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Infant exposure of perfluorinated compounds: Levels in breast milk and commercial baby food

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ABSTRACT

In this study, an analytical method to determine six perfluorinated compounds (PFCs) based on alkaline digestion and solid phase extraction (SPE) followed by liquid chromatography-quadrupole-linear ion trap mass spectrometry (LC-QqLIT-MS) 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.

The method was applied to investigate the occurrence of PFCs in 20 samples of human breast milk, and 5 samples of infant formulas and cereal baby food (3 brands of commercial milk infant formulas and 2 brands of cereals baby food). Breast milk samples were collected in 2008 from donors living in Barcelona city (Spain) on the 40 days postpartum. Perfluorooctanesulfonate (PFOS) and perfluoro-7-methyloctanoic acid (i, p-PFNA) were predominant being present in the 95% of breast milk samples. Perfluorooctanoic acid (PFOA) was quantified in 8 of the 20 breast milk samples at concentrations in the range of 21–907 ng/L. Commercial formulas and food were purchased also in 2009 from a retail store. The six PFCs were detected in all brands of milk infant formulas and cereals baby food analyzed, being perfluorodecanoic acid (PFDA), PFOS, PFOA and i, p-PFNA the compounds detected in higher concentrations (up to 1289 ng/kg). PFCs presence can be associated to possible migration from packaging and containers during production processes.

Finally, based on estimated body weight and newborn intake, PFOS and PFOA daily intakes and risk indexes (RI) were estimated for the firsts 6 month of life. We found that ingestion rates of PFOS and PFOA, with exception of one breast milk sample did not exceed the tolerable daily intake (TDI) recommended by the EFSA. However, more research is needed in order to assess possible risk associated to PFCs contamination during early stages of life.

1. 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. PFCs have been also used as surfactants and lubricants in fire-fighting foams, pesticides and personal care products (Voogt and Saez, 2006). Until the last years the environmental occurrence of PFCs has received little attention, mainly because of their low acute toxicity and difficulties in their chemical analysis. However, their manufacture, use and disposal have led to their widespread distribution in the environment. PFCs have been detected in different water matrices (as rivers (Kannan et al., 2002), lakes (Furdui et al., 2007), rainwater (Kim and Kannan, 2007), and wastewater (Bossi et al., 2008)), in the air (Kim and Kannan, 2007), wildlife (Giesy and Kannan, 2001; Kelly et al., 2009) and humans (Kärrman et al., 2009; Tao et al., 2008a,b). These compounds are biomagnified in the aquatic food chains (Kannan et al., 2005; Kelly et al., 2009) and are highly persistent (Olsen et al., 2007). A study performed with serum of retired production workers showed that half-life elimination of PFOS, perfluoro hexanesulfonate (PFHxS) and PFOA appears to occur over a long period of time. Differences in species-specific pharmacokinetics may be due, in part, to a saturable renal resorption process (Olsen et al., 2007). On the other hand,

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results obtained in epidemiological studies in PFOS and PFOA exposed workers have not shown concluding evidences of increased cancer risk (Olsen et al., 2003). However, different studies have revealed toxicological effects of PFCs, such as the suppression of humoral immunity in mice (Peden-Adams et al., 2008). The toxicology of PFCs has been reviewed (Kudo and Kawashima, 2003; Lau et al., 2007). One of the more relevant observations is the decrease of the thyroid hormone levels in the serum of monkeys and rodents after PFC exposure (Benvenga et al., 2002; Lau et al., 2007; Luebker et al., 2002; Weiss et al., 2009). PFCs are structurally homologous to free fatty acids (Holmes et al., 2009; Luebker et al., 2002), and as such they bind to liver fatty acids-binding protein and albumin which is mainly in blood and liver (Jones et al., 2003). By altering thyroid hormone levels, PFCs may affect fetal and neonatal development (Larsen and Delallo, 1989).

During the last years different studies have assessed the levels of PFCs in human breast milk (Kärrman et al., 2009, 2007; Kishikawa and Kuroda, 2009; Völkel et al., 2009), 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 (Inoue et al., 2004; Tao et al., 2008a,b), 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 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); and 3) to evaluate the Risk Index (RI) for daily childhood intake based on the guidelines of the European Food Safety Authority (EFSA).

2. Materials and methods

2.1. 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.

2.2. 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 −20 °C. Before extraction, samples were lyophilized, homogenized and stored at −36 °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 400 g 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 250 g plastic bag packages with composition in proteins, fat and carbohydrates in the range of 6–6.5%, 1–1.2% and 87.4–88%. The composition of the studied infant formulas as given on the label was:

Baby Cereal (A) — Made with cereal grains (rice) that have been enzymatically hydrolyzed to be easy to digest without gluten. This cereal contains specially adapted milk, made with skim milk and a blend of vegetable oils, to suit babies' nutritional needs.

Baby Cereal (B) — Made with wheat based infant cereal grain (rice and corn) enzymatically hydrolyzed without milk, lactose and gluten.

Sample pre-treatment and extraction procedure was based on an alkaline digestion according to a protocols described before (Llorca et al., 2009; Ye et al., 2008, Taniyasu et al., 2005) followed by a clean-up using solid phase extraction (SPE) with C_{18} Sep-Pack cartridges.

Briefly, approximately 1 g of each lyophilized sample was weighted and transferred into a 15 mL PP tube, then 2 mL of ultrapure water were added and shaken. In order to evaluate the recoveries sample homogenates were fortified with the surrogate internal standards and digested with 8 mL of NaOH (10 mM in MeOH) during 3 h at 125 u/min on an orbital shaker table at room temperature. After the orbital digestion the samples were centrifuged during 10 min. at 4000 rpm and 3 mL of supernatant was taken and diluted with 27 mL of water in a 50 mL PP tube and vortexed during 5 min. A SPE was performed using Sep-Pak cartridges preconditioned with 5 mL of MeOH and 5 mL of water. Then, pre-treated samples were loaded onto the cartridge, under gravity conditions, and dried under vacuum in a J.T. Baker (Phillipsburg, NJ EEUU). The elution was carried out with 5 mL of MeOH in a 15 mL of PP tube and reduced to dryness under a gentle stream of nitrogen. The extracts were reconstituted with 150 µL of mobile phase at its initial gradient conditions, and in order to account the matrix effects during the analysis the instrument performance internal standard was introduced (at 1.5 ng/mL level in vial). The extraction procedures were carried out working in triplicate for all samples.

2.3. Instrumental analysis

The analysis of PFCs was performed by LC-ESI-MS/MS. LC was performed using a Symbiosis ™-Pico (Spark Holland, Emmen, The Netherlands) with a C18 LiChroCART® Purosphere Star-18e analytical column (125 mm × 4 mm i.d., 5 µm) from Merck (Darmstadt, Germany) at room temperature. The mobile phase consisted of (A) aqueous ammonium acetate 20 mM (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.