Perfluorinated compounds in pooled human milk from developing countries

Kärrman A[1], Mattioli, L[1], Malisch, R[2], Magulova, K[3], van Bavel, B[1], Fiedler, H[4]

[1]MTM Research Centre, Örebro University Sweden; [2]State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany; [3]UNEP Stockholm Convention Secretariat, Châtelaine, Switzerland [4]UNEP Chemicals, Châtelaine, Switzerland

Introduction

Geographical gaps in the current literature have been identified when establishing baseline levels for future evaluation of the effectiveness of the Stockholm Convention on persistent organic pollutants (POPs). Nineteen (19) pooled milk samples were collected during 2008-2010 from 19 countries covered by the milk survey programme under WHO coordination (Antigua and Barbuda, Chile, Congo - Dem. Rep., Cote d'Ivoire, Georgia, Ghana, India, Kenya, Lithuania, Mali, Mauritius, Moldova, Nigeria, Senegal, Syria, Tajikistan, Togo, Uganda and Uruguay). These were analysed for concentrations of the twelve legacy POPs listed initially under the Stockholm Convention on POPs[1]. In 2009, nine new chemicals were added to Annexes A (elimination), B (restriction), and C (unintentional production) of the Convention[2]. One of these new chemicals is perfluorooctane sulfonate (PFOS, Annex B), which has been detected in humans world-wide, is biologically active and toxic, is used in many industrial and consumer products, and is extremely persistent[3,4]. This study presents level of PFOS and related perfluorinated compounds (PFCs) in pooled human milk samples from 19 developing countries collected by WHO in cooperation with United Nations Environmental Programme (UNEP).

Materials and methods

The samples were extracted using weak anion exchange, solid-phase extraction (Waters Oasis® WAX)[5]. Labelled internal standards ($^{18}O_2$PFHxS, $^{13}C_4$PFOS, $^{13}C_2$PFHxA, $^{13}C_4$PFOA, $^{13}C_5$PFNA, $^{13}C_2$PFDA, $^{13}C_2$PFUnDA) and 2 mL formic acid/water (1:1) were added to 1 mL milk. The solution was sonicated for 15 min and centrifuged at 10 000 x g for 30 minutes. The supernatant was extracted and the perfluorinated compounds were eluted with 1 mL 2% ammonium hydroxide in methanol, after washing the sorbent with 2 mL sodium acetate buffer solution, pH 4, and 2 mL 40% methanol in water. The volume of the extracts was reduced to 20 µL by using nitrogen, and 30 µL 2 mM ammonium acetate in water was added. Performance standards, $^{13}C_8$PFOA, $^{13}C_8$PFOS, and *7H*-PFHpA, were added to the extracts before injection. Analysis was performed using an Acquity UPLC coupled to a Quattro Premier XE MS/MS (Waters Corporation, Milford, US) with an atmospheric electrospray interface operating in negative ion mode. Separation was performed on an Acquity BEH C18 2.1 x 100 mm, 1.7 µm kept at 50°C. An extra guard column (PFC isolator, Waters Corporation, Milford, US) was inserted between the pump and injector to trap contaminants originating from the LC system. Injection volume was 10 µL and the flow rate was set to 300 µL/min. A gradient program was employed delivering mobile phases consisted of 2 mM ammonium acetate in methanol, and 2 mM ammonium acetate in water.

Quality assurance and control

The method used has been validated and described earlier [5]. Concentration of the analytes in the samples was calculated using internal standard quantification. A minimum of five-point calibration curve was used. The internal standard closest in retention time was used for those compounds that did not have a corresponding labeled internal standard (PFBS, PFDS, PFPeA, PFHpA). Two product ions were monitored for each compound, when possible. The ratio between the two product ions in the samples were calculated and compared to an authentic standard, and the difference did not exceed 50%. Recoveries of internal standards were monitored for each sample. The recovery of $^{13}C_4$PFOS and $^{13}C_4$PFOA were 56-83% (average 77 and 73%, respectively) for all milk samples. In addition, several other PFCs were determined in some samples given that the product ion ratio

and internal standard recovery was acceptable (50-150%). Reproducibility expressed as relative standard deviation (RSD) for a quality control sample extracted on eight different days was 3.5% for PFOS, 3.0% for PFOA, and 5.0% for PFHxS. The quality control sample also contained relatively low levels of PFNA and PFUnDA, and the RSD for those compounds were 10% and 40%, respectively. One procedural blank was performed for each six milk samples. Milk levels are reported when the signal in the sample is higher than the average+ 3 standard deviations of the signal measured in procedural blanks (n=6). Empty sample containers used to ship and store the milk samples were tested by adding Milli-Q water which was extracted in the same way as the milk samples. No contamination from the containers could be detected above the limit of detection. Successful participation in an interlaboratory study on PFCs in milk took place in 2009/2010[6].

Results and discussion

Levels of PFCs in human milk are relatively low, and previous studies have shown that mother´s milk contain approximately 1% of the corresponding PFOS maternal serum concentration[5]. Even so, PFOS was still quantified in 84% of the milk samples from developing countries, concentrations ranging from <9 to 65 ng/L (Table 1). The detection limit for PFOA was higher than levels of PFOS found in the samples, notably PFOA was still quantified in 31% of the samples (<80 – 192 ng/L). In addition, PFHxS, PFNA, PFDA, and PFUnDA were quantified in some samples.

The milk samples contained relatively low levels compared to studies reported from other countries. PFOA and PFOS levels in samples from Moldova, Antigua, Congo and Cote d'Ivoire were in parity with reported levels from example China and Malaysia[7]. PFUnDA was found in samples from Ghana (552 ng/L) and Kenya (110 ng/L) at relatively high levels compared to previously reported studies. The tubes used for sample storage were tested and showed no contamination, however previously used tubes and handling steps have not been evaluated for possible PFC contamination.

References

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Table 1. Concentrations (ng/L) of perfluorinated compounds in pooled human milk from developing countries, collected 2008-2010.

	Concentrations (ng/L)										
	PFBuS	**PFHxS**	**PFOS**	**PFHxA**	**PFPeA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFDS**	**PFUnDA**
Antigua	<10	*NQ*	32	<100	<20	<35	192	*<25**	*NQ*	<10	*NQ*
Chile	<10	<9	11	<100	<20	<35	91	<25	<25	<10	NQ
Congo	<10	<9	11	<100	<20	<35	146	*NQ*	*NQ*	<10	*NQ*
Cote d´Ivoire	<10	<9	32	<100	<20	<35	116	<25	*NQ*	<10	*NQ*
Georgia	<10	<9	27	<100	<20	<35	<80	*<25**	*NQ*	<10	*NQ*
Ghana	<10	<9	24	<100	<20	<35	89	55	<25	<10	552*
India	<10	<9	<9	<100	<20	<35	<80	<25	<25	<10	41*
Kenya	<10	<9	9.8	<100	<20	<35	<80	<25	25	<10	110*
Lithuania	<10	<9	29	<100	<20	<35	<80	<25	<25	<10	<30*
Mali	<10	14	25	<100	<20	<35	<80	<25	<25	<10	41
Mauritius	<10	*NQ*	21	<100	<20	<35	<80	<25*	*NQ*	<10	*NQ*
Moldova	<10	*NQ*	65	<100	<20	<35	108	38*	*NQ*	<10	*NQ*
Nigeria	<10	<9	29	<100	<20	<35	<80	<25*	<25*	<10	*NQ*
Senegal	<10	<9	16	<100	<20	<35	<80	<25	<25*	<10	*NQ*
Syria	<10	<9	<9	<100	<20	<35	<80	29*	<25*	<10	*NQ*
Tajiki-stan	<10	*NQ*	11	<100	<20	<35	<80	31*	*NQ*	<10	*NQ*
Togo	<10	11	31	<100	<20	<35	<80	<25*	<25*	<10	*NQ*
Uganda	<10	<9	<9	<100	<20	<35	<80	<25	<25*	<10	*NQ*
Uruguay	<10	<9	50	<100	<20	<35	<80	<25*	<25*	<10	*NQ*

NQ not quantified (recovery <25% or >150%

* recovery 25-50%