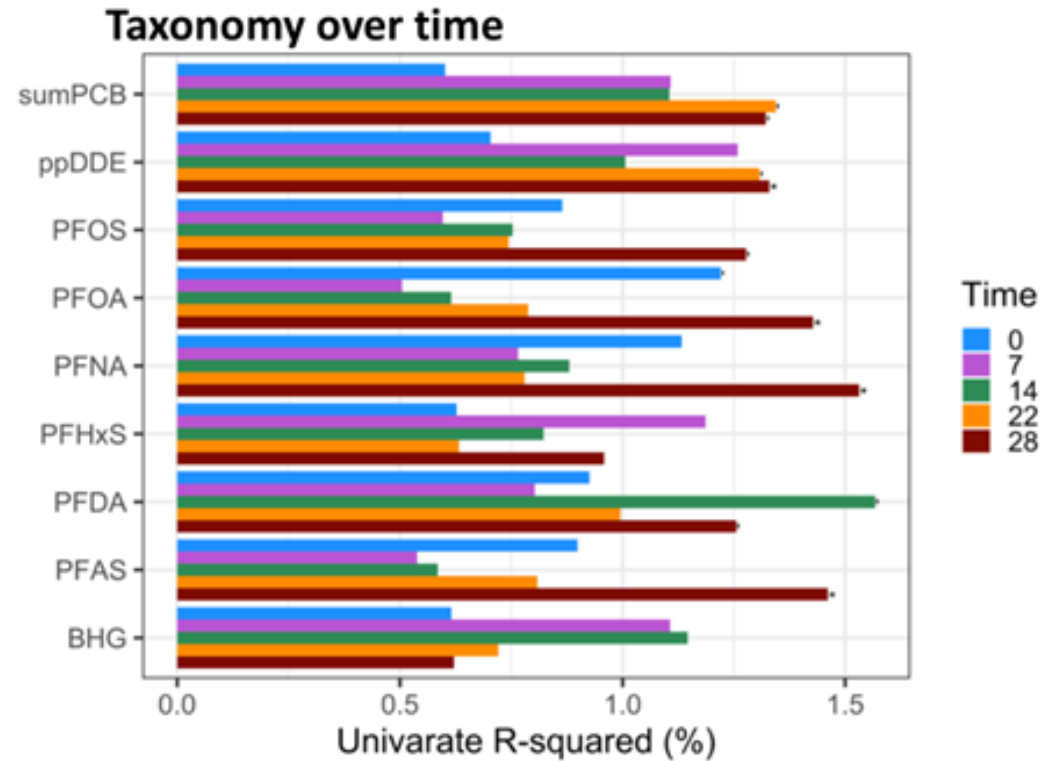
RESULTS
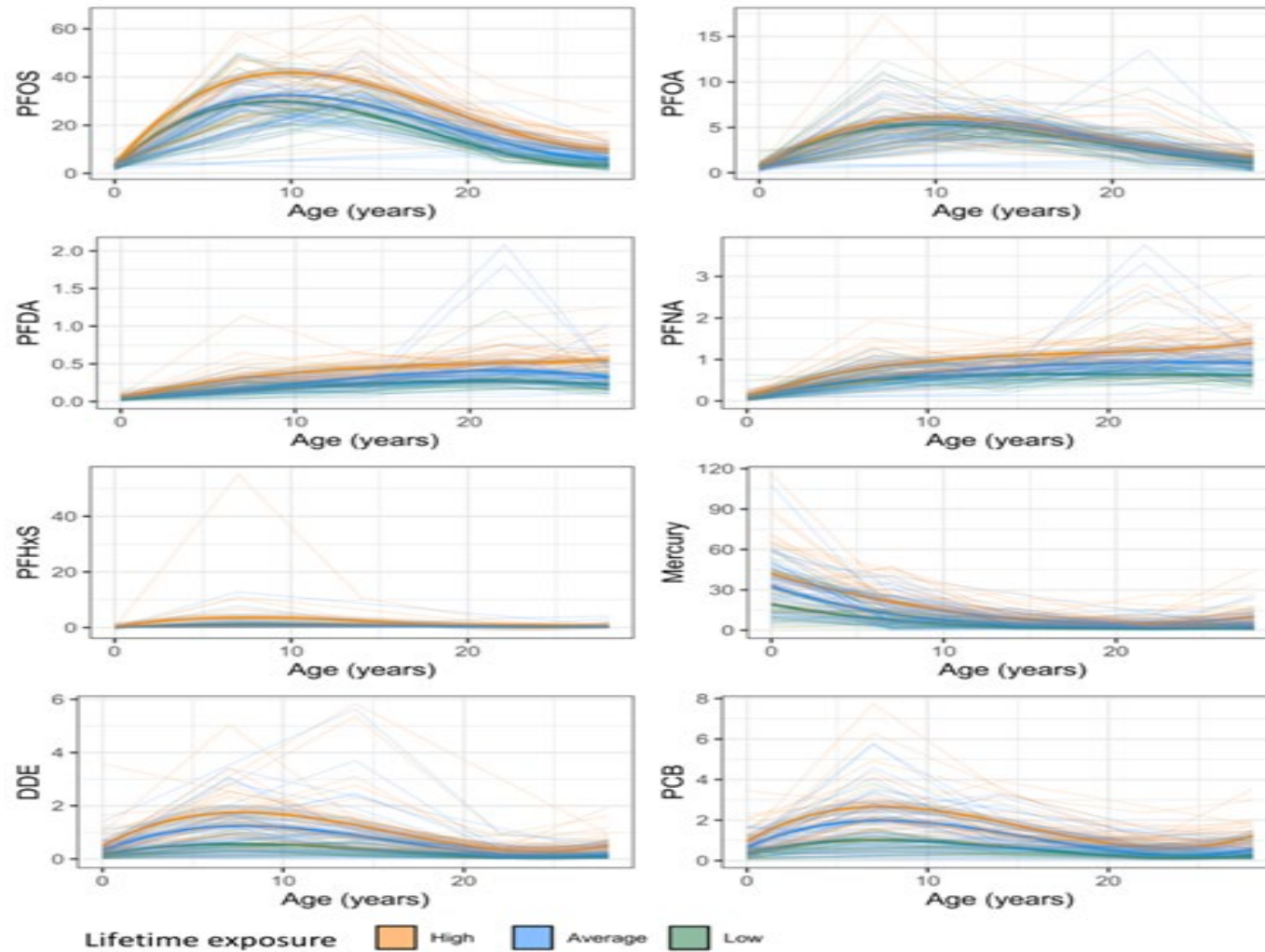
Summary of the chemical contaminants found in this population over time. Subject exposures were determined for PFASs, PCBs, DDE, and methylmercury at all timepoints.
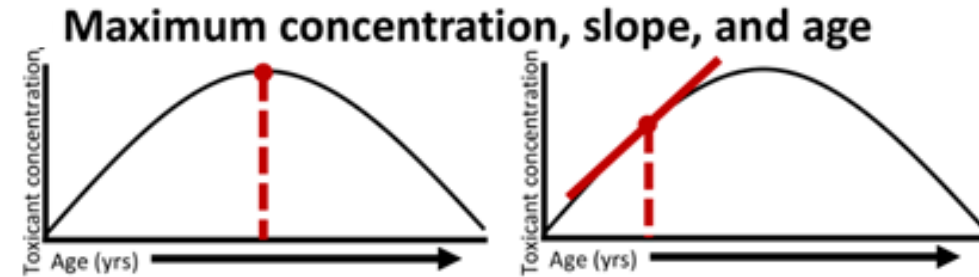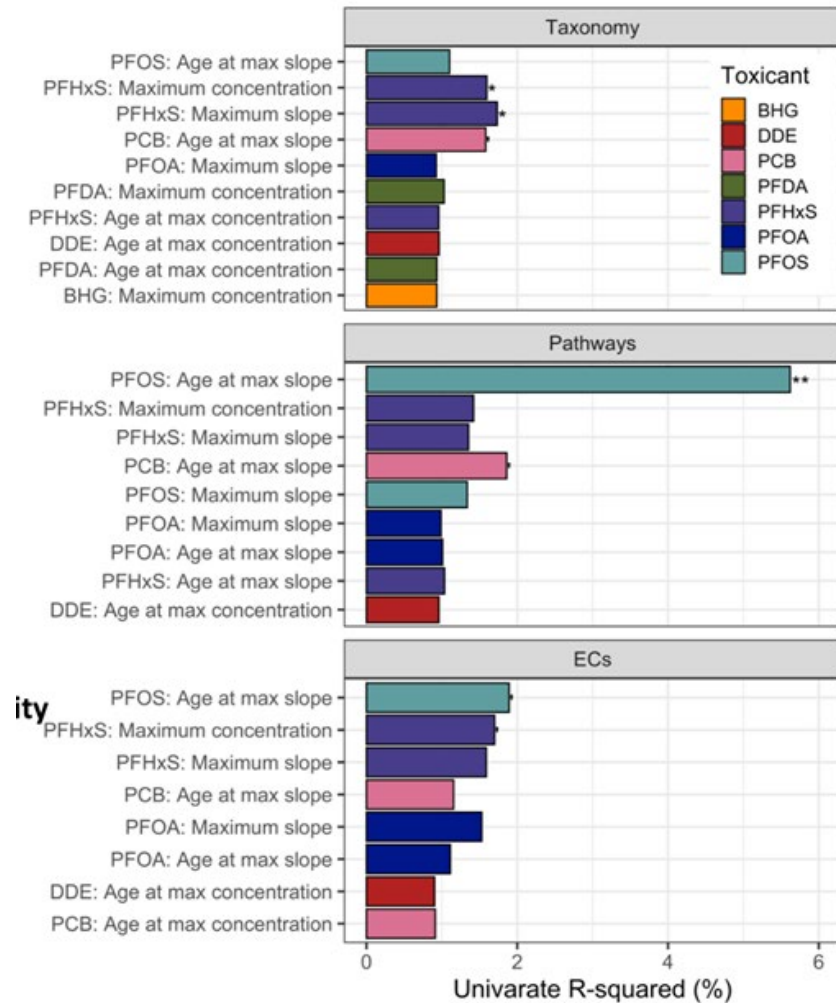
RESULTS

Taxonomy over time

The associations between chemical concentrations at each sampling time-point (birth, 7yrs, 14yrs, 22yrs, and 28 yrs) and the gut microbiome taxonomic profile at 28 yrs. Univariable PERMANOVA on Bray-Curtis dissimilarity

RESULTS

Community composition versus trend metrics


Maximum concentration, slope, and age

Summary of the findings of the associations between the generated summary statistics of chemical trends with the overall taxonomic composition and functional landscape of the microbiome (as MetaCyc pathways and Enzyme Commission category [EC] abundances.
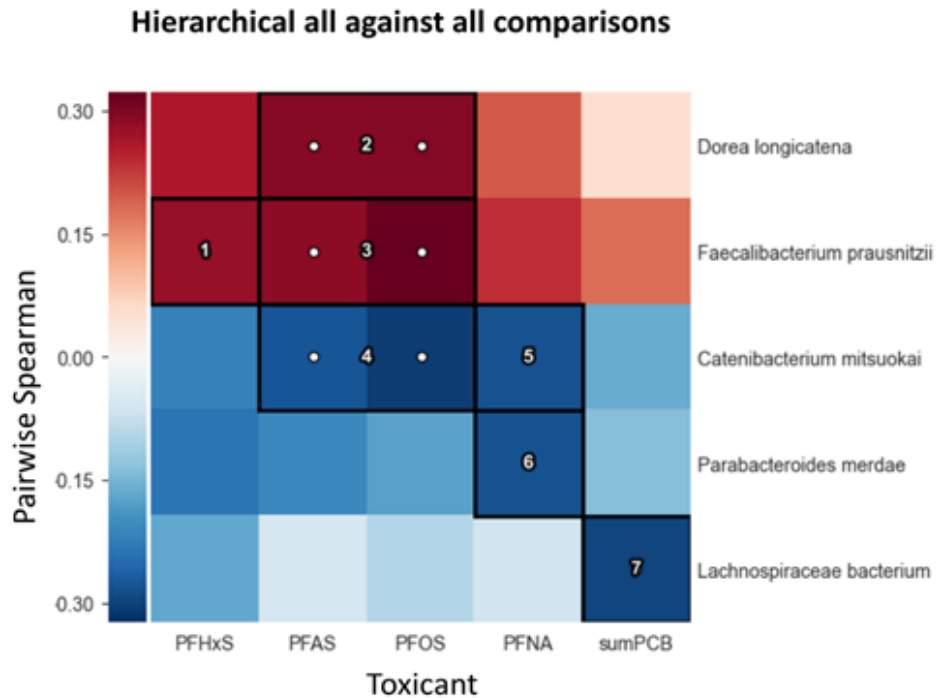
RESULTS

Quantiles of toxicants versus the overall dissimilarity
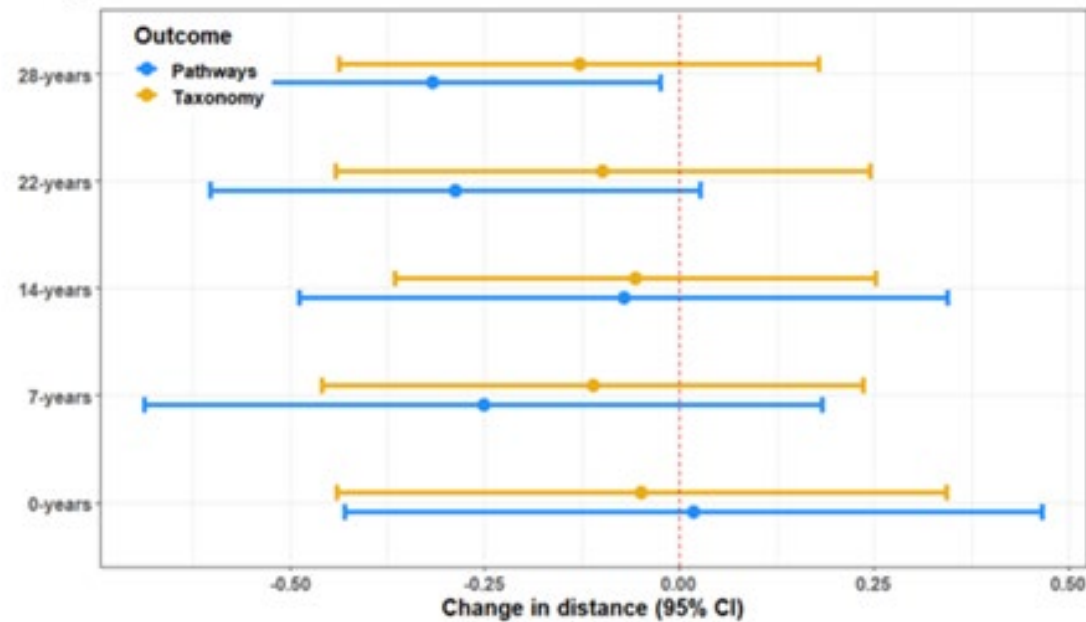
Estimates of the joint effect of a one quartile increase in the mixture of chemicals on pathways and taxonomy distances at each time point using quantile G-computation.

RESULTS

Hierarchical all against all comparisons

Significant associations (Spearman p FDR <0.05, FNT 0.2 for formation of cluster) between the contaminant concentrations at 28 years and the microbial composition of the gut microbiome. **Concentrations of several of the PFAS compounds are associated with abundance, but not prevalence of the short-chain fatty acid producing bacteria**

RESULTS


Per-feature taxonomic alterations with PFAS compounds
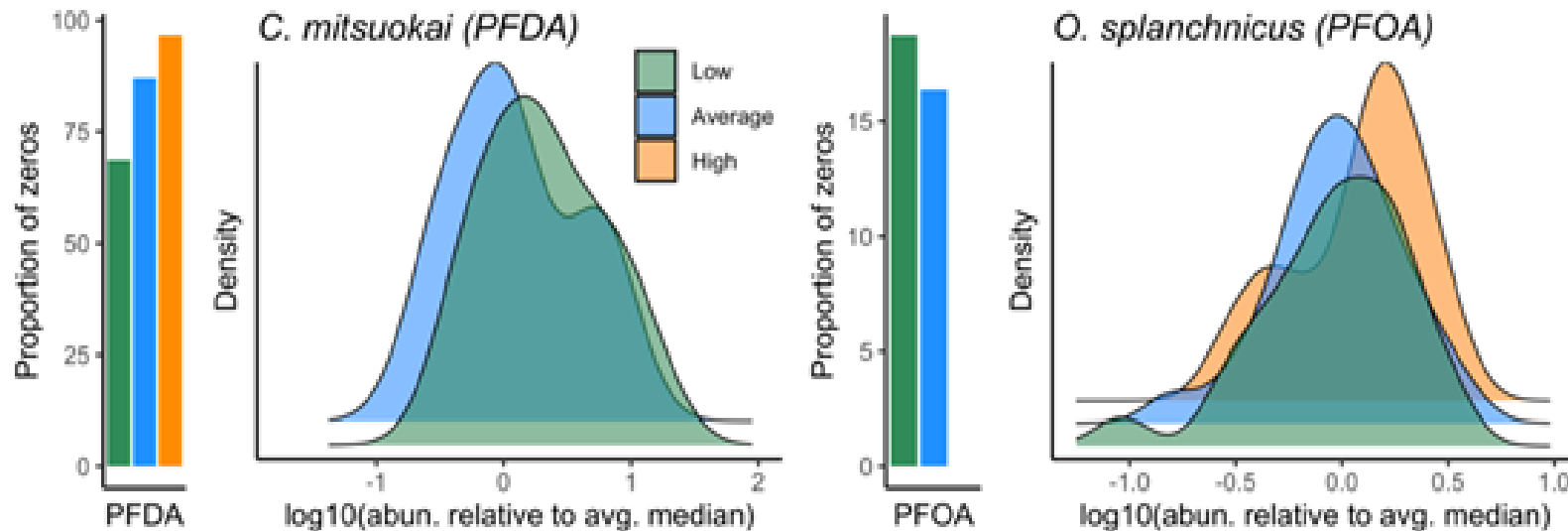
Catenibacterium mitsuokai and Odoribacter splanchnicus were both associated with PFAS exposure. Specifically, Odoribacter splanchnicus abundance and prevalence were both increased at 28 years in association with categorized PFOA exposure. Catenibacterium mitsuokai showed decreased abundance and prevalence with increasing concentrations of PFDA
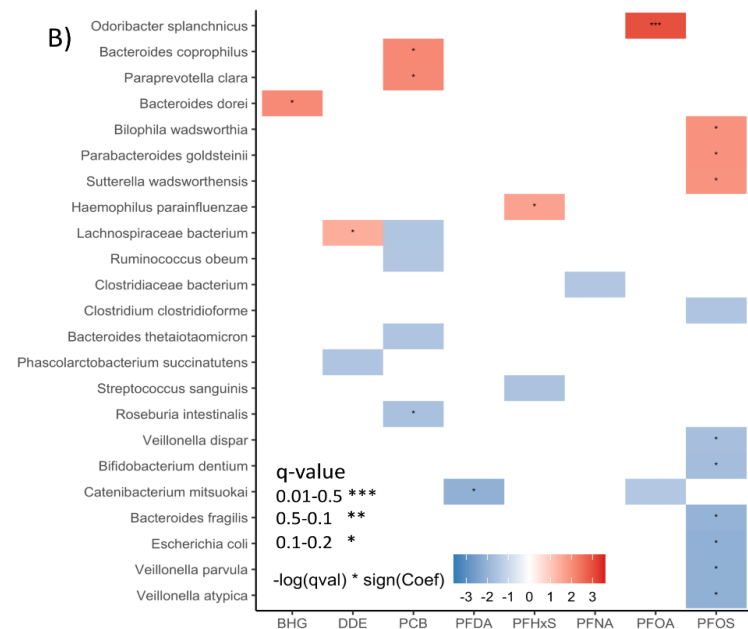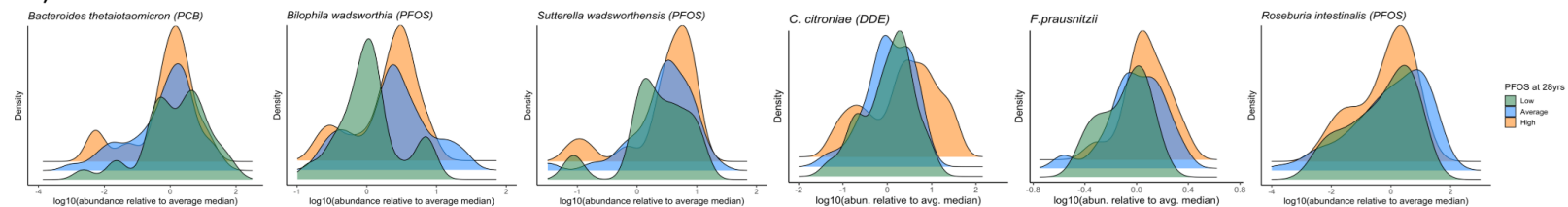
RESULTS

A) Overall composition and per-feature statistics at 28 yrs
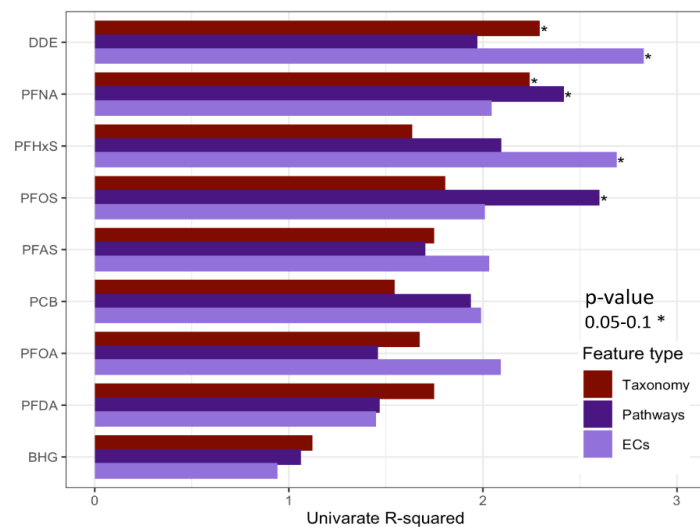
B)

C)

A) Nominal overall variations were identified for DDE, PFNA, PFHxS, and PFOS on the overall clade and functional landscape of the adult gut microbiome. **B)** Several features were identified as associated with many of the PFAS compounds (Mixed linear models MaAsLin 2; q-value <0.25). **C)** Examples of taxonomic features identified by either HAllA or MaAsLin 2 as being significantly associated with environmental chemicals (MaAsLin 2 q<0.25; HAllA q<0.05)

SUMMARY

- Only proximal exposures at age 28 years appeared to be associated with key measures of microbiome diversity and taxonomic profiles

- Associations were apparent mainly for several species previously associated with intestinal inflammation and inflammatory bowel disease

- Limited statistical power may have hampered our ability to identify any significant impact of early-life exposure to priority environmental chemicals on the composition of the adult gut microbiome

- The effects of early-life exposures might rather affect the microbiome indirectly, via innate immune priming, adaptive immunity, epigenetics, or microbial functional activities (e.g. transcriptional regulation) not captured here

- Extend the methodological toolbox for microbiome epidemiology with environmental chemicals, historical exposures, and deep metagenomic sequencing