Pregnancy complications and birth outcomes following low-level exposure to per- and polyfluoroalkyl substances in the Vitamin D Antenatal Asthma Reduction Trial

Authors: Sofina Begum[1,2], Nicole Prince[1], Lidia Mínguez-Alarcón[1,3], Yulu Chen[1], Djøra I. Soeteman[1,4], Vrushali Fangal[1], Mengna Huang[1], Craig Wheelock[5], Scott T.Weiss[1], Jessica Lasky-Su[1], Rachel S Kelly[1*]

[1]*Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA*
[2]*Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, London, United Kingdom*
[3]*Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA*
[4]*Center for Health Decision Science, Harvard T. H. Chan School of Public Health, Boston, MA, USA*
[5]*Department of Medical Biochemistry and Biophysics, Division of Physiological Chemistry 2, Karolinska Institute, Stockholm, Sweden*

Corresponding Author:
Rachel S. Kelly, Ph.D.
Channing Division of Network Medicine
Department of Medicine
Brigham and Women's Hospital
181 Longwood Avenue, Boston, MA, 02115
Telephone: (617) 525-0065
Email: hprke@channing.harvard.edu

Supplementary Materials

Table S1: Cohort Demographics by Study Site

Study Site	Boston	San Diego	St.Louis
Total number of participants	128	157	174
Pre-pregnancy BMI kg/m^2, Mean (SD)[1]	30.1 (7.5)	27.2 (6.8)	29.5 (9.0)
Age at collection, Mean (SD)	27.0 (5.4)	30.3 (4.6)	25.1 (5.1)
Race			
Black	74 (57.8)	3 (1.9)	127 (73.0)
White	46 (35.9)	90 (57.3)	40 (23.0)
Other	8 (6.2)	64 (40.8)	7 (4.0)
Household Income Category, n (%)[2]			
Low	62 (80.5)	42 (29.8)	90 (70.3)
Medium	13 (16.9)	68 (48.2)	25 (19.5)
High	2 (2.6)	31 (22.0)	13 (10.2)
Maternal Education Category, n (%)[3]			
Low	102 (79.7)	56 (35.7)	129 (74.1)
Medium	19 (14.8)	65 (41.4)	26 (14.9)
High	7 (5.5)	36 (22.9)	19 (10.9)
Vitamin D Level at Time of Collection (ng/mL), Mean (SD)	31.1 (12.4)	35.6 (12.9)	30.4 (15.4)

[1]BMI in mothers was reported prior to pregnancy.
[2]Income was categorized based on reported household yearly income in USD: Low (<$50,000/year), Medium ($50,000-$100,000/year), or High (>$100,000/year)
[3]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)

Table S2: PFAS Levels Stratified by Study Site.

Study Site			Boston (n=128)		San Diego (n=157)		St Louis (n=174)		
PFAS Analyte	Abbreviation	Limit of Detection (ng/ml)	Median (ng/ml)	25th-75th Percentiles	Median (ng/ml)	25th-75th Percentiles	Median (ng/ml)	25th-75th Percentiles	*ANOVA q^a*
2-(N-methyl-perfluorooctane sulfonamido) acetate	ME-PFOSA-ACOH	0.1	0.07	0.07,0.10	0.07	0.07,0.10	0.10	0.07,0.30	9.27×10^{-5}***
Perfluorohexane sulfonate	PFHXS	0.1	0.60	0.40,1.00	0.90	0.60,1.40	0.90	0.60,1.70	0.05*
n-Perfluorooctanoate	n-PFOA	0.1	1.20	0.90,1.70	1.40	1.10,2.00	1.10	0.80,1.60	1.18×10^{-5}***
Sum of branched perfluorooctanoates[c]	Sb-PFOA	0.1	0.07	0.07,0.07	0.07	0.07,0.07	0.07	0.07,0.07	0.87
Perfluorodecanoate	PFDEA	0.1	0.20	0.20,0.30	0.20	0.20,0.30	0.20	0.10,0.20	1.17×10^{-6}***
Perfluoroundecanoate	PFUA	0.1	0.20	0.10,0.30	0.10	0.07,0.20	0.10	0.07,0.20	3.96×10^{-7}***
Perfluoromethylheptane sulfonates	Sm-PFOS	0.1	0.90	0.60,1.40	1.20	0.80,1.70	1.10	0.72,1.50	2×10^{-3}**
n-Perfluorooctane sulfonate	n-PFOS	0.1	2.50	1.70,3.42	2.60	1.80,3.60	2.95	2.20,3.80	0.64
Perfluorononanoate	PFNA	0.1	0.60	0.40,0.80	0.50	0.40,0.70	0.40	0.30,0.60	4.91×10^{-6}***
NASEM Total PFAS[b]	-	0.1	18.00	13.07,22.02	18.07	14.07,23.07	17.00	13.02,22.00	0.22

[a] One-Way ANOVA, *q* value (*q<0.05, **q<0.01, ***q<0.001).

[b] Cumulative sum of all PFAS (PFOA, n-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH), as per NASEM report guidelines.

Table S3: Multivariate Logistic and Linear Regression results of PFAS plasma profiling associated with pregnancy and maternal health outcomes in VDAART mothers stratified by study site: Boston.

Boston VDAART pregnant mothers (32-38 weeks' gestation) and PFAS-Plasma profiling

PFAS Analyte	Gestational Diabetes (n=2 cases, total cohort=128)		Preeclampsia (n=2 cases, total cohort=128)		Low Birth Weight (n=8 cases, total cohort=128)		Gestational Weeks at Delivery (n=128)		Birth-Weight-for-Gestational Age (n=128)		Birth-Length-for-Gestational Age (n=128)		Head-Circumference-for-Gestational Age (n=128)	
	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	-[d]	-	-	-	-	-	0.25 (-0.46, 0.96)	0.62	0.12 (-0.46, 0.70)	0.82	0.29 (-0.34, 0.91)	0.87	0.15 (-0.57, 0.87)	0.69
PFOA	-	-	-	-	-	-	-0.32 (-1.24, 0.6)	0.62	-0.36 (-1.11, 0.39)	0.82	0.03 (-0.78, 0.85)	0.96	-0.75 (-1.66, 0.16)	0.69
PFOS	-	-	-	-	-	-	-0.19 (-0.90, 0.51)	0.62	0.29 (-0.28, 0.87)	0.82	0.31 (-0.31, 0.92)	0.87	0.17 (-0.54, 0.88)	0.69
PFDEA	-	-	-	-	-	-	0.19 (-0.57, 0.96)	0.62	0.19 (-0.44, 0.82)	0.82	0.12 (-0.56 0.79)	0.96	0.18 (-0.59, 0.96)	0.69
PFHXS	-	-	-	-	-	-	-0.53 (-1.17, 0.10)	0.41	-0.14 (-0.67, 0.40)	0.82	-0.18 (-0.75, 0.40)	0.87	-0.16 (-0.82, 0.5)	0.69
PFNA	-	-	-	-	-	-	-0.63 (-1.48, 0.22)	0.41	-0.37 (-1.07, 0.34)	0.82	-0.30 (-1.06, 0.46)	0.87	-0.57 (-1.43, 0.3)	0.69
PFUA	-	-	-	-	-	-	0.19 (-0.41, 0.80)	0.62	0.04 (-0.46, 0.54)	0.87	-0.01 (-0.55, 0.52)	0.96	0.35 (-0.26, 0.96)	0.69
Sm-PFOS	-	-	-	-	-	-	-0.71 (-1.46, 0.05)	0.41	0.12 (-0.52, 0.76)	0.82	0.32 (-0.36, 1.00)	0.87	-0.37 (-1.16, 0.41)	0.69
Qg-comp Total PFAS[b]	-	-	-	-	-	-	0.91 (0.58, 1.44)	0.70	1.39 (1.01, 1.92)	0.05*	1.34 (0.96, 1.88)	0.09	1.15 (0.79, 1.68)	0.47
NASEM Total PFAS[c]	-	-	-	-	-	-	-0.43 (-1.55, 0.68)	0.62	0.13 (-0.79, 1.05)	0.87	0.26 (-0.73, 1.25)	0.91	-0.06 (-1.20, 1.07)	0.91

[a]p value corrected for multiple testing (*p<0.05, FDR, Benjamini-Hochberg). Models adjusted for maternal age, BMI, site, socioeconomic factors, maternal alcohol use, household smoking, parity, 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]Cumulative sum of all PFAS (PFOA, n-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH), as per NASEM report guidelines.
[d] Models do not converge due to low number of cases of gestational diabetes and preterm birth from the Boston site.

Table S4: Multivariate Logistic and Linear Regression results of PFAS plasma profiling associated with pregnancy and maternal health outcomes in VDAART mothers stratified by study site: San Diego

San Diego VDAART pregnant mothers (32-38 weeks' gestation) and PFAS-Plasma profiling

PFAS Analyte	Gestational Diabetes (n=15 cases, total cohort=157)		Preeclampsia (n=7 cases, total cohort=157)		Low Birth Weight (n=5 cases, total cohort=157)		Gestational Weeks at Delivery (n=157)		Birth-Weight-for-Gestational Age (n=157)		Birth-Length-for-Gestational Age (n=157)		Head-Circumference-for-Gestational Age (n=157)	
	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	1.28 (0.55, 3.01)	0.84	-[d]	-	-	-	0.07 (-0.33, 0.47)	0.81	0.20 (-0.07, 0.48)	0.59	0.09 (-0.15, 0.33)	0.98	0.22 (-0.12, 0.55)	0.30
PFOA	0.83 (0.22, 3.16)	0.90	-	-	-	-	0.46 (-0.04, 0.97)	0.15	0.10 (-0.26, 0.46)	0.70	0.11 (-0.20, 0.42)	0.98	0.03 (-0.41, 0.47)	0.90
PFOS	0.42 (0.09, 2.04)	0.76	-	-	-	-	0.20 (-0.36, 0.76)	0.77	-0.17 (-0.57, 0.22)	0.70	-0.04 (-0.38, 0.3)	0.98	-0.40 (-0.87, 0.07)	0.30
PFDEA	0.25 (0.06, 0.97)	0.36	-	-	-	-	0.63 (0.12, 1.13)	0.07[§]	0.09 (-0.27, 0.46)	0.70	-0.02 (-0.34, 0.29)	0.98	0.29 (-0.15, 0.73)	0.30
PFHXS	1.24 (0.51, 3.05)	0.84	-	-	-	-	-0.04 (-0.39, 0.3)	0.81	-0.22 (-0.45, 0.02)	0.59	-0.17 (-0.38, 0.04)	0.86	-0.36 (-0.65, -0.07)	0.12
PFNA	0.98 (0.26, 3.64)	0.97	-	-	-	-	0.49 (0.00, 0.98)	0.13	0.05 (-0.30, 0.40)	0.78	0.00 (-0.30, 0.31)	0.98	0.24 (-0.18, 0.67)	0.30
PFUA	0.31 (0.07, 1.28)	0.42	-	-	-	-	0.60 (0.18, 1.02)	0.05*	0.08 (-0.22, 0.39)	0.70	-0.12 (-0.38, 0.14)	0.98	0.22 (-0.14, 0.59)	0.30
Sm-PFOS	0.59 (0.15, 2.31)	0.84	-	-	-	-	0.10 (-0.41, 0.61)	0.81	-0.16 (-0.51, 0.19)	0.70	-0.01 (-0.32, 0.30)	0.98	-0.30 (-0.73, 0.13)	0.30
Qg-comp Total PFAS[b]	1.19 (0.34, 4.23)	0.78	-	-	-	-	1.02 (0.72, 1.45)	0.91	1.09 (0.86, 1.41)	0.46	1.05 (0.83, 1.33)	0.70	0.89 (0.66, 1.21)	0.46
NASEM Total PFAS[c]	0.47 (0.08, 2.57)	0.81	-	-	-	-	0.90 (0.19, 1.60)	0.06	0.06 (-0.45, 0.56)	0.82	-0.04 (-0.47, 0.40)	0.98	0.03 (-0.58, 0.65)	0.91

[a] p value corrected for multiple testing (*p<0.05, FDR, Benjamini-Hochberg). Models adjusted for maternal age, BMI, site, socioeconomic factors, maternal alcohol use, household smoking, parity, 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] Cumulative sum of all PFAS (PFOA, n-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH), as per NASEM report guidelines.
[d] Models do not converge due to low number of cases of preeclampsia from the San Diego site.
[§] These p values were nominally significant (<0.05) prior to multiple correction testing.

Table S5: Multivariate Logistic and Linear Regression results of PFAS plasma profiling associated with pregnancy and maternal health outcomes in VDAART mothers stratified by study site: St Louis

St Louis VDAART pregnant mothers (32-38 weeks' gestation) and PFAS-Plasma profiling

PFAS Analyte	Gestational Diabetes (n=7 cases, total cohort=174)		Preeclampsia (n=7 cases, total cohort=174)		Low Birth Weight (n=17 cases, total cohort=174)		Gestational Weeks at Delivery (n=174)		Birth-Weight-for-Gestational Age (n=174)		Birth-Length-for-Gestational Age (n=174)		Head-Circumference-for-Gestational Age (n=174)	
	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	-[d]	-	-	-	1.17 (0.16, 8.68)	0.96	-0.08 (-0.44, 0.29)	0.88	-0.06 (-0.30, 0.19)	0.84	-0.20 (-0.48, 0.07)	0.92	-0.12 (-0.41, 0.17)	0.56
PFOA	-	-	-	-	0.06 (0.00, 12.55)	0.95	-0.10 (-0.56, 0.36)	0.88	-0.05 (-0.36 0.26)	0.84	-0.09 (-0.44, 0.26)	0.92	0.13 (-0.24, 0.49)	0.56
PFOS	-	-	-	-	0.01 (0.00, 3.08)	0.89	0.18 (-0.43, 0.80)	0.88	0.20 (-0.21, 0.62)	0.84	0.07 (-0.40, 0.54)	0.92	0.15 (-0.35, 0.65)	0.56
PFDEA	-	-	-	-	0.52 (0.02, 16.57)	0.95	0.07 (-0.50, 0.63)	0.88	0.11 (-0.28, 0.49)	0.84	0.06 (-0.38, 0.49)	0.92	0.14 (-0.32, 0.61)	0.56
PFHXS	-	-	-	-	0.21 (0.00, 12.81)	0.95	-0.24 (-0.62, 0.14)	0.88	0.03 (-0.23, 0.29)	0.84	0.02 (-0.27, 0.32)	0.92	0.18 (-0.13, 0.48)	0.56
PFNA	-	-	-	-	1.13 (0.01, 117.23)	0.96	0.28 (-0.32, 0.88)	0.88	0.32 (-0.08 0.72)	0.84	0.12 (-0.34, 0.58)	0.92	0.42 (-0.06, 0.90)	0.56
PFUA	-	-	-	-	0.23 (0.00, 60.03)	0.95	-0.20 (-0.87, 0.47)	0.88	0.05 (-0.41, 0.50)	0.84	-0.03 (-0.55, 0.49)	0.92	-0.18 (-0.72, 0.37)	0.56
Sm-PFOS	-	-	-	-	0.50 (0.01, 16.78)	0.95	0.04 (-0.51, 0.59)	0.88	0.12 (-0.26, 0.50)	0.84	0.02 (-0.41, 0.46)	0.92	0.27 (-0.19, 0.72)	0.56
Qg-comp Total PFAS[b]	-	-	-	-	1.14 (0.57, 2.27)	0.72	0.98 (0.64, 1.50)	0.91	1.20 (0.92, 1.58)	0.18	0.87 (0.64, 1.20)	0.41	1.11 (0.79, 1.56)	0.54
NASEM Total PFAS[c]	-	-	-	-	0.86 (0.04, 138.21)	0.87	0.09 (-0.69, 0.87)	0.88	0.24 (-0.30, 0.77)	0.84	0.04 (-0.57, 0.65)	0.92	0.18 (-0.46, 0.82)	0.58

[a] p value corrected for multiple testing (*p<0.05, FDR, Benjamini-Hochberg). Models adjusted for maternal age, BMI, site, socioeconomic factors, maternal alcohol use, household smoking, parity, 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] Cumulative sum of all PFAS (PFOA, n-PFOS and Sm-PFOS, PFHXS, PFNA, PFDEA, PFUA, ME-PFOSA-ACOH), as per NASEM report guidelines.
[d] Models do not converge due to low number of cases of preeclampsia from the St. Louis site.