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Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case–cohort study

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BACKGROUND: The associations between prenatal exposure to endocrine disruptive chemicals (EDCs) and fetal growth are inconsistent, and few studies have considered small-for-gestational-age (SGA) birth as an outcome. Our current study of Scandinavian parous women aimed to address these inconsistencies and gaps in the literature.

METHODS: This case–cohort study included 424 mother–child pairs who participated in a prospective, multi-center study of parous women in Norway (Trondheim and Bergen) and Sweden (Uppsala). We used linear and logistic regression with 95% confidence intervals (CIs) to analyze the associations between two perfluoroalkyl substances (PFASs) and five organochlorines (OCs) from early second trimester and indices of fetal growth.

RESULTS: Among Swedish women, prenatal exposure to perfluorooctanoate (PFOA), polychlorinated biphenyl (PCB) 153 and hexachlorobenzene (HCB) were associated with higher odds for SGA birth. We found stronger associations among Swedish male offspring. In the Norwegian cohort, we found no significant associations between EDC exposure and indices of fetal growth.

CONCLUSIONS: Some populations may be more vulnerable to EDCs, possibly due to differences in exposure levels, exposure sources and/or modifiable lifestyle factors. Male offspring may be more vulnerable to endocrine disruption.

Fetal growth restriction (FGR) is defined as a pathologic inhibition of intrauterine fetal growth and failure to achieve the fetus' growth potential. FGR is associated with perinatal mortality and morbidity. Small-for-gestational-age (SGA) is a proxy for FGR and defined as birth weight below the 10th percentile for gestational age, sex, and parity (1).

Perfluoroalkyl substances (PFASs) and organochlorines (OCs) are persistent, bio-accumulative chemicals that have been detected in maternal blood during pregnancy and in cord blood at delivery. PFASs and OCs may act as endocrine disrupting chemicals (EDCs), and *in utero* exposure to EDCs may have consequential developmental effects on the fetus (2). A United Nations Environment Program/World Health Organization (UNEP/WHO) report from 2012 identifies human exposure to EDCs as an emerging challenge (3). Increased knowledge is required to ensure better protection for the most vulnerable members of society. Pregnant mothers and children are particularly vulnerable to developmental exposures like EDCs, and fetal programming may increase susceptibility to diseases later in life (3).

The associations between EDCs and indices of fetal growth have been studied in different populations, although results are inconsistent (4,5). Diet is considered an important exposure route for PFASs and OCs even in periods with simultaneous direct exposure from production and emission of chemicals (6). In Scandinavian populations, consumption of seafood have been associated with high PFAS and OC serum levels (7,8). The Baltic Sea, which is situated on the East coast of Sweden, has been vastly contaminated with OCs (9), and a study of births in Sweden from 1973–1991 found associations between high maternal intake of fish from the Baltic Sea and restricted fetal growth (10). However, most other studies report positive associations between higher maternal fish intake and better fetal growth (11). Dietary intake of fish from different water sources may result in population-specific EDC exposures. Taken together, source-specific fish intake may confound associations between EDCs and fetal growth.

Most observational studies have been underpowered to detect significant associations between EDCs and SGA birth, or only included birth weight as an outcome. A most recent systematic review of prenatal serum levels of PFASs and human fetal growth published in 2015, recommended that future EDC exposure studies should focus on SGA birth as an outcome of interest (4).

In the current study, we used a case–cohort design to study the association between maternal serum levels of PFASs and OCs and indices of fetal growth including birth weight, birth

length, head circumference, and SGA birth in Scandinavian women from Norway and Sweden. We also explored possible effect modification by country and offspring sex.

RESULTS

Maternal and Offspring Baseline Characteristics

Overall, Swedish women had, on average, higher prepregnancy BMI (22 vs. 21 kg/m²) compared to Norwegian women. A greater proportion of Swedish women were nonsmokers at the time of conception compared to their Norwegian peers (67 vs. 47% nonsmokers) (**Table 1**). The total cohort of Swedish mothers compared to Norwegian mothers did not differ significantly in other baseline characteristics (maternal age, height, education, parity, and interpregnancy interval). Swedish offspring were on average longer at birth compared to Norwegian offspring, but did not differ significantly in other characteristics (birth weight, head circumference, and gestational age).

Swedish women who gave birth to SGA offspring (SGA mothers) were on average shorter, had lower prepregnancy BMI, fewer years of education, and lower parity compared to Swedish women with non-SGA births (non-SGA mothers). A higher proportion of Swedish SGA mothers reported smoking at conception compared to Swedish non-SGA mothers (60 vs. 21% smokers). We observed a similar pattern in baseline characteristics when Norwegian SGA and non-SGA mothers were compared. Norwegian SGA mothers were also significantly younger than Norwegian non-SGA mothers (28 vs. 29 y of age). Swedish SGA offspring had, on average, lower birth weight, shorter birth length and smaller head circumference compared to Swedish non-SGA offspring. Norwegian SGA offspring had in addition significantly lower gestational age compared to the Norwegian non-SGA offspring.

PFAS and OC Levels

Overall, Swedish mothers had significantly higher median serum levels of PFOA (2.33 vs. 1.62 ng/ml), perfluoroctane sulfonate (PFOS) (16.4 vs. 9.74 ng/ml), PCB 153 (117 vs. 80.1 ng/g lipid), and β-HCH (25.0 vs. 21.2 ng/g lipid) compared to the total Norwegian cohort (**Table 2**, **Figure 1**). Swedish mothers had significantly lower median serum levels of *t*-NC (6.28 vs. 6.74 ng/g lipid) compared to Norwegian mothers (**Table 2**, **Figure 1**).

Median levels of PFOA and PCB 153 were significantly higher among Swedish SGA mothers compared to Swedish non-SGA mothers (**Figure 1**). Differences in levels of other EDCs (PFOS, *p,p*'-DDE, HCB, β-HCH and *t*-NC) were not statistically significant when Swedish SGA mothers and Swedish non-SGA mothers were compared (**Figure 1**).

We observed no significant differences in PFAS or OC levels between Norwegian SGA mothers and Norwegian non-SGA mothers.

Overall, there were medium to high correlations within the PFASs (ρ = 0.56–0.73) and within the OCs (ρ = 0.29–0.70) (**Supplementary Figure S1** online). However, the PFASs were not highly correlated with the OCs (ρ = −0.01–0.22)

(**Supplementary Figure S1** online). The same pattern was observed in both Norwegian and Swedish cohorts.

Associations Between PFAS and OC Levels and Indices of Fetal Growth

In the pooled analyses including data from both Norway and Sweden, we found no significant associations between EDCs and indices of fetal growth after adjustment for important covariates (**Supplementary Table S1** online).

Table 3 shows associations between serum levels of PFASs and OCs and indices of fetal growth stratified by country of residence. In adjusted linear models for the Swedish cohort, birth weight decreased significantly by −359 g (95% CI: −596, −122) and −292 g (95% CI: −500, −84) per ln-unit increase in PFOA and PFOS, respectively. Birth length was also negatively associated with increasing levels of PFOA and PFOS. For each ln-unit increase in HCB levels, a significant decrease in head circumference (β = −1.0 cm (95% CI: −1.7, −0.2)) was observed after adjustment.

In multivariate logistic regression models for the Swedish cohort, we observed a significant increase in adjusted odds for SGA birth per ln-unit increase in PFOA (aOR = 5.25 (95% CI: 1.68–16.4)), PCB 153 (aOR = 5.59 (95% CI: 1.05–29.9)) and HCB (aOR = 5.62 (95% CI: 1.26–25.1)). We also observed increased aOR for SGA birth per ln-unit increase in PFOS (aOR = 2.51 (95% CI: 0.93–6.77)), although nonsignificant. Among Swedish women, there were no significant associations between levels of PFASs or OCs and gestational age (**Table 3**). Among Norwegian women, we observed no statistically significant associations between PFASs or OCs and indices of fetal growth (**Table 3**).

In the Swedish cohort, the *P* value for interaction between PFOA and infant sex was 0.046 in multivariate linear regression models with birth weight as an outcome. No other interaction terms between EDCs and infant sex were statistically significant (*P* < 0.10) (data not shown). In Swedish male offspring, we observed a significant decrease in birth weight (β = −526 g; 95% CI: −828, −222) and birth length (β = −1.6 cm; 95% CI: −2.9, −0.4) for each ln-unit increase in PFOA (**Table 4** and **Figure 2**). We also observed a significant increase in adjusted odds for SGA birth (aOR = 6.55; 95% CI: 1.14–37.5) among male Swedish offspring (**Table 4** and **Figure 2**). No statistically significant associations between PFOA and indices of fetal growth were observed among Swedish female offspring. We found no significant interaction terms between PFASs or OCs and offspring sex in the Norwegian cohort (data not shown).

DISCUSSION

In this case–cohort study of 424 Scandinavian parous women, we observed increased odds of SGA birth for each ln-unit increase in PFOA, PCB 153 and HCB, among Swedish but not Norwegian mothers. Increasing levels of PFOA, PFOS and HCB were negatively associated with other indices of fetal growth including birth weight, birth length and head circumference among the Swedish mothers. The associations

Table 1. Maternal and offspring baseline characteristics by country and SGA status

	Norway (Trondheim: N = 137, Bergen: N = 128)			Sweden (Uppsala: N = 159)		
	Non-SGA (N = 174)	SGA (N = 91)	Total (N = 265)	Non-SGA (N = 107)	SGA (N = 52)	Total (N = 159)
	Mean (95% CI) or n (%)	Mean (95% CI) or n (%)	Mean (95% CI) or n (%)	Mean (95% CI) or n (%)	Mean (95% CI) or n (%)	Mean (95% CI) or n (%)
Maternal characteristics						
High-risk group	0 (0)	74 (81)*	74 (28)	0 (0)	43 (83)*	43 (27)
10% random sample	174 (100)	17 (19)*	191 (72)	107 (100)	9 (17)*	115 (73)
Maternal age	29.3 (28.6–29.9)	27.9 (27.0–28.7)*	28.8 (28.3–29.3)	29.5 (28.7–30.3)	28.9 (27.5–30.4)	29.3 (28.6–30.0)
Maternal height	167.0 (166.1–167.9)	164.3 (163.2–165.4)*	166.1 (165.3–166.8)	166.6 (165.5–167.7)	164.2 (162.7–165.7)*	165.8 (164.9–166.7)
Maternal prepregnancy BMI	21.6 (21.2–22.0)	20.2 (19.7–20.7)*	21.1 20.8-21.4	22.6 (22.1–23.1)	20.9 (20.2–21.6)*	22.1 (21.6–22.5)*
Education (years)						
9 or less	20 (12)	19 (21)*	39 (15)	16 (15)	15 (29)*	31 (20)
10–12	93 (53)	54 (60)	147 (56)	46 (44)	17 (33)	63 (41)
13 or more	61 (35)	17 (19)*	77 (29)	42 (40)	19 (37)	60 (39)
Smoking at conception (number of cigarettes)						
0	103 (59)	20 (22)*	123 (47)	85 (79)	21 (40)*	106 (67)*
1–9	21 (12)	14 (16)	35 (13)	4 (3.7)	3 (5.8)	7 (4.4)
10 or more	50 (29)	56 (62)*	106 (40)	18 (17)	28 (54)*	46 (29)
Parity						
1	122 (70)	65 (71)	186 (71)	66 (62)	39 (75)*	104 (66)
2	52 (30)	26 (29)	78 (29)	41 (38)	13 (25)	54 (34)
Interpregnancy interval (months)						
18 or less	43 (25)	14 (15)	57 (22)	26 (24)	20 (39)*	46 (29)
19–60	98 (56)	56 (62)	154 (58)	58 (54)	21 (40)	79 (50)
61 or more	33 (19)	21 (23)	54 (20)	23 (22)	11 (21)	34 (21)
Offspring characteristics						
Gender						
Male	87 (50)	47 (52)*	133 (50)	52 (49)	29 (56)*	81 (51)
Female	87 (50)	44 (48)	131 (50)	55 (51)	23 (44)	77 (49)
Weight (g)	3661 (3590–3732)	2882 (2815–2949)*	3402 (3335–3469)	3790 (3680–3900)	2891 (2820–2963)*	3503 (3403–3601)
Length (cm)	50.8 (50.5–51.1)	48.3 (47.9–48.8)*	50.0 (49.8–50.3)	51.4 (50.9–51.9)	48.4 (48.0–48.8)*	50.5 (50.1–50.8)*
Head circumference (cm)	35.4 (35.2–35.5)	34.0 (33.7–34.2)*	34.9 (34.7–35.1)	35.5 (35.2–35.8)	33.7 (33.4–33.9)*	34.9 (34.7–35.1)
Gestational age	40.0 (39.8–40.2)	39.7 (39.4–40.0)	39.9 (39.8–40.1)	40.2 (40.0–40.6)	39.5 (39.1–39.9)*	40.1 (39.9–40.3)

***Total** group: Significant difference between the total group in Norway vs. the total group in Sweden (*P* < 0.05) using Student *t*-test for normally distributed variables and Mann-Whitney *U*-test for categorical variables. **SGA**-group: Significant difference between SGA vs. non-SGA group within country strata (*P* < 0.05) using Student *t*-test for normally distributed variables and Mann-Whitney *U*-test for categorical variables.
SGA, small for gestational age.

between PFOA and SGA birth, birth weight, birth height and head circumference were stronger among male offspring in the Swedish cohort. We found no statistically significant associations between EDCs and indices of fetal growth among Norwegian women in this study population.

Birth weight, adjusted for gestational age at birth, is often used as a proxy for fetal growth in observational studies. However, adjusting for gestational age, which acts like a

mediator in the association between birth weight and health outcomes, may introduce bias (12). SGA birth is a better marker for fetal growth restriction because it identifies babies with low birth weight accounting for the gestational age at birth. Only four studies have assessed associations between PFASs and SGA birth (13–16) with conflicting results. One study found increased odds for SGA with higher PFOS (13), and none found statistically significant associations with

Table 2. Wet-weight levels of PFASs, and wet-weight and lipid-adjusted levels of OCs in serum by country from the study population in the SGA-study, *N* = 424

	Norway		Sweden			
	Median (range)	AM (SD)	Median (range)	AM (SD)	LOD	%>LOD
Wet-weight (ng/ml)						
PFOA	1.62 (0.31–7.97)	1.83 (1.00)	2.33 (0.60–6.70)	2.42 (1.00)	0.03	100
PFOS	9.74 (0.95–59.6)	11.3 (7.02)	16.4 (2.28–55.2)	17.3 (7.45)	0.03	100
PCB 153	0.46 (0.17–1.30)	0.49 (0.18)	0.68 (0.25–1.40)	0.69 (0.21)	0.01	100
p,p'-DDE	1.20 (0.10–11.0)	1.65 (1.43)	1.30 (0.39–9.20)	1.62 (1.13)	0.09	100
HCB	0.10 (0.04–0.33)	0.11 (0.04)	0.10 (0.04–0.38)	0.11 (0.04)	0.04	100
t-NC	0.04 (0.01–0.14)	0.04 (0.02)	0.04 (0.01–0.10)	0.04 (0.02)	0.01	100
β-HCH	0.12 (<LOD-0.39)	0.13 (0.06)	0.13 (<LOD-0.76)	0.15 (0.08)	0.01	99
Lipid-adjusted (ng/g lipids)						
PCB 153	80.1 (31.0–212)	84.4 (28.4)	117 (45.4–212)	121 (32.8)	–	–
p,p'-DDE	209 (16.7–1791)	285 (238)	244 (71.2–1223)	284 (175)	–	–
HCB	17.2 (7.1–48.2)	18.4 (6.27)	18.3 (6.98–73.0)	19.2 (7.56)	–	–
t-NC	6.74 (2.37–25.2)	7.55 (3.52)	6.28 (1.82–17.3)	6.79 (2.79)	–	–
β-HCH	21.2 (<LOD-57.2)	22.7 (9.07)	25.0 (<LOD-134)	26.5 (13.5)	–	–

AM, arithmetic mean; HCB, hexachlorobenzene; LOD, limit of detection; %>LOD, percentage of samples over limit of detection. Levels below LOD were set to LOD/$\sqrt{2}$; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluoroctane sulfonate.

PFOA. In contrast, we observed increased odds for SGA birth with increasing levels of PFOA in our Swedish study population. Our finding of increased odds for SGA birth with increasing levels of PCB 153 in the Swedish cohort has not previously been reported, but is consistent with a recent meta-analysis that established an association between PCB 153 and birth weight (5). The increased odds for SGA birth with increasing levels of HCB among our Swedish women has not been established in previous studies.

The possible biological mechanisms for the effects of PFASs and OCs on fetal growth are uncertain, but their endocrine-disruptive properties may be involved. Normal development is highly dependent on thyroid and sex steroid hormones, and the prenatal period is a critical period for hormonal changes. Thyroid hormones are important for somatic growth and are involved in differentiation and functions of target tissues during development (17). Prenatal estrogens are also important in promoting fetal growth (18). Both PFASs and OCs have been associated with lower levels of circulating thyroid hormones (19,20). Competitive binding to estrogenic receptors, disruption of enzymes or inhibition of the effects of endogenous estrogens are hypothesized actions of estrogenic and antiestrogenic PCB congeners, and may lead to inhibition of fetal growth (21). Moreover, human *in vitro* studies have found that PFASs interfere with the estrogen and androgen receptor (22). Since sex steroids might be disrupted by PFASs and OCs, there could be different effects in male and female fetuses which may explain our findings of stronger negative associations between PFOA and indices of restricted fetal growth among male but not female offspring in the Swedish cohort. Our sex specific finding differs from a Japanese study from 2009 that showed stronger negative associations between PFOS and birth weight among girls (*n* = 428 mother–child pairs) (23). The biological mechanisms for the sex difference in the toxicity for PFOA remains to be established, and it is possible that decreasing the sample size by stratifying our groups by sex reduced the precision of our results. Thus, further studies are warranted to elucidate any sex-related differences in fetal growth indices based on *in utero* exposure to PFASs.

The conflicting estimates on fetal growth between the Norwegian and Swedish cohort may be explained by differences in exposure levels and heterogeneity in exposure routes. Seafood and particularly fatty fish have been associated with high PFAS and OC levels in Scandinavian populations (7,8). In our study, Norwegian women with fish consumption > 50 g/d in the second trimester had 20 and 19% higher PFOA and PFOS levels, respectively, compared to women who did not eat fish (**Supplementary Table S2** online). However, other food items, like meat, may be more important in other populations with less fish intake (24). Moreover, we cannot discount the possibility of differences in dietary patterns between Scandinavian countries, which may partially account for our results. Since fish consumption is associated with both EDC levels and fetal growth, this may introduce bias in epidemiological studies. A recent report from the Norwegian Scientific Committee for Food Safety concluded that overall, there is a *beneficial* effect of fish consumption on birth weight, because fatty acids and vitamins important for enhancing fetal growth outweigh potential negative effects from EDCs (11). However, it is important to note that this evaluation was based on evidence from studies conducted when EDC levels were lower than the EDC levels found during our study period (1986–88). Our estimates in the Norwegian cohort did not substantially change when we adjusted for fish consumption (data not
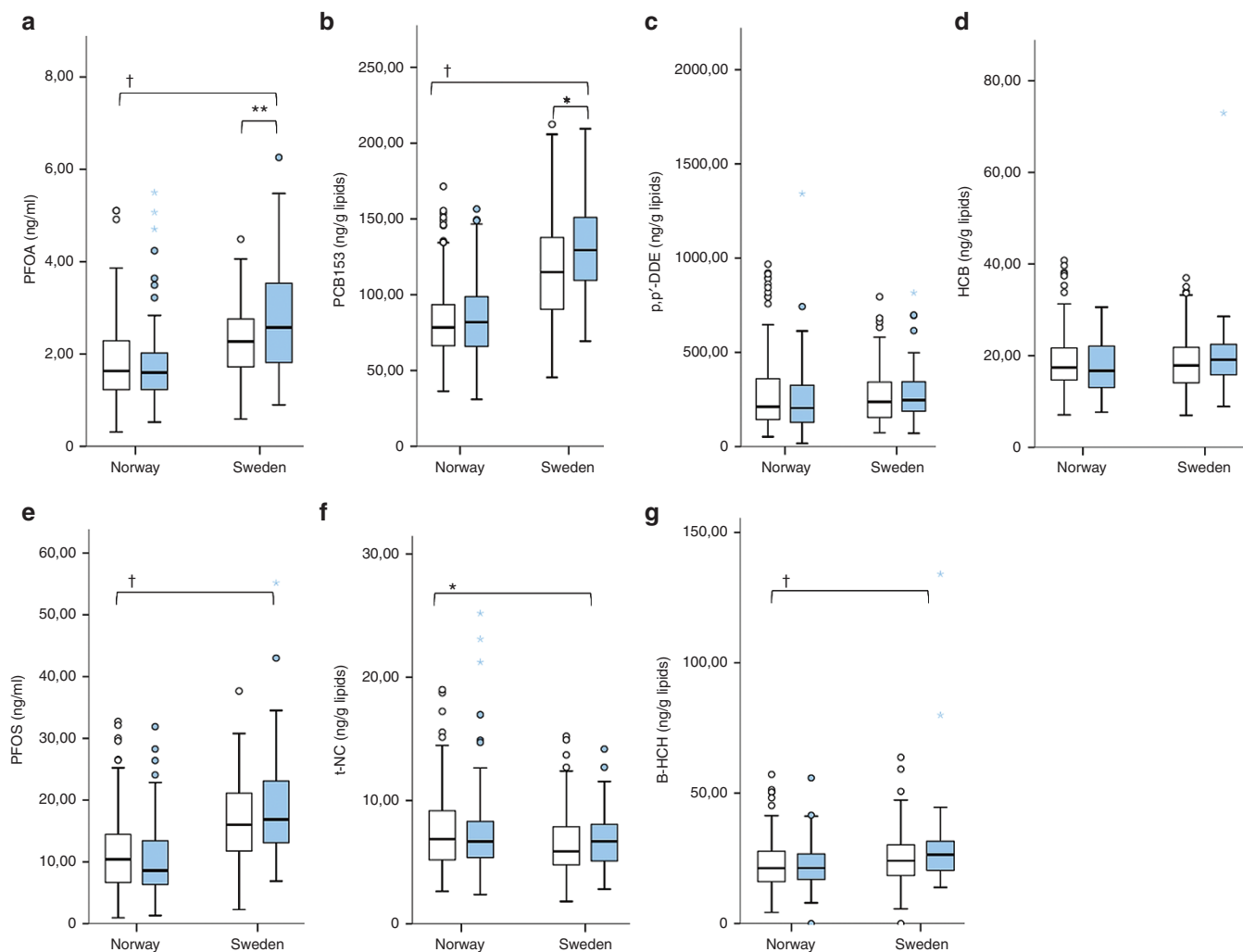
Figure 1. Levels of perfluoroalkyl substances (ng/ml) and organochlorines (ng/g lipids) analyzed in maternal serum in second trimester, stratified by country of residence and SGA status (**a**: PFOA, **b**: PCB 153, **c**: p,p'-DDE, **d**: HCB, **e**: PFOS, **f**: *t*-NC, **g**: β-HCH). White boxes: non-SGA mothers, light blue boxes: SGA-mothers. Boxes represent the 25th–75th percentiles, horizontal lines represents the median, whiskers indicate 1.5 times the length of the interquartile range above and below the 75th and 25th percentiles, respectively, and outliers are represented as data points. *$P \leq 0.05$, **$P \leq 0.01$, and †$P \leq 0.001$ for comparisons of levels between countries and between SGAs and non-SGAs within each country. HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluoroctane sulfonate; SGA, small for gestational age.

shown). Seasonal or daily variations in dietary intake of fish may introduce differential misclassification bias when using 3 d dietary intake as a measure of the overall consumption pattern, and we may not be able to capture the "real" fish eaters by this approach. We did not have information on fish consumption in the Swedish cohort.

In addition to different dietary patterns between populations and temporal trends of EDCs, there might be differences in contamination levels between fish sources. Studies have shown high OC contamination of the fish in the Baltic Sea on the East Coast of Sweden (9). Another study (1973–1991) that compared fishermen's pregnant wives from the East and West coast of Sweden, found that higher fish consumption from the East Coast resulted in higher maternal serum levels of PCB 153, which again resulted in increased risk of having an infant with low birth weight (25).

Smoking is often closely associated with socio-economic, dietary and lifestyle variables. In the Swedish cohort,

smokers had 20% higher PFOA levels compared to non-smokers (**Supplementary Table S2** online). Whereas, in the Norwegian cohort, smokers had 19% lower PFOS levels compared to nonsmokers. The fact that PFASs were higher among smokers in Sweden, but lower among smokers in Norway, speaks against a potential direct biologic effect of smoking on PFAS levels. In addition, we found no evidence of stronger associations between PFASs and fetal growth among the smokers compared to the non-smokers in Norway or Sweden, respectively. Taken together, this suggests that there might be differences in socio-economic status, lifestyle, and dietary variables between the women with high PFAS levels in Norway and Sweden, respectively, which will further confound the analyses. Problems with individual and local differences in EDC levels will be greater during time periods with continued use of contaminants, and possible contamination of the drinking water with PFASs in Sweden (26) may account

Table 3. Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFAS and organochlorines and indices of fetal growth (Norway: N = 265, Sweden: N = 159)

	Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	OR (95% CI)	P
Norway										
PFOA	37 (−99, 174)	0.590	−0.1 (−0.7, 0.4)	0.656	0.2 (−0.2, 0.5)	0.354	−0.2 (−0.6, 0.2)	0.431	0.66 (0.33–1.33)	0.246
PFOS	74 (−31, 178)	0.167	−0.0 (−0.4, 0.4)	0.987	0.2 (−0.1, 0.4)	0.189	−0.01 (−0.3, 0.3)	0.952	0.71 (0.42–1.20)	0.201
PCB 153	100 (−104, 304)	0.334	−0.1 (−1.0, 0.7)	0.772	0.2 (−0.3, 0.7)	0.507	0.1 (−0.5, 0.7)	0.693	0.70 (0.25–1.98)	0.502
p,p′-DDE	46 (−47, 139)	0.327	0.2 (−0.2, 0.6)	0.306	0.1 (−0.2, 0.3)	0.531	−0.04 (−0.3, 0.2)	0.747	0.73 (0.46–1.15)	0.174
HCB	61 (−132, 255)	0.534	0.1 (−0.7, 0.9)	0.792	0.1 (−0.4, 0.6)	0.626	−0.1 (−0.6, 0.5)	0.756	0.52 (0.20–1.34)	0.176
B-HCH	−73 (−231, 86)	0.368	−0.5 (−1.1, 0.2)	0.152	−0.1 (−0.5, 0.3)	0.614	−0.1 (−0.6, 0.3)	0.592	0.76 (0.33–1.71)	0.500
t-NC	98 (−56, 253)	0.212	0.2 (−0.5, 0.8)	0.605	0.4 (−0.02, 0.8)	0.065	0.2 (−0.2, 0.7)	0.383	0.86 (0.40–1.84)	0.699
Sweden										
PFOA	−359 (−596, −122)	0.003	−1.3 (−2.3, −0.3)	0.010	−0.4 (−1.0, 0.1)	0.115	−0.3 (−0.9, 0.3)	0.318	5.25 (1.68–16.4)	0.004
PFOS	−292 (−500, −84)	0.006	−1.2 (−2.1, −0.3)	0.007	−0.4 (−0.9, 0.04)	0.073	−0.4 (−0.9, 0.2)	0.201	2.51 (0.93–6.77)	0.068
PCB 153	−11 (−374, 352)	0.953	0.1 (−1.4, 1.6)	0.891	−0.2 (−1.1, 0.6)	0.558	0.6 (−0.4, 1.6)	0.222	5.59 (1.05–29.9)	0.044
p,p′-DDE	38 (−147, 223)	0.688	0.3 (−0.4, 1.1)	0.400	0.2 (−0.2, 0.7)	0.273	0.4 (−0.04, 0.9)	0.072	1.70 (0.75–3.85)	0.200
HCB	−269 (−595, 57)	0.105	−1.0 (−2.4, 0.3)	0.134	−1.0 (−1.7, −0.2)	0.011	0.1 (−0.7, 1.0)	0.742	5.62 (1.26–25.1)	0.024
B-HCH	−161 (−446, 125)	0.268	−0.4 (−1.6, 0.8)	0.537	−0.4 (−1.1, 0.2)	0.209	0.1 (−0.7, 0.8)	0.852	3.20 (0.84–12.1)	0.087
t-NC	−92 (−379, 195)	0.529	−0.2 (−1.4, 1.0)	0.766	−0.4 (−1.1, 0.2)	0.213	0.3 (−0.5, 1.1)	0.452	2.04 (0.64–6.55)	0.229

[a]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m²), education (<9, 9–12, >12 y), parity (1 or 2), smoking status at conception (0, 1–9, >10 cig/d), interpregnancy interval (<18, 19–60, >60 mo) and offspring sex (male/female). [b]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m²), education (<9, 9–12, >12 y), smoking status at conception (0, 1–9, >10 cig/d), and interpregnancy interval (<18, 19–60, >60 mo).
HCB, hexachlorobenzene; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluoroctane sulfonate.

Table 4. Beta coefficients (β) and odds ratios (ORs) with 95% confidence intervals (95% CI) for associations between PFOA and indices of fetal growth in Sweden by offspring sex (N = 159)

		Birth weight (g)[a]		Birth length (cm)[a]		Head circumference[a] (cm)		Gestational age[a] (weeks)		SGA[b]	
		β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	OR (95% CI)	P
PFOA	N										
Boys	81	−526 (−828, −222)	0.001	−1.6 (−2.9, −0.4)	0.012	−0.6 (−1.3, 0.1)	0.103	−0.4 (−1.2, 0.5)	0.365	6.55 (1.14–37.45)	0.035
Girls	78	−156 (−541, 228)	0.419	−0.8 (−2.4, 0.8)	0.340	−0.1 (−1.0, 0.7)	0.728	−0.1 (−1.1, 0.9)	0.802	4.73 (0.79–28.3)	0.089

[a]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m²), education (<9, 9–12, >12 y), parity (1 or 2), smoking status at conception (0, 1–9, >10 cig/d), inter-pregnancy interval (<18, 19–60, >60 mo), and offspring sex (male/female). [b]Adjusted for maternal age (years), height (cm), prepregnancy BMI (kg/m²), education (<9, 9–12, >12 y), smoking status at conception (0, 1–9, >10 cig/d), and interpregnancy interval (<18, 19–60, >60 mo).
PFOA, perfluorooctanoate.

for some differences between populations. Although we did not find evidence of effect modification by smoking in our study, the high proportion of smokers in our study population may introduce bias and thereby underestimate true effects of the contaminants. In addition, due to the strong associations between smoking and fetal growth, the true effects of EDCs could be overshadowed and hard to detect, even in adjusted analyses.

Assessing potential causal effects of multiple EDCs on health outcomes are complicated because of the complex correlation of exposures. Associations found in some studies may largely reflect correlated EDC exposures instead of a specific EDC under study. In our study, we found moderate or high correlations within the PFASs (ρ = 0.56–0.73) and within the OCs (ρ = 0.35–0.70) (**Supplementary Figure S1** online), but only weak correlations between the PFASs and

OCs (ρ = −0.01–0.22). The lack of correlation between PFASs and OCs made it possible to distinguish between the independent effects of PFASs and OCs. When assessing individual EDCs, we demonstrated positive associations between PFOA, PCB 153 and HCB and SGA birth (**Table 3**). However, the only association that remained statistically significant when including the other contaminants as covariates in final models was the association between PFOA and SGA birth (**Supplementary Table S3** online). This further confirms that the association between PFOA and SGA birth is not explained by correlated exposures. The association between HCB and SGA birth was also significant when we adjusted for PFOA. Based on these analyses, it seems like both PFOA and HCB are independently associated with SGA birth. Accounting for correlations within and between different EDCs strengthens our findings by distinguishing between contaminants
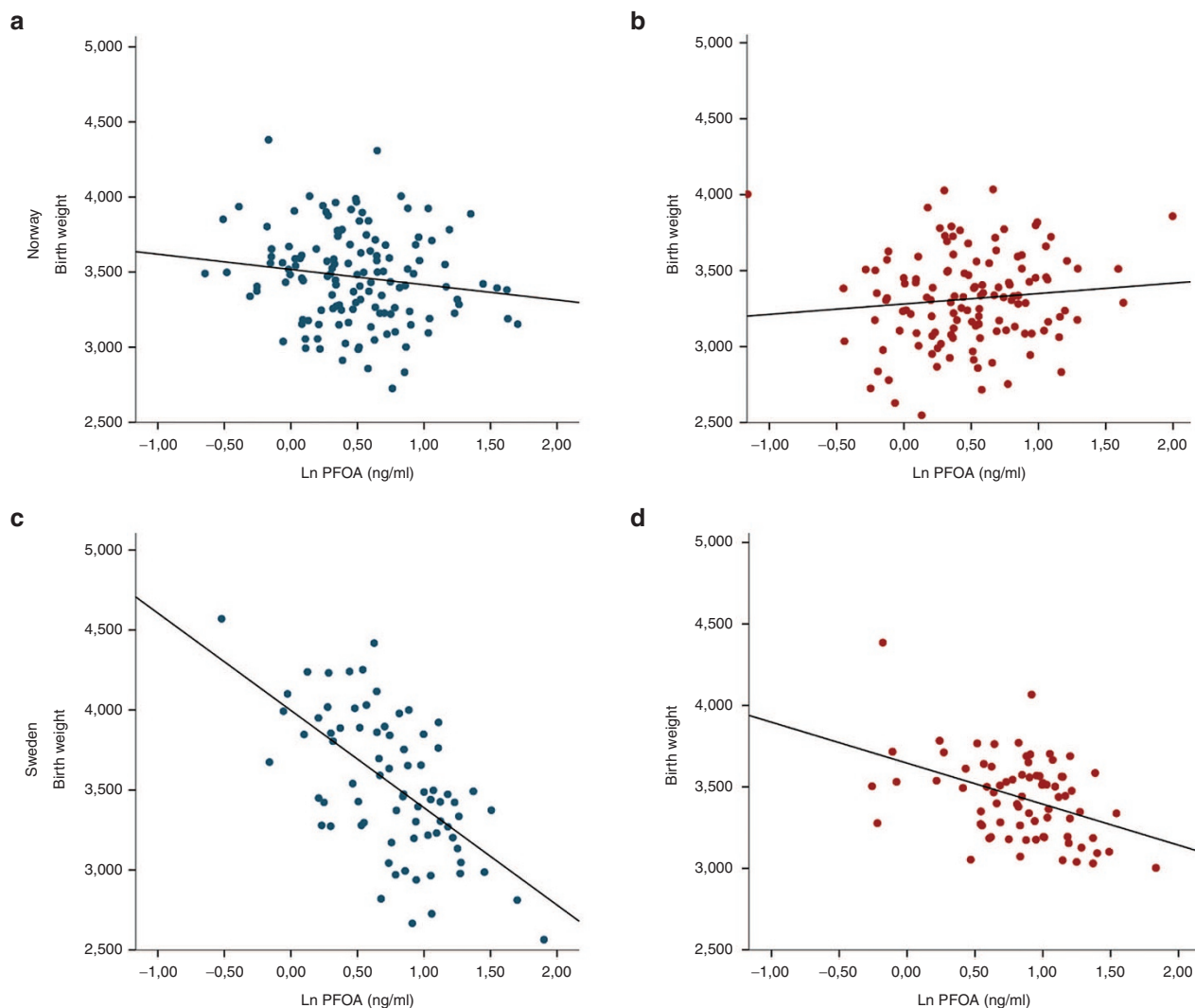
Figure 2. Scatter plot with ln (PFOA (ng/ml)) values on the X-axis and adjusted values of birth weight (g) on the Y-axis, stratified by country and offspring sex. Blue dots: boys, red dots: girls. (**a**) Norwegian boys, (**b**) Norwegian girls, (**c**) Swedish boys, (**d**) Swedish girls. PFOA, perfluorooctanoate.

that may have independent causal effects on restricted fetal growth.

This study has several strengths including a large, homogeneous population of mother–child pairs (*n* = 424). Ours is one of few studies to investigate a variety of PFAS and OC exposures. Since PFASs and OCs were not highly correlated, our results show an independent association between PFOA and fetal growth. The unique design of this case-cohort study accounts for a high proportion of SGA births as a marker for FGR, rather than birth weight alone. Therefore, this study has greater power to detect clinically relevant associations between EDCs and FGR using SGA birth as an outcome. We further tested the generalizability of our results by excluding the women from the high-risk group in all the linear and logistic models, and the associations in the Swedish cohort persisted (data not shown). The use of lipid-adjusted serum levels of OCs may be prone to bias in epidemiological studies (27). We therefore analyzed all linear and logistic models using wet weight values adjusted for total lipids as an independent variable with

no considerable change in point estimates (data not shown). Maternal weight gain during pregnancy is suggested to be an important confounder in studies with lipophilic chemicals, but mainly in studies using serum from late pregnancy or cord blood (28). Since maternal weight gain also represents blood volume expansion, it may also be an important confounder in studies with PFASs, which largely are bound to albumin in the blood (4). Alcohol consumption may also be a confounder because it has been associated with higher levels of OCs (29). However, sensitivity analyses revealed that the estimates did not change when we included weight gain or alcohol consumption as covariates in the analyses (**Supplementary Tables S4 and S5** online).

This study also has some weaknesses. We have not corrected for multiple comparisons, which increases the chance of a rare event and enables false-positive results (i.e., Type I error). Measures of renal filtration have been proposed to be important confounders in studies with PFASs and fetal growth (30). Unfortunately, we only had glomerular filtration
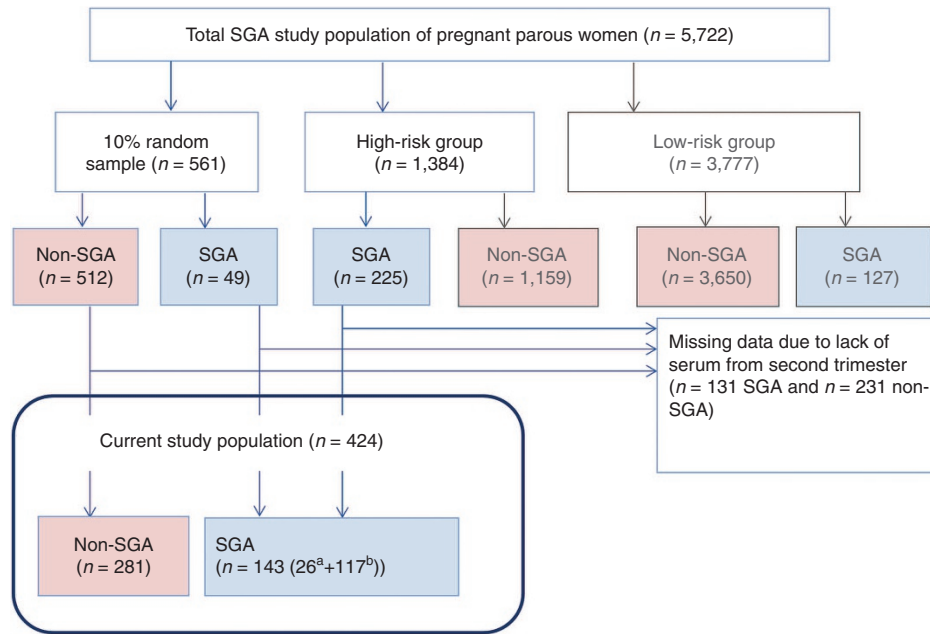
Figure 3. Flow chart of the study selection. [a]Number of participants from the 10% random sample. [b]Number of participants from the high-risk group.

rate (GFR) for 88 women, and could not include GFR as a covariate in adjusted models due to problems with missing data. Primiparous women were not eligible for study inclusion, which may contribute to some selection bias. However, excluding these women may largely reduce confounding due to parity because first time mothers have a higher risk of delivering small babies (31). At the same time, primiparous women may have higher serum EDC levels, because they lack previous excretion through placenta and breastmilk (2). Persistent and bio-accumulative chemicals with the same properties are highly correlated. Therefore, our point estimates may be subject to residual confounding due to some unmeasured chemicals (e.g., lead) in our analyses.

Conclusion

We found higher odds for SGA birth with increasing serum levels of PFOA, PCB 153 and HCB, but only in the Swedish cohort. Both PFOA and HCB were associated with SGA birth after accounting for correlation between EDCs. We observed stronger associations between increasing levels of PFOA and SGA birth in male offspring from the Swedish cohort. Associations between EDCs and indices of fetal growth in the Norwegian cohort were null. Our results suggest that some populations may be more vulnerable to EDCs, possibly due to differences in EDC levels, exposure sources and/or potentially modifiable lifestyle factors. The study also suggests that male offspring may be more vulnerable to endocrine disruption than female offspring.

METHODS
Ethics
The project is approved by the Central Norway Regional Committee for Medical and Health Science Research Ethics (REK Midt 2010/1449-5).

Study Population
Participants were from the US National Institute of Child Health and Human Development (NICHD) Scandinavian Successive Small-for-Gestational Age (SGA) births study; a population-based prospective multicenter study conducted in Trondheim and Bergen (Norway) and Uppsala (Sweden). Participant recruitment occurred over a 27-mo period (1986–1988). The SGA births study was designed to study the etiology and consequences of intrauterine growth restriction (32). Since the study also examined the tendency for individual women to repeat an SGA birth outcome in consecutive pregnancies, first time mothers were ineligible for study participation. Eligible participants were second and third time mothers of Caucasian origin who spoke one of the Scandinavian languages, had a singleton pregnancy, and were registered by the study center prior to the 20th gestational week. In total, we recruited 5,722 women, from which we defined three groups: a 10% random sample representative of the parous population at each study site ($n = 561$); a group at high risk for SGA birth ($n = 1,384$), and a low risk group ($n = 3,777$) (**Figure 3**). Both the random sample and high-risk group were included for detailed follow-up throughout pregnancy and at birth. The high risk group was defined by one or more of the following risk factors: (i) a prior SGA or low-birth-weight (LBW) child, (ii) maternal cigarette smoking at conception, (iii) low prepregnancy weight (<50 kg), (iiii) a previous perinatal death, or (iiiii) the presence of chronic maternal disease including essential hypertension or renal disease. In the current study, we used a case–cohort design (33) to estimate the associations between prenatal EDC exposure and indices of fetal growth. In total, 143 SGA births were selected as cases, and 281 non-SGA controls were selected from the 10% random sample group (total $n = 424$) (**Figure 3**). Selection was based on the availability of second trimester maternal serum samples.

Exposure Assessment
Serum samples were collected in second trimester (gestational week 17–20) in the SGA study (1986–1988) and serum was stored at −80 °C for later analysis. All 424 samples were included for PFAS and OC analyses. All chemicals presented were detected in 100% of the samples, except β-hexachlorohexane (β-HCH) that was detected in 99%. Limits of detection (LODs) are listed in **Table 2**, and values below LOD were replaced by LOD/$\sqrt{2}$.

Chemical Analyses of PFAS
Analyses were performed at the laboratories of Norwegian Institute for Air Research, Tromsø, Norway (NILU). All serum samples were

quantified for two target analytes; perfluorooctanoate (PFOA) and PFOS. They were analyzed using sonication-facilitated liquid-liquid extraction, activated ENVI-carb clean-up (34), quantified and analyzed by ultrahigh pressure liquid chromatography triple-quadruple mass-spectrometry (UHPLC-MS/MS). Sample preparation and extraction were performed as described by Hanssen *et al* (35) except for minor volume changes. The quantification was conducted with the LC Quan software, version 2.6.0 (Thermo Fisher Scientific, Waltham, MA). The internal-standard addition method with isotope-labeled PFASs was used to quantify the contaminants (35). Participation in the AMAP Ring Test (36) indicates that the uncertainties of the analysis are within ± 15–20% of the assigned values.

Chemical Analyses of OCs

OCs were analyzed at the Institut National de Santé Publique du Quebec, Centre Toxicologie, Quebec. This laboratory is the organizer of the AMAP Ring Test (36). OCs measured were hexachlorobenzene (HCB), oxychlordane, polychlorinated biphenyl (PCB) 52, 101, 118, 153, 156, 170, and 180, *p,p'*-dichlorodiphenyldichloroehylene (*p,p'*-DDE), *p,p'*-dichlorophenyltrichloroethane (*p,p'*-DDT), *β*-hexachlorohexane (*β*-HCH) and *trans*-nonachlor (*t*-NC). We chose to report PCB 153 representing total PCBs, and excluded *p,p*-DDT because of low detection limit (>50% of samples <LOD). In short, 0.5–1 ml serum sample was extracted using hexane (2 × 6 ml), ethanol (2 ml) and saturated ammonium sulphate solution (2 ml), a slight modification of Sandanger *et al.* (37).

Outcome Assessment

Indices of fetal growth including birth weight (continuous; grams (g)), birth length (continuous; centimeters (cm)) and head circumference (continuous; cm) were measured and recorded at birth. Gestational age (continuous; completed weeks) was determined by ultrasound scan at 17 wk of gestation. SGA birth was defined as birth weight below the 10th percentile adjusted for gestational age, parity and sex of child (32).

Covariates

Based on prior knowledge of PFAS and OC properties, and known risk factors for SGA birth, we included potential confounders from data collected at first study visit in gestational week 17. These involved maternal age (continuous; years), maternal height (continuous; cm), maternal prepregnancy BMI (continuous; kg/m^2), education level (categorical; 9 y or less, 10–12 y, or 13 y or more), smoking status at conception (categorical; 0, 1–9 or >10 cigarettes per day), parity (binary; 1 or 2) and interpregnancy interval (categorical; 18 mo or less, 19–60 mo, 61 mo or more). We categorized the interpregnancy interval based on a known J-shaped association to adverse perinatal outcomes including restricted fetal growth (38). Offspring sex (male/female) was registered at birth. Weight gain up to 17 wk was calculated based on recorded weight measurements done by midwives at the regular prenatal visits throughout pregnancy. Alcohol consumption was self-reported in gestational week 33. Dietary information from the Norwegian women consisted of a self-reported food frequency questionnaire over a 3-d period in week 17 and 33.

Statistical Analyses

PFAS and OC levels were logarithmically (ln) transformed to obtain normal distribution. We used wet weight concentrations of PFAS and lipid-adjusted serum concentrations of OCs (39). Total lipid values were calculated based on measurements of triglycerides and cholesterol:

Total lipids = 1.33*triglycerides + 1.12*cholesterol + 1.48 (g/l) (39). This formula showed good correlation with complete formulas including phospholipids (40).

In a complete case analysis we used uni- and multivariate linear regression with 95% confidence intervals (CIs) to estimate the adjusted associations between natural log-transformed (ln) serum levels of seven individual EDCs (*PFASs:* PFOA, PFOS; *OCs:* PCB153, *p,p'*-DDE, HCB, *t*-NC, and *β*-HCH) and indices of fetal growth including birth weight, birth length, head circumference and gestational age at birth. We evaluated linear model assumptions using diagnostic plots of the residuals. In all linear regression analyses we adjusted for the same covariates (maternal age, maternal height, prepregnancy BMI, maternal education level, smoking at conception, parity, interpregnancy interval, and offspring sex). We conducted uni- and multivariate logistic regression to estimate the crude and adjusted odds ratios (ORs and aORs) for SGA birth per unit increase in natural log-transformed (ln) serum levels of each PFAS and OC. In all the logistic regression analyses we adjusted for the same covariates (maternal age, maternal height, prepregnancy BMI, maternal education level, smoking at conception, and interpregnancy interval). SGA birth was defined as birthweight below the 10th percentile adjusted for gestational age, parity and offspring sex; hence, we did not adjust for parity or offspring sex in the models with SGA birth as an outcome. Based on the evidence of effect modification by geography, we stratified our results by country of residence. In multivariate logistic models with SGA as outcome variable, we found significant *P* values for interaction between the different EDCs and country of residence (PFOA *P* = 0.043; PCB 153 *P* = 0.073; HCB *P* = 0.008). In multivariate linear regression models with birth weight as an outcome, *P* values for the interaction term were 0.004, 0.001, and 0.036 for PFOA, PFOS, and HCB, respectively. Within country-specific strata, we further considered possible effect modification by offspring sex, because proposed endocrine disrupting properties might be sex-specific. Missing data was less than 10% for included covariates. All statistical analyses were conducted with SPSS statistical software, version 22 (IBM SPSS, Chicago, IL).

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