Environmental Science Advances

PAPER

View Article Online
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Cite this: *Environ. Sci.: Adv.*, 2024, **3**, 1426

Pregnancy complications and birth outcomes following low-level exposure to per- and polyfluoroalkyl substances in the vitamin D antenatal asthma reduction trial†

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic, highly fluorinated aliphatic compounds, commonly utilised in a wide variety of consumer products with diverse applications. Since the genesis of these compounds, a growing body of evidence has demonstrated adverse health effects associated with PFAS exposure. In a racially diverse cohort of 459 pregnant mothers, demographically weighted towards minority representation (black 44.4%, white 38.4%, other 17.2%), across three major populous cities of the US, PFAS profiling was performed. Nine distinct PFAS species were quantified using mass spectrometry in plasma samples collected during the third trimester. Multivariable logistic and linear regression analyses were conducted to interrogate the associations of PFAS with gestational and birth outcomes: gestational diabetes, preeclampsia, gestational age at delivery, low birth weight, birth weight-, birth length- and head circumference-for-gestational-age. Detectable levels for eight out of nine profiled PFAS species were found in the plasma of pregnant mothers with a median range of 0.1–2.70 ng ml^{-1}. Using a mixtures approach, we observe that increased quantile-based g-computation (Qg-comp) "total" PFAS levels were associated with increased newborn birth-weight-for-gestational-age (β 1.28; 95% CI 1.07–1.52; FDR p 0.006). In study centre-stratified analyses, we observed a similar trend in Boston pregnant mothers, with Qg-comp total PFAS associated with higher newborn birth-weight-for-gestational-age (β 1.39; 95% CI 1.01–1.92, FDR p 0.05). We additionally found elevated PFUA concentrations were associated with longer gestational terms in San Diego pregnant mothers (β 0.60; 95% CI 0.18–1.02, FDR p 0.05). In this multi-city study, we detected lower levels of PFAS than in many previous US environmental studies, concordant with current US trends indicating environmental PFAS levels are falling, and we note geographical variation in the associations between PFAS levels and birth outcomes.

Received 4th January 2024
Accepted 12th August 2024

DOI: 10.1039/d4va00001c

rsc.li/esadvances

Environmental significance

This study provides an important perspective to the changing PFAS landscape, and the subsequent impact on human health, in the context of a critical window of development, the prenatal environment and late gestational pregnancy. In this PFAS profiling study, we investigate PFAS exposure and adverse prenatal and birth outcomes in an understudied racially diverse and socioeconomically vulnerable population from a multi-centre study. The findings of this study, compared to temporally older studies that have reported positive associations with PFAS exposure and increased risk of pregnancy complications, suggest that the decreasing environmental exposure to particular PFAS species may reduce adverse effects on pregnancy and birth health outcomes, but there is still some risk when considered at the cumulative level.

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4va00001c

Introduction

Per- and polyfluoroalkyl substances are a diverse class of synthetic compounds, defined by the possession of at least one aliphatic perfluoroalkyl moiety.[1] Since conception, these substances have been broadly utilised for both industrial and household consumer products, including but not limited to fire-fighting foams, non-stick cookware and water-resistant coatings on carpets and furniture.[2] The Organisation for Economic Co-operation and Development (OECD) and the United Nations Environment Programme (UNEP) have estimated that over 4700 unique PFAS-related chemical substances have been utilized globally since the conception and introduction of PFAS to global markets.[3] Each PFAS is structurally distinct, and consequently demonstrates differences in properties, including solubility, polymerisation, charge, volatility, and physical state.[4] Improvements in techniques and development of high-resolution mass spectrometry technologies for PFAS profiling has enabled enhanced structural separation and identification of these different sub-species.[5]

Regulation of PFAS was driven by growing evidence over four decades, linking exposure to a wide variety of disorders, diseases, and adverse health outcomes.[6–10] Additionally, PFAS, have demonstrated the capacity to bioaccumulate in specific tissues,[11] and to interfere with liver, thyroid, and renal function; with landmark studies noting hepatotoxicity and immunosuppressive properties of PFAS.[12–14] The first major regulation of PFAS began in May 2000, with 3 M, the principal global manufacturer of PFOS, negotiating with the US Environmental Protection Agency (USEPA) to phase out the manufacture of Perfluorooctanesulfonate (PFOS) and related compounds.[15] Since 2002, the USEPA has implemented multiple Significant New Use Rules (SNURs) under the Toxic Substances Control Act (TSCA) to require notification to USEPA before any manufacture, use, and/or import of certain chemically related PFAS.[15] On an international level, The Stockholm Convention on Persistent Organic Pollutants (POPs) is a United Nations treaty signed in 2001 aimed at reducing or eliminating the production, use, and release of key POPs, which was subsequently amended to include PFOS and perfluorooctanoic acid (PFOA).[16] However, despite being phased out in US manufacturing, this does not prevent the importation of products containing prohibited PFAS pollutants, nor the shift towards the use of newer shorter chain PFAS in industry.[17,18]

Consequently, despite these regulations, PFAS exposure remains a public health issue, with exposure during pregnancy a particular area of concern.[6–14] Prenatal PFAS levels in pregnant women have previously been associated with poorer birth outcomes such as low birth weight and size,[19] both of which are associated with excess child adiposity during childhood, and subsequently increased risk of secondary comorbidities such as obesity and diabetes.[20–22] Furthermore, exposure to specific PFAS, including perfluorooctane sulfonic acid (PFOS), during pregnancy has been linked to immunosuppression in offspring at age five, with a substantially sequestered response to childhood vaccination.[23] The risk of pregnancy complications and PFAS exposure is also well documented, with substantial evidence demonstrating an association between PFAS exposure and miscarriage,[24] reduced fetal growth,[25,26] preterm birth,[26,27] and preeclampsia.[28] Despite this growing body of work, there remains inconsistency and disparities in observations from PFAS studies with respect to direction and magnitude of effect, for example, with some population studies observing no association between PFAS exposure and poorer prenatal outcomes.[29,30] An additional contributing factor is the complexity of pregnancy development, with a myriad of physiological changes occurring at specific stages during gestation. Broad differences in association by timing of blood sampling during pregnancy and PFAS profiling have also been noted.[31] Consequently, a better understanding of the effects of PFAS during the complex *in utero* period is necessary to elucidate their role in early life health and childhood disease.

Efforts to better monitor the clinical implications and consequences of PFAS exposure are ongoing. The National Academies of Science, Engineering and Medicine (NASEM) report published in July 2022, provided important clinical guidance and recommendations on community monitoring of PFAS, particularly for those at risk of elevated exposure with known high levels of environmental contamination.[32] The NASEM report defines clinical risk thresholds based on the cumulative sum of seven legacy PFAS species currently monitored by the Centers for Disease Control (CDC): perfluorooctanoic acid (PFOA), linear and branched perfluorooctanesulfonic acid (*n*-PFOS; Sm-PFOS), perfluorohexane-1-sulphonic acid (PFHXS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDEA) perfluoroundecanoate (PFUA), and 2-*N*-methyl-perfluorooctane sulfonamido acetate (ME-PFOSA-ACOH). This report recommends that individuals with a cumulative PFAS blood concentration below 2 nanograms per millilitre (ng mL^{-1}) are not expected to have adverse health effects. At cumulative PFAS levels of 2 and 20 ng ml^{-1}, there may be potential for adverse effects, especially in vulnerable populations (pregnant individuals, pre-existing conditions), and these individuals should be prioritised for health screening of dyslipidemia, hypertensive disorders of pregnancy, and breast cancer based on age and other risk factors. At cumulative PFAS levels above 20 ng ml^{-1}, individuals may have increased risk of adverse effects, with recommended routine testing for dyslipidemia, thyroid function, and assessed for kidney cancer, testicular cancer and ulcerative colitis.[32] This report underscores the necessity for routine monitoring of cumulative PFAS exposure, and highlights the continued risk posed to communities, despite efforts to reduce environmental exposure.

In this study, we investigated associations between prenatal PFAS levels and adverse pregnancy and birth outcomes in 459 pregnant women and their offspring in a racially, geographically and socioeconomically diverse population from the Vitamin D Antenatal Asthma Reduction Trial (VDAART).

2 Materials and methods

2.1 Vitamin D antenatal asthma reduction trial cohort, blood collection and clinical measurements

The Vitamin D Antenatal Asthma Reduction Trial Cohort (VDAART) was a randomized, double-blind, parallel-design

study conducted at three sites across the United States (https://clinicaltrials.gov/ identifier: NCT00920621), previously described by Litonjua *et al.*[33] The VDAART protocol for the primary study was approved by the Institutional Review Board (IRB) at Brigham and Women's Hospital (IRB Protocol Number: 2009P000557) and this ancillary profiling study was further approved (IRB Protocol Number: 2018P000478). All research was performed in accordance with relevant regulations and guidelines. Informed consent was obtained from all human subjects participating in this study. VDAART began recruitment in 2009, with biofluid samples collected for this study during the years 2010–2011. Pregnant mothers were randomized to Vitamin D supplementation at 4000 IU per day or placebo to determine whether supplementation was protective for offspring asthma; all women received 400 IU per day vitamin D supplementation as part of usual pregnancy care. Mothers returned for a follow-up clinical study visit at 32–38 weeks, during which blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes. Plasma was separated through centrifugation at 2000 rpm at 4 °C, and samples were stored at −80 °C. Clinical phenotyping data were collected at research visits, including at the time of blood sampling (32–38 weeks), using comprehensive questionnaires, from which outcomes of interest were derived.[33] Follow up of the offspring is currently ongoing.

2.2 Measurement of PFAS in VDAART plasma samples

PFAS levels were quantified from third trimester (32–38 gestational weeks) blood samples of a subset of 459 mothers based on availability of suitable plasma sample volume. PFAS quantification was subsequently performed on the stored samples in 2019, as part of an initiative funded by the National Institutes of Health Child Health Exposure Analysis Resource (NIH-CHEAR), now part of the NIH-Human Health Exposure Analysis Resource (NIH-HHEAR) and analysed at the Division of Laboratory Sciences at the Centres for Disease Control and Prevention (CDC) (Atlanta, Georgia). Quantitative, targeted measurement of nine PFAS were included for this study, using online solid-phase extraction, coupled with isotope dilution high-performance liquid chromatography-tandem mass spectrometry for plasma samples, originally developed by Kato *et al.*[34] for serum samples, and amended for plasma samples as reported in Sagiv *et al.*[35] In these analyses, 8 PFAS were quantified in ≥50% of samples: perfluorooctanoic acid (PFOA), linear and branched perfluorooctanesulfonic acid (*n*-PFOS; Sm-PFOS), perfluorohexane-1-sulphonic acid (PFHXS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDEA) perfluoroundecanoate (PFUA), and 2-*N*-methyl-perfluorooctane sulfonamido acetate (ME-PFOSA-ACOH). Values below the limit of detection (LOD) were not reported by the CDC, and thus imputed as the LOD divided by the square root of 2.[36] The LOD was established by replicate injections of low concentration chemical standards. The reported LOD is also the run limit of quantitation (LOQ), and this level is always included as the low-level standard in the calibration curve. This value is not determined by a statistical evaluation of the data at that point, but rather as an actual standard used as part of the calibration curve, dependent on meeting the calibration curve requirements. As per the NASEM report recommendation, NASEM Total PFAS is defined as the cumulative sum of PFOA, *n*-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH. Based on the cumulative sum of all measured PFAS (NASEM Total PFAS): low risk PFAS exposure is defined as <2 ng ml^{-1}, moderate risk is defined as 2–20 ng ml^{-1}, high risk is defined as >20 ng mL^{-1}.[32]

2.3 Multivariate statistical analysis

VDAART is a well-characterised cohort, with comprehensive clinical metadata. Specific outcomes of interest for the current analysis were gestational diabetes, pre-eclampsia, gestational weeks at birth, low birth weight (<2.5 kg), and birth weight-, birth length- and head circumference-for gestational age. The birth outcomes: birth weight, birth length and head circumference, were converted to *z*-scores for gestational age based on World Health Organisation (WHO) standard curves using the 'growthstandards' *R* package.[37,38]

One-Way Analysis of Variance (ANOVA) was used to explore differences in PFAS concentrations and health outcomes between the three study sites. Regression analyses were performed with plasma log-scaled PFAS concentrations as continuous predictors (PFOA, PFOS, PFDEA, PFHXS, PFNA, Sm-PFOS, PFUA, ME-PFOSA-ACOH) of these outcomes with adjustment for maternal age at delivery, pre-pregnancy BMI, study site, vitamin D levels (ng ml^{-1}) at blood collection, socio economic factors (income and mothers' education), parity, maternal alcohol use, household smoking and race (black, white, other). The "Other" race category was concatenated due to low sampling numbers and representation, including: Asian, American Indians and Alaska Natives, Hispanics (or Latinos), Native Hawaiians and other Pacific Islanders. Household income was categorized based on reported household yearly income in USD: low (<$50 000 per year), medium ($50 000–$100 000 per year), or high (>$100 000 per year). Maternal education was categorized based on maximum education level reported: low (elementary school, high school, or some college/junior college), medium (technical/trade school or bachelor's degree), or high (graduate degree). Gestational diabetes, preeclampsia and low birth weight models are also adjusted for gestational age. Models were further stratified based on study site (San Diego, Boston, St. Louis), to investigate potential geographical differences.

For all single PFAS models logistic and linear regression analyses were performed using the 'glm' package in *R* for binary and continuous outcomes respectively. The Benjamini–Hochberg procedure was used to correct for multiple testing.[39]

2.4 Quantile based g-computation (Qg-comp) total PFAS mixtures approach

A cumulative PFAS measure was calculated to assess the effect of global PFAS burden using quantile based g-computation;[40] a mixtures-based approach which assess the burden of several exposures simultaneously, estimating both independent and

joint effects of PFAS species in this case. This approach estimates a regression line based on expected change in any outcome, given a simultaneous increase in the quantile-based category for PFAS exposures conditional on confounding variables (maternal age, BMI, site, socioeconomic factors, parity, alcohol use, household smoking, vitamin D level and race). This method has been found to be more suitable for studying PFAS exposure than other methods such as weighted quantile sum (WQS) regression which has predefined directionality assumptions.[40,41] Categorical (gestational diabetes, pre-eclampsia, low birth weight) and continuous (gestational weeks at delivery, birth weight-, birth length- and head circumference-for-gestational-age) outcomes were included in mixture modelling. Due to the low percentage of detection of PFUA and ME-PFOSA-ACOH (<50%), these PFAS species were excluded from the mixtures analysis.[40,42]

3 Results

3.1 VDAART cohort description and clinical characteristics

PFAS profiling was conducted on third trimester samples (32–38 weeks), from 459 pregnant mothers in the VDAART cohort (Table 1). The cohort was racially and ethnically diverse, with a distribution of 44.4% black, 38.3% white and 17.2% other race mothers. Participants were recruited across 3 sites in the US, with near equivalent numbers of total participants from all sites. A substantial proportion of mothers in VDAART were categorised as having a lower level of education (primary school, secondary school, or some college/junior college), and a low annual household income level (<$50 000 per year). Demographics stratified by study site can be found in ESI Table S1.†

Table 2 summarises all pregnancy and birth covariates investigated, with generally low numbers of adverse events (preeclampsia, gestational diabetes, low birth weight).[37] In VDAART, the maximum gestation period reported was 42 weeks, with 110 mothers (23.97%) reaching full-term (40 weeks) and 42 mothers (9.15%) reaching post-term (>40 weeks). St. Louis was found to be the city with the lowest number of mothers reaching full-term (33 mothers, 18.97%) and post-term (9 mothers, 5.17%), followed by Boston; full-term (29 mothers, 22.66%), post-term (19 mothers, 14.84%), with San Diego reporting the highest number of full-term pregnancies, (48 mothers, 30.57%), post-term (14 mothers, 8.92%). The CDC reports an average 38.50 gestational weeks at birth in the US,[43] with VDAART mothers in this study averaging at 38.69 gestational weeks at birth. An average birth weight of 3.28 kg (±0.52) was reported among the offspring; 30 children from this cohort are defined as low birth weight based on the World Health Organisation (WHO) standard (<2.50 kg).[44] A mean head circumference of 34.02 cm was reported in new-borns from this cohort of pregnant mothers, in line with the 50th percentile for the WHO Child Growth Standards.[44]

In VDAART, we observe broad variation in pregnancy and birth outcomes between study sites. Significant variation ($p < 0.05$) was observed in incidence of gestational diabetes, with the highest burden in San Diego. Amongst the 3 sites, St. Louis reports the highest incidence of preeclampsia ($p < 0.001$), preterm birth ($p < 0.05$) and low birth weight ($p < 0.05$). Additionally, we also observe significant variance between study sites in gestational weeks at birth, birth weight, birth length (all $p < 0.001$) and head circumference ($p < 0.05$).

Table 1 Maternal cohort characteristics for VDAART pregnant mothers with PFAS measured in the third trimester

VDAART maternal cohort characteristics	
Total number of participants	459
Pre-pregnancy BMI kg m^{-2}, mean (SD)[a]	28.8 (8.0)
Age at collection, mean (SD)	27.4 (5.5)
Race, *n* (%)	
Black	204 (44.4)
White	176 (38.4)
Other	79 (17.2)
Study site, *n* (%)	
Boston	128 (27.9)
San Diego	157 (34.2)
St. Louis	174 (37.9)
Household income category, *n* (%)[b]	
Low	194 (56.1)
Medium	106 (30.6)
High	46 (13.3)
Maternal education category, *n* (%)[c]	
Low	287 (62.5)
Medium	110 (24.0)
High	62 (13.5)
Vitamin D level at time of collection (ng mL^{-1}), mean (SD)	32.4 (13.9)

[a] BMI in mothers was reported prior to pregnancy. [b] Income was categorized based on reported household yearly income in USD: low (<$50 000 per year), medium ($50 000–$100 000 per year), or high (>$100 000 per year). [c] Maternal educational status was categorized based on maximum education level reported: low (primary school, secondary school, or some college/junior college), medium (technical/trade school or bachelor's degree), or high (graduate degree).

3.2 PFAS profiling in VDAART mothers

Of 9 quantified PFAS, 8 PFAS had >50% detection coverage, with branched perfluorooctanoates (sb-PFOA) measured below the Limit of Detection (LoD) in >99% of samples. Subsequently sb-PFOA was excluded from these analyses. Of the 8 PFAS included in these analyses, levels were generally low, with a median concentration range of 0.10–2.70 ng ml^{-1} (Table 3). When stratifying by study site, variability in PFAS concentrations is observed (ESI Tables S2†), with significant differences in levels of ME-PFOSA-ACOH, *n*-PFOA, PFDEA, PFUA, PFNA ($p < 0.001$), sm-PFOS($p < 0.01$) and PFHXS ($p < 0.05$) between the sites. Of these PFAS, the greatest differences were observed in concentrations of *n*-PFOA and *n*-PFOS. Significantly higher levels of *n*-PFOA ($p < 0.001$) were observed in San Diego mothers (median: 1.40 ng ml^{-1}, 25–75% percentiles: 1.10–2.00 ng ml^{-1}) compared to Boston and St Louis mothers (median: 1.20 ng ml^{-1}, 25–75% percentiles: 0.90–1.70 ng ml; median: 1.10 ng ml^{-1}, 25–75% percentiles: 0.80–1.60 ng ml^{-1} respectively). Variability was also observed for *n*-PFOS between sites, with the highest levels

Table 2 Pregnancy and perinatal outcomes for VDAART pregnant mothers (32–38 weeks' gestation) with PFAS measured in the third trimester

VDAART clinical measures and diagnoses	VDAART mothers ($N = 459$)	FDR p^b
Gestational diabetes (%)	24 (5.20)	
Boston ($n = 128$)	2 (1.60)	0.03*
San Diego ($n = 157$)	15 (9.60)	
St. Louis ($n = 174$)	7 (4.00)	
Preeclampsia (%)	24 (5.20)	
Boston ($n = 128$)	7 (5.50)	5.40×10^{-3}**
San Diego ($n = 157$)	2 (1.30)	
St. Louis ($n = 174$)	15 (8.60)	
Low birth weight (%)[a]	30 (6.54)	
Boston ($n = 128$)	8 (6.20)	0.02*
San Diego ($n = 157$)	5 (3.20)	
St. Louis ($n = 174$)	17 (9.80)	
Gestational weeks at birth, mean (SD)	38.70 (1.69)	
Boston ($n = 128$)	38.85 (1.61)	3.07×10^{-4}***
San Diego ($n = 157$)	39.01 (1.36)	
St. Louis ($n = 174$)	38.30 (2.18)	
Birth weight kg, mean (SD)	3.28 (0.52)	
Boston ($n = 128$)	3.23 (0.49)	8.02×10^{-8}***
San Diego ($n = 157$)	3.47 (0.48)	
St. Louis ($n = 174$)	3.14 (0.54)	
Birth length cm, mean (SD)	50.69 (3.03)	
Boston ($n = 128$)	50.41 (2.70)	3.05×10^{-4}***
San Diego ($n = 157$)	51.49 (2.37)	
St. Louis ($n = 174$)	50.17 (3.61)	
Head circumference cm, mean (SD)	34.02 (1.90)	
Boston ($n = 128$)	34.14 (1.80)	0.03*
San Diego ($n = 157$)	34.22 (1.61)	
St. Louis ($n = 174$)	33.76 (2.18)	

[a] Low birth weight was defined as birth weight <2.5 kg, as defined by the World Health Organisation. [b] All p values are from One-Way ANOVA are corrected for multiple testing (*$p < 0.05$, ** < 0.01, ***0.001; FDR, Benjamini–Hochberg).

observed in St. Louis mothers (median: 2.95 ng ml^{-1}, 25–75% percentiles: 2.20–3.80 ng ml^{-1}) compared to Boston and San Diego mothers (median: 2.50 ng ml^{-1}, 25–75% percentiles: 1.70–3.42 ng ml; median: 2.60 ng ml^{-1}, 25–75% percentiles: 1.80–3.60 ng ml^{-1} respectively), although this difference was non-significant after multiple correction testing.

When applying the NASEM PFAS exposure guidelines, no VDAART pregnant mothers classified at the low risk (total NASAM PFAS <2 ng ml^{-1}), 282 (61.4%) of VDAART pregnant mothers were defined as moderate risk (total NASAM PFAS 2 to 20 ng ml^{-1}), and 177 (38.6%) were defined as high risk of adverse effects from PFAS exposure (total NASAM PFAS >20 ng

Table 3 Results of quantified targeted PFAS-plasma profiling of VDAART pregnant mothers (32–38 weeks' gestation)

PFAS analyte	Abbrev.	N^a	Limit of Detection (LOD, ng ml^{-1})	Total detection above LoD[b] (%)	Median (ng ml^{-1})	25–75th percentile (ng ml^{-1})	Range (ng ml^{-1})
2-(*N*-Methyl-perfluorooctane sulfonamido) acetate	ME-PFOSA-ACOH	459	0.1	50	0.10	0.07–0.20	0.07–2.90
n-Perfluorooctanoate	*n*-PFOA	459	0.1	99	1.30	0.90–1.80	0.07–7.10
n-Perfluorooctane sulfonate	*n*-PFOS	459	0.1	100	2.70	1.95–3.70	0.30–11.50
Perfluorodecanoate	PFDEA	459	0.1	90	0.20	0.20–0.30	0.07–1.10
Perfluorohexane sulfonate	PFHXS	459	0.1	99	0.80	0.50–1.40	0.07–31.90
Perfluorononanoate	PFNA	459	0.1	99	0.50	0.40–0.70	0.07–2.70
Perfluoroundecanoate	PFUA	459	0.1	68	0.10	0.07–0.20	0.07–0.90
Perfluoromethylheptane sulfonates	Sm-PFOS	459	0.1	100	1.10	0.70–1.50	0.20–5.10
Sum of branched perfluorooctanoates[c]	Sb-PFOA	459	0.1	0.8	0.07	0.07–0.07	0.07–0.10
NASEM total PFAS[d]	-	459	0.1	100	18.07	13.07–22.07	4.28–32.00

[a] Denotes number of samples analysed. [b] Denotes percentage of samples with detected PFAS analyte above the limit of detection in the total population (≥50%). [c] Excluded from further analyses due to high number of missingness (below LoD <50%). [d] Cumulative sum of all PFAS (PFOA, *n*-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH), as per NASEM report guidelines.

ml^{-1}). We observe no significant difference in NASEM Total PFAS levels across all three sites (ESI Table S2†), San Diego mothers had a median: 18.07 ng ml^{-1}, 25–75% percentiles: 14.07–23.07, with comparable levels in Boston and St. Louis mothers (median: 18.00 ng ml^{-1}, 25–75% percentiles: 13.07–22.00 ng ml; median: 17.00 ng ml^{-1}, 25–75% percentiles: 13.02–22.00 ng ml^{-1} respectively).

3.3 Associations between PFAS and pregnancy/birth outcomes

Elevated Qg-comp total PFAS concentrations were found to be significantly associated with higher birth-weight-for-gestational age (β 1.28, 95%CI 1.07–1.52, FDR $p = 0.006$), with all individual PFAS observations indicating a similar trend but were found to be statistically non-significant post correction for multiple testing (Table 4). At nominal significance, higher levels of ME-PFOSA-ACOH were found to be associated with risk of a birth weight <2500 g (OR 2.97, 95% CI 1.33–6.65, FDR $p = 0.06$), although it should be noted this PFAS was only quantifiable above the limit of detection in 50% of the total VDAART population. PFDEA, PFNA and PFUA concentrations were found to be nominally significantly associated with a higher number of gestational weeks at delivery (PFDEA: β 0.36, 95% CI 0.03–0.69, $p = 0.04$; PFNA: β 0.34, 95%CI 0.01–0.66, $p = 0.04$; PFUA: β 0.34, 95% CI 0.05–0.63, $p = 0.04$) but were not robust to multiple testing correction (Table 4). No significant associations were observed between the NASEM Total PFAS index and outcomes of interest (Table 4).

In analyses stratified by site, gestational weeks at delivery was significantly associated with PFUA levels after correction for multiple testing in 157 mothers from San Diego (β 0.60; 95% CI 0.18–1.02, FDR $p = 0.05$, ESI Table S4†), with higher PFAS associated with longer gestational periods. Additionally, maternal PFDEA levels were found to be nominally significantly associated (β 0.63, 95% CI 0.12–1.13, $p = 0.03$) with increased gestational weeks at delivery in newborns from San Diego, however this was not robust against multiple correction testing. In Boston pregnant mothers, we observed higher newborn birth-weight-for-gestational-age associated with Qg-comp Total PFAS (β 1.39; 95%CI 1.01–1.92, FDR p 0.05). No further significant associations were found (ESI Tables S3–S5†).

4 Discussion

In this study, we interrogated the associations between PFAS exposure and adverse pregnancy and birth outcomes in a well-characterised mother-child cohort. We found that using a mixtures approach, elevated Qg-comp total PFAS levels in plasma during the third trimester, were significantly associated with higher birth-weight-for-gestational age. We found no other significant associations between prenatal PFAS levels and maternal pregnancy (gestational diabetes and pre-eclampsia) or birth (gestational weeks at delivery, low birth weight, birth weight-, birth length- and head circumference-for-gestational-age) outcomes after correction for multiple testing. In site-stratified analyses, we observe increased risk for higher birth-weight-for-gestational-age with higher cumulative PFAS levels in Boston mothers; and higher levels of PFUA were found to be significantly associated with longer gestational periods in San Diego mothers. Higher levels of some PFAS species, including PFUA, have been reported in Californians, compared to other regions of the U.S and the world, which is hypothesized to be due to the states' stringent flammability regulations and common use of PFAS in fire suppression strategies.[45] We observe no significant associations with the NASEM Total PFAS level.

In VDAART, San Diego mothers were found to have the largest proportion of mothers reaching 40–42 weeks' gestation, in addition to a higher proportion of white, higher income and higher education mothers. Conversely, Boston and St. Louis VDAART mothers tended to be of an alternative demographic, with higher proportions of black, lower income and lower education mothers. Prior research has observed that a normal gestational length is on average shorter in black and Asian mothers, compared to White mothers,[46] which may contribute to the complexity in understanding PFAS exposure during pregnancy. As these city-stratified analyses were based on only 157 mothers in San Diego, further research in a larger sample size is required to determine whether this represents a true association and whether geographical and demographical variation, may modify the relationship between PFAS exposure and prenatal outcomes in pregnant mothers.

Whilst levels have fallen since the implementation of reforms surrounding PFAS use, certain PFAS species such as PFHXS and PFNA have been found to be persistent,[47] and detectable in human serum, including in pregnant mothers.[48] However, in this pregnancy cohort collected between the years 2010–11, detection rates of plasma PFAS levels in VDAART mothers were lower in comparison to levels in non-pregnant women reported by the 2011–2012 National Health and Nutrition Examination Survey (NHANES), a cross-sectional study of the general population overseen by the US Centres for Disease Control (CDC). Notably, Higher levels of PFOS in serum were reported by non-pregnant women in NHANES[49,50] compared to plasma[51] from VDAART pregnant mothers. However, it is noteworthy that they observed generally higher levels in non-hispanic white populations, whereas VDAART is nearly 45% Black, which may help explain the PFAS exposure disparities. Furthermore, NHANES captures a broader representative US population, as opposed to VDAART which is anchored by three study sites, and thus this may impact the trends observed. However, generally across the US, monitored PFAS levels have been observed to be falling with the Agency for Toxic Substances and Disease Registry (ATSDR) noting from 1999–2018, blood PFOS levels have declined by 85%, and blood PFOA levels declined by 70%.[52]

The effect of pregnancy haemodynamics on low PFAS serum levels has been previously reported, thought to be convoluted by increased blood volume during pregnancy[53] which may also explain the lower PFAS levels observed in this study. Although some previous studies have reported PFAS levels are not affected by pregnancy,[54] it should be noted these studies were conducted in earlier stages of pregnancy. While some studies

Table 4 Multivariate logistic and linear regression results of PFAS plasma profiling associated with pregnancy and maternal health outcomes in VDAART mothers

VDAART pregnant mothers (32–38 weeks' gestation) and PFAS-Plasma profiling														
	Gestational diabetes (n = 24 cases, total cohort = 459)		Preeclampsia (n = 24 cases, total cohort = 459)		Low birth weight (n = 30 cases, total cohort = 459)		Gestational weeks at delivery (n = 459)		Birth-weight-for-gestational age (n = 459)		Birth-length-for-gestational age (n = 459)		Head-circumference-for-gestational age (n = 459)	
PFAS analyte	OR (95% CI)	FDR p^a	OR (95% CI)	FDR p^a	OR (95% CI)	FDR p^a	β (95% CI)	FDR p^a	β (95% CI)	FDR p^a	β (95% CI)	FDR p^a	β (95% CI)	FDR p^a
ME-PFOSA-ACOH	1.15 (0.59, 2.26)	0.94	0.88 (0.41, 1.87)	0.79	2.97 (1.33, 6.65)	0.06[d]	−0.04 (−0.29, 0.20)	0.82	0.07 (−0.10, 0.24)	0.99	−0.01 (−0.19, 0.17)	0.98	0.01 (−0.20, 0.21)	0.95
PFOA	0.96 (0.36, 2.56)	0.94	0.61 (0.27, 1.36)	0.78	1.40 (0.41, 4.75)	0.68	0.17 (−0.14, 0.48)	0.44	−0.03 (−0.25, 0.18)	0.99	0.01 (−0.21, 0.24)	0.98	0.03 (−0.23, 0.29)	0.94
PFOS	1.14 (0.38, 3.42)	0.94	0.57 (0.19, 1.71)	0.78	0.38 (0.10, 1.37)	0.56	0.12 (−0.22, 0.47)	0.64	0.04 (−0.20, 0.28)	0.99	0.10 (−0.15, 0.35)	0.98	−0.05 (−0.34, 0.24)	0.94
PFDEA	0.63 (0.23, 1.74)	0.94	0.72 (0.24, 2.15)	0.78	0.61 (0.19, 1.94)	0.68	0.36 (0.03, 0.69)	0.12[d]	0.07 (−0.17, 0.30)	0.99	−0.02 (−0.27, 0.22)	0.98	0.18 (−0.10, 0.46)	0.59
PFHXS	1.57 (0.80, 3.10)	0.94	0.75 (0.33, 1.67)	0.78	1.05 (0.41, 2.71)	0.91	−0.19 (−0.42, 0.04)	0.22	−0.07 (−0.24, 0.09)	0.99	−0.05 (−0.22, 0.12)	0.98	−0.10 (−0.30, 0.09)	0.59
PFNA	0.96 (0.36, 2.56)	0.94	0.73 (0.24, 2.24)	0.78	0.73 (0.22, 2.35)	0.68	0.34 (0.01, 0.66)	0.12[d]	0.06 (−0.17, 0.29)	0.99	0.00 (−0.24, 0.24)	0.98	0.17 (−0.11, 0.44)	0.59
PFUA	0.57 (0.21, 1.54)	0.94	0.86 (0.28, 2.62)	0.79	0.66 (0.22, 1.97)	0.68	0.34 (0.05, 0.63)	0.12[d]	−0.01 (−0.22, 0.20)	0.99	−0.12 (−0.33, 0.10)	0.98	0.14 (−0.11, 0.39)	0.59
Sm-PFOS	1.30 (0.47, 3.58)	0.94	0.48 (0.17, 1.33)	0.78	0.62 (0.18, 2.11)	0.68	−0.02 (−0.34, 0.30)	0.89	0.00 (−0.23, 0.22)	0.99	0.12 (−0.12, 0.35)	0.98	−0.07 (−0.34, 0.20)	0.94
Qg-comp total PFAS[b]	1.46 (0.68, 3.11)	0.33	1.02 (0.53, 1.99)	0.94	0.91 (0.46, 1.81)	0.79	1.09 (0.84, 1.42)	0.50	1.28 (1.07, 1.52)	0.006 **	1.14 (0.94, 1.37)	0.18	1.11 (0.90, 1.37)	0.32
NASEM total PFAS[c]	1.01 (0.24, 4.22)	0.99	0.95 (0.07, 2.08)	0.66	0.99 (0.23, 31.18)	0.88	0.44 (−0.02, 0.90)	0.15	0.09 (−0.23, 0.42)	0.98	0.05 (−0.29, 0.39)	0.98	0.06 (−0.33, 0.45)	0.92

[a] All p values are from multivariate regression models are corrected for multiple testing (FDR, Benjamini–Hochberg, * <0.05, ** <0.01, ***0.001). Models adjusted for maternal age, BMI, site, socioeconomic factors, parity, alcohol use, household smoking, vitamin D level and race. Gestational diabetes, preeclampsia and low birth weight models are also adjusted for gestational age. [b] Calculated by Quantile-based g-computation (Qg-comp) of all PFAS (>50% LoD threshold) as a cumulative measure of Total PFAS. [c] Calculated sum of all PFAS as recommended by the NASEM report guidelines as a cumulative measure of Total PFAS. [d] These p values were nominally significant (<0.05) prior to multiple correction testing.

have stated that renal function and glomerular filtration are not significant confounders in studies of PFAS and prenatal health outcomes during early pregnancy,[55] Steenland *et al.*, (2028) in a systematic review, reports that late pregnancy sampling is susceptible to confounding by high glomerular filtration rate (GFR), which increases by 50% during pregnancy, paralleled to increase in blood volume,[31] which may contribute to the associations observed in this study. In the Vanguard Pilot Study of the National Children's Study (NCS), PFAS levels measured primarily in third trimester pregnancy were found to be comparable with those in VDAART, with recruitment for both studies during a similar time period, 2009–2010 for NCS and 2010–2011 for VDAART. They similarly found no significant association of individual PFAS levels with birth-weight-for-gestational-age.[56] A growing body of research suggests the potential for maternal offloading of PFAS, transferred in utero *via* the placental barrier, which may contribute to the lower levels of PFAS detected in this study.[57,58] However, given our small window of third trimester sampling (36–38 weeks), we are limited to investigate this in the scope of this work.

Project VIVA, a maternal and early prenatal (9 weeks gestation) PFAS exposure study based in Massachusetts, USA, also used comparable analytical methods for PFAS profiling to this study.[59] Project VIVA reported substantially elevated levels of three PFAS species in first trimester pregnant mothers compared to VDAART third trimester mothers. When comparing Project VIVA to VDAART PFAS concentrations, PFOS levels were reported to be nearly 10 times greater; PFOA levels nearly 5 times greater and PFHXS levels four times greater. However, it is worthwhile noting that PFDEA was detected in <50% of Project VIVA mothers, and in 90% of VDAART mothers, although at low levels, (25–75% percentiles: 0.2–0.3 ng ml; LoD: 0.1 ng ml^{-1}). Interestingly, PFNA was reported to be largely comparable in both Project VIVA and VDAART Boston mothers, but a lower 75% percentile was observed in VDAART (Project VIVA; median: 0.6 ng ml^{-1}, 25–75% percentiles: 0.5–0.8 ng ml^{-1}. VDAART; median: 0.5 ng ml^{-1}, 25–75% percentiles: 0.5–0.7 ng ml^{-1}).[59] It is noteworthy to additionally consider the time period of sampling, with Project VIVA samples collected during 1999–2002, NCS samples collected 2009–2010 and VDAART samples collected during 2010–2011. From the early 2000s, stricter regulation of PFAS was implemented in the US to reduce the prevalence of PFAS in the environment and subsequent exposure.[60] As such, PFAS exposure has generally declined in the US over time due to discontinued industrial production of some PFAS species,[61] which may contribute to the some of the disparities in findings from these two studies from different timepoints, demonstrating reduced PFAS exposure over a decade and consistent null effect on prenatal outcomes, regardless of gestational period.

Few cases of gestational diabetes and preeclampsia were reported in VDAART. Whilst there are few cases of adverse pregnancy outcomes within this cohort, this is representative of the healthcare in the USA and high-income countries, with improved pregnancy education and prenatal care/interventions.[62] Whilst few robust associations were found regarding adverse birth outcomes such as low birth weight, it is also worthwhile noting that previous studies have reported a positive association between high birth weight and high PFAS exposure,[63,64] a trend that is also observed in this study. Given the cross-sectional design of this study, there is an argument for reverse causality when investigating outcomes such as gestational diabetes which is diagnosed typically between 24 and 28 weeks of pregnancy, with samples in this study collected between 34–38 weeks pregnancy.[65,66] However, studies have also found that irrespective of plasma sampling time during pregnancy, the impact of PFAS exposure on risk of gestational diabetes was consistent.[66] Further studies investigating longer-chain PFAS, such as PFOA, establish that these species have longer half-lives than their short chain PFAS counterparts,[67] and are subsequently more robust to longitudinal study designs given the bioaccumulative nature of PFAS.

Overall, these findings are encouraging, demonstrating that in this population, at the individual PFAS level exposure there is no significant association with adverse birth outcomes. When assessing the burden of Qg-comp total PFAS, we do observe a significant association with higher birth-weight-for-gestational-age, although we note no such association was observed when considering an alternative cumulate metric of PFAS, the NASAM total level. To our knowledge, this VDAART maternal PFAS study is one of the largest racially diverse prenatal PFAS profiling studies in pregnant mothers to date, with the low numbers of adverse pregnancy events representative of the current US birth and pregnancy statistics. Nevertheless, there are some limitations; maternal PFAS were measured only at a single timepoint, however, given the half-lives of PFAS *in vivo*, which for some species can be months to years,[68] we are able to observe the residual accumulation of PFAS over time from a single sampling. Additionally, significant advancements in PFAS separation and chromatographic methods have since enabled deeper deconvolution of the PFAS exposome, and subsequently a large number of PFAS species could not be captured using this targeted legacy PFAS panel. Given the changing uses of PFAS in industry, and the ongoing usage of PFAS internationally, it is imperative to assess PFAS exposure at a more comprehensive level. Despite the encouraging findings in this study, it is important to consider the broader scope of health and exposure to PFAS, with PFOA recently classified as a class IA carcinogen and PFOS also suspected as such.[69] The NASEM report guidelines, as observed in this study, highlight the need for longitudinal follow-up of at-risk individuals who have been exposed to higher levels of PFAS, and prerequisite to implement clinical monitoring into communities at higher exposure to PFAS given the broadly severe adverse implications for health.

The application and understanding of PFAS toxicology and exposomics remain in their infancy, and as such exploring associations between PFAS exposure and health outcomes in diverse ethnically and socioeconomically cohorts is crucial to identify vulnerable populaces. In this well-characterised cohort, we report a geographically varied response to PFAS exposure in pregnant mothers. Further work is required in order to fully understand the life cycle of PFAS exposure in the complex

landscape of pregnancy, in racially and geographically diverse populations.

Data availability

This dataset is openly available *via* the Human Health Exposure Analysis Resource at **https://hheardatacenter.mssm.edu/**.

Author contributions

SB, JLS, and RSK designed the study. SB performed statistical analysis and prepared the original draft of the manuscript. JLS, RSK, NP, YC, VF, CEW, LMA, DS, and STW reviewed and revised the original manuscript. STW oversaw the cohort, design, and funding acquisition for VDAART. JLS, STW and RSK oversaw the cohort, design, and funding acquisition for CHEAR-VDAART analyses. All authors gave final approval for the submitted version.

Conflicts of interest

No authors declare no competing interests.

Acknowledgements

The Vitamin D Antenatal Asthma Reduction Trial (**https://clinicaltrials.gov/** identifier: NCT00920621) was supported by grant U03HL091528 from NHLBI. This data was generated by funding provided by the NIH Child Health Exposure Analysis Resource. NP was supported by NIH T32 HL007427. RSK was supported by K01 HL146980 from the NHLBI. The funders played no role in the design or conduct of these analyses or in the decision to publish this manuscript. We thank all VDAART mothers and children for their participation in the study and would like to acknowledge all VDAART study staff for their work with data collection. We thank Dr Antonia Calafat and Dr Julianne Botelho for their crucial expertise in PFAS biochemistry.

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