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Prenatal Exposure to Perfluoroalkyl Substances and Birth Outcomes; A Pooled Analysis in the Danish National Birth Cohort

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Prenatal Exposure to Perfluoroalkyl Substances and Birth Outcomes; A Pooled Analysis in the Danish National Birth Cohort

A thesis submitted in partial satisfaction

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by

Qi Meng

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ABSTRACT OF THE THESIS

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A Pooled Analysis in the Danish National Birth Cohort

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Master of Science in Epidemiology

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Professor Beate Ritz, Chair

Perfluoroalkyl substances (PFASs) are widespread industrial pollutants that are extremely persistent in the environment. Animal studies have indicated that in-utero PFAS exposures can affect fetal growth, but findings from human studies are inconclusive. Few human studies have sufficient sample size to study the influence of PFASs on adverse birth outcomes. Here, we conducted a pooled analysis using data of 3,535 mothers and infant pairs using three sub-samples originating from the Danish National Birth Cohort (DNBC), and we evaluated the associations between prenatal PFASs exposures and birth outcomes. Maternal plasma concentrations of six types of PFASs in early pregnancy (around 8.7 gestational weeks) were studied. We found that each LN-ng/ml increase in PFOS, PFOA, PFNA and PFHpS was associated with a 65g, 51g, 52g or 56g decrease in birth weight respectively. Moreover, we also found that prenatal PFOS, PFHpS, PFDA levels were associated with the risks for preterm

birth (< 37 completed gestational weeks). Our findings strengthen the evidence that in-utero PFAS exposures may affect fetal growth. These findings raise concerns considering the ubiquitous contamination of the environment by PFASs. Public health strategies to prevent or lower PFASs exposures in pregnant women are needed.

The thesis of Qi Meng is approved.

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2018

Table of Contents

List of Tables

List of Figures

1. Introduction

Perfluoroalkyl substances (PFASs) are industrial persistent pollutants that are wide-spread in the environment.[1] The most commonly used PFASs are perfluorooctanote (PFOA), perflourooctanesulfonate (PFOS),[2] perfluorohexane sulfonate (PFHxS) and perfluorononanoic acid (PFNA).[3] According to the 1999-2000 National Health and Nutrition Examination Survey (NHANES) analyzing representative samples in the United States, PFOA, PFOS and PFHxS were detected in all (100%) samples while PFNA was found in 95%.[4]

Animal studies suggested that prenatal PFASs exposure can affect fetal growth i.e. PFOS and PFOA exposures in-utero can reduce birth weight and gestational age of deliveries in rodents.[5-11] Several potential mechanisms have been suggested, including a disturbance of lipid and glucose homeostasis, effects on cell proliferation and differentiation, suppression of primary antibody response, or altered glucocorticoids and reproductive hormones levels.[12-19]

While high PFOA and PFOS exposures in pregnancy have been reported to be associated with lower average birth weights in human newborns in epidemiological studies,[20-23] most studies analyzed small sample sizes with very few numbers of infants born low birth weight or preterm. Moreover, evidence about the possible influence of other types of PFASs on fetal growth is also sparse and needs to be investigated.

A previousl study conducted in the DNBC found an inverse association between maternal plasma PFOA levels and birth weight,[23] however, the study alone has insufficient power to evaluate adverse birth outcomes such as low birth weight and preterm birth. Here, we conducted additional pooled analysis utilizing 3 sub-study samples with a total of 3,535 prenatal PFASs measures in the Danish National Birth Cohort (DNBC) and evaluated the associations between prenatal exposure to several types of PFASs and birth weight and preterm birth.

2. Methods

2.1 Study population

The Danish National Birth Cohort (DNBC) is a nationwide follow-up study of pregnant women and their offspring in Denmark.[24] Includion to the DNBC was given by general practitioners from 1996-2002 and a total of 101,042 pregnancies were initially enrolled. About 50% of all pregnant women during the study period in Denmark were invited and 60% accepted. After informed consent, four computer-assisted telephone interviews based on structured questionnaires were conducted – approximately at the gestational weeks 12 and 30, and when the child was 6 and 18 months old. Moreover, two maternal blood samples were taken during pregnancy (once in the first and once in the second trimester), and one umbilical cord blood sample was obtained at birth and stored in a biobank. Blood samples were transported at room temperatures for about 4 - 48 hours, but most samples arrived and were processed within 28 hours.

The source population for this study are N=83,389 mother-child pairs enrolled in the DNBC who completed interview 1 and for whom blood samples were available for PFASs analyses. Three sub-studies measured PFASs samples in the DNBC.[23,25,26] A selection flowchart for these samples is provided in figure 1.

We analyzed each of these sub-samples separately and also conducted a pooled analysis combining all samples. Samples included only live-born children. All sub-samples have first trimester maternal plasma concentrations for PFOS and PFOA, and 4 additional PFAS (PFHxS, PFNA, PFHpS and PFDA) were measured in sub-sample 2 and 3. Sample 1 randomly selected 1,398 mothers-child pairs among those who had all four computer-assisted telephone interviews and a 7-year follow up questionnaire completed by the mothers.[23] Sample 2 includes 545 population controls frequency matched by sex who were selected at random from the

DNBC cohort among those who completed interview 1 for a case-cohort study originally designed to study attention-deficit/hyperactivity disorder (ADHD), autism and cerebral palsy in children.[25,27] Sample 3 included 1,592 participants enrolled in the Lifestyle During Pregnancy Study (LDPS),[26] a DNBC sub-cohort with two-stage design and sampling strategies based on prenatal alcohol exposure categories with the aim to study early life influences on brain functions in children at age 5.[26]

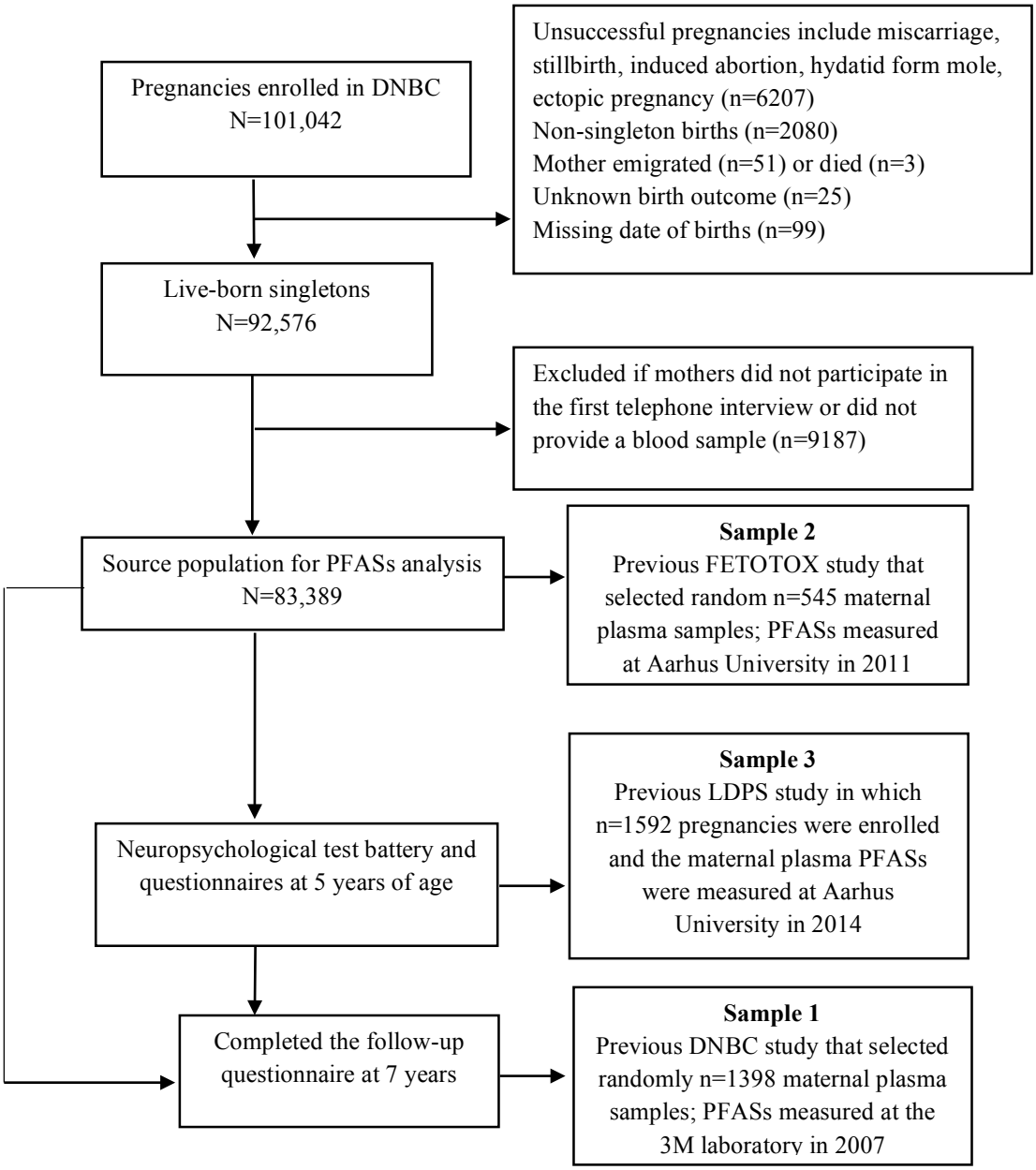

Figure 1. Sample selection for the three sub-study samples

Information on potential confounders, including infant sex, infant birth year, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy and drinking during the pregnancy, was collected by highly structured questionnaires (available at http://www.bsmb.dk).[24] Birth weight and gestational age at birth were obtained from the National Hospital Discharge Register at the National Board of Health in Denmark. The assessment of gestational age was based on either the first day of the last menstrual period (LMP) or from ultrasound examination done before 24 weeks of gestation [28] conducted by midwives. Infants with recorded birth weight <500g or >6800g (n=6) or gestational age <140days or >315days (n=4) were considered extreme values and these measures were assigned as missing and excluded from the analyses for that specific outcome.

Low birth weight (LBW) was defined as birth weight < 2,500 g. Preterm birth was defined as the birth of an infant before 37 completed weeks of gestation (259 days). Small for gestational age (SGA) was defined as an infant with birth weight below the 10th percentile at a specific gestational age in weeks, based on the distribution of all singleton live births of the same sex and birth year in Denmark generated from register data.

2.2 Exposure Assessment

Details about our analytic methods for PFASs have been described elsewhere.[23,25,27] Briefly, all blood samples collected in the DNBC were sent by mail to Statens Serum Institute in Copenhagen, separated and stored in freezers at -20°C or -80°C. For study sample 1, plasma concentrations of PFOS and PFOA were measured in the 3M Toxicology Laboratory,[29] and the study sample 2 and 3 were analyzed at the Department of Environmental Science at Aarhus University. Both laboratories were blinded to the exposures and the outcomes. A total of 0.1 ml stored maternal plasma were sent to the laboratories. Solid Phase Extraction (SPE)

technique was used for sample extraction and purification. PFAS concentrations were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Only PFOS and PFOA were measured in sample 1 while 16 PFASs were measured in both sample 2 and 3, because sample 1 measured in year 2007 when only those 2 compounds can be measured in the laboratory, while for sample 2 and 3, the measures were conducted in later years (in 2011 and 2014 respectively) and 16 PFASs were mesured then. In sample 2 and 3, we focused on 6 types of PFASs that were previously found to be quantifiable in >90% measured samples including PFOS 100%, PFOA 100%, PFHxS 98%, PFHpS 96%, PFNA 92%, PFDA 90%.[25,27] We used multiple imputations to account for PFASs values below quantitation limits.

2.3 Statistical Analysis

We used multivariable linear regression to evaluate the associations between continuous birth weight and length of gestation (continuous gestational age in days) and maternal plasma PFASs level. We used logistic regression to estimate odds ratio (OR) and 95% confidence interval (CI) for low birth weight, preterm birth and SGA. The PFAS levels were analyzed as continuous values or categorized in tertiles (< 33th, 33th–< 67th, ≥ 67th). For continuous PFAS values, both natural log-transformed (LN) and untransformed concentrations were tested in the statistical models, and the findings were similar. The PFAS tertile classifications were based on untransformed PFAS values and those below the 33th percentile were used as the reference group. The PFAS tertile was generated based on the study-sample specific cut-off in order to account for laboratory differences or the "batch effect". We also used the PFAS tertile analyses to evaluate potential non-linear exposure and outcome response.

We adjusted for potential confounders that could influence fetal growth or length of gestation at birth. These factors included infant sex, infant birth year, gestational age at blood draw (in weeks), maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy, and drinking during the pregnancy. All covariates but infant birth year and gestational age at blood draw were introduced into models as categorical variables. We used multiple imputations to account for the missing values for all above mentioned covariates (<10% of the sample had at least 1 missing value). Stratified analyses by parity, pre-pregnancy BMI and sex were performed to evaluate effect measure modifications. We also conducted analyses separately for each study sample to examine the consistency of the results across strata. Moreover, we employed weighted regression analysis taking into account the sampling fractions and selection probabilities from each study sub-samples.[27,30]

3. Results

Demographic and other characteristics of the study participants are presented in Table 1. Almost half of the women were having their first babies. There were more male infants (55.9%) than female (44.1%) due to over-sampling of males in sample 2. During pregnancy, 28.3% ever smoked, and only 21.7% did not drink in pregnancy primarily because of the over-sampling of alcohol intake during pregnancy in sample 3. Nearly 30% mothers had a pre-pregnancy BMI of ≥ 24 kg/m^2. The mean birth weight was 3,614 g, and 1.7% infants were born LBW. The mean gestational age at birth was 280 days, and 3.2% of infants were born preterm.

On average, birth weight was lower among these first born, with low maternal pre-pregnancy BMI, lower socio-occupational status, and if the mothers were drinkers or smokers (Table 1). The mean birth weight was 96g higher in male infants compared with female. The

mean of gestational week of blood-draw in sample 1 was 8.5 weeks with a standard deviation (SD) of 2.1 weeks. Sample 2 was 9.6 (SD 4.7) and sample 3 was 8.7 (SD 2.4) weeks. Most PFASs levels decreased with increasing maternal age and parity. High levels of PFOS were also observed in obese women (Table 2).

Table 1. Mean and standard deviations (mean±SD) of birth weight and gestational age by study characteristics (n=3,535)

	No. (%)	Birth weight (g)	Gestational age (days)
All participants	3,535 (100)	3614.0 ± 526.9	280.5 ± 10.8
Study sample			
Sample1	1,398 (39.5)	3626.3 ± 529.8	280.4 ± 11.0
Sample2	545 (15.4)	3608.3 ± 555.2	280.1 ± 11.2
Sample3	1592 (45.0)	3605.2 ± 514.4	280.7 ± 10.5
Infant sex			
Female	1559 (44.1)	3560.1 ± 507.3	280.8 ± 10.6
Male	1976 (55.9)	3656.5 ± 538.2	280.3 ± 10.9
Maternal age			
<30	1638 (46.3)	3580.7 ± 515.4	280.6 ± 11.0
30-34	1721 (48.7)	3649.2 ± 529.3	280.8 ± 10.7
35-39	176 (5.0)	3628.0 ± 548.4	279.7 ± 10.5
Socio-occupational status			
High	2366 (67.2)	3624.1 ± 520.6	280.7 ± 14.9
Medium	1057 (30.0)	3601.6 ± 539.0	280.2 ± 11.0
Low	100 (2.8)	3515.2 ± 544.8	279.2 ± 11.4
Missing	12	3528.5 ± 491.4	279.3 ± 14.9
Parity			
1	1622 (47.1)	3518.5 ± 498.4	280.7 ± 12.3
2	1212 (35.2)	3680.3 ± 522.6	280.6 ± 12.3
>=3	610 (17.7)	3753.7 ± 551.7	280.0 ± 10.6
Missing	91	3487.5 ± 568.7	279.8 ± 12.3
Alcohol intake during pregnancy			
Never	766 (21.7)	3641.2 ± 537.6	280.2 ± 11.4
Once or less per week	629 (17.8)	3651.3 ± 554.2	280.2 ± 10.8
More than once per week	2140 (60.5)	3593.4 ± 513.9	280.7 ± 10.6
Smoking during pregnancy			
No	2534 (71.7)	3656.4 ± 516.9	280.8 ± 10.4
Yes	1001 (28.3)	3506.0 ± 537.0	279.6 ± 11.7
Pre-pregnancy BMI (kg/m^2)			
<18.5	143 (4.1)	3367.9 ± 691.3	279.1 ± 12.4
18.5-23.9	2355 (68.0)	3596.0 ± 502.7	280.5 ± 10.6
24.0-29.9	705 (20.4)	3668.4 ± 552.1	280.5 ± 11.3
>=30.0	258 (7.5)	3749.3 ± 558.8	281.3 ± 10.7
Missing	74	3674.6 ± 691.3	280.8 ± 11.1

Table 2. Mean and standard deviations (mean±SD) of plasma concentrations of PFASs (ng/ml) by characteristics of study partisans (n=3,535*)

	No.	PFOS	PFOA	PFHxS	PFNA	PFHpS	PFDA
All participants*	3535	32.0 ± 13.1	4.9 ± 2.2	1.1 ± 0.8	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Study sample							
Sample1	1398	35.3 ± 13.0	5.6 ± 2.5	N/A	N/A	N/A	N/A
Sample2	545	29.3 ± 13.2	4.3 ± 2.0	1.1 ± 1.0	0.5 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
Sample3	1592	30.1 ± 12.5	4.4 ± 1.8	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Infant sex							
Female	1559	32.3 ± 13.3	4.9 ± 2.2	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Male	1976	31.8 ± 12.9	4.8 ± 2.3	1.1 ± 0.8	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Maternal age							
19-29	1638	33.5 ± 13.3	5.2 ± 2.4	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
30-34	1721	31.4 ± 13.0	4.6 ± 2.0	1.1 ± 0.9	0.5 ±0.2	0.4 ± 0.2	0.2 ±0.1
35-39	176	29.3 ± 12.0	4.4 ± 2.1	1.1 ± 0.7	0.5 ± 0.2	0.3 ± 0.2	0.2 ±0.1
Socio-occupational status							
High	2366	31.1 ± 12.9	4.8 ± 2.2	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Medium	1057	34.2 ± 13.1	5.0 ± 2.2	1.1 ± 1.0	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Low	100	31.5 ± 13.4	4.5 ± 2.1	0.9 ± 0.4	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.1
Missing	12	30.7 ± 13.5	4.2 ± 2.0	1.5 ± 0.7	0.4 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Parity							
1	1622	33.4 ± 13.4	5.7 ± 2.3	1.3 ± 0.9	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
2	1212	30.7 ± 12.8	4.2 ± 1.8	1.0 ± 0.5	0.5 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
>=3	610	30.5 ± 12.3	4.0 ± 2.0	0.9 ± 1.1	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
Missing	91	35.1 ± 12.4	5.6 ± 2.2	1.2 ± 0.4	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Alcohol intake during pregnancy							
Never	766	32.1 ± 12.8	4.8 ± 2.2	1.0 ± 0.6	0.5 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
<=1 per week	629	33.9 ± 13.4	5.1 ± 2.3	1.2 ± 1.3	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
>1 per week	2140	31.4 ± 13.0	4.9 ± 2.2	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Smoking during pregnancy							
No	2534	32.6 ± 13.2	4.9 ± 2.3	1.1 ± 0.7	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Yes	1001	30.5 ± 12.6	4.8 ± 2.1	1.1 ± 1.0	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Pre-pregnancy BMI (kg/m^2)							
<18.5	143	31.3 ± 15.5	4.7 ± 2.1	1.2 ± 0.8	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
18.5-23.9	2355	31.3 ± 12.8	4.8 ± 2.3	1.1 ± 0.8	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
24.0-29.9	705	33.3 ± 12.4	4.9 ± 2.1	1.1 ± 0.8	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
>=30.0	258	35.5 ± 15.0	5.1 ± 2.3	1.0 ± 0.5	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1
Missing	74	30.0 ± 12.7	4.9 ± 2.1	1.2 ± 0.6	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.1

* For PFOS and PFOA, the total is 3535, for other 4 PFASs, the total number is 2137.

We observed that each LN-ng/ml increase in PFOS, PFOA, PFNA and PFHpS was associated with a 65g, 51g, 52g or 56g decrease in average birth weight (Table 3). PFOS, PFNA and PFHpS were also associated with a small decrease in the gestational age at delivery (Table 3). We did not find consistent differences in the associations between PFASs and birth weight and gestational age comparing the sex, parity and pre-pregnancy BMI strata (Table 3).

We also found that each LN-ng/ml increase in PFOS, PFHpS, PFDA were associated with preterm birth, i.e. the adjusted ORs for preterm birth were 1.8 (95% CI 1.1, 2.9), 1.7 (95% CI 1.0, 3.0) and 2.2 (95% CI 1.3, 3.7) for PFOS, PFHpS and PFDA, respectively (Table 5). We did not observe consistent associations between each of the PFAS and LBW, and the estimates were null for any of the PFASs and SGA. In PFAS tertiles, higher PFOS and PFHpS tertiles were also associated with risks for preterm birth. The estimates for PFOS and LBW were elevated (i.e. the second and the third tertile both had an estimated OR 1.7 95% CI 0.9, 3.1 compared with the lowest tertile) (Table 5). Similarly, no associations were found for PFAS tertiles and SGA. The PFAS tertiles analyses also did not suggest strong non-linearity between each of the PFAS exposure and outcome responses.

Table 3. Adjusted regression coefficients (β) and 95% confidence interval (CI) for continuous birth weight (g) according to PFASs (LN-ng/ml) in maternal plasma during pregnancy.

	Adjusted difference in birth weight (β and 95%CI), pooled sample 1, 2 and 3			Adjusted difference in birth weight (β and 95%CI), pooled sample 2 and 3				
Strata	**No.**	**PFOS**	**PFOA**	**No.**	**PFHxS**	**PFNA**	**PFHpS**	**PFDA**
Crude [a]	3507	-63.2 (-108.6, -17.9)	-130.0 (-170.1, -89.8)	2120	-85.5 (-127.4, -43.6)	-87.1 (-135.7, -38.5)	-92.5 (-140.1, -44.8)	-26.9 (-77.2, 23.4)
All [b]	3507	-65.2 (-110.9, -19.6)	-51.4 (-95.7, -7.1)	2120	1.8 (-40.8, 44.3)	-52.3 (-101.8, -2.8)	-56.1 (-104.8, -7.4)	-13.0 (-62.4, 36.3)
Sex [c]								
Female	1547	-94.2 (-161.1, -27.3)	-36.0 (-103.0, 31.0)	865	-6.4 (-69.7, 57.0)	-38.2 (-113.8, 37.5)	-88.0 (-162.8, -13.2)	-23.8 (-95.6, 48.0)
Male	1960	-35.0 (-96.9, 26.8)	-59.9 (-118.5, -1.3)	1255	20.2 (-37.0, 77.5)	-64.3 (-130.1, 1.4)	-27.9 (-91.8, 35.9)	-3.3 (-70.7, 64.2)
Parity [d]								
1	1653	-55.5 (-121.2, 10.2)	-42.8 (-111.7, 26.0)	1039	-67.7 (-124.6, -10.9)	-54.5 (-135.6, 26.6)	-83.8 (-152.7, -14.8)	-57.4 (-125.8, 11.0)
2	1241	-50.5 (-123.0, 22.0)	-49.6 (-119.1, 19.9)	734	26.5 (-50.9, 103.9)	-66.4 (-139.3, 6.5)	-67.4 (-145.9, 11.1)	-3.5 (-80.8, 73.9)
>=3	613	-105.3 (-226.8, 16.2)	-65.8 (-171.1, 39.6)	347	57.2 (-57.0, 171.5)	24.6 (-104.3, 153.5)	36.5 (-94.6, 167.5)	227.0 (72.2, 381.7)
BMI [e]								
<18.5	150	83.9 (-106.0, 273.8)	-18.4 (-233.6, 196.8)	88	-16.0 (-212.5, 180.6)	-150.8 (-346.5, 44.8)	-10.0 (-247.7, 227.7)	-113.0 (-352.0, 126.0)
18.5-23.9	2379	-77.2 (-129.8, -24.6)	-59.0 (-109.4, -8.7)	1459	-8.7 (-59.8, 42.3)	-47.2 (-107.6, 13.2)	-72.2 (-129.4, -15.1)	17.2 (-41.2, 75.7)
23.9-30	720	-50.0 (-170.9, 71.0)	-38.4 (-153.1, 76.2)	416	6.8 (-108.7, 122.2)	-77.5 (-202.9, 47.9)	12.5 (-109.0, 134.0)	-15.8 (-153.3, 121.6)
>30	258	-148.2 (-302.7, 6.3)	-62.9 (-227.2, 101.4)	157	-4.1 (-125.5, 117.3)	63.0 (-127.8, 253.8)	-39.4 (-193.6, 114.8)	18.8 (-120.4, 157.9)
Study sample [f]								
Sample1	1387	-43.3 (-118.2, 31.5)	-78.1 (-148.2, -8.0)	N/A	N/A	N/A	N/A	N/A
Sample2	540	-73.5 (-184.1, 37.0)	-99.2 (-211.3, 13.0)	540	-70.8 (-143.7, 2.2)	-14.0 (-108.4, 80.4)	-75.8 (-162.3, 10.7)	-12.9 (-96.3, 70.5)
Sample3	1580	-58.4 (-126.9, 10.1)	10.2 (-56.9, 77.3)	1580	45.7 (-6.8, 98.2)	-60.2 (-119.0, -1.5)	-37.6 (-97.2, 22.0)	-7.6 (-69.4, 54.3)

a Adjusted for study sample.
b Adjusted for study sample, infant sex, infant birth year, blood-drawn week, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy and drinking during the pregnancy.
c The models did not include infant sex.
d The models did not include parity.
e The models did not include pre-pregnancy body mass index (BMI).
f The models did not include study sample.

Table 4. Adjusted regression coefficients (β) and 95% confidence interval (CI) for continuous gestational age (days) according to PFASs (LN-ng/ml) in maternal plasma during pregnancy.

	Adjusted difference in gestational age (β and 95%CI), pooled sample 1, 2 and 3			Adjusted difference in gestational age (β and 95%CI), pooled sample 2 and 3				
Strata	**No.**	**PFOS**	**PFOA**	**No.**	**PFHxS**	**PFNA**	**PFHpS**	**PFDA**
Crude [a]	3526	-1.1 (-2.0, -0.2)	-0.3 (-1.1, 0.5)	2132	-0.5 (-1.3, 0.3)	-1.4 (-2.3, -0.4)	-1.3 (-2.2, -0.3)	-1.0 (-1.9, 0.0)
All [b]	3526	-1.6 (-2.5, -0.6)	-0.5 (-1.4, 0.4)	2132	-0.3 (-1.1, 0.6)	-1.4 (-2.4, -0.4)	-1.7 (-2.7, -0.8)	-0.8 (-1.8, 0.2)
Sex [c]								
Female	1555	-1.5 (-2.9, -0.1)	-0.2 (-1.6, 1.2)	869	0.1 (-1.2, 1.4)	0.1 (-1.5, 1.6)	-1.6 (-3.1, -0.1)	0.1 (-1.3, 1.5)
Male	1971	-1.7 (-2.9, -0.4)	-0.8 (-2.0, 0.4)	1263	-0.6 (-1.8, 0.5)	-2.7 (-4.1, -1.4)	-1.9 (-3.1, -0.6)	-1.7 (-3.1, -0.3)
Parity [d]								
1	1663	-1.7 (-3.2, -0.3)	-0.3 (-1.8, 1.3)	1045	-0.9 (-2.2, 0.3)	-0.5 (-2.3, 1.2)	-1.9 (-3.4, -0.4)	-0.7 (-2.2, 0.8)
2	1249	-0.9 (-2.4, 0.5)	-0.4 (-1.7, 1.0)	739	0.1 (-1.4, 1.7)	-1.7 (-3.1, -0.2)	-1.9 (-3.4, -0.3)	-0.4 (-2.0, 1.1)
>=3	614	-1.7 (-4.0, 0.6)	-0.6 (-2.6, 1.4)	348	-0.3 (-2.3, 1.6)	-1.5 (-3.7, 0.7)	-1.0 (-3.2, 1.2)	-0.8 (-3.5, 1.8)
BMI [e]								
<18.5	150	-0.7 (-5.5, 4.1)	-3.2 (-8.5, 2.2)	88	-1.3 (-7.0, 4.4)	-6.9 (-12.5, -1.3)	-1.4 (-8.3, 5.5)	-4.6 (-11.5, 2.4)
18.5-23.9	2395	-1.0 (-2.1, 0.0)	0.0 (-1.1, 1.0)	1469	-0.6 (-1.6, 0.4)	0.0 (-1.2, 1.2)	-1.6 (-2.7, -0.4)	0.7 (-0.5, 1.8)
23.9-30	722	-1.7 (-4.1, 0.6)	-0.9 (-3.1, 1.4)	418	1.6 (-0.3, 3.6)	-3.4 (-5.5, -1.2)	0.3 (-1.8, 2.4)	-2.6 (-5.0, -0.3)
>30	259	-4.0 (-7.2, -0.7)	-0.7 (-4.2, 2.7)	157	-0.3 (-2.7, 2.1)	-3.3 (-7.0, 0.4)	-4.3 (-7.3, -1.3)	-2.3 (-5.0, 0.4)
Study sample [f]								
Sample1	1394	-0.9 (-2.5, 0.7)	-0.2 (-1.7, 1.3)	N/A	N/A	N/A	N/A	N/A
Sample2	545	-4.6 (-6.9, -2.3)	-3.3 (-5.6, -0.9)	545	-1.9 (-3.5, -0.4)	-1.3 (-3.2, 0.7)	-3.6 (-5.4, -1.8)	-1.9 (-3.6, -0.1)
Sample3	1587	-0.8 (-2.1, 0.6)	0.5 (-0.8, 1.8)	1587	0.7 (-0.4, 1.7)	-1.5 (-2.7, -0.4)	-0.9 (-2.1, 0.3)	-0.5 (-1.7, 0.7)

a Adjusted for study sample.
b Adjusted for study sample, infant sex, infant birth year, blood-drawn week, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy and drinking during the pregnancy.
c The models did not include infant sex.
d The models did not include parity.
e The models did not include pre-pregnancy body mass index (BMI).
f The models did not include study sample.

Table 5. Adjusted OR (95% CI) for low birth weight(LBW), preterm birth (PTB) and small for gestational age (SGA) according to prenatal PFASs.

		Adjusted OR and 95%CI, pooled Sample 1, 2 and 3			Adjusted OR and 95%CI, pooled sample 2 and 3			
Outcome	**No.**	**PFOS**	**PFOA**	**No.**	**PFHxS**	**PFNA**	**PFHpS**	**PFDA**
LBW								
Crude[a]	61	1.6 (0.9, 2.7)	1.5 (0.9, 2.5)	37	1.7 (1.0, 2.8)	1.8 (0.9, 3.4)	1.0 (0.6, 1.7)	1.3 (0.7, 2.4)
All[b]	61	1.5 (0.8, 2.8)	1.0 (0.6, 1.9)	37	1.3 (0.8, 2.2)	1.7 (0.8, 3.4)	1.0 (0.5, 1.7)	1.4 (0.7, 2.5)
PTB								
Crude[a]	113	1.8 (1.2, 2.8)	1.4 (1.0, 2.1)	59	1.3 (0.9, 2.0)	1.9 (1.1, 3.3)	1.6 (1.0, 2.7)	2.3 (1.4, 3.9)
All[b]	113	1.8 (1.1, 2.9)	1.2 (0.7, 1.8)	59	1.1 (0.7, 1.7)	1.6 (0.9, 2.9)	1.7 (1.0, 3.0)	2.2 (1.3, 3.7)
SGA								
Crude[a]	240	0.7 (0.6, 1.0)	1.3 (0.9, 1.7)	151	1.7 (1.3, 2.2)	0.9 (0.7, 1.3)	1.0 (0.7, 1.3)	0.7 (0.5, 1.0)
All[b]	240	0.7 (0.5, 0.9)	0.7 (0.5, 1.0)	151	1.2 (0.9, 1.6)	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.7 (0.5, 0.9)

a Adjusted for study sample.

b Adjusted for study sample, infant sex, infant birth year, blood-drawn week, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy and drinking during the pregnancy.

Table 6. Adjusted OR (95% CI) for low birth weight (LBW), preterm birth (PTB) and Small for gestational age (SGA) according to prenatal PFASs levels (in tertiles).

Outcome	Exposure Tertile	LBW		PTB		SGA	
		N	OR[a] and 95% CI	N	OR[a] and 95% CI	N	OR[a] and 95% CI
PFOS	T1	13 (1.1%)	-	29 (2.5%)	-	84 (7.2%)	-
	T2	23 (1.9%)	1.7 (0.9, 3.1)	41 (3.4%)	1.6 (1.0, 2.5)	70 (5.9%)	0.6 (0.5, 0.9)
	T3	25 (2.2%)	1.7 (0.9, 3.1)	43 (3.7%)	2.0 (1.2, 3.2)	86 (7.5%)	0.7 (0.5, 0.9)
PFOA	T1	18 (1.6%)	-	32 (2.8%)	-	63 (5.4%)	-
	T2	18 (1.5%)	1.1 (0.6, 2.1)	40 (3.3%)	1.1 (0.7, 1.7)	86 (7.2%)	1.0 (0.7, 1.3)
	T3	25 (2.2%)	1.1 (0.6, 2.0)	41 (3.5%)	0.9 (0.6, 1.5)	91 (7.9%)	0.8 (0.5, 1.1)
PFHxS	T1	12 (1.7%)	-	21 (2.9%)	-	42 (5.9%)	-
	T2	9 (1.3%)	0.9 (0.4, 1.8)	18 (2.5%)	1.0 (0.6, 1.8)	48 (6.7%)	0.9 (0.6, 1.3)
	T3	16 (2.3%)	0.9 (0.4, 1.8)	20 (2.9%)	0.7 (0.4, 1.4)	61 (8.9%)	1.1 (0.7, 1.6)
PFNA	T1	10 (1.3%)	-	20 (2.7%)	-	47 (6.3%)	-
	T2	12 (1.7%)	1.3 (0.6, 2.6)	15 (2.1%)	0.5 (0.2, 0.9)	51 (7.2%)	0.8 (0.6, 1.2)
	T3	15 (2.3%)	1.3 (0.6, 2.8)	24 (3.6%)	1.4 (0.8, 2.6)	53 (8.0%)	0.6 (0.4, 0.9)
PFHpS	T1	15 (2.1%)	-	18 (2.5%)	-	43 (6.1%)	-
	T2	11 (1.5%)	2.1 (1.0, 4.2)	19 (2.5%)	1.9 (1.0, 3.5)	53 (7.1%)	1.1 (0.8, 1.7)
	T3	11 (1.7%)	0.6 (0.2, 1.6)	22 (3.3%)	1.8 (0.9, 3.6)	55 (8.3%)	0.8 (0.5, 1.2)
PFDA	T1	15 (1.9%)	-	21 (2.6%)	-	61 (7.7%)	-
	T2	7 (1.1%)	0.6 (0.3, 1.3)	15 (2.3%)	1.3 (0.7, 2.4)	43 (6.5%)	1.1 (0.8, 1.6)
	T3	15 (2.3%)	1.0 (0.5, 1.9)	23 (3.4%)	1.6 (0.9, 2.9)	47 (7.1%)	0.5 (0.4, 0.8)

a Adjusted for infant sex, infant birth year, blood-drawn week, maternal age, parity, socio-occupational status, pre-pregnancy body mass index (BMI), smoking during pregnancy and drinking during the pregnancy.

4. Discussion

In this large nationwide population-based cohort study in Denmark, we found that prenatal exposure to several PFASs were associated with adverse birth outcomes. Our findings were consistent with previous reports that suggested PFOS and PFOA exposures were associated with decreased length of gestation and birth weight.[20,21,23,31-34] In addition, we provided further evidence that several PFASs may influence the risks of preterm birth, for which none of the previous epidemiological studies have had sufficient statistical power.

There are moderate to high correlations between difference PFASs, which makes it difficult to disentangle the specific exposure effects to one chemical or from the mixtures. The effect estimates for PFOS, PFOA and PFHpS were often in the same direction; possibly driven by the correlations among these chemicals ($r > 0.71$). When we mutually adjusted for all six chemicals in the same model, the effect estimates for all PFASs became less precise, while only PFNA was still associated with lower birth weight, and PFHpS was related to reduced gestational age. Although experimental studies have indicated that mixtures of several PFASs have additive or more than additive effects,[35] further research is needed to explore the mechanism of the interaction among different PFASs.

Our study has several strengths. All of the three sub-samples were selected from a nationwide well-described cohort of pregnant women and their infants.[24] The PFASs measures were obtained when available at the time using state-of-the-art laboratory facilities, and the laboratory personnel were blinded for exposure and outcome status. Data on birth weight and gestational age originated from the Danish Hospital Discharge Register based on standard clinic procedures. Most importantly, we took full advantage of the existing PFASs biomarker measures generated in the DNBC and conducted this pooled analyses with a sample size sufficient to evaluate some adverse birth outcomes that were not well studied previously.

The effect estimates were largely consistent across study samples, but some small variations were observed. This could be due to different sampling and selection criteria for each study sample, influence of measurement errors, or by chance. We have in our analyses adjusted for the sampling and selection probabilities using weighted regressions throughout, but some differences in the study characteristics across study samples may still remain. The correlations between PFOA and PFOS values measured at the two laboratories have previously found to be very high (Pearson correlation r=0.94 for PFOS and r=0.95 for PFOA). We adjusted for the study sample using an indicator in all regression models that analyzed continuous PFAS. Moreover, we used study-sample specific cut-offs in the PFAS quartile analyses. Any measurement errors in the exposures and outcomes were expected to be non-differential and might likely bias the association towards the null.

The observed association may not be causal and can possibly be influenced by biases. There could be unmeasured confounding factors that we could not take into considerations. When using biomarkers of PFASs, physiological factors that affect accumulation or excretions of PFASs should also be considered. For instance, lower glomerular filtration rate (GFR) in mid- or late-pregnancy has been suggested to be such a possible confounding factor.[36] Mothers with lower GFR might possibly have lower PFASs excretion, and a lower GFR in pregnancy has been linked with adverse birth outcomes. However, our PFASs measures were measured in first trimester plasma samples (mean 8.7 gestational week) and PFAS measures in early pregnancy are less likely to be influenced by the changes of GFR in pregnancy.[36] Participants were unlikely to be aware of their PFAS levels which limits the possibility of self-selection bias. However, prenatal PFASs exposures may increase risk of miscarriages.[37,38] Our previous study has demonstrated that "live-birth selection bias" may occur if PFASs cause fetal losses

and infants born alive are studied. In such scenario, the true effect estimates might be biased towards the null or in a negative direction.[39]

In conclusion, our pooled analyses demonstrated that several prenatal PFASs are inversely associated with birth weight and gestational age, and prenatal exposure to several PFASs may increase the risks for preterm birth. Our findings strengthen the evidence that in-utero PFAS exposures affect fetal growth. These findings raise concerns considering the ubiquity of PFASs contamination in the environment and in humans. Strategies to prevent or lower PFASs exposures in pregnant women and young infants might be needed.

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