Title

Associations of per- and polyfluoroalkyl substances (PFAS) and their mixture with oxidative stress biomarkers during pregnancy

Authors

Taibl, Kaitlin R
Schantz, Susan
Aung, Max T
et al.

Publication Date

2022-11-01

DOI

10.1016/j.envint.2022.107541

Peer reviewed

Environment International 169 (2022) 107541

Contents lists available at ScienceDirect

Environment International

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

Full length article

Associations of per- and polyfluoroalkyl substances (PFAS) and their mixture with oxidative stress biomarkers during pregnancy

Kaitlin R. Taibl [a], Susan Schantz [b,c], Max T Aung [d], Amy Padula [e], Sarah Geiger [b,f], Sabrina Smith [g], June-Soo Park [e,g], Ginger L. Milne [h], Joshua F. Robinson [e], Tracey J. Woodruff [e], Rachel Morello-Frosch [e,i], Stephanie M. Eick [a,*]

[a] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA, USA
[b] Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Champaign, IL USA
[c] Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, Champaign, IL, USA
[d] Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
[e] Program on Reproductive Health and the Environment, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, San Francisco, CA, USA
[f] Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, Champaign, IL, USA
[g] Environmental Chemistry Laboratory, Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, CA, USA
[h] Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[i] Department of Environmental Science, Policy and Management and School of Public Health, University of California, Berkeley, Berkeley, CA, USA

ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
PFAS
Oxidative stress
Mixtures
Maternal and child health

ABSTRACT

Background: Oxidative stress from excess reactive oxygen species (ROS) is a hypothesized contributor to preterm birth. Per- and polyfluoroalkyl substances (PFAS) exposure is reported to generate ROS in laboratory settings, and is linked to adverse birth outcomes globally. However, to our knowledge, the relationship between PFAS and oxidative stress has not been examined in the context of human pregnancy.

Objective: To investigate the associations between prenatal PFAS exposure and oxidative stress biomarkers among pregnant people.

Methods: Our analytic sample included 428 participants enrolled in the Illinois Kids Development Study and Chemicals In Our Bodies prospective birth cohorts between 2014 and 2019. Twelve PFAS were measured in second trimester serum. We focused on seven PFAS that were detected in >65 % of participants. Urinary levels of 8-isoprostane-prostaglandin-$F_{2\alpha}$, prostaglandin-$F_{2\alpha}$, 2,3-dinor-8-*iso*-$PGF_{2\alpha}$, and 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ were measured in the second and third trimesters as biomarkers of oxidative stress. We fit linear mixed-effects models to estimate individual associations between PFAS and oxidative stress biomarkers. We used quantile g-computation and Bayesian kernel machine regression (BKMR) to assess associations between the PFAS mixture and averaged oxidative stress biomarkers.

Results: Linear mixed-effects models showed that an interquartile range increase in perfluorooctane sulfonic acid (PFOS) was associated with an increase in 8-isoprostane-prostaglandin-$F_{2\alpha}$ ($\beta = 0.10$, 95 % confidence interval = 0, 0.20). In both quantile g-computation and BKMR, and across all oxidative stress biomarkers, PFOS contributed the most to the overall mixture effect. The six remaining PFAS were not significantly associated with changes in oxidative stress biomarkers.

Conclusions: Our study is the first to investigate the relationship between PFAS exposure and biomarkers of oxidative stress during human pregnancy. We found that PFOS was associated with elevated levels of oxidative stress, which is consistent with prior work in animal models and cell lines. Future research is needed to understand how prenatal PFAS exposure and maternal oxidative stress may affect fetal development.

* Corresponding author at: Gangarosa Department of Environmental Health, Emory University Rollins School of Public Health, 1518 Clifton Rd, Atlanta, GA 30322, USA.
E-mail address: stephanie.marie.eick@emory.edu (S.M. Eick).

https://doi.org/10.1016/j.envint.2022.107541
Received 1 June 2022; Received in revised form 21 September 2022; Accepted 22 September 2022
Available online 27 September 2022

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are environmental chemicals of public health concern and clinical significance because of their ubiquity, toxicity, and persistence in humans (Sunderland et al., 2019). Over 9,000 PFAS are in existence, and they are used in the manufacturing of consumer goods, where they are released into the environment and slow to break down (Sunderland et al., 2019). The most common exposure route is ingestion of water and food contaminated with PFAS, which poses a health risk for the mother and fetus during pregnancy. (Sunderland et al., 2019). An estimated 99 % of pregnant people are exposed to perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) (Woodruff et al., 2011). These PFAS have also been found in follicular fluid, amniotic fluid, cord blood, fetal tissues, placenta, and breastmilk (Petro et al., 2014; Zhang et al., 2013; Tao et al., 2008; Panagopoulos Abrahamsson et al., 2021). Studies show that these toxicants increase risk of reproductive disorders, including preeclampsia, miscarriage, preterm birth, and reduced fetal growth, which are the leading morbidities among mothers and infants worldwide (Chambers et al., 2021; Bommarito et al., Oct 2021; Gao et al., 2021). The cause of such adverse pregnancy outcomes is multifactorial, and therefore the underlying molecular mechanisms have been difficult to elucidate, but mounting evidence suggests prenatal PFAS exposure is a contributing factor (Gao et al., 2021).

Oxidative stress *in utero* impacts the integrity and function of biomolecules and cells, thereby altering the normal course of pregnancy (Go and Jones, 2017; Marseglia et al., 2014). Environmental toxicants are a source of excess reactive oxygen species (ROS) in the perinatal period, as evidenced by recent population-based studies that demonstrate metals, phthalates, organophosphate esters, polycyclic aromatic hydrocarbons, parabens, and phenols are associated with oxidative stress and its sequelae, oxidative damage and inflammation (Aung et al., 2021; Ingle et al., 2020; Ferguson et al., Dec 2016; Watkins et al., 2015). Several adverse pregnancy and birth outcomes that are characterized by elevated levels of oxidative stress have also been linked to a high PFAS body burden (Aouache et al., 2018; Lembo et al., 2021; Eick et al., 2020). Hence, there is growing interest in determining if oxidative stress arises from PFAS exposure during pregnancy.

Experimental research and preliminary epidemiologic studies suggest a relationship between PFAS exposure and oxidative stress based on systemic responses and cellular reactions. For example, blood PFAS levels perturbed redox-related pathways and biomarkers in proteomic, metabolomic, and lipidomic studies conducted in adults, including pregnant people (Salihovic et al., Dec 2020; Lu et al., 2019; Chang et al., 2022). Cross-sectional studies have found that PFAS exposure is positively correlated with circulating levels of inflammatory and prooxidant markers in humans, albeit none have used isoprostanes, well-studied biomarkers of lipid peroxidation (Lu et al., 2019; Omoike et al., 2021; Lin et al., 2020). Other studies have reported higher immune cell counts and altered inflammatory responses among pregnant people, postpartum mothers, and children environmentally exposed to PFAS (Omoike et al., 2021; Zota et al., 2018). In laboratory settings, PFAS have been shown to generate ROS and elicit inflammation *in vivo* and *in vitro* (Jiao et al., 2021; Kleszczynski et al., 2009; Ojo et al., 2021; Xu et al., 2022). Taken altogether, the growing body of literature indicates that PFAS exposure promotes oxidative stress, which may be particularly detrimental to maternal and fetal health.

To our knowledge, no study has investigated PFAS as promoters of oxidative stress during pregnancy, an important developmental period that necessitates improved risk assessment. We sought to address this gap by leveraging two integrated, prospective birth cohorts, the Illinois Kids Development Study (IKIDS) and Chemicals In Our Bodies (CIOB), to test the hypothesis that prenatal PFAS exposure is associated with elevated levels of oxidative stress biomarkers during pregnancy. Oxidative stress was assessed through the quantification of urinary F_2-Isoprostanes (F_2-IsoPs), which are often considered the "gold standard" biomarkers of oxidative stress since they indicate lipid peroxidation. The NIEHS-sponsored Biomarkers of Oxidative Stress Study (BOSS) compared commonly used biomarkers of oxidative stress and F_2-IsoPs were shown to reflect endogenous lipid peroxidation – a hallmark of oxidative stress – in a time- and dose-dependent manner. Importantly, urinary F_2-IsoPs are stable and specific molecules that are not affected by dietary lipids in humans, allowing for direct comparison across different populations, which is particularly advantageous for epidemiological work (Roberts and Morrow, 2000). Herein, we included 8-iso-prostaglandin-$F_{2\alpha}$ (8-*iso*-$PGF_{2\alpha}$), an abundant and the most well-studied F_2-IsoP isomer (Roberts and Morrow, 2000). We additionally included two of the major 8-*iso*-$PGF_{2\alpha}$ metabolites, as they may be more sensitive than the parent compound when measured in urine (Dorjgochoo et al., 2012). Finally, we included prostaglandin F-$_{2\alpha}$ ($PGF_{2\alpha}$) as the fourth biomarker since it is isomeric to 8-*iso*-$PGF_{2\alpha}$ and enzymatically derived, thereby reflecting inflammation pathways.

2. Methods

2.1. Study design and population

Participants included in our study were a subset of participants enrolled in CIOB and IKIDS, which together make up the ECHO.CA.IL cohort (Eick et al., 2021). This subset includes those for whom serum PFAS and urinary levels of oxidative stress biomarkers were available (Total N = 428 with 225 from IKIDS and 203 from CIOB; Figure S1).

A detailed description of the ECHO.CA.IL participants is provided elsewhere (Eick et al., 2021). Briefly, our study population is comprised of pregnant people with diverse sociodemographic backgrounds and geographic locations. Recruitment for IKIDS took place in Urbana-Champaign, Illinois at Christie Clinic and Carle Physicians Group while recruitment for CIOB took place in San Francisco, California at Zuckerberg San Francisco General, Moffitt Long, and Mission Bay Hospitals. Inclusion criteria for IKIDS were as follows: less than 15 weeks gestation, between 18 and 40 years of age, not pregnant with multiples, spoke English as a primary language, low-risk pregnancy, not already enrolled in the study with another child, and resided within a 30-minute drive of Champaign, IL. Pregnant people were eligible for inclusion in CIOB if they were in their second trimester, at least 18 years of age, English or Spanish speaking, and not pregnant with multiples. The Institutional Review Boards at the University of Illinois at Urbana-Champaign approved IKIDS and the University of California, San Francisco, and Berkeley approved CIOB (approval numbers 09498, 10-00861, 2010-05-04, respectively).

Upon enrollment in both cohorts, participants completed self-reported interview questionnaires that were used to ascertain information on maternal age at delivery (years), maternal education (<college degree, college degree, or graduate degree), maternal race and ethnicity (white, Black, Asian/Pacific Islander, Latina, other/multi-racial), current smoking status (yes, no), marital status (married or cohabitating with partner, single). Information regarding parity (no prior births, at least one prior birth) and pre-pregnancy body mass index (BMI, kg/m^2) was obtained via medical record abstraction in CIOB and calculated from pre-pregnancy weight and height obtained via questionnaire in IKIDS.

2.2. Measurement of PFAS exposure

Serum samples were obtained from participants during the second trimester (median: 18 weeks gestation) and frozen at −80 °C until analysis. Twelve PFAS were quantified at the Environmental Chemical Laboratory at the California Department of Toxic Substances Control (DTSC) using methods previously described in detail (Morello-Frosch et al., 2016). Briefly, the serum was thawed, then spiked with internal standards, and denatured. Extractions were subsequently obtained from

an automated Biotage rapid trace SPE Work Station and injected onto an automated on-line solid phase extraction method coupled to liquid chromatography and tandem mass spectrometry.

A limit of detection (LOD) was calculated for each PFAS equal to three times the standard deviation of the blank concentration (Morello-Frosch et al., 2016). For values below the LOD, the machine read value was used if a signal was detected. If a machine read value was unavailable, measurements were imputed with LOD/$\sqrt{2}$ (Hornung and Reed, 1990). A total of twelve PFAS were measured, including PFNA, PFOA, PFHxS, PFOS, 2-*N*-methyl-perfluorooctane sulfonamido acetic acid (Me-PFOSA-AcOH), perfluorodecanoic acid (PFDeA), and perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorooctane sulfonamide (PFOSA), perfluorobutane sulfonic acid (PFBS), 2-*N*-ethyl-perfluorooctane sulfonamido acetic acid (Et-PFOSA-AcOH), perfluoroheptanoic acid (PFHpA). We restricted our analysis to PFAS analytes detected in ≥65% of participants, which included PFNA, PFOA, PFHxS, PFOA, Me-PFOSA-AcOH, PFDeA, and PFUdA.

2.3. Measurement of oxidative stress biomarkers

Urine samples collected at up to two timepoints during pregnancy were analyzed for oxidative stress biomarkers by the Eicosanoid Core Laboratory at Vanderbilt University Medical Center (median 18 and 26 weeks gestation, respectively). Prior to analysis, urine samples were frozen at −80 °C. Liquid chromatography–triple quadrupole tandem mass spectrometry (LC/MS-MS) was used to quantify urinary levels of 8-*iso*-$PGF_{2\alpha}$, its two major metabolites 2,3-dinor-5,6-dihydro-8-iso-prostane-prostaglandin-$F_{2\alpha}$ (2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$) and 2,3-dinor-8-isoprostane-prostaglandin-$F_{2\alpha}$ (2,3-dinor-8-*iso*-$PGF_{2\alpha}$), and $PGF_{2\alpha}$.

Prior to LC/MS analysis, the samples were purified by extraction on a Waters HLB 96-well microelution plate (Waters Corporation, Milford, MA USA). Sample wells were first washed methanol (200uL × 2) followed by 25 % methanol in water (200uL × 2). The sample was then loaded into the well and washed with 400uL of a solution of 0.1 % formic acid/methanol (95/5, v/v). The wells were then washed with 200uL hexanes. Isoprostanes and metabolites were eluted from the plate with 30uL 2-propanol/acetonitrile (50/50, v/v) into a 96-well collection plate containing 30uL water in each well. LC/MS was performed on a Waters Xevo TQ-XS triple quadrupole mass spectrometer connected to a Waters Acquity I-Class UPLC (Waters Corp., Milford, MA USA). Separation of analytes was obtained using a Waters BEH C18 UPLC column (1.0 × 100 mm, 1.7um) with mobile phase A being 0.01 % formic acid in water and mobile phase B acetonitrile. The gradient elution began with 30 % B and progressed linearly to 95 % B over 8 min at a flow rate of 0.300 mL/min.

Because 8-*iso*-$PGF_{2\alpha}$ is synthesized from both non-enzymatic lipid peroxidation and prostaglandin-endoperoxide synthases pathways, we conducted a sensitivity analysis where we quantified the proportions of 8-*iso*-$PGF_{2\alpha}$ derived from each of the respective pathways using a ratio formula of 8-*iso*-$PGF_{2\alpha}$ to $PGF_{2\alpha}$ (van 't Erve et al., 2015). First, we calculated the ratio of chemical lipid peroxidation (CLP) to prostaglandin-endoperoxide synthases (PGHS) with the equation $\frac{CLP}{PGHS} = \frac{-0.996*(8-iso-PGF2\alpha/PGF2\alpha)+0.004}{0.507*(8-iso-PGF2\alpha/PGF2\alpha)-0.493}$ (van 't Erve et al., 2015). Second, we calculated the absolute contribution of PGHS and CLP using a custom interface for the R package "Constrained Linear Mixed Effects" (CLME) that has been described in detail by (van 't Erve et al., 2015). The "chemical fraction" reflects non-enzymatic lipid peroxidation resulting from oxidative stress involving free radicals, whereas the "enzymatic fraction" reflects prostaglandin-endoperoxide synthases and is more reflective of inflammation (van 't Erve et al., 2017).

Measured oxidative stress biomarkers with values below the LOD were imputed with LOD/$\sqrt{2}$ (Hornung and Reed, 1990). We accounted for urinary dilution by correcting for specific gravity with the equation $Ox_c = Ox\left(\frac{SpG_{Median}-1}{SpG-1}\right)$; where SpG_{Median} is the SpG population median (1.012 and 1.015 for CIOB and IKIDS, respectively). *Ox* is the uncorrected oxidative stress biomarker level and Ox_c is the SpG-corrected oxidative stress biomarker level. For participants with oxidative stress biomarkers available at both timepoints (N = 428 unique participants, of which 225 had a measurement available at both timepoints; Figure S1), we used the geometric mean (GM) of the biomarker concentrations (Eick et al., 2020) as the primary outcome in our mixture analyses. If oxidative stress biomarkers were measured once, we used only that measure.

2.4. Statistical analysis

We analyzed the distribution of demographic characteristics using descriptive statistics and reported them as mean (±standard deviation [SD]) or frequency (%). The distribution of serum PFAS and urinary oxidative stress biomarkers were examined with detection frequency percentiles, GMs, and geometric standard deviations (GSDs). We natural log-transformed PFAS for downstream analyses to address positive skewness and improve model fits. Pearson correlation coefficients were estimated for PFAS concentrations and averaged oxidative stress biomarkers.

We first modeled the unadjusted and adjusted associations between serum PFAS concentrations and urinary oxidative stress biomarkers levels using linear mixed-effects models with a random intercept for participant ID. This analytical approach allowed us to account for the repeated measures of oxidative stress biomarkers and maximize the usage of all available data. Covariates retained in adjusted models were selected based on the construction of a directed acyclic graph (DAG; Figure S2) and included maternal age, education, parity, pre-pregnancy BMI, gestational week of sample collection, and cohort. In linear mixed-effects models, PFAS were standardized to the population's interquartile range (IQR). All statistical analyses were performed in R (Boston, MA, USA, Version 4.1.0).

2.5. Mixture analysis

We examined the joint effects of seven PFAS in relation to oxidative stress biomarkers using two approaches for modeling and parametrizing exposure mixtures, quantile g-computation and Bayesian kernel machine regression (BKMR). Together, these models inform the partial and cumulative dose–response relationships between a mixture of environmental chemicals and health outcomes, which are often obscured by single pollutant models. Mixture models can also account for highly correlated co-exposures. All mixture models were adjusted for maternal age, education, parity, pre-pregnancy BMI, and cohort, and run on complete cases (N = 428; Figure S1).

Our first approach, quantile g-computation, uses a parametric, generalized linear model-based implementation of g-computation (Keil et al., 2020). Quantile g-computation estimates the overall change in an oxidative stress biomarker corresponding to a simultaneous one-quartile increase in all PFAS included in the mixture. Quantile g-computation also allows each exposure in the mixture to have a positive or negative effect on the outcome, which is reflected with relative weights for each exposure that sum to one (Keil et al., 2020).

In our second approach, we implemented BKMR to identify potential linear and non-linear associations and interactions between exposures. BKMR estimates a non-parametric high-dimensional exposure–response function using kernel machine regression (Bobb et al., 2015). BKMR was performed with 10,000 iterations and checked for convergence with the Markov chain Monte Carlo procedure. Linearity was assessed by examining univariate exposure–response functions, which reflect the relationship between levels of a single PFAS and levels of oxidative stress biomarkers, holding the remaining PFAS in the mixture constant at the median value. We assessed interaction using bivariate

exposure–response functions, which indicate evidence of interaction when lines are not parallel and/or overlapping. We calculated posterior inclusion probabilities (PIPs) to determine the relative importance of a given PFAS exposure on oxidative stress biomarkers. A traditional threshold of 0.5 was used to identify "important" predictors (Cathey et al., 2021). We then estimated the overall effect of the PFAS mixture by comparing the expected change in individual oxidative stress biomarkers when all PFAS exposures were set at the first and third quartiles versus fixed at the median value.

To determine if our results were robust against the influence of one of the two cohorts in ECHO.CA.IL, we conducted a series of sensitivity analyses in which linear mixed-effects, quantile g-computation, and BKMR models were stratified by cohort. Cohort-specific models were adjusted for maternal age, education, parity, and pre-pregnancy BMI. We also analyzed the single pollutant associations with 8-*iso*-$PGF_{2\alpha}$ using the LOD/$\sqrt{2}$ imputation procedure for PFAS detected below the LOD.

3. Results

The sociodemographic and clinical characteristics of the pregnant people included in this analysis were representative of all pregnant people enrolled in the ECHO.CA.IL cohort (Table 1). Of the 428 participants included in our analytic sample, 63.6 % self-identified as white and 16.1 % self-identified as Latina. Most participants were married or cohabitating with their partner (93.2 %) and had attained a college or graduate degree (75.3 %). On average, the participants were 32 years of age and delivered at 39 weeks gestation (SD = 2.1) (Table 1). Approximately half of the participants had at least one prior pregnancy (53.5 %) and delivered a female infant (54.4 %). The percentage of participants with gestational diabetes and gestational hypertension in the analytic sample was 15.7 % and 7.0 %, respectively. The sociodemographic characteristics were similar across the cohorts, although CIOB was more racially and ethnically diverse and a larger percentage of IKIDS participants had a graduate degree (Table S1).

Concentrations of PFNA, PFOA, PFHxS, PFOS, Me-PFOSA-AcOH, PFDeA, and PFUdA were detected in > 65 % of serum samples (Table 2). The GM concentration for these PFAS ranged from 0.05 ng/mL to 2.03 ng/mL, with the highest observed for PFOS and the lowest observed for Me-PFOSA-AcOH. Oxidative stress biomarkers had a GM concentration between 0.89 ng/mL (for 8-*iso*-$PGF_{2\alpha}$) and 4.13 ng/mL (for 2,3-dinor-8-*iso*-$PGF_{2\alpha}$). The distribution of PFAS were similar when stratified by cohort (Table S2). Levels of 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ were higher in CIOB, while levels of additional oxidative stress biomarkers were higher in IKIDS (Table S3). PFAS were moderately correlated with one another and weakly correlated with oxidative stress biomarkers (Figure S3).

In adjusted linear mixed-effects models, we observed that an IQR increase in PFOS was marginally associated with elevated levels of 8-*iso*-$PGF_{2\alpha}$ ($\beta = 0.10$, 95 % CI = 0, 0.20) and $PGF_{2\alpha}$ ($\beta = 0.12$; 95 % CI = -0.27, 0.51) (Fig. 1**A**; Table S4). An IQR increase in PFOA was moderately associated with elevated levels of 8-*iso*-$PGF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ ($\beta = 0.05$, 95 % CI = -0.09, 0.19 and $\beta = 0.56$, 95 % CI = -0.34, 1.46, respectively) (Fig. 1**A**; Table S4). In contrast, the enzymatic fraction was reduced by each IQR increase in PFOA, PFNA, PFDeA, and PFUdA (Fig. 1**A**; Table S4). In models minimally adjusted for cohort, effects were similar to those observed in fully adjusted models (Table S4). Stratification by cohort revealed an IQR increase in most PFAS was consistently associated with elevated levels of oxidative stress biomarkers in IKIDS only, although confidence intervals were imprecise (Table S4). Lastly, the effect estimates obtained from linear mixed-effects models that included 8-*iso*-$PGF_{2\alpha}$ as the outcome were similar across imputation methods, including machine read values versus LOD/$\sqrt{2}$ (Table S5).

In quantile g-computation, a simultaneous one-quartile increase in all PFAS was associated with a modest increase in 8-*iso*-$PGF_{2\alpha}$ ($\beta = 0.09$, 95 % CI = -0.07, 0.25) and $PGF_{2\alpha}$ ($\beta = 0.09$, 95 % CI = -0.39, 0.56). Based on the magnitude of the weights obtained from quantile g-computation, the chemical fraction contributed the most to the overall mixture effect on 8-*iso*-$PGF_{2\alpha}$, which reflects the contribution of oxidative stress (Table 3). Both of the 8-*iso*-$PGF_{2\alpha}$ metabolites were inversely associated with the PFAS mixture, but 95 % confidence intervals included the null. Across oxidative stress biomarkers, PFOS was consistently identified as having the largest positive weight while PFNA was only assigned a negative weight (Fig. 1**B**). PFUdA had a positive partial effect, as suggested by its quantile g-computation weight, on the enzymatic fraction, which contrasted with the linear mixed-effect model results. This difference may be due to the consideration of co-exposures

Table 1
Characteristics of the pregnant people in this analysis and all pregnant people in the ECHO.CA.IL cohorts, 2014 – 2019.

Characteristic	Included (N = 428)	Overall (N = 1,462)
Maternal Age at Delivery (years)		
Mean (SD)	32 (4.9)	32 (5.1)
Missing	0 (0)	46 (3.1)
Pre-pregnancy Body Mass Index (kg/m^2)		
Mean (SD)	26 (6.0)	26 (6.1)
Missing	0 (0)	201 (13.7)
Gestational Age at Delivery (weeks)		
Mean (SD)	39 (2.1)	39 (1.8)
Missing	0 (0)	170 (11.6)
Birth Weight (grams)		
Mean (SD)	3400 (590)	3400 (530)
Missing	39 (9.1)	222 (15.2)
Maternal Education		
< College Degree	106 (24.8 %)	405 (27.7 %)
College Degree	130 (30.4 %)	412 (28.2 %)
Graduate Degree	192 (44.9 %)	577 (39.5 %)
Missing	0 (0 %)	68 (4.7 %)
Maternal Race/Ethnicity		
White	272 (63.6 %)	787 (53.8 %)
Black	20 (4.7 %)	81 (5.5 %)
Asian/Pacific Islander	47 (11.0 %)	190 (13.0 %)
Latina	69 (16.1 %)	321 (22.0 %)
Other/Multi-Racial	20 (4.7 %)	63 (4.3 %)
Missing	0 (0 %)	20 (1.4 %)
Infant Sex		
Male	193 (45.1 %)	664 (45.4 %)
Female	233 (54.4 %)	682 (46.6 %)
Missing	2 (0.5 %)	116 (7.9 %)
Parity		
1 + Births	229 (53.5 %)	726 (49.7 %)
No Prior Births	199 (46.5 %)	612 (41.9 %)
Missing	0 (0 %)	124 (8.5 %)
Smoking Status		
Not Current Smoker	424 (99.1 %)	1236 (84.5 %)
Current Smoker	4 (0.9 %)	30 (2.1 %)
Missing	0 (0 %)	196 (13.4 %)
Marital Status		
Married or Living Together	399 (93.2 %)	1234 (84.4 %)
Single	29 (6.8 %)	112 (7.7 %)
Missing	0 (0 %)	116 (7.9 %)

Abbreviations: SD, standard deviation.

Table 2
Distributions of second trimester serum levels of per- and polyfluoroalkyl substances (PFAS; ng/mL) and second and third trimester urinary oxidative stress biomarkers corrected with specific gravity (ng/mL) in the ECHO.CA.IL cohort, 2014 – 2019.

					Percentile				
Biomarker	**N**	**% Above LOD**	**% Machine readable**	**Geometric Mean (Geometric SD)**	**5**	**25**	**50**	**75**	**95**
PFAS									
PFNA	428	97.90	99.53	0.28 (2.14)	0.08	0.18	0.29	0.42	0.79
PFOA	428	99.77	99.77	0.75 (2.14)	0.21	0.45	0.81	1.23	2.17
PFHxS	428	98.60	98.83	0.44 (2.87)	0.09	0.24	0.46	0.83	2.41
PFOS	428	99.07	99.53	2.03 (2.47)	0.49	1.32	2.20	3.55	7.01
Me-PFOSA-AcOH	428	90.42	95.79	0.05 (3.20)	0.01	0.02	0.05	0.09	0.25
PFDeA	428	67.99	92.99	0.10 (2.50)	0.03	0.06	0.10	0.18	0.38
PFUdA	428	70.33	92.52	0.06 (3.17)	0.01	0.03	0.07	0.14	0.36
PFDoA	428	3.27	44.63	0.05 (3.66)	0	0.04	0.08	0.14	0.14
PFOSA	428	7.71	41.36	0.01 (2.81)	0	0.01	0.01	0.02	0.02
PFBS	428	1.17	42.06	0.01 (2.64)	0	0.01	0.02	0.02	0.02
Et-PFOSA-AcOH	428	9.11	37.62	0.01 (2.23)	0	0.01	0.01	0.01	0.02
PFHpA	428	15.19	54.21	0.02 (2.71)	0	0.02	0.02	0.04	0.07
Oxidative Stress									
Measured									
8-*iso*-$PGF_{2\alpha}$	653	97.24	–	0.89 (2.29)	0.26	0.61	0.91	1.45	2.95
2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$	653	78.10	–	1.09 (8.02)	0.03	0.56	1.45	4.16	21.40
2,3-dinor-8-*iso*-$PGF_{2\alpha}$	653	98.93	–	4.13 (2.42)	1.21	2.77	4.32	6.78	13.13
$PGF_{2\alpha}$	653	96.48	–	1.57 (3.77)	0.11	0.81	2.21	3.73	7.21
Derived									
Chemical fraction	653	–	–	0.52 (2.76)	0.07	0.32	0.57	0.96	2.36
Enzymatic fraction	653	–	–	0.09 (13.58)	0	0.03	0.32	0.53	1.08

Abbreviations: SD, standard deviation; LOD, limit of detection.
Note: There were 425 samples from visit 1 and 228 samples from visit 2. The geometric mean, standard deviation, and percentiles urine concentrations were corrected for specific gravity. Geometric mean, geometric SD, and percentile values use the machine read value if it was available. If there was no machine read value, missing values were replaced with LOD/square root of 2.

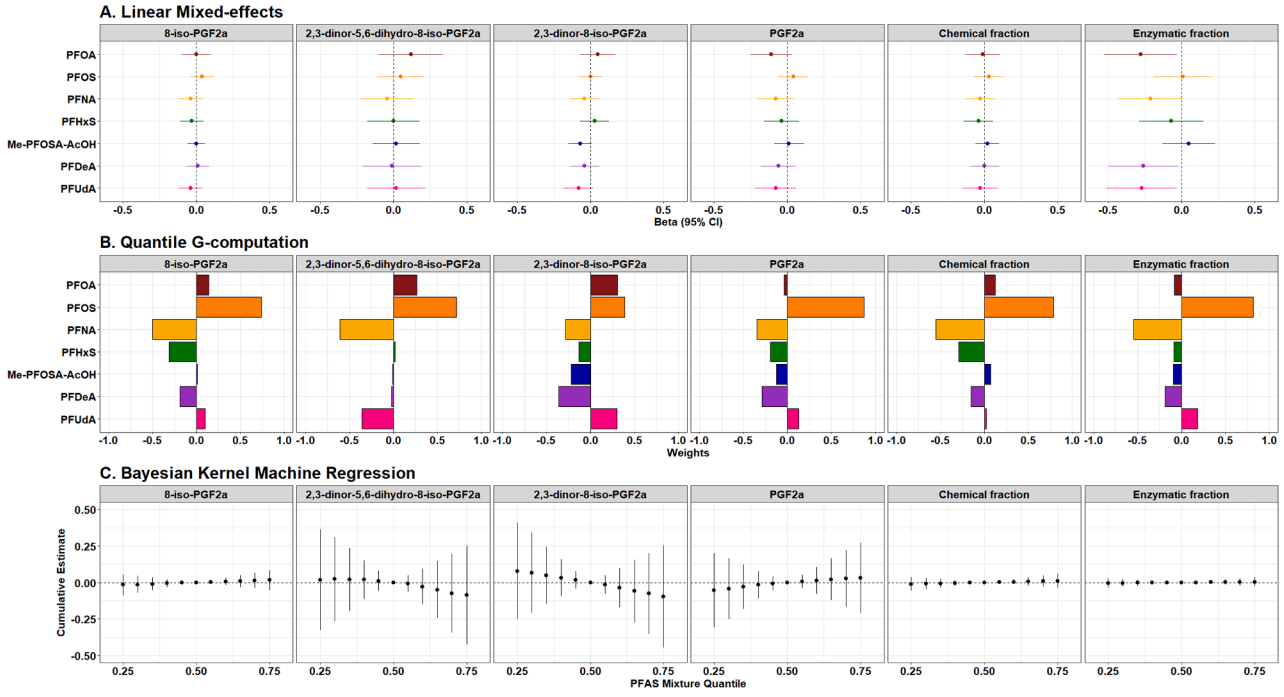

Fig. 1. Associations between serum levels of per- and polyfluoroalkyl substances (PFAS; ng/mL) and urinary levels of specific gravity corrected oxidative stress biomarkers (ng/mL) during pregnancy in the ECHO.CA.IL cohort, 2014 – 2019 (N = 428). (A) Beta and 95 % confidence interval indicating the change in oxidative stress biomarkers in association with an interquartile range increase in individual PFAS, estimated using linear mixed-effect models; (B) Positive and negative weights representing the partial effects of PFAS in the mixture on individual oxidative stress biomarkers, estimated using quantile g-computation; (C) Cumulative effect and 95 % credible intervals of the PFAS mixture on individual oxidative stress biomarkers, estimated using Bayesian kernel machine regression (BKMR). Note: Linear mixed-effects models adjusted for maternal age, education, parity, pre-pregnancy body mass index, cohort, gestational weeks at sample collection, and including a random intercept included for participant ID. Quantile g-computation and BKMR models are adjusted for maternal age, education, parity, pre-pregnancy body mass index, and cohort.

Table 3
Quantile g-computation estimates and 95 % confidence intervals for the change in urinary oxidative stress biomarkers corrected with specific gravity for a one quantile increase in the per- and polyfluoroalkyl substances mixture in the ECHO.CA.IL cohort, 2014 – 2019 (N = 428).

Oxidative Stress Biomarker	β (95 % CI)
Measured	
8-*iso*-$PGF_{2\alpha}$	0.09 (-0.07, 0.25)
2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$	−0.04 (-0.69, 0.61)
2,3-dinor-8-*iso*-$PGF_{2\alpha}$	−0.33 (-1.01, 0.34)
$PGF_{2\alpha}$	0.09 (-0.39, 0.56)
Derived	
Chemical fraction	0.07 (-0.04, 0.17)
Enzymatic fraction	0.02 (-0.06, 0.10)

Abbreviations: CI, confidence interval.
Note: Beta estimates are interpreted as the effect on oxidative stress biomarker of increasing every exposure in the mixture by one quantile. Models are adjusted for maternal age, education, parity, pre-pregnancy BMI, and cohort.

in the PFAS mixture analysis. In models stratified by cohort, increasing all PFAS in the mixture by one quartile was associated with slightly elevated levels of oxidative stress biomarkers among IKIDS only (Table S6). We observed PFOS to be the strongest driver of the overall mixture effect in both ECHO.CA.IL cohorts. Similarly, PFOA was assigned a positive weight across all oxidative stress biomarkers among IKIDS participants (Figure S4A).

Using BKMR, we observed a non-significant, inverse association between the PFAS mixture and levels of 2,3-dinor-8-*iso*-$PGF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ (Fig. 1**C**). This trend was pronounced in CIOB relative to IKIDS (Figure S4B). The univariate exposure–response functions showed that for all oxidative stress biomarkers, PFOS had a positive, linear relationship whereas PFNA had a negative, linear relationship (Fig. 2). We did not observe interaction, as all PFAS had parallel bivariate exposure–response functions (Figures S5 – S10). Finally, BKMR did not identify any PFAS as being "important" exposures since all PIPs were < 0.5 and ranged from 0.01 to 0.49 in the overall sample (Table S7).

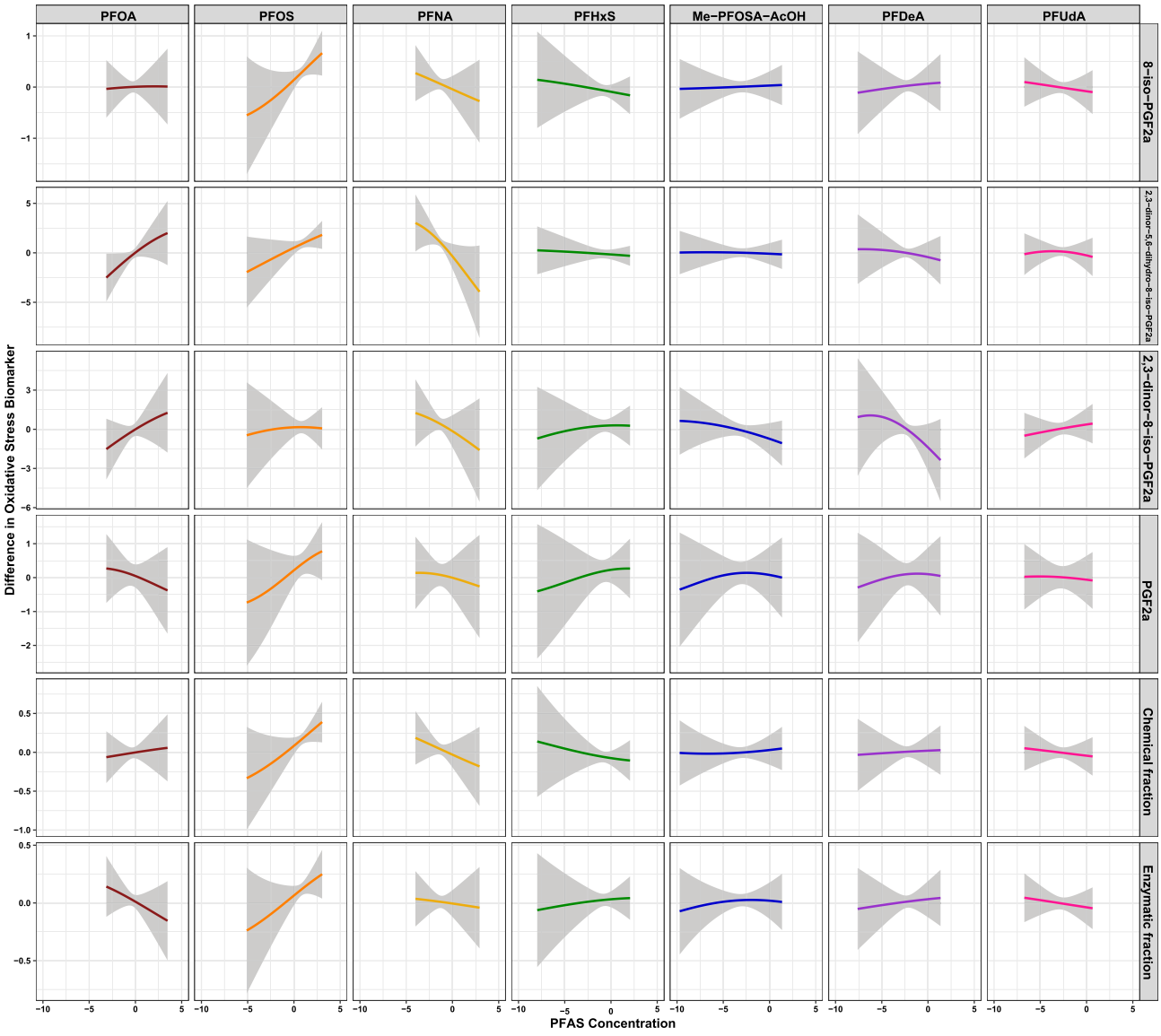

Fig. 2. Univariate exposure–response functions and 95 % credible intervals for the change in urinary oxidative stress biomarker levels resulting from individual PFAS while holding all the remaining exposures in the mixture fixed at their median concentration, estimated using Bayesian kernel machine regression (BKMR) (N = 428).
Note: Models are adjusted for maternal age, education, parity, pre-pregnancy body mass index, and cohort.

4. Discussion

The objective of our study was to examine the associations between prenatal PFAS exposure and oxidative stress biomarkers among pregnant people enrolled in the ECHO.CA.IL cohort. We found the PFAS exposure mixture was not strongly related to urinary levels of isoprostanes during pregnancy. However, there was a consistent and positive trend between PFOS and biomarkers of oxidative stress throughout our single pollutant and mixture analyses, which warrants further investigation.

In the present analysis, we estimated a modest, yet non-significant association, between the PFAS mixture and 8-*iso*-$PGF_{2\alpha}$. Our findings point to the difficulty of studying oxidative stress from environmental chemical exposure in humans. It is well-documented that PFAS induce ROS and propagate oxidative stress events, including lipid peroxidation, DNA damage, mitochondrial destabilization, reduced antioxidant capacity, and cytotoxicity *in vivo* and *in vitro*. For example, a mouse study found exposure to PFOA elevated pancreatic and hepatic levels of 8-*iso*-$PGF_{2\alpha}$ (Kamendulis et al., 2014). In primary tilapia hepatocytes, PFOA also induced maleic dialdehyde, a biomarker of lipid peroxidation, in association with increased cytotoxicity, ROS, and activities of key antioxidant enzymes (e.g., superoxide dismutase, catalase and glutathione reductase) (Liu et al., 2007). The two most commonly detected PFAS in humans, PFOA and PFOS, generate ROS and impact antioxidant defenses in zebrafish embryos and human cells from the liver, microvascular endothelium, and umbilical vein (Shi and Zhou, 2010; Wielsoe et al., 2015; Qian et al., 2010; Liao et al., 2012). Exposure to PFAS is hypothesized to overwhelm and destabilize the mitochondria, thereby limiting the effective management of ROS (Jiao et al., 2021; Souders et al., 2021). Prolonged dysregulation of free radicals and antioxidant defenses in the mitochondria may prompt a cascade of cell death signals and pathways, ultimately resulting in cytotoxic effects (Kleszczynski et al., 2009). Select studies have suggested PFAS toxicity occurs independently of oxidative stress but there are multiple lines of evidence to support these toxicants induce oxidative stress (Ojo et al., 2021; Crebelli et al., 2019). Given the inconsistencies in experimental literature and paucity of epidemiologic studies, additional research is needed to improve our understanding of the redox mechanisms impacted by PFAS exposure.

A limited body of research has investigated the relationships between PFAS exposure and oxidative stress in humans, with no studies conducted among pregnant people or with 8-*iso*-$PGF_{2\alpha}$, a highly sensitive and specific biomarker of oxidative stress. Prior work using National Health and Nutrition Examination Survey data from 2005 and 2012 found that circulating levels of PFOS, PFNA, and PFHxS were positively correlated with iron, albumin, bilirubin, which suggests that PFAS exposure leads to an increase in circulating antioxidants, presumably to counteract oxidative stress (Omoike et al., 2021). Another cohort of adults from the general population in Taiwan demonstrated that serum PFAS concentrations increase levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-nitroguanine, two biomarkers of oxidative and nitrative stress (Lin et al., 2020). Similarly, a randomized controlled trial of 141 senior Koreans with elevated serum PFOS and PFDoDA concentrations also had higher urinary levels of malondialdehyde and 8-OHdG (Kim et al., 2016). Epidemiologic studies conducted in occupational settings have found comparable results. For example, forty adult workers at a Chinese fluorochemical manufacturing plant had depleted methionine sulfoxide activity, a potent scavenger of ROS (Lu et al., 2019). A separate investigation performed in China identified several relationships between environmental exposure to PFAS and perturbed metabolomic pathways involved in oxidative and nitrative stress among 181 adult males (Wang et al., 2017). While our study used different biomarkers of oxidative stress, our results build upon previous findings that exposure to certain PFAS may be associated with a rise in oxidative stress.

Prior work, including work in this study population, has shown that prenatal PFAS exposure is linked to an increased risk of preterm birth and restricted fetal growth (Eick et al., 2022; Eick et al., 2020). Systematic reviews and meta-analyses of pregnancy cohorts have additionally found mothers exposed to higher levels of PFAS are more likely to experience reproductive tract dysfunction and give birth to children with acute and chronic health issues (Chambers et al., 2021; Rickard et al., 2022; Starnes et al., 2022; Ding et al., 2020; Liew et al., 2018). Induction of oxidative stress may provide a mechanistic explanation for these associations. Urinary levels of isoprostanes in our study population are comparable to other studies which have found increased risk of adverse birth outcomes, including preterm birth (Eick et al., 2020; Arogbokun et al., 20212021; Morales et al., 2022; Eick et al., 2022). For example, prospective cohort studies of pregnant people in the mainland United States and Puerto Rico have demonstrated that elevated levels of 8-*iso*-$PGF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ are associated with any preterm birth as well as preterm birth of spontaneous origin (Eick et al., 2020; Rosen et al., 2019). Second trimester 8-*iso*-$PGF_{2\alpha}$ levels are also higher in preeclamptic pregnancies compared to normotensive pregnancies. (Ferguson et al., 2017) Pregnant people with increased levels of 8-*iso*-$PGF_{2\alpha}$, $PGF_{2\alpha}$, and 2,3-dinor-5,6-dihydro-8-*iso*-$PGF_{2\alpha}$ have a greater risk of developing intra-amniotic infection and gestational diabetes and delivering small-for-gestational age and lower birthweight infants (Arogbokun et al., 20212021; Hsieh et al., 2012; Park et al., 2016; Kapustin et al., 2020). Collectively, these adverse pregnancy and birth outcomes constitute a substantial portion of the disease burden in pregnant populations and many have been linked to prenatal PFAS exposure. Further, infants may be uniquely sensitive to changes in levels of isoprostanes, which have bioactive roles in vascularization and respiratory development in the postnatal period (Matthews et al., 2016; Chen et al., 2012). Hence, it remains an important public health initiative to identify risk factors for elevated oxidative stress during pregnancy, as this information may aid in the development of preventive, diagnostic, and therapeutic targets for improved maternal and child health.

The present analysis has several strengths. First, we leveraged single pollutant models to account for the repeated measures of oxidative stress biomarkers in the second and third trimesters among almost half of the pregnant participants. Evidence obtained from the linear mixed-effects models was further enriched by the two mixture modeling approaches, which enabled us to characterize partial and cumulative dose–response relationships. Single pollutant models may not accurately portray real-life exposures, as in reality, we are exposed to complex mixtures of environmental chemicals, the components of which may interact or have a joint effect on health outcomes. Second, urinary oxidative stress biomarkers were assessed using mass spectrometry. This method is preferred over blood samples analyzed by enzyme-linked immunoassays, which are subject to auto-oxidation during storage (Morrow et al., 1990; Klawitter et al., 2011). Third, we included two metabolites of 8-*iso*-$PGF_{2\alpha}$ as they are hypothesized to be more sensitive than the parent compound when measured in urine (Dorjgochoo et al., 2012). Lastly, we examined the chemical and enzymatic fractions of 8-*iso*-$PGF_{2\alpha}$, which allowed us to quantify the proportion of 8-*iso*-$PGF_{2\alpha}$ derived from oxidative stress and inflammation pathways.

We also acknowledge several limitations in our study. Our sample size is relatively small, which limited our statistical power, and this imprecision is reflected in our confidence intervals. Short chain PFAS, including PFDoA, PFOSA, PFBS, Et-PFOSA-AcOH, and PFHpA, were also detected in less than 50 % of our participants, which hindered our ability to examine their effects on oxidative stress biomarkers. Further, we did not correct for multiple comparisons, but rather focused on identifying patterns, as opposed to an overreliance on statistical significance (Rothman, Jan 1990). The mixture methods applied in our analysis do not account for toxicological effects of each PFAS congener. We also do not have information on participants' physical activity, which may have an effect on oxidative stress levels. Lastly, as with all observational studies, our results may not be generalizable to other populations and may be subject to residual confounding.

5. Conclusion

To our knowledge, this is the first study to examine associations between prenatal PFAS exposure mixtures and oxidative stress biomarker levels. Among participants enrolled in the ECHO.CA.IL birth cohort, we observed that prenatal PFOS exposure was associated with modestly elevated levels of oxidative stress biomarkers. The six additional PFAS included in our analysis had null and/or weak associations with isoprostanes. Our findings advance the current state of science on how PFAS, in particular PFOS, may affect maternal redox homeostasis during pregnancy. Quantification of additional PFAS not measured in our study may reveal greater insight into cumulative effects on oxidative stress. Further research is needed to confirm our findings in other populations with larger sample sizes and should investigate oxidative stress as a mediating pathway between PFOS exposure and adverse reproductive and perinatal health outcomes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank our clinical research coordinators for collecting the data and the data analysis teams for helping to enter and compile the data, particularly Aileen Andrade, Cheryl Godwinde Medina, Cynthia Melgoza Canchola, Tali Felson, Harim Lee, Maribel Juarez, Lynn Harvey and Allison Landowski from the CIOB group and Ana Lucic, Shuk Han Ng, Mary Wakefield, Jeni Bushman, Mindy Howe, and Darcie Reckers from the IKIDS group. We also thank the research team in the Eicosanoid Core Lab, including Stephanie Sanchez, Warda Amin, Holly Borntrager, and Sarah Thomas for the analysis of oxidative stress biomarkers. We also thank the study participants who participated in the CIOB and IKIDS studies. Lastly, we would like to thank the DTSC biomonitoring team for the laboratory analysis of PFAS in serum.

Funding Sources

This work was supported by grants RD83543301 AND RD83543401 from the United States Environmental Protection Agency Children's Environmental Health and Disease Prevention Research Center, P30ES019776, P30ES030284, P01ES022841, P01ES022848 and R01ES02705 from the National Institute of Environmental Health Sciences, and UG3OD023272, UH3OD023272, and 5U2COD023375-05 from the National Institutes of Health Environmental influences on Child Health Outcomes (ECHO) program.

Data** Availability **Statement

Per University of California, San Francisco Institutional Review Board approval, the data that support the findings of this study are restricted for transmission to those outside the primary investigative team. Data sharing with investigators outside the team requires IRB approval. Requests may be submitted to the Program on Reproductive Health and the Environment (PRHE).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107541.

References

Aouache, R., Biquard, L., Vaiman, D., Miralles, F., 2018. Oxidative stress in preeclampsia and placental diseases. Int J Mol Sci. May 17 2018;19(5)doi:10.3390/ijms19051496.

Arogbokun, O., Rosen, E., Keil, A.P., et al., 2021. Maternal oxidative stress biomarkers in pregnancy and child growth from birth to age 6. J. Clin. Endocrinol. Metab. 2021; 106(5):1427–1436. doi:10.1210/clinem/dgab018.

Aung, M.T., Yu, Y., Ferguson, K.K., Cantonwine, D.E., Zeng, L., McElrath, T.F., Pennathur, S., Mukherjee, B., Meeker, J.D., 2021. Cross-sectional estimation of endogenous biomarker associations with prenatal phenols, phthalates, metals, and polycyclic aromatic hydrocarbons in single-pollutant and mixtures analysis approaches. Environ. Health Perspect. 129 (3) https://doi.org/10.1289/EHP7396.

Bobb, J.F., Valeri, L., Claus Henn, B., et al., 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics. 16(3), 493–508. doi:10.1093/biostatistics/kxu058.

Bommarito, P.A., Ferguson, K.K., Meeker, J.D., McElrath, T.F., Cantonwine, D.E., 2021. Maternal levels of perfluoroalkyl substances (PFAS) during early pregnancy in relation to preeclampsia subtypes and biomarkers of preeclampsia risk. Environ. Health Perspect. 129 (10), 107004 https://doi.org/10.1289/EHP9091.

Cathey, A.L., Eaton, J.L., Ashrap, P., Watkins, D.J., Rosario, Z.Y., Vélez Vega, C., Alshawabkeh, A.N., Cordero, J.F., Mukherjee, B., Meeker, J.D., 2021. Individual and joint effects of phthalate metabolites on biomarkers of oxidative stress among pregnant women in Puerto Rico. Environ. Int. 154, 106565.

Chambers, W.S., Hopkins, J.G., Richards, S.M., 2021. A review of per- and polyfluorinated alkyl substance impairment of reproduction. Front Toxicol. 3, 732436 https://doi.org/10.3389/ftox.2021.732436.

Chang, C.-J., Barr, D.B., Ryan, P.B., Panuwet, P., Smarr, M.M., Liu, K., Kannan, K., Yakimavets, V., Tan, Y., Ly, ViLinh, Marsit, C.J., Jones, D.P., Corwin, E.J., Dunlop, A. L., Liang, D., 2022. Per- and polyfluoroalkyl substance (PFAS) exposure, maternal metabolomic perturbation, and fetal growth in African American women: a meet-in-the-middle approach. Environ. Int. 158, 106964.

Chen, J.-X., O'Mara, P.W., Poole, S.D., Brown, N., Ehinger, N.J., Slaughter, J.C., Paria, B. C., Aschner, J.L., Reese, J., 2012. Isoprostanes as physiological mediators of transition to newborn life: novel mechanisms regulating patency of the term and preterm ductus arteriosus. Pediatr. Res. 72 (2), 122–128.

Crebelli, R., Caiola, S., Conti, L., Cordelli, E., De Luca, G., Dellatte, E., Eleuteri, P., Iacovella, N., Leopardi, P., Marcon, F., Sanchez, M., Sestili, P., Siniscalchi, E., Villani, P., 2019. Can sustained exposure to PFAS trigger a genotoxic response? A comprehensive genotoxicity assessment in mice after subacute oral administration of PFOA and PFBA. Regul. Toxicol. Pharm. 106, 169–177.

Ding, N., Harlow, S.D., Randolph Jr., J.F., Loch-Caruso, R., Park, S.K., 2020. Perfluoroalkyl and polyfluoroalkyl substances (PFAS) and their effects on the ovary. Hum Reprod Update. 26 (5), 724–752. https://doi.org/10.1093/humupd/dmaa018.

Dorjgochoo, T., Gao, Y.T., Chow, W.H., et al., 2012. Major metabolite of F2-isoprostane in urine may be a more sensitive biomarker of oxidative stress than isoprostane itself. Am. J. Clin. Nutr. 2012;96(2):405-14. doi:10.3945/ajcn.112.034918.

Eick, S.M., Hom Thepaksorn, E.K., Izano, M.A., Cushing, L.J., Wang, Y., Smith, S.C., Gao, S., Park, J.-S., Padula, A.M., DeMicco, E., Valeri, L., Woodruff, T.J., Morello-Frosch, R., 2020. Associations between prenatal maternal exposure to per- and polyfluoroalkyl substances (PFAS) and polybrominated diphenyl ethers (PBDEs) and birth outcomes among pregnant women in San Francisco. Environ. Health 19 (1).

Eick, S.M., Ferguson, K.K., Milne, G.L., Rios-McConnell, R., Vélez-Vega, C., Rosario, Z., Alshawabkeh, A., Cordero, J.F., Meeker, J.D., 2020. Repeated measures of urinary oxidative stress biomarkers and preterm birth in Puerto Rico. Free Radic. Biol. Med. 146, 299–305.

Eick, S.M., Enright, E.A., Padula, A.M., Aung, M., Geiger, S.D., Cushing, L., Trowbridge, J., Keil, A.P., Gee Baek, H., Smith, S., Park, J.-S., DeMicco, E., Schantz, S.L., Woodruff, T.J., Morello-Frosch, R., 2022. Prenatal PFAS and psychosocial stress exposures in relation to fetal growth in two pregnancy cohorts: applying environmental mixture methods to chemical and non-chemical stressors. Environ. Int. 163, 107238.

Eick, S.M., Geiger, S.D., Alshawabkeh, A., Aung, M., Barrett, E., Bush, N.R., Cordero, J.F., Ferguson, K.K., Meeker, J.D., Milne, G.L., Nguyen, R.H.N., Padula, A.M., Sathyanarayana, S., Welch, B.M., Schantz, S.L., Woodruff, T.J., Morello-Frosch, R., 2022. Associations between social, biologic, and behavioral factors and biomarkers of oxidative stress during pregnancy: findings from four ECHO cohorts. Sci. Total Environ. 835, 155596.

Eick, S.M., Enright, E.A., Geiger, S.D., et al., 2021. Associations of Maternal Stress, Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFAS), and Demographic Risk Factors with Birth Outcomes and Offspring Neurodevelopment: An Overview of the ECHO.CA.IL Prospective Birth Cohorts. Int. J. Environ. Res. Public Health. 16, 18 (2). doi: 10.3390/ijerph18020742.

Ferguson, K.K., Cantonwine, D.E., McElrath, T.F., Mukherjee, B., Meeker, J.D., 2016. Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy. Reprod. Toxicol. 66, 93–98. https://doi.org/10.1016/j.reprotox.2016.10.002.

Ferguson, K.K., Meeker, J.D., McElrath, T.F., Mukherjee, B., Cantonwine, D.E., 2017. Repeated measures of inflammation and oxidative stress biomarkers in preeclamptic and normotensive pregnancies. Am J Obstet Gynecol. 216(5), 527 e1–527 e9. doi: 10.1016/j.ajog.2016.12.174.

Gao, X., Ni, W., Zhu, S., Wu, Y., Cui, Y., Ma, J., Liu, Y., Qiao, J., Ye, Y., Yang, P., Liu, C., Zeng, F., 2021. Per- and polyfluoroalkyl substances exposure during pregnancy and adverse pregnancy and birth outcomes: a systematic review and meta-analysis. Environ. Res. 201, 111632.

Go, Y.M., Jones, D.P., 2017. Redox theory of aging: implications for health and disease. Clin. Sci. (Lond). 131 (14), 1669–1688. https://doi.org/10.1042/CS20160897.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg. 5 (1), 46–51. https://doi.org/10.1080/1047322X.1990.10389587.

Hsieh, T.T., Chen, S.F., Lo, L.M., Li, M.J., Yeh, Y.L., Hung, T.H., 2012. The association between maternal oxidative stress at mid-gestation and subsequent pregnancy complications. Reprod Sci. 19 (5), 505–512. https://doi.org/10.1177/1933719111426601.

Ingle, M.E., Watkins, D., Rosario, Z., VélezVega, C.M., Calafat, A.M., Ospina, M., Ferguson, K.K., Cordero, J.F., Alshawabkeh, A., Meeker, J.D., 2020. An exploratory analysis of urinary organophosphate ester metabolites and oxidative stress among pregnant women in Puerto Rico. Sci. Total Environ. 703, 134798.

Jiao, X., Liu, N., Xu, Y., Qiao, H., Sep 2021. Perfluorononanoic acid impedes mouse oocyte maturation by inducing mitochondrial dysfunction and oxidative stress. Reprod. Toxicol. 104, 58–67. https://doi.org/10.1016/j.reprotox.2021.07.002.

Kamendulis, L.M., Wu, Q., Sandusky, G.E., Hocevar, B.A., 2014. Perfluorooctanoic acid exposure triggers oxidative stress in the mouse pancreas. Toxicol. Rep. 1, 513–521. https://doi.org/10.1016/j.toxrep.2014.07.015.

Kapustin, R., Chepanov, S., Kopteeva, E., Arzhanova, O., 2020. Maternal serum nitrotyrosine, 8-isoprostane and total antioxidant capacity levels in pre-gestational or gestational diabetes mellitus. Gynecol. Endocrinol. 36 (sup1), 36–42. https://doi.org/10.1080/09513590.2020.1816727.

Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environ. Health Perspect. 128 (4), 47004. https://doi.org/10.1289/EHP5838.

Kim, J.H., Park, H.Y., Jeon, J.D., Kho, Y., Kim, S.-K., Park, M.-S., Hong, Y.-C., 2016. The modifying effect of vitamin C on the association between perfluorinated compounds and insulin resistance in the Korean elderly: a double-blind, randomized, placebo-controlled crossover trial. Eur. J. Nutr. 55 (3), 1011–1020.

Klawitter, J., Haschke, M., Shokati, T., Klawitter, J., Christians, U., 2011. Quantification of 15–F2t-isoprostane in human plasma and urine: results from enzyme-linked immunoassay and liquid chromatography/tandem mass spectrometry cannot be compared. Rapid Commun. Mass Spectrom. 25 (4), 463–468. https://doi.org/10.1002/rcm.4871.

Kleszczynski, K., Stepnowski, P., Skladanowski, A.C., 2009. Mechanism of cytotoxic action of perfluorinated acids II. Disruption of mitochondrial bioenergetics. Toxicol. Appl. Pharmacol. 235 (2), 182–190. https://doi.org/10.1016/j.taap.2008.11.021.

Lembo, C., Buonocore, G., Perrone, S., 2021. Oxidative stress in preterm newborns. Antioxidants (Basel). 10(11). doi:10.3390/antiox10111672.

Liao, Y., Wang, J., Huang, Q.-S., Fang, C., Kiyama, R., Shen, H., Dong, S., 2012. Evaluation of cellular response to perfluorooctane sulfonate in human umbilical vein endothelial cells. Toxicol. In Vitro 26 (3), 421–428.

Liew, Z., Goudarzi, H., Oulhote, Y., 2018. Developmental exposures to perfluoroalkyl substances (PFASs): an update of associated health outcomes. Curr. Environ. Health Rep. 5 (1), 1–19. https://doi.org/10.1007/s40572-018-0173-4.

Lin, C.Y., Lee, H.L., Hwang, Y.T., Su, T.C., 2020. The association between total serum isomers of per- and polyfluoroalkyl substances, lipid profiles, and the DNA oxidative/nitrative stress biomarkers in middle-aged Taiwanese adults. Environ. Res. 182, 109064 https://doi.org/10.1016/j.envres.2019.109064.

Liu, C., Yu, K., Shi, X., Wang, J., Lam, P., Wu, R., Zhou, B., 2007. Induction of oxidative stress and apoptosis by PFOS and PFOA in primary cultured hepatocytes of freshwater tilapia (Oreochromis niloticus). Aquat. Toxicol. 82 (2), 135–143.

Lu, Y., Gao, K., Li, X., et al., 2019. Mass spectrometry-based metabolomics reveals occupational exposure to per- and polyfluoroalkyl substances relates to oxidative stress, fatty acid beta-oxidation disorder, and kidney injury in a manufactory in China. Environ. Sci. Technol. 53 (16), 9800–9809. https://doi.org/10.1021/acs.est.9b01608.

Marseglia, L., D'Angelo, G., Manti, S., et al., 2014. Oxidative stress-mediated aging during the fetal and perinatal periods. Oxid. Med. Cell Longev. 2014;2014:358375. doi:10.1155/2014/358375.

Matthews, M.A., Aschner, J.L., Stark, A.R., Moore, P.E., Slaughter, J.C., Steele, S., Beller, A., Milne, G.L., Settles, O., Chorna, O., Maitre, N.L., 2016. Increasing F2-isoprostanes in the first month after birth predicts poor respiratory and neurodevelopmental outcomes in very preterm infants. J. Perinatol. 36 (9), 779–783.

Morales, E., García-Serna, A.M., Larqué, E., Sánchez-Campillo, M., Serrano-Munera, A., Martinez-Graciá, C., Santaella-Pascual, M., Suárez-Martínez, C., Vioque, J., Noguera-Velasco, J.A., Avilés-Plaza, F.V., Martínez-Villanueva, M., Ballesteros-Meseguer, C., Galdo-Castiñeira, L., García-Marcos, L., 2022. Dietary patterns in pregnancy and biomarkers of oxidative stress in mothers and offspring: the NELA birth cohort. Front Nutr. 9, 869357 https://doi.org/10.3389/fnut.2022.869357.

Morello-Frosch, R., Cushing, L.J., Jesdale, B.M., Schwartz, J.M., Guo, W., Guo, T., Wang, M., Harwani, S., Petropoulou, S.-S., Duong, W., Park, J.-S., Petreas, M., Gajek, R., Alvaran, J., She, J., Dobraca, D., Das, R., Woodruff, T.J., 2016. Environmental chemicals in an urban population of pregnant women and their newborns from San Francisco. Environ. Sci. Technol. 50 (22), 12464–12472.

Morrow, J.D., Hill, K.E., Burk, R.F., Nammour, T.M., Badr, K.F., Roberts 2nd, L.J., 1990. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc. Natl. Acad. Sci. USA 87 (23), 9383–9387. https://doi.org/10.1073/pnas.87.23.9383.

Ojo, A.F., Xia, Q., Peng, C., Ng, J.C., 2021. Evaluation of the individual and combined toxicity of perfluoroalkyl substances to human liver cells using biomarkers of oxidative stress. Chemosphere 281, 130808. https://doi.org/10.1016/j.chemosphere.2021.130808.

Omoike, O.E., Pack, R.P., Mamudu, H.M., Liu, Y., Strasser, S., Zheng, S., Okoro, J., Wang, L., 2021. Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. Environ. Res. 196, 110361.

Panagopoulos Abrahamsson, D., Wang, A., Jiang, T., Wang, M., Siddharth, A., Morello-Frosch, R., Park, J.-S., Sirota, M., Woodruff, T.J., 2021. A comprehensive non-targeted analysis study of the prenatal exposome. Environ. Sci. Technol. 55 (15), 10542–10557.

Park, J.Y., Romero, R., Lee, J., Chaemsaithong, P., Chaiyasit, N., Yoon, B.H., 2016. An elevated amniotic fluid prostaglandin F2alpha concentration is associated with intra-amniotic inflammation/infection, and clinical and histologic chorioamnionitis, as well as impending preterm delivery in patients with preterm labor and intact membranes. J. Matern. Fetal. Neonatal. Med. 29 (16), 2563–2572. https://doi.org/10.3109/14767058.2015.1094794.

Petro, E.M.L., D'Hollander, W., Covaci, A., Bervoets, L., Fransen, E., De Neubourg, D., De Pauw, I., Leroy, J.L.M.R., Jorssen, E.P.A., Bols, P.E.J., 2014. Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence. Sci. Total Environ. 496, 282–288.

Qian, Y., Ducatman, A., Ward, R., Leonard, S., Bukowski, V., Lan Guo, N., Shi, X., Vallyathan, V., Castranova, V., 2010. Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. J. Toxicol. Environ. Health A. 73 (12), 819–836.

Rickard, B.P., Rizvi, I., Fenton, S.E., 2022. Per- and poly-fluoroalkyl substances (PFAS) and female reproductive outcomes: PFAS elimination, endocrine-mediated effects, and disease. Toxicology 465, 153031.

Roberts, L.J., Morrow, J.D., 2000. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic. Biol. Med. 28 (4), 505–513. https://doi.org/10.1016/s0891-5849(99)00264-6.

Rosen, E.M., van 't Erve, T.J., Boss, J., Sathyanarayana, S., Barrett, E.S., Nguyen, R.H.N., Bush, N.R., Milne, G.L., McElrath, T.F., Swan, S.H., Ferguson, K.K., 2019. Urinary oxidative stress biomarkers and accelerated time to spontaneous delivery. Free Radical. Biol. Med. 130, 419–425.

Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology. 1 (1), 43–46.

Salihovic, S., Lind, L., Larsson, A., Lind, P.M., 2020. Plasma perfluoroalkyls are associated with decreased levels of proteomic inflammatory markers in a cross-sectional study of an elderly population. Environ. Int. 145, 106099 https://doi.org/10.1016/j.envint.2020.106099.

Shi, X., Zhou, B., 2010. The role of Nrf2 and MAPK pathways in PFOS-induced oxidative stress in zebrafish embryos. Toxicol. Sci. 115 (2), 391–400. https://doi.org/10.1093/toxsci/kfq066.

Souders 2nd, C.L., Sanchez, C.L., Malphurs, W., Aristizabal-Henao, J.J., Bowden, J.A., Martyniuk, C.J., 2021. Metabolic profiling in human SH-SY5Y neuronal cells exposed to perfluorooctanoic acid (PFOA). Neurotoxicology. 85, 160–172. https://doi.org/10.1016/j.neuro.2021.05.009.

Starnes, H.M., Rock, K.D., Jackson, T.W., Belcher, S.M., 2022. A critical review and meta-analysis of impacts of per- and polyfluorinated substances on the brain and behavior. Front Toxicol. 4, 881584 https://doi.org/10.3389/ftox.2022.881584.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J Expo Sci Environ Epidemiol. 29 (2), 131–147. https://doi.org/10.1038/s41370-018-0094-1.

Tao, L., Kannan, K., Wong, C.M., Arcaro, K.F., Butenhoff, J.L., 2008. Perfluorinated compounds in human milk from Massachusetts, USA. Environ. Sci. Technol. 42 (8), 3096–3101. https://doi.org/10.1021/es702789k.

van 't Erve, T.J., Kadiiska, M.B., London, S.J., Mason, R.P., 2017. Classifying oxidative stress by F2-isoprostane levels across human diseases: a meta-analysis. Redox Biol. 2017;12:582–599. doi:10.1016/j.redox.2017.03.024.

van 't Erve, T.J., Lih, F.B., Kadiiska, M.B., Deterding, L.J., Eling, T.E., Mason, R.P., 2015. Reinterpreting the best biomarker of oxidative stress: The 8-iso-PGF(2alpha)/PGF (2alpha) ratio distinguishes chemical from enzymatic lipid peroxidation. Free Radic. Biol. Med. 83, 245–51. doi:10.1016/j.freeradbiomed.2015.03.004.

Wang, X., Liu, L., Zhang, W., Zhang, J., Du, X., Huang, Q., Tian, M., Shen, H., 2017. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ. Pollut. 229, 168–176.

Watkins, D.J., Ferguson, K.K., Anzalota Del Toro, L.V., Alshawabkeh, A.N., Cordero, J.F., Meeker, J.D., 2015. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. Int. J. Hyg. Environ. Health 218 (2), 212–219. https://doi.org/10.1016/j.ijheh.2014.11.001.

Wielsoe, M., Long, M., Ghisari, M., Bonefeld-Jorgensen, E.C., 2015. Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. Chemosphere 129, 239–245. https://doi.org/10.1016/j.chemosphere.2014.10.014.

Woodruff, T.J., Zota, A.R., Schwartz, J.M., 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ. Health Perspect. 119 (6), 878–885. https://doi.org/10.1289/ehp.1002727.

Xu, M., Legradi, J., Leonards, P., 2022. Using comprehensive lipid profiling to study effects of PFHxS during different stages of early zebrafish development. Sci. Total Environ. 808, 151739.

Zhang, T., Sun, H., Lin, Y., Qin, X., Zhang, Y., Geng, X., Kannan, K., 2013. Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environ. Sci. Technol. 47 (14), 7974–7981.

Zota, A.R., Geller, R.J., Romano, L.E., Coleman-Phox, K., Adler, N.E., Parry, E., Wang, M., Park, J.-S., Elmi, A.F., Laraia, B.A., Epel, E.S., 2018. Association between persistent endocrine-disrupting chemicals (PBDEs, OH-PBDEs, PCBs, and PFASs) and biomarkers of inflammation and cellular aging during pregnancy and postpartum. Environ. Int. 115, 9–20.