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Fetal exposure and maternal elimination of PFOS and PFOA during pregnancy were quantified.

Environmental impact

Although few studies have shown that perfluoroalkyl substances (PFASs) can be transferred by placental routes from mother to fetus, little is known about the extent of PFASs exposure during pregnancy. In the present study, fetal exposure and maternal elimination of PFOS and PFOA during pregnancy were quantified. This work contributes to provide tool for assessment of prenatal exposure risks. The average daily fetal exposure dose via placental transfer were approximately 10 ng for PFOS and PFOA; Pregnancy and child birth may reduce the PFASs levels in female adults due to maternal elimination. Further, paired maternal-placenta-cord samples gave additional information about trans-placental transfer of PFOS isomers to foetus.

Assessment of fetal exposure and maternal elimination to perfluoroalkyl substances

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Abstract

In the study, we estimated the body burden (*BB*) of perfluoroalkyl substances (PFASs) in fetus at the time of delivery, and elimination of PFASs for female adults during pregnancy; and explored isomer branching pattern-related placental transfer of perfluorooctane sulfonate (PFOS). The mean *BB* of PFASs were 3980 ng for PFOS and 2320 ng for perfluorooctanoic acid (PFOA), therefore, the average daily exposure doses via placental transfer were estimated to be 13.7 and 8.32 ng day^{-1} for PFOS and PFOA, respectively, by dividing the *BB* of PFASs by gestational age. The total daily elimination of PFOS and PFOA in female adults through pregnancy was 30.1 and 11.4 ng day^{-1}, which indicates that pregnancy and child birth may reduce the PFASs levels in female adults. Further, branched PFOS was more readily transferred through placenta than linear PFOS.

Keywords: perfluoroalkyl substances; isomer-specific transfer; body burden; pregnant elimination

Introduction

Prenatal exposure to perfluoroalkyl substances (PFASs) have become an important public health concern because of their possible developmental toxicity, immunotoxicity and hormonal changes.[1,2] Recent studies have shown that PFASs can be transferred by placental routes from mother to fetus, and the trans-placental transfer efficiencies (TTEs) of PFASs from various countries ranged from 0.31 to 0.54 for perfluorooctane sulfonate (PFOS), and 0.55 to 1.02 for perfluorooctanoic acid (PFOA).[3-12] However, little is known about the extent of PFASs exposure during pregnancy. Quantitative assessment of prenatal exposure to PFASs is necessary for the assessment of risks.

Furthermore, there is an incomplete understanding of how PFASs are excreted in humans. Several studies have estimated that the urinary elimination of PFOS and PFOA by adults were in the range of 1.4 to 36.2 ng day^{-1} and 8.7 to 39.0 ng day^{-1}, respectively.[13-15] However, elimination of PFASs during pregnancy in adult females was not reported previously.

Electrochemical fluorination manufacturing method was used to produce PFOS and its precursors beginning in 1949.[16] This apparent preferential bioaccumulation of branched PFOS isomer (B-PFOS) in humans is opposite to what is anticipated from PFOS isomer pharmacokinetic studies in rodents, whereby B-PFOS are excreted more efficiently in urine than their linear counterparts (L-PFOS).[17,18] From a public health perspective, recognizing that PFOS occur as multiple isomers of unknown relative toxicity, it may be important to characterize the maternal-fetal transmission of PFOS and its isomers.

Eleven PFASs were measured in 27 matched mother-fetal samples collected in Tianjin, China.[19] In the present study, we were in an effort to quantify prenatal

exposure and elimination during pregnancy, and examine the isomer-specific transfer of PFOS across the placenta.

Materials and Methods

Study Subjects and Sample Sampling

We collected 27 matched maternal samples including maternal blood (MB), cord blood (CB), placenta and amniotic fluid (AF), from pregnant women at hospital located in Tianjin, China. The sample collection was done by well-trained nurses. Whole blood was collected in this study. MB samples were collected from antecubital vein in the preoperative holding area within one hour of delivery; and placenta, AF and CB were collected at the time of delivery. Placenta was stored in hermetic polyethylene bag; AF samples were collected in 50 mL polypropylene tube; blood samples were collected into heparinized plastic vacutainers (BD Vacutainer, Franklin Lakes, NJ). Placenta was freeze-dried immediately and stored at - 20 °C; AF and blood samples were frozen at - 20 °C until analysis.

The demographic data of pregnant women and newborn babies including age, weight, parity, body mass index (BMI) etc. were recorded at the time of sampling (**Table S1**). All mothers aged from 21 to 39 yrs (median: 30 yrs); gestational age ranged from 35 to 47 weeks (median: 39 weeks). The BMI ranged from 19.6 to 47.3 kg/m^2 for mothers, and from 9.96 to 15.2 kg/m^2 for fetus. All participants were healthy, and none reported occupational exposure to PFASs. Detailed demographic information of the subjects is shown in **Table S1**. The Institutional Review Board of School of Environmental Science and Engineering, Sun Yat-Sen University approved this study and informed consent was obtained from participating women.

Sample Extraction, Instrumental Analysis and QA/QC

Prior to extraction, samples of whole blood and AF were thawed and allowed to return to room temperature, and dried placenta samples were homogenized. All blood and placenta samples were extracted by ion-pair extraction method as reported earlier.[19,20] AF samples were extracted using Oasis WAX SPE cartridge (Waters Corp, Milford, MA, USA), and were cleaned up using Envi-carbon cartridge (Supelco, Inc., Bellefonte, PA, USA).[19] Concentrations of 11 PFASs were analyzed with Waters Acquity ultra performance liquid chromatography equipped with Waters Acquity TQD triple quadrupole mass spectrometer (UPLC-MS/MS). Good quality assurance quality control (QA/QC) were obtained in this study for PFASs in placenta and blood, and for PFOS and PFOA in AF samples (**Table S2**). All instrumental blanks and procedural blanks were free of detectable concentrations of the target PFASs analyzed. Details regarding reagents and chemicals, sample preparation, instrumental analysis and QA/QC are given in the Supplementary Material.

Body Burden of PFASs in Fetus at Delivery

Quantification of daily exposure to PFASs through placental pathway cannot be made directly. Assuming placenta is the sole exposure source to PFASs in fetuses during gestation, the body burden of PFASs (*BB*) in newborns can be estimated by combining the amount of PFOS and PFOA in blood (i.e., CB) and each organs and tissues. Maestri et al. measured the PFOS and PFOA in matched human blood, liver, kidney, adipose tissue, brain, basal ganglia, hypophysis, thyroid, gonads, pancreas, lung and skeletal muscle; and reported the ratios of PFASs levels between blood and other tissues.[21] Therefore, the distribution ratio in PFAS concentrations among human tissues reported by Maestri et al. was used for estimation of body burden in fetus in this study.[21] The *BB* of PFOS and PFOA in fetuses at delivery can be estimated as the sum of PFOS and PFOA in blood and each organs (or tissues):

$$BB = C_{blood} \times V_{blood} + \sum i(C_{tissue} \times M_{tissue})$$

where C_{blood} is concentration of PFAS in whole blood (i.e., CB) of newborns (ng mL^{-1}), C_{tissue} is concentration of PFAS in tissues such as liver, kidney and muscle, etc (ng g^{-1} fresh weight), V_{blood} is the volume of whole blood (mL), and M_{tissue} is the mass of organs (g). Concentrations of PFOS and PFOA in different tissues can be estimated based on the concentrations measured in whole blood (i.e., CB), and the tissue distribution ratios reported.[21] Due to the slow elimination of PFOS and PFOA in humans, the *BB* of newborn babies could represent the integrated exposure over the whole pregnant process.[22]

Results and Discussion

Prenatal Exposure to PFASs

The *BB* of PFASs were estimated to be 3980 (range: 324-13100) ng for PFOS and 2320 (837-5130) ng for PFOA (**Table 1**). By dividing the *BB* of PFASs by gestational age (**Table S1**) at delivery, the average daily exposure doses via placental transfer were estimated to be 13.7 (range: 1.22-48.0) ng day^{-1} for PFOS, and 8.32 (3.89-20.4) ng day^{-1} for PFOA (**Table 1**).

A major limitation in this study was the lack of matched breast milk samples. For the comparison of prenatal and postnatal exposure of PFOS and PFOA, postnatal exposure was estimated based on PFAS concentrations in human milk reported in a recent study in China and milk consumption of newborn baby (600 mL day^{-1}).[23] The estimated postnatal exposure dose of PFOS and PFOA via breastfeeding was 33.6 and 109 ng day^{-1} (**Table 1**), respectively. This indicates that the postnatal exposure through breastfeeding is 2.5 and 13 times higher than the prenatal trans-placental exposure for PFOS and PFOA, respectively. Fromme et al. found a significant

increase in blood PFOS and PFOA levels during the first 6 months in newborns, which indicated that breastfeeding is a major source of exposure in early life stages.[4]

Very few studies have reported the prenatal/placental exposure dose of PFASs to fetus.[9] The *BB* of PFOS and PFOA reported for newborn babies from Korea and the U.S. were approximately 3 times lower than the burdens reported for Chinese newborn babies. This can be due to low concentrations of PFOS and PFOA detected in CB from Korea, and in blood spot from newborn babies in the U.S. Furthermore, our results on *BB* of PFASs in newborns were higher than those reported in another study from China, because only blood and liver were considered in that study.[9]

In this study, no associations between *BB* of PFOS in fetus and fetal BMI, head circumference, chest circumference, gender; and maternal BMI, age, parity, and gestational age were found. Nevertheless, fetal *BB* of PFOA was positively correlated (Spearman Rank Correlation: $r = 0.497$, $p < 0.05$) with maternal BMI, and primiparities had significantly higher (One way ANOVA: $p < 0.05$) *BB* of PFOA than the women who gave birth to their second children. Furthermore, female fetus had significantly higher (One way ANOVA: $p < 0.05$) *BB* of PFOA than male fetus, when the *BB* of PFOA was estimated on a body weight basis.

Elimination to PFASs during Gestation

Based on the PFAS concentrations measured in placenta and AF, and the reported average weight (or volume) of placenta (550 g) and AF (1000 mL), PFAS burdens were estimated to be 4500 ng for PFOS, 869 ng for PFOA in placenta, and 21.1 ng for PFOS, 44.5 ng for PFOA in AF (**Table 1**). We estimated the elimination of PFOS and PFOA in female adults during gestation, based on several pathways such as burdens estimated in fetus, placenta and AF. The total daily elimination of PFOS and PFOA through pregnancy was 30.1 and 11.4 ng day^{-1} (**Table 1**), on average. Our

results indicate that pregnancy and child birth may reduce the PFASs levels in female adults.

Isomer-specific maternal-fetal transfer of PFOS

Isomer-specific maternal-fetal transfer of PFOS was also examined in this study, and the UPLC-MS/MS chromatograms of B-PFOS and L-PFOS are shown in **Figure 1**. The ratio of concentrations between B-PFOS and total PFOS (T-PFOS = sum of B-PFOS and L-PFOS) (B-PFOS:T-PFOS) was 0.18 in MB; this value was nearly the same in placenta (0.18) and in the analytical standard solution (0.20) (**Figure 2**). However, a statistically greater (One way ANOVA: $p < 0.01$) B-PFOS:T-PFOS in CB than that in corresponding MB and placenta was observed. The mean B-PFOS:T-PFOS in CB was 0.27 (**Figure 2**). Our results suggest that B-PFOS was more efficiently transferred through placenta than L-PFOS. The higher percentage of B-PFOS relative to the T-PFOS in CB compared to the MB is consistent with other reports from Canada, Norway and South Africa.[3,5,6] This showed that B-PFOS contributed to a significant proportion of T-PFOS in CB than in MB. B-PFOS is expected to be more hydrophilic than L-PFOS, and that may have contribution for high placental transfer efficiency.

Conclusions

The *BB* in fetus at delivery via placental pathway were estimated to be 3980 and 2320 ng for PFOS and PFOA, respectively. The daily maternal elimination during pregnancy were estimated to be 30.1 ng day^{-1} for PFOS, and 11.4 ng day^{-1} for PFOA, based on several pathways such as burdens estimated in fetus, placenta and AF. The B-PFOS:T-PFOS ratio in CB was significant greater than that in MB and placenta,

189 which indicates B-PFOS was more efficiently transferred through placenta than
190 L-PFOS.
191
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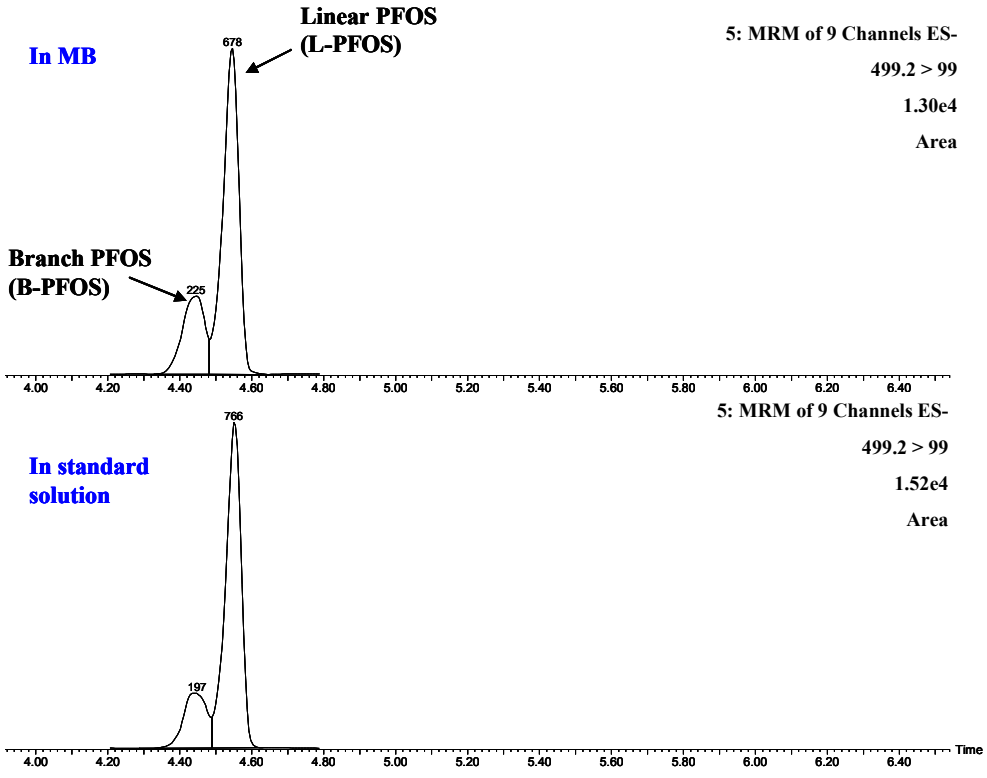
Table 1 Prenatal exposure and elimination of PFOS and PFOA by fetuses or their mothers.

	PFOS	PFOA
Body burden in fetus (ng)	3980	2320
Prenatal exposure dose by fetus (ng day^{-1})	13.7	8.32
Elimination during pregnancy by pregnant women [a] (ng day^{-1})	30.1	11.4
Mean (median) concentrations in placenta [b] (ng g^{-1} fresh weight)	8.18 (7.32)	1.58 (1.41)
Mean (median) concentrations in AF [b] (ng mL^{-1})	0.020 (< LOQ)	0.044 (0.043)

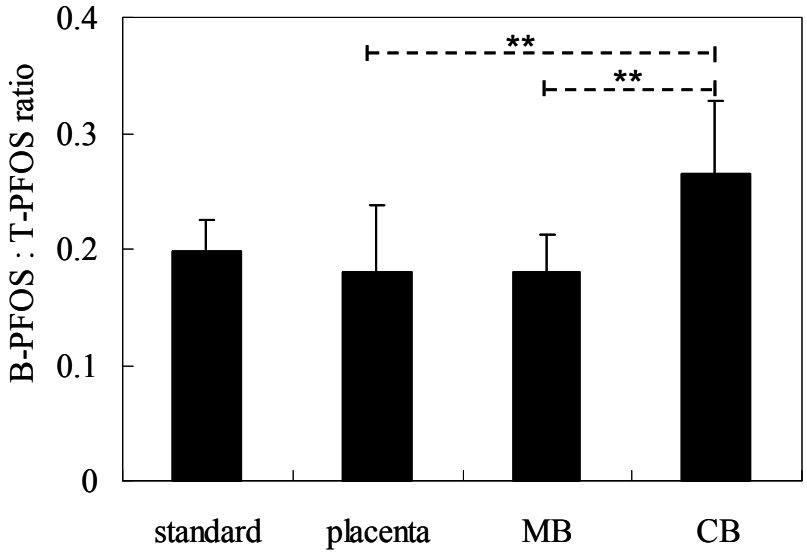
[a] We estimated the elimination of PFOS and PFOA in pregnant women during gestation, based on several pathways such as burdens estimated in fetus, placenta and AF; [b] mean (median) concentrations of PFOS and PFOA were reported in our other study.[19]



283

284 **Fig. 1** UPLC-MS/MS chromatograms of obtained in this study. Examples of B-PFOS

285 and L-PFOS in MB and standard solution are given.



286

287 **Fig. 2** The ratio of concentrations quantified by 499.2 > 99.0 transition between
288 branched PFOS (B-PFOS) and total PFOS (T-PFOS) in analytical standard, MB,
289 placenta and CB, respectively. Asterisk indicates a statistical significance ($p < 0.01$).