J. Dairy Sci. 97:3339–3343
http://dx.doi.org/10.3168/jds.2014-8005

Short communication: Monitoring the presence of perfluoroalkyl substances in Italian cow milk

Andrea Barbarossa, Teresa Gazzotti, Elisa Zironi, Andrea Serraino, and Giampiero Pagliuca[1]
Laboratorio di Chimica Analitica Bio-Agroalimentare (CABA-Lab), Department of Veterinary Medical Sciences Alma Mater Studiorum, University of Bologna Via Tolara di Sopra 50, 40064 Ozzano dell'Emilia (BO), Italy

ABSTRACT

Perfluoroalkyl substances (PFAS) are fully fluorinated compounds widely used during the last 60 yr in the production of multiple industrial and consumer applications, such as food packaging, nonstick cookware, cleaning agents, and many more. These emerging contaminants have recently become of concern for human health because of their potential negative effects. The risk of exposure to PFAS for humans is mainly related to diet, and the increasing interest in food safety has led the European Commission to call Member States to monitor these contaminants in food matrices. The purpose of the present work was to perform the first monitoring on the presence of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the 2 main and most widely investigated molecules of this family, in cow milk commercially available in Italy. We used an analytical protocol consisting of liquid-liquid extraction followed by 2 purification steps through solid-phase extraction cartridges and injection on an ultra-performance liquid chromatography-tandem mass spectroscopy system. The analysis of 67 samples of different types of cow milk from Italy demonstrated that contamination by PFOS was often present, although at relatively low concentrations (up to 97 ng/L), whereas PFOA was rarely found. On the basis of these results and data reported in the literature on this matrix, milk does not seem to be a major source of PFAS compared with other food categories such as fish and seafood. However, variability among different types of milk must be taken into account, and surveys of milk-derived products would be helpful to better define the risk for consumers.

Key words: perfluoroalkylated substances, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), cow milk

Short Communication

Perfluoroalkylated substances (**PFAS**) is the collective name for a large group of fully fluorinated compounds, which includes various neutral and anionic surfactants characterized by high resistance to thermal, chemical, and biological degradation. The PFAS have almost exclusively anthropogenic origin and have been widely used in industrial applications, such as stain-resistant coating for fabrics and carpets, oil-resistant claddings for paper products used in food packaging, firefighting foams, floor detergents, and formulations for insecticides (Prevedouros et al., 2006). These compounds have been often detected in the environment and in animals, including fish, birds, and mammals, and in areas far from anthropogenic activities (Hu et al., 2005; EFSA, 2008). However, their potential to accumulate is not completely clear, being related in part to the chemical structure of each compound (Conder et al., 2008).

Recently, interest in the potential negative effects of these emerging pollutants on human health has increased and they have been linked to various pathologies in exposed organisms, including immunotoxicity, lung toxicity, hepatotoxicity, reproductive toxicity, and hormonal effects (OECD, 2002). The PFAS have been demonstrated to have high absorption and slow elimination. Renal clearance in humans is low compared with that observed in laboratory animals; the half-life of PFAS in humans is estimated to be around 5 to 8 yr (EFSA, 2008). The most important and studied substances of this family are perfluorooctanoic acid (**PFOA**) and perfluorooctane sulfonate (**PFOS**). Because of its persistence in the environment, bioaccumulation potential, and toxic activity, PFOS has been included in Annex B of the 2009 Stockholm Convention as a persistent organic pollutant and its production and use consequently restricted (Wang et al., 2009).

Due to their oleophobic character, PFAS bind to proteins rather than accumulating in fatty tissues, and they have often been detected in human blood. These chemicals tend to concentrate along the food chain and the main route of exposure is dietary, particularly in foods with a high protein content, such as fish, meat,

Received January 13, 2014.
Accepted February 24, 2014.
[1]Corresponding author: giampiero.pagliuca@unibo.it

eggs, and milk (Haug et al., 2010; Cornelis et al., 2012; EFSA, 2012; Hlouskova et al., 2013). Moreover, their presence can derive from the materials used in food packaging (Tittlemier et al., 2006). The need to assess the risk of exposure for consumers recently led the European Commission to call Member States to perform surveys in as many food categories as possible to collect extensive data and enable accurate estimations (European Commission, 2010). According to various investigations, fish and seafood appear to be the most contaminated matrices, constituting a potentially relevant risk for consumer health in those areas where they represent a main component of the diet (UK Food Standards Agency, 2006; EFSA, 2012; Cornelis et al., 2012). At lower concentrations, PFAS have also been detected in different types of meat and in eggs (Wang et al., 2008; Zhang et al., 2010; D'Hollander et al., 2011). The few studies that have investigated milk have proven that it can be a source of exposure of these pollutants. The PFAS can be accumulated by milk-producing animals and easily eliminated through mammary excretion because of their binding to β-lactoglobulins (Cornelis et al., 2012; Domingo et al., 2012; Hlouskova et al., 2013; Vestergren et al., 2013). The aim of this work was to monitor the presence of PFOA and PFOS in commercially available cow milk in northern Italy to provide data from this part of Europe, taking into account as many types of product as possible.

All analytical standards of perfluoro-n-octanoic acid (PFOA), sodium perfluoro-1-octanesulfonate (PFOS), and their corresponding $^{13}C_4$-labeled internal standards (purity >98%) were from Wellington Laboratories (Guelph, ON, Canada). Ammonium acetate and methanol (liquid chromatography-mass spectrometry grade), as well as formic acid, glacial acetic acid, and ammonia solution (33%), were purchased from Sigma Aldrich (St. Louis, MO), and acetone was from VWR (Radnor, PA). Ultrapure water was produced in the laboratory with a Human Power I system (Seoul, Korea). Oasis HLB 500-mg solid-phase extraction (**SPE**) cartridges were from Waters (Milford, MA), and ENVI-Carb 500-mg SPE cartridges were purchased from Supelco (Sigma Aldrich).

Sixty-seven dairy milk samples of different types (full-cream, part-skim, organic, raw, and high quality) were collected between 2011 and 2012 in northern Italy. Twenty-two samples were purchased from different stores in the Bologna area, packed in tetra-pack bricks or plastic bottles; the remaining 45 samples (8 of organic milk and 37 of milk produced on farms with high hygienic and nutritional standards) were representative of all producers delivering milk to 2 dairy plants located in Lombardia and Emilia Romagna regions. Samples were stored in polypropylene tubes at −20°C in the dark.

Samples were extracted following the protocol described by Kadar et al. (2011), fully validated in accordance with the guidelines set by Commission Decision 2002/657/EC (European Commission, 2002). After the addition of the 2 internal standards to 3 mL of milk, a liquid-liquid extraction with 9 mL of acetone was performed and the sample was mixed by vortex for 30 s, placed in an ultrasonic bath for 10 min, and centrifuged for 10 min at 2,000 × *g*. The supernatant was concentrated to around 3 mL at 45°C under nitrogen stream and acidified by addition of 8 mL of 0.1 *M* formic acid before undergoing a 2-step clean-up on SPE columns. The first purification step was performed on a Waters Oasis HLB cartridge, previously activated with 10 mL of methanol followed by 10 mL of 0.1 *M* formic acid. Once the sample was loaded, a first washing with 5 mL of 0.1 *M* formic acid and a second washing with 5 mL of 0.1 *M* formic acid:methanol (50:50, vol/vol) were performed; then, vacuum was applied for 5 min and the analytes were eluted with 6 mL of a methanol:ammonia 33% (99:1, vol/vol) solution. After concentrating the eluate to about 2 mL, it was loaded on a Supelco ENVI-Carb cartridge (activated by means of 10 mL of methanol) followed by 6 mL of methanol:glacial acetic acid (80:1, vol/vol) to guarantee complete recovery of the analytes. The extracted sample was then evaporated to dryness, reconstituted in 200 μL of methanol:water (50:50, vol/vol) and transferred to a microtube. After centrifugation for 45 min at 10,000 × *g*, 150 μL was placed into a polypropylene vial together with 50 μL of water before being injected onto the ultra-performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) system.

Analysis was conducted on a UPLC-MS/MS system, composed of a Waters Acquity UPLC binary pump including built-in vacuum degasser, thermostated autosampler and column heater, equipped with a Waters Acquity BEH C18 reversed-phase column (50 × 2.1 mm, 1.7 μm) coupled to a VanGuard guard column with identical packaging (Waters). Methanol (solvent A) and 20 m*M* ammonium acetate aqueous solution (solvent B) were used as the mobile phase under programmed conditions at a constant flow rate of 0.5 mL/min. After 30 s at 30% A and 70% B, the ratio was switched to 100% A over 1 min, held for 1.5 min, and was then returned to initial conditions for 0.5 min and finally held for further 1.5 min so that the column could equilibrate before the next injection. The column heater temperature was set at 45°C to reduce backpressure; sample vials were kept in the autosampler at 6°C and the volume injection, in "full loop" mode, was 10 μL.