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Perfluorinated carboxylic acids in human breast milk from Spain and estimation of infant's daily intake

Miguel Motas Guzmàn [a], Chiara Clementini [b], Maria Dolores Pérez-Cárceles [c], Sandra Jiménez Rejón [c], Aurora Cascone [d], Tania Martellini [d], Cristiana Guerranti [b,e], Alessandra Cincinelli [d,*]

[a] Área de Toxicología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain
[b] University of Siena, Department of Physical Sciences, Earth and Environment, Via Mattioli, 4, 53100 Siena, Italy
[c] Department of Legal Medicine, School of Medicine, University of Murcia & Instituto Murciano de Investigacion Biomedica (IMIB), (IMIB-VIRGEN DE LA ARRIXACA), Murcia, Spain
[d] Department of Chemistry "Ugo Schiff", via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy
[e] Bioscience Research Center, Via Aurelia Vecchia 32, 58015 Orbetello, GR, Italy

HIGHLIGHTS

- Perfluorinated carboxylic acids were analyzed in a set of 67 breast milk samples collected from Spanish women.
- PFOA appeared as the major contributor to the total perfluorinated carboxylic acids.
- PFOA concentrations were significantly higher in milk of primiparous participants.
- PFOA daily intake and risk index were estimated for the firsts six month of life.

GRAPHICAL ABSTRACT

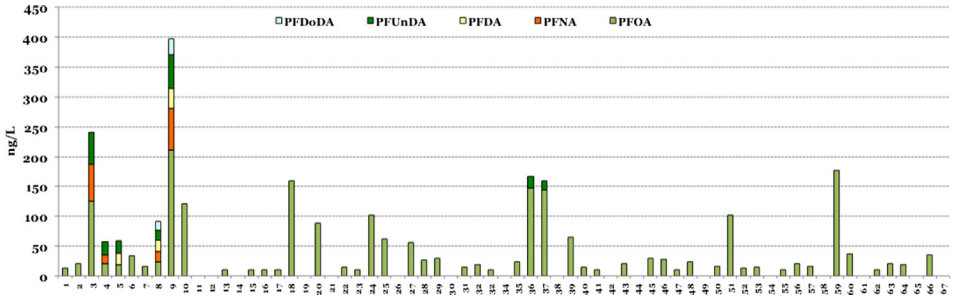

Figure SI 1. Concentrations (ng/L) of PFCs recovered in 67 samples of human breast milk.

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ABSTRACT

Human milk samples were collected from 67 mothers in 2014 at a Primary Care Centre in Murcia (Spain) and analyzed for perfluorinated carboxylic acids (PFCAs). Concentrations measured for perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA) and perfluorododecanoic acid (PFDoDA) ranged from <LOQ (<10 ng/L) to 397 ng/L with a mean concentration of 66 ± 68 ng/L and a median of 29 ng/L. The presence of these compounds was revealed in 50 samples out of 67 analyzed. Influence of number of pregnancies and food habits on PFCAs concentrations was also investigated. Statistically significant differences in PFCA levels were found when the women were divided into maternal age classes and into the categories primiparae and multiparae. A greater transfer of PFC during breastfeeding by primiparous was evidenced and thus a higher exposure to these contaminants for the first child. Moreover, it was possible to hypothesize that the content of PFCs is in general correlated to the eating habits of donors and, in particular, with the fish consumption. Finally, PFOA daily intakes and risk index (RI) were estimated for the first six months of life and we found that ingestion rates of PFOA did not exceed the tolerable daily intake (TDI) recommended by the European Food Safety Authority (EFSA).

1. Introduction

Perfluoalkyl substances (PFAS) are a large group of manufactured organic chemicals which have been widely used for several decades in

* Corresponding author.
E-mail address: acincinelli@unifi.it (A. Cincinelli).

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numerous consumer products and variety of industrial applications such as food packaging material and fire-fighting foams, in making products water- and oil-repellents, in the surface coatings of cooking pans, in the protective coating for textiles, carpets, papers, electronic and photographic devices, and as surfactant in diverse cleaning agents (OECD, 2002).

Regarding all PFAS, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are the compounds which have been most extensively studied and received worldwide attention, mainly due to their global occurrence in different environmental matrices, biopersistence and toxicity. As a consequence of these chemical–physical characteristics and their widespread presence in environmental media (Kim and Kannan, 2007; Bossi et al., 2008), biota (Kelly et al., 2009) and humans (Tao et al., 2008a, 2008b), the EU restricted PFOS production and use from 2008 onward (Directive 2006/122/EC of the European Parliament and of the Council) and in 2009, PFOS was included in Annex B of the Stockholm Convention on Persistent Organic Pollutants. Moreover, PFOA, its salts and related compounds were identified in the EU as substances of very high concern (SVHCs), having been shown to be both toxic for reproduction and substances that are persistent, bioaccumulative and toxic (PBTs) (CW 20 June 2013). Recently, the EU Council of Ministers adopted the proposal for the listing of perfluorooctanoic acid and its compounds in Annex A of the Stockholm Convention on POPs.

PFAS exposure in human primarily originates through the ingestion of contaminated foodstuffs and/or drinking water (Ericson et al., 2008), even if recent studies have revealed not only that dust and air are also exposure pathways for certain PFAS, i.e. PFOS (Gebbink et al., 2015), but also that exposure varies depending on the type of PFAS, geographical locations, type of food and food consumption pattern (Ullah et al., 2014). Recently, the European Food Safety Authority (EFSA) has recognized PFAS as emerging contaminants in the food chain and established tolerable daily intakes (TDI) of 150 ng/kg body weight (bw)/day for PFOS and 1500 ng/kg bw/day for PFOA.

Several studies have suggested that breast milk is the primarily route of exposure of environmental chemicals for breastfed infants. In fact, even if breast milk is a natural and complete food and provides almost all the necessary nutrients for the babies and a protection against a number of diseases (diarrhea, otitis, acute respiratory infections), it could also be a potential excretion route for mothers that may contain toxic compounds due to the exposure mainly from dietary sources. Thus, the presence of toxic compounds in breast milk and their potential adverse effects on infant development and health are of concern and have been extensively investigated in the last decades. However, while persistent lipophilic organic compounds such as PAHs, PCBs, PBDEs bind to lipids, PFAS strongly bind to the protein fraction in blood, notably albumin (Völkel et al., 2008) and their lactational transfer is believed to be caused by binding to milk protein (Fromme et al., 2010). For this reason, the determination of levels of organic compounds in mother's milk can be very useful to estimate the exposure of the growing fetus and the breastfeeding infants to contaminants.

In this context, the main objective of the present study was to determine the occurrence and concentration levels of perfluorinated carboxylic acids (PFCAs) in 67 Spanish breast milk samples and compare the results with those previously obtained in other countries. In order to evaluate the infant exposure to these contaminants, the daily childhood intake through breast milk was also estimated. In fact, in many cases, human milk is the only food that newborns consume in the first months of life, thus the analysis of human breast milk allows to estimate the intake of xenobiotic compounds in a more simple and accurate manner than in organisms with a more complex diet.

2. Materials and methods

2.1. Milk samples

In May 2014, 67 individual breast milk samples were obtained from nursing mothers (22 primiparous and 45 multiparous) at a Primary Care Centre, in the area of Portman Bay (Murcia, Spain), that is one of the most degraded zones of the Mediterranean due to the mining impact. The women donated milk samples on the first weeks after delivery. The criteria and approach for donor selection and human milk sampling were based on the "Guidelines for Developing a National Protocol" of the Fourth WHO-Coordinated Survey of Human milk for Persistent Organic Pollutants in Cooperation with UNEP (WHO, 2007). The guideline recommended recruiting a minimum of 50 individual donors in order to get statistically reliable data. Possible donors were contacted before giving birth. Once a participant indicates the willingness to take part in the survey, she was invited to complete a detailed questionnaire to provide confidential information on her age, number of births, number of infant previously breast fed, occupation, smoking habits, lifestyle factors and dietary habits (mixed diet, vegetarian but with milk and eggs, strictly vegetarian, other). Moreover, participants had to provide information on how often on average (once a week, twice a week, more than twice a week but not every day, every day) they ate the following foods: fish and fish products from the sea, freshwater fish, sea-food (i.e. shrimps, mussels), milk and milk products, meat, poultry, eggs. The questionnaire was completed through a personal interview at the prenatal clinic. Mothers, who donated breast milk, were fully informed of the nature and purpose of the study, about the benefits of breast feeding and invited to sign an informed consent form.

Donors in the study were 33 ± 5 years old and had resided in the represented area for at least the previous 5 years. All the mothers chosen for the study were not occupationally exposed to chemicals.

Milk samples (about 50 mL) were hand-expressed into 100 mL precleaned polypropylene pots, and appropriate precautions (such as previous rinse with hexane) were taken to prevent the contamination of the samples. All samples were immediately frozen and kept at −20 °C until analysis.

2.2. Standards

Perfluoroheptanoic acid (PFHpA, <95%), perfluorooctanoic acid (PFOA, 95%), perfluorononanoic acid (PFNA, 97%), perfluorodecanoic acid (PFDA, 98%), perfluoroundecanoic acid (PFUnA, 95%), and perfluorododecanoic acid (PFDoA, 95%) were purchased from Sigma-Aldrich (Germany).

2.3. Sample extraction and analysis

The analytical procedure for the extraction of PFAS from human milk samples was similar to that described by Guerranti et al. (2013). Briefly, the extraction was performed using an ion-pairing extraction procedure and measured using Gas Chromatography–Mass Spectrometry (GC/MS). 1 mL of 0.5 M tetrabutylammonium (TBA) hydrogen sulfate solution (97%, Sigma Aldrich, Germany) and 2 mL of sodium carbonate buffer (0.25 M, pH 10) (Sigma Aldrich, Germany) were added to 0.5 mL of the samples in a polypropylene tube and thoroughly mixed for extraction. 5 mL of methyl tert-butyl ether (MTBE) were added to the above mixture and shaken for 20 min. The organic and aqueous layers were separated by centrifugation, and an exact volume of MTBE (4 mL) was removed from the solution. The aqueous mixture was rinsed with MTBE and separated twice; both the rinses were combined in a second polypropylene tube. The solvent was evaporated under nitrogen up to 1 mL. This extract was passed through a Nylon mesh filter (0.2 μm) into an autosample vial and then evaporated under nitrogen up to a drop volume before being reconstituted in 0.5 mL of methanol. At the time of extraction blank sample were prepared every ten samples according to the same procedure. PFAS were analyzed adopting the procedure proposed by Dufková et al. (2009, 2012). The procedure consists in the esterification of PFC due to the reaction with the alkyl chloroformates. The alkyl is first bound to the acid, with liberation of HCl and the formation of the labile acid anhydride which rapidly decarboxylates and provides the ester and carbon dioxide, according to the