SURVEY OF PFCs PRECURSORS IN PAIRED MATERNAL AND CORD SERUM FORM JIANGSU PROVINCE, CHINA

Yang L[1], Shi Y[1], Li JG[1]*, Wang YX[1], Zhao YF[1], Wu YN[1]

[1] Key Laboratory of Food Safety Risk Assessment, Ministry of Health and China National Center for Food Safety and Risk Assessment, No. 7, Panjiayuannanli, 100021, Beijing, China

Introduction

Perfluorinated compounds (PFCs) have been widely used for many industrial and consumer applications due to their unique physico-chemical characteristics, such as chemical and thermal stability, surface active properties, hydrophobicity and lipophobicity[1-2]. Widespread exposure of those compounds, especially perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) have been reported in wildlife and humans around the world[3]. Lots of studies have been conducted to show that hepatotoxicity, developmental toxicity, immunotoxicity, hormonal changes and a carcinogenic potency are the main PFCs toxicity effects[4-5]. In May 2009, PFOS and perfluorooctane sulfonyl fluoride were listed as "restricted use" compounds in Annex B under the Stockholm Convention on persistent organic pollutants[6].

PFCs precursors, as one potential indirect source of PFSAs and PFCAs contamination[7], have been observed in environmental and human samples worldwide[8-9]. Perfluoroalkylsulfonamide (PFSAm) based PFSAs precursors, such as N-methyl or N-ethyl perfluorooctane sulfonamide (NMeFOSA or NEtFOSA), N-methyl or N-ethyl perfluorooctane sulfonamidoethanol (NMeFOSE or NEtFOSE) and N-EtFOSE based polyfluoroalkyl phosphate esters (SAmPAPs), are mass produced and used in texile, agriculture and food packaging applications. Biotransformation of N-EtFOSE to PFOS has been observed in rat liver microsomes, cytosol fractions, and liver slices by Xu et al [10] and the conversion of NEtFOSA to PFOS has been found in Rainbow Trout (*Onchorhynchus mykiss*) Liver Microsomes[11]. Perfluorooctanesulfonamidoacetates (FOSAAs), as the metabolites of SAmPAPs and FOSEs, have been commonly detected in human blood[8]. Fluorotelomer alcohols (x:2 FTOHs), fluorotelomer-based phosphate surfactants (polyfluoroalkyl phosphate esters, PAPs; perfluorophosphates, PFPAs; perfluorophosphinates, PFPiAs), fluorotelomer sulfonates (FTSs) are kinds of PFCAs precursors. These fluorotelomer-based materials are largely applicated in grease proofing food contact papers, wax, leveling and wetting agents. Cell and rat studies showed that 8:2 FTOH biotransformation proceeds via a β-oxidation-like mechanism to produce the PFOA as the major oxidation product[12-13]. These results were consistent with the wok reported by Nilsson et al that the ski wax technicians exposed to very high indoor air 8:2 FTOH concentration have significantly elevated PFOA level (nearly 45 folds higher) in their sera compaired to the general Swedish population[14]. DiPAPs can be hydrolyzed into FTOHs and were established biological precursors of PFCAs in microbial and mammalian systems[15-16]. FTSs have been shown to biodegrade to the PFCAs by desulfonation and oxidation reactions[17]. Apart from transformation into PFCs, some of these precursors themselves or their metabolic intermediates also have toxicity effects, such as estrogen-like properties, protein binding, cytotoxicity and so on[18-20].

However, most researches about PFCs precursors exposure focus on adults population. Maternal-fetal transfer of these chemicals has not been well examined, and there might be a potential harmful impact on fetus growth and development due to prenatal exposure. In this study, 50 paired maternal and cord serum samples were analyzed for fifteen different PFSAs and PFCAs precursors. This is the first investigation of PFCs precursors prenatal exposure in China. The objective is to characterize the human exposure of the PFCs precursors in China, paving the way for the studies of PFCs precursor placenta transfer and newborns exposure risk assessment.

Materials and methods

Ion-pair extraction[21] and UPLC-MS/MS were used to analyze 50 pairs of maternal and cord serum after ethics approval, which were collected in Jinhu county, Jiang Su province of China. Maternal blood was collected within the first week after delivery and cord blood was collected immediately after delivery. All of the newborn babies were healthy and without congenital anomalies. Serum sample (0.5 mL), internal standard solution (100 pg), 0.5 M TBA solution (1 mL, adjusted to pH 10 with 2 mM sodium hydroxide solution) and 0.25 M sodium carbonate buffer (2 mL) were mixed in a 15 mL polypropylene tube. MTBE (5 mL) was added to the solution for extraction. The organic and aqueous layers were separated by centrifugation. The aqueous mixture was

rinsed twice with MTBE. All rinses were combined in a second polypropylene tube and evaporated at ambient temperature under nitrogen gas flow, and then reconstituted in 0.25 mL of methanol/water (1:1). The supernatant was filtered through a 0.2 μm nylon filter (Sartorius, Goettingen, Germany) before analysis. Analytes were separated and quantified using an ultra-performance liquid chromatography system coupled to a triple quadrupole MS system (ACQUITY UPLC-TQS, Waters, USA). A gradient of 2 mM aqueous ammonium acetate solution and methanol were used as mobile phases at a flow rate of 0.4 mL/min. The triple-quadrupole mass spectrometer was operated in the negative electrospray mode with multiple-reaction-monitoring (MRM).
Fifteen PFCs precursors that included the FTSs, FOSAs, FOSAAs, fluorotelomer unsaturated carboxylate (FTUCAs), diPAPs, PFPiAs were analyzed. The limits of detection (LOD) and limits of quantitation (LOQ) were defined as the concentrations producing a signal-to-noise (S/N) ratio of equal to or greater than 3 and 10, respectively. The recovery test was conducted by analyzing calf serum recovery matrix. Analyte recoveries were corrected for background concentrations present in the unspiked matrix and ranged from 74% to 128%. Data from the single donor samples were largely non-normally distributed (~80% of the analytes). Non-normally distributed data were logarithmically transformed, but normality only improved for ~10% of the transformed data. The assumption of normality in the data was minimized by using nonparametric methods, such as the Mann-Whitney *U* test to compare analyte concentrations and the Spearman rank correlation test to test for possible correlations among the target analytes. A *p*-value of 0.05 was chosen as the criterion for statistical significance in all analyses. All the statistical analyses were performed using the software of SPSS 19.

Results and discussion

6:2 FTS, 8:2 FTS, FOSA, NMeFOSAA, NEtFOSAA were detected in both maternal and cord serum samples with different concentration levels and detection frequencies as shown in Table 1.

Table 1. Summary statistics of the PFCs precursors in 50 matched maternal and cord serum samples from Jinhu, China[a].

	6:2 FTS	8:2 FTS	FOSA	NMeFOSAA	NEtFOSAA
Maternal serum (pg/mL)					
Detection frequency	64%	34%	88%	78%	44%
GM	7.74	4.28	0.85	1.47	0.67
Median	11.55	2.83	0.95	1.85	0.28
Mean	13.39	6.27	1.32	2.74	1.35
Range	<3.00~48.24	<4.00~72.34	<0.20~8.15	<0.30~13.47	<0.40~8.32
Cord serum (pg/mL)					
Detection frequency	68%	14%	38%	70%	50%
GM	10.45	*[b]	0.25	1.18	0.75
Median	13.53	*[b]	0.14	1.54	0.37
Mean	20.99	*[b]	0.40	2.64	1.53
Range	<3.00~90.21	<4.00~33.61	<0.20~2.35	<0.30~11.44	<0.40~8.92

a. Undetectable concentration was accounted as a value equal to the LOD divided by the square root of 2 and values below the LOQ were used unaltered.

b. Geometric mean, median and arithmetic mean concentrations were not reported due to the low frequency of detection in the samples (<20%).

Among five detected analytes, 6:2 FTS has the highest level (median concentration: 11.55 pg/mL maternal; 13.53 pg/mL cord) and similar detection frequency (64% and 68%) in both maternal and cord serum. 8:2 FTS were also detected, but much less frequently (34% maternal; 14% cord). FOSA has the higest detection frequency in maternal serum (88%, 0.95 pg/mL), which significantly decreased to 38% in cord serum. Both NMeFOSAA and NEtFOSAA have similar detection frequency between maternal and cord serum (78% and 70%, 44% and 50%, respectly). The median concentration of NMeFOSAA was 1.85 pg/mL in materanl serum and 1.54 pg/mL in cord serum, while NEtFOSAA was 0.28 pg/mL in maternal serum and 0.37 pg/mL in cord serum (Table 1).

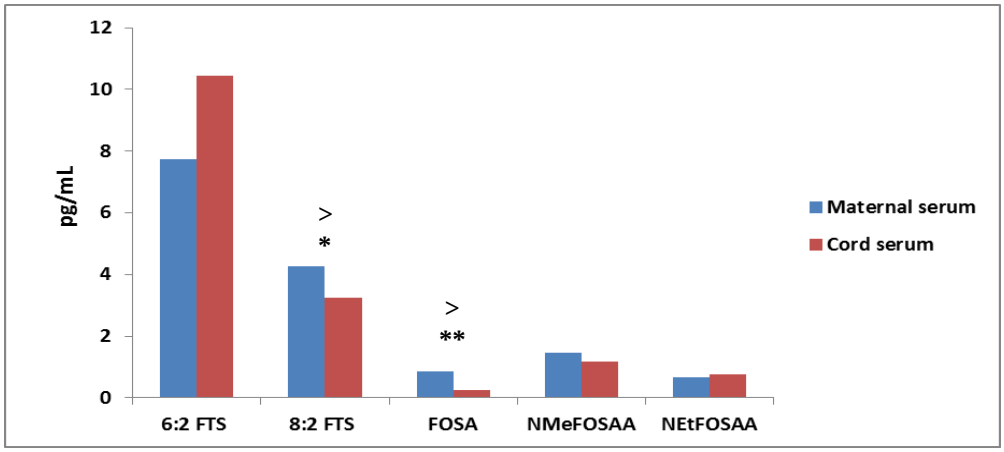

Figure 1. GM concentration of 6:2 FTS, 8:2 FTS, FOSA, NMeFOSAA, NEtFOSAA in maternal and cord serum. (Statistic significant difference: *, $p < 0.05$; **, $p < 0.01$)

Besides the decreased detection frequency, the concentrations of 8:2 FTS and FOSA measured in cord serum were also significantly lower than that in maternal serum samples (Mann-Whitney *U* test, $p < 0.05$, 8:2 FTS; $p < 0.01$, FOSA), while no significant change (Mann-Whitney *U* test, $p > 0.05$) was observed between the concentrations of 6:2 FTS, NMeFOSAA and NEtFOSAA measured in cord and maternal serum samples (Figure 1). This result indicated that there were differences of placenta transfer efficiency among these five compounds. The smaller molecular size and/or the relative higher maternal serum concentration might be the reason for the higher placenta transfer rate of 6:2 FTS compared to 8:2 FTS. For FOSA and FOSAAs, the transfer rate difference may be based on the changed lipid solubility of the N-substituted functional groups.

The concentrations of these PFCs precursors from two infant sex groups were compared. No gender difference was observed among the groups (Mann-Whitney *U* test, $p > 0.05$) except the cord serum 8:2 FTS ($p = 0.039$). However, due to the low detection frequency of 8:2 FTS (7/50) in cord serum, this gender difference (6/24 female; 1/26 male) needs further investigation and larger sample scale to test its validation. Three age groups were divided by the mothers' age, namely, 20-24, 25-30, older than 30. ANOVA (normally distributed data) and Kruskal-Wallis Test (non-normally distributed data) were used to analysis the age differeces. Only the concentrations of cord serum 6:2 FTS showed difference among the three age groups (Table 2. Left, ANOVA $P = 0.039$). The LSD multiple comparisons (Table 2. Right) showed group 20-24 was significantly different with the other two age groups ($p < 0.05$), which has the highest level of 6:2 FTS. This result might be inferred that the placenta transfer rate of 6:2 FTS would decrease as the mother's age gets increased.

Table 2. Left: Concentrations of cord serum 6:2 FTS in different age groups; Right: LSD multiple comparisons results.

Mothers' age	**20-24**	**25-30**	**>30**
Cord serum 6:2 FTS (pg/mL)			
GM	16.65	8.46	5.18
Mean	30.24	16.67	7.46
Median	32.91	7.91	6.57
Range	<3.00~90.21	<3.00~83.46	<3.00~15.80

LSD Multiple Comparisons		
Age Groups	25-30	>30
20-24	$P = 0.044$	$P = 0.028$

As shown in Table 3, spearman rank correlation test showed that concentrations of NMeFOSAA, NEtFOSAA and 8:2 FTS from maternal ans cord sera were significantly related. Materanl serum 6:2 FTS and 8:2 FTS has significant correlation (r=0.369, p=0.008) may imply a similar emission or exposure source for the two compounds. More correlations between these PFCs precursors and relative PFCAs and PFSAs final biotransformation products are under investigation.

Table 3. Spearman rank correlation test results. (M: Maternal serum; C: Cord serum)

Spearman test	M-8:2 FTS	M-FOSA	M-NMeFOSAA	M-NEtFOSAA
M-6:2FTS	r=0.369, p=0.008**	$p > 0.05$	$p > 0.05$	$p > 0.05$
C-8:2FTS	r=0.542, p=0.000**	$p > 0.05$	$p > 0.05$	$p > 0.05$
C-FOSA	r=0.334, p=0.018*	$p > 0.05$	$p > 0.05$	$p > 0.05$
C-NMeFOSAA	$p > 0.05$	r=0.331, p=0.019*	r=0.335, p=0.017*	$p > 0.05$
C-NEtFOSAA	$p > 0.05$	r=0.292, p=0.040*	$P > 0.05$	r=0.328, p=0.020*

Conclusion: Five PFCs precursors were detected in the paired maternal and cord serum samples. 6:2 FTS, NMeFOSAA and NEtFOSAA have moderate detection frequency in both maternal and cord sera, while the mean concentration and detection frequency of 8:2 FTS and FOSA were significantly reduced in cord sera. Concentrations of cord serum 6:2 FTS showed difference among the three age groups. The 20-24 age group turned out to have the highest concentration level. Several correlations between the compouds were also observed.

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