ANALYSIS, OCCURRENCE AND RISK OF PERFLUORINATED COMPOUNDS IN BREAST MILK AND COMMERCIAL BABY FOOD.

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Introduction

Perfluorinated compounds (PFCs) have been manufactured since the 1960s for a wide range of industrial and consumer applications. The strong carbon fluorine (C-F) bonds of PFCs give them a high thermal, chemical and biological stability. These compounds have been employed in textiles and food packaging due to their unique properties as repellents of water and oils.

During the last years different studies have assessed the levels of PFCs in human breast milk (1-4), reporting levels of concentration in the range of ng/mL. Different investigations have studied possible relations between different factors as mother ages, birth weight, infant sex, or the levels of thyroid hormone in infant's blood (5,6), but not correlations were established. All these results indicate that further studies are needed to determine how a long exposure in humans can result in reproductive impairments.

In this context, the aims of the current study were: 1) To validate an analytical method based on solid phase extraction followed by liquid chromatography coupled to tandem mass spectrometry (SPE-LC-MS/MS) for the analysis of six perfluorinated compounds (PFCs) in breast milk samples and baby food; 2) To analyze the PFCs in different types of childbirth food (human breast milk, milk infant formulas and cereals baby food); 3) To evaluate the Risk Index (RI) for daily childhood intake based on the guidelines of the European Food Safety Authority (EFSA).

Materials and methods

Chemicals and standards Perfluoro-n-octanoic acid (PFOA) [MW: 414; > 99%], perfluoro-n-nonanoic acid (PFNA) [MW: 464; > 99 %], perfluoro-7-methyl octanoic acid (i,p-PFNA) [MW: 464], perfluoro-n-decanoic acid (PFDA) [MW: 514; > 99 %], potassium perfluoro octanesulfonate (PFOS) [MW: 538.22], sodium perfluoro-1-decanesulfonate (PFDS) [MW: 622.13; > 99 %] were purchased from Wellington Laboratories Inc., Canada. Internal standard sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octane sulfonate ($^{13}C_4$-PFOS) [MW: 526.08; > 99 %] and perfluoro-n-[1,2,3,4-$^{13}C_4$] octanoic acid ($^{13}C_4$-PFOA) [MW: 418; > 99 %] and the surrogate perfluoro-n-[1,2-$^{13}C_2$] decanoic acid ($^{13}C_2$-PFDA) [MW: 516; > 99 %] were also purchased from Wellington Laboratories Inc. Water and Methanol (MeOH) were of HPLC grade and they were from Merck (Darmstadt, Germany). Ammonium acetate salt (AcNH4: MW, 77.08; ≥98 %) was obtained from Sigma-Aldrich, Steinheim, Germany. Sodium hydroxide base (NaOH: MW, 39.997; >97 %) was from Merck.

Sample collection and sample preparation Following institutional review board approval, 20 samples from women residing in Barcelona city (Spain) were included in this study. The experimental protocol was approved by a local ethical committee in accordance with the Spanish regulation, and the informed consent was obtained from all participating subjects. After signing the informed consent, the mothers were asked to complete a questionnaire for information about residence, age, number of infants previously breast fed, newborn weight and newborn sex, mother habits, type of work and diet.

Breast milk samples were collected either using a breast pump or by hand expressing the milk into the pre-washed polypropylene (PP) tubes containers on the 40 days postpartum at the hospital. Aliquots of 25-30 ml of breast milk were collected into 50 mL PP tubes, stored at -20º C. Before extraction, samples were lyophilized, homogenized and stored at -36º C.

3 brands of powdered milk based infant formulas and 2 brands of dry cereals baby food from retail store were included in this study. Powdered milk infant formulas were supplied in 400g tin packing, and the composition in proteins, fat and carbohydrates were in the range of 10.5-11%, 27.5-29% and 55-56.9%, respectively. Dry cereals baby food were supplied in 250g plastic bag packages with composition in proteins, fat and carbohydrates in the range of 6-6.5 %, 1-1.2% and 87,4-88%.

Sample pre-treatment and extraction procedure was based on an alkaline digestion according to a protocols described before (7-9) followed by a clean-up using solid phase extraction (SPE) with C_{18} Sep-Pack cartridges.

The analysis of PFCs was performed by LC-ESI-MS/MS. LC using a Symbiosis ™-Pico (Spark Holland, Emmen, The Netherlands) with a C18 LiChroCART® Purosphere Star–18e analytical column (125mm x 4mm i.d., 5µm) from Merck (Darmstadt, Germany) at room temperature. The mobile phase consisted of (A) aqueous ammonium acetate 20mM (B) methanol. The elution gradient conditions for the LC mobile phase were as follows: 10–80% B over 5 min, then 80–90% B over other 5 min followed by an isocratic hold at 90% B for 8 min. At 18 min, B was returned to 10% in 2 min. The total run time for each injection was 20 min. The flow rate was kept at 0.5 mL/min throughout the run, and the sample volume injected was 20 µL. The LC system was coupled to a quadrupole-linear ion trap mass spectrometer (QLIT-MS/MS) 4000 QTRAP (Applied Biosystems), equipped with a Turbo Ion Spray source employed in the negative electrospray ionization mode (ESI(-)). Acquisition was performed in multiple reaction monitoring (MRM) mode to obtain sufficient quantification points for confirmation of each analyte. Identification and quantification of target analytes were carried out using m/z transitions and retention times. Optimized parameters were as follows: curtain gas (CUR), 30 (arbitrary units); ion source gas 1 (GS1), 25 (arbitrary units); ion source gas 2 (GS2), 25 (arbitrary units); source temperature (TEM), 350 ◦C; ion spray (IS), −4500V; entrance potential (EP), −10 V, collision cell exit potential (CXP) −10 C and declustering potential (DP) −25 V. The dwell time of each MRM transition was 150ms.

The analytical method was validated for the analysis of human breast milk, milk infant formulas and cereals baby food. The average recoveries of the different matrices were in general higher than 70% with a relative standard deviation (RSD) lower than 21% and method limits of detection (MLOD) ranging from 1.2 to 362 ng/L for the different compounds and matrices.

As an additional feature, in this instrument, the SRM mode can be combined with attractive working modes the Enhanced Product Ion Scan (EPI) and MS^3 modes, for the unambiguous confirmation of compounds. Operating with the EPI mode, Q1 filters the desired parent ion which is fragmented in the Q2 region. Fragment ions are trapped in the Q3 region for a specified time prior to being scanned out. The main limitation is the low stability of fragment ions because the isolation and fragmentation steps are both occurring in the LIT, only fragment ions produced with *m*/*z* values of 30% of the parent mass and higher are stable in the ion trap. However, the EPI and MS^3 modes were used for confirmatory purposes.

Results and Discussion

Table 1 summarizes the results obtained for breast milk samples. PFOS, i,p-PFNA and PFOA were the compounds more frequently found. PFOS and i,p-PFNA were detected and quantified in 95% of the 20 samples analyzed. PFOA was quantified in 45% of samples. Concentrations measured were in the range of 28-865ng/L and 21 to 260ng/L for PFOS and i,p-PFNA, respectively. PFOS was ranging between 100 and 200 ng/L and i,p-PFNA was below 100 ng/L in agreement with previously reported studies (1,10). PFOA was present in less number of samples than PFOS and i,p-PFNA, but some of the samples with PFOA were in high concentrations. In most of the samples PFNA, PFDA and L-PFDS were also detected but in concentrations below the MLOQ.

The six PFCs included in this work were detected in all of the infant milk formula samples and baby food analyzed. For milk infant formulas the compound detected in higher concentrations was PFDA with concentration ranging 693-1289 ng/Kg followed by PFOS, PFOA and i,p-PFNA. The presence of PFCs in the milk could be associated to possible a migration/contamination from packaging and production processes. This conjecture is supported by the fact that the pattern of PFCs present in these products is different of that present in the human milk. In the case of cereals baby food concentrations in general were lower, but again all compounds were quantified. Predominant compounds were PFOS, PFOA and i,p-PFNA with concentrations ranging 300-430 ng/Kg. It is noted that there is a general predominance of acid forms (PFOA, i,p-PFNA and PFDA). Sulphonated forms, although being present, represent approximately 30% of the total PFCs measured. The levels of PFOS and PFOA were similar in milk infant formulas and in cereals baby food.

In order to evaluate possible risks to infant health associated to PFCs intake the Risk Index (RI) was calculated for human breast milk, cereals baby food and milk infant formulas according to the EFSA guidelines (European Food Safety Authority, 2008).The Daily Intake (DI) was calculated according to:

DI (ng of PFC/Kg of body weight /day)
Where, ng of PFCs = (Consumption[a] $_X$ PFCconcentration[b])
[a]Consumption expressed in mL of milk or g of milk based infant formula or baby food per day
[b]PFCs concentration in ng/mL or ng/g.

DI of PFOS and PFOA through mother´s breast were calculated based on the general infant's milk ingestion rate during the first 6 months of life.

The risk index (RI) was calculated according to the expression:

RI = DI/ TDI

Where, TDI is the tolerable daily intake. According to the EFSA guideline (European Food Safety Authority, 2008), TDI for PFOS was 150 ng/kg of b.w. and for PFOA 1500 ng/kg of b.w.

Finally, RIs calculated for breast milk samples and baby food did not exceed maximum limit according to the EFSA recommendations with exception of 1 sample of breast milk. Lactation is a considerable source of PFCs exposure for infants, and according to data obtained from this study approximately 300 ng of PFCs per day may transferred from lactating mother to infants. These results justify further investigation on human monitoring of PFCs and their possible toxicological effects.

Table 5: **Human Breast Milk results:**

Sample	**Human Breast Milk (ng/L)**					
	PFOA	**T-PFOS**	**i,p-PFNA**	**PFNA**	**PFDA**	**L-PFDS**
1	78 ±	52	< LOQ	<LOQ	<LOQ	<LOQ
2	176	60	34	<LOQ	<LOQ	43
3	< LOQ	28	95	<LOQ	<LOQ	<LOQ
4	907	111	260	<LOQ	<LOQ	<LOQ
5	609	52	35	<LOQ	<LOQ	<LOQ
6	289	84	83	<LOQ	237	<LOQ
7	604	865	40	<LOQ	<LOQ	<LOQ
8	21	185	27	<LOQ	<LOQ	<LOQ
9	< LOQ	89	134	<LOQ	<LOQ	<LOQ
10	291	101	72	<LOQ	<LOQ	<LOQ
11	< LOQ	41	93	<LOQ	1095	58
12	15	32	21	<LOQ	<LOQ	<LOQ
13	< LOQ	84	57	<LOQ	<LOQ	<LOQ
14	< LOQ	99	59	<LOQ	<LOQ	<LOQ
15	< LOQ	28	53	<LOQ	<LOQ	59
16	< LOQ	56	52	<LOQ	<LOQ	<LOQ
17	< LOQ	99	46	<LOQ	<LOQ	40
18	< LOQ	< LOQ	34	<LOQ	<LOQ	<LOQ
19	< LOQ	97	52	<LOQ	<LOQ	54
20	< LOQ	156	178	<LOQ	<LOQ	70

<*LOQ*: lower than limit of quantification

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References

1. Kärrman A, Domingo J, Llebaria X, Nadal M, Bigas E, van Bavel B, Lindström G. (2010); *Environ. Sci. and Poll. Res. Int.* On line.
2. Kärrman A, Ericson I, van Bavel B, Darnerud PO, Aune M, Glynn A, Lignell S, Lindström G. (2007); *Environ. Health Perspec.* 115:226-230.
3. Kishikawa N, Kuroda N. (2009); *J. Health Sci.* 55:1-10.
4. Völkel W, Moscha C, Kiranoglua M, U. V-R, H. F. (2009); *Toxicol. Lett.*189:S151-S152.
5. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, Uno A, Saijo Y, Sata F, Yoshimura Y, Kishi R, Nakazawa H. (2004); *Environ. Health Perspec.* 112:1204-1207
6. Tao L, Kannan K, Wong CM, Arcaro KF, Butenhoff JL. (2008); *Environ. Sci. Technol.* 42:3096-3101.
7. Llorca M, Farré M, Picó Y, Barceló D. (2009); *J. Chromatogr. A.* 1216: 7195-7204
8. Ye X, Schoenfuss HL, Jahns ND, Delinsky AD, Strynar MJ, Varns J, Nakayama SF, Helfant L, Lindstrom AB. (2008); *Environ. Int.* 34:932-938.
9. Taniyasu S, Kannan K, Man KS, Gulkowska A, Sinclair E, Okazawa T, Yamashita N. (2005); *J. Chromatogr. A.* 1093:89-97.
10. So MK, Yamashita N, Taniyasu S, Jiang Q, Giesy JP, Chen K, Lam PK.(2006); *Environ. Sci. Technol.* 40:2924-2929.