PRELIMINARY RESULTS ON LEVELS OF PERFLUORINATED COMPOUNDS IN HUMAN MILK SAMPLES FROM CENTRAL ITALY

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Introduction

Perfluorinated compounds (PFCs) are synthetic substances widely used in several fields for covering plastic tissues, plastic materials and electric products, antispot compounds, non-stick coverings and photographic films. PFCs include compounds based on perfluottan sulphonylfluorur (POSF) that, upon being degraded, e.g. by environmental factors or metabolized by the organisms, lead to the formation of substances of toxicological importance such as perfluoctane sulphonate (PFOS) and perfluoroctanoic acid (PFOA).

The high stability of these molecules makes them able to accumulate in the organisms, for which they turn out to be toxic. PFOA and PFOS are endocrine disrupting chemicals (EDC) and researchers are trying to highlight possible correlations between PFOS and PFOA exposure and the presence of some diseases.

Extensive screening analyses of PFOS and PFOA in wildlife samples from all over the world have identified these compounds as global pollutants and have shown their bioaccumulation into higher trophic levels in the food chains[1,2].

PFOS and PFOA have been identified also in human samples, such as blood, blood serum and milk, from general population[3, 4].

Concentrations of PFOS and PFOA in human milk have been examined in several studies[5, 6, 7, 8,9,10]. The mechanism by which perfluorinated substances are transferred from mother's blood to breast milk is not clear, but it is well known that those perfluorinated compounds are strongly bound to the protein fraction in blood[11]. The possibility of PFCs entering the milk and accumulating to levels observed in maternal plasma is therefore limited.

As these substances are found in environmental biota, it is likely that food is a human exposure route. The relative contribution of the various foodstuffs to the total human exposure is, however, not known.

Despite the environmental importance of PFCs and the detection of these compounds in many countries around the world, little is known on their occurrence and distribution in Italy. This study reports the results of a research on the distribution and levels of PFOS and PFOA in human milk samples from the city and province of Siena (Tuscany, Central Italy).

Materials and methods

Forty-nine milk samples from women living in the city of Siena and its province were analyzed for this study. Information such as age, delivery frequency, occupation, smoking, dietary habits etc. was recorded for all the mothers. The participants in the study were on average 31±4 years of age and had lived in the respective areas for at least 5 years.

Precautions were taken to prevent contamination of the samples. All samples were kept frozen at −20°C until analysis.

The analytical procedure for the extraction of PFOS and PFOA from samples was similar to that described by Corsolini et al.[12]. PFOS and PFOA were extracted using an ion-pairing extraction procedure and measured using high performance liquid chromatography (HPLC) with electrospray ionization (ESI) tandem mass spectrometry. Analytes separation was performed using a Finnigan Surveyor Plus HPLC System, consisting of a quaternary pump, vacuum degasser, and autosampler. Chromatographic separation was achieved using a Betasil C18 column (50 × 2.1mm i.d., 5 μm) supplied by Thermo Electron Corporation, San Jose, CA. For quantitative determination, the HPLC system was interfaced to a Finnigan LTQ linear ion trap mass spectrometer (Thermo Electron Corporation, San Jose, CA) operated in negative electrospray mode. Primary and product ions monitored for PFOS and PFOA determinations were 412.8>168.8, 218.8, and 498.8>368.9, respectively.

Data quality assurance and quality control protocols included matrix spikes, laboratory blanks, and regular calibration verification.

Blanks were analyzed with each set of five tissue samples as a check for possible laboratory contamination and interferences.

Matrix spikes were also analyzed.
LOD, determined as three times the signal-to-noise (S/N) ratio, was 0.5 ng/g wet wt (w.w).

Results and discussion

PFOS levels detected in 20 samples were often below the LOD; the values of PFOS ranged from 1.06 ng/g w.w. (1.02 ng/mL) to 4.43 ng/g w.w. (4.28 ng/mL). The mean value of the concentration of PFOS was of 0.88 ng/g w.w. (0.85 ng/mL) while the median was <LOD; due to the small percentage of samples that resulted contaminated, the value of the standard deviation was somewhat larger than the average, 1.20 ng/g w.w. (1.16 ng/mL).
PFOA was found in just one sample, from a primipara at an appreciably higher level than PFOS: 8.04 (7.78 ng/mL). The average PFOA value calculated on the total sample set was 0.16 ng/g w.w. (0.16 ng/mL). The presence of PFOA in only a milk sample of those considered in the study is also reported by Kärrman et al. [7]; this work reports PFOA at concentrations above the LOD in only a sample out of the 12 analyzed.
Mean PFOS values obtained milk samples from women of the Sienese area were high when compared with those from reported for samples of breast milk from Sweden[9] and from Massachusetts (USA)[10] (mean values for PFOS 0.20 ng/mL and 0.13 ng/mL and PFOA <LOD and 0.04 ng/mL, respectively for Sweden and Massachusetts).
However, it should be noted that the distribution of results between this study and the one mentioned was different, since according to the above reported studies almost all the samples resulted contaminated with PFOS and PFOA.
Splitting the sample set in primiparae and multiparae women, the milk samples showed slightly higher mean values of PFOS for the batch of multiparae (table 1), but this difference was not statistically significant (Mann-Whitney Wilcoxon). Further statistical tests performed (Spearman correlation), have not shown correlations between levels of contaminants in the samples and the prevalence of the most contaminated foods in the diet or age or occupation of the mothers.

Table 1: Mean values of PFOS in samples of milk from primipara or multipara Sienese women.

	Milk samples from primiparae	**Milk samples from multiparae**
ng/g w.w.	0.68±1.24	1.03±1.10
ng/mL	0.65±1.19	1.00±1.07

Given the low number of milk samples that resulted contaminated, it is difficult, at least at this preliminary stage, to extrapolate considerations on the state of contamination of the group of donor women and the more of the environment in which they live.
Mean PFOS value found is higher than the results of studies on breast milk from other countries; in particular the maximum values of both PFOS and PFOA are higher than the comparison data, perhaps highlighting the particular mother's exposure (i.e. food and/or indoor contamination) which deserve further investigation.
The estimated dietary intakes (EDI) of PFCs for breastfeeding infants in Sienese area were calculated on the basis of PFCs levels in human milk and the average infant daily consumption data (742 mL/day) given by the U.S. EPA[13] and a body mass of 6 kg[6].
TDIs of 150 ng/kg/day for PFOS and 1500 ng/kg/d for PFOA were established by the scientific panel on Contaminants in the Food Chain requested by the European Food Safety Authority in 2008[14]. The EDIs of PFOS and PFOA by breastfeeding infants were lower than these TDIs or RfDs, if calculated on average values of contaminants. However, the EDI of PFOS, calculate on single samples was over to the TDI for several samples (for milk samples with PFOS concentration over 1.21 ng/ml) (table 2).
Although the TDI refers to the lifetime tolerable daily intake and the breastfeeding period is relatively short, infants are at an increased risk due to their susceptibility to chemical contaminants, such as PFOS.

Table 2: EDIs of PFOS and PFOA by breastfeeding infants from Sienese area, calculated on average and maximum values of contaminants; TDIs for a 6 kg child, indicated by EFSA are also reported. In bold the value exceeding the safe dose.

Contaminant	**average intake** (ng/die)	**maximum intake** (ng/die)	**daily safe dose for a 6 Kg infant** (ng)
PFOS	631	**3175**	900
PFOA	119	5779	9000

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