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Reference:

D' Hollander Wendy, Herzke Dorte, Huber Sandra, Hajslova Jana, Pulkabova Jana, Brambilla Gianfranco, De Filippis Stefania Paola, Bervoets Lieven, de Voogt Pim.- Occurrence of perfluorinated alkylated substances in cereals, salt, sweets and fruit items collected in four European countries
Chemosphere - ISSN 1879-1298 - 129(2015), p. 179-185
DOI: <http://dx.doi.org/doi:10.1016/j.chemosphere.2014.10.011>
Handle: <http://hdl.handle.net/10067/1244990151162165141>

Occurrence of perfluorinated alkylated substances in cereals, salt, sweets and fruit items collected in four European Countries

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Abstract

In the context of a European project, 12 perfluoroalkyl acids (PFAAs) were determined in 14 food items collected in four European Countries representing northern, southern, eastern and western Europe. This study presents the results of PFAAs measured in fruit, cereals, sweets and salt. Out of the 12 PFAAs, 10 PFAAs were detected in 67% of the samples. Overall, PFOA was the most abundant compound and the highest concentrations were found for PFOS but all were less than 1 ng/g. When comparing the four countries, highest levels and detection frequencies were observed in Belgium (Western Europe), followed by the Czech Republic (Eastern Europe), Italy (Southern Europe) and finally Norway (Northern Europe). Comparison of profiles and levels is difficult due to variations in constitution of the food categories in the investigated countries and countries of origin of the food items. Dietary intake assessments for PFOS and PFOA show that the daily intake of PFAAs is far below the existing tolerable levels. However, they contribute to the total dietary intake and should therefore be included in future dietary exposure assessments.

Keywords: PFAAs, fruit, cereals, PFOS, PFOA, diet

Introduction

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants with unique physico-chemical properties. Due to the strong C-F bond and both hydrophobic and lipophilic properties, PFAAs have been used in broad range of industrial applications and consumer products such as surfactants for lowering surface tensions, firefighting foams, floor polish, water- and oil repellents for textiles and additives for food contact paper (Buck et al., 2011; Kiss, 2001). As a consequence of their widespread use, persistence and potential to bioaccumulate in the environment, PFAAs are globally detected in wildlife and humans. The presence of PFAAs in serum or blood from non-occupationally exposed humans is well documented (reviewed by Fromme et al., 2009). The ingestion of dust, dermal adsorption, inhalation via air and dietary intake have been described as possible exposure routes for the general population (Cornelis et al., 2012; D'Hollander et al., 2010; Klenow et al., 2013). The intake through the diet and water has been reported by several papers as one of the most important intake routes (e.g. Fromme et al., 2009; Vestergren and Cousins, 2009). In recent years the literature on PFAAs in food and/or dietary intake has increased rapidly but data necessary to estimate the intake via food remain scarce (Cornelis et al., 2012; Ericson et al., 2008; Ericson-Jogsten et al., 2009; Klenow et al., 2013; Noorlander et al., 2011; Vestergren et al., 2012b). However, PFAAs in food items in which high levels are expected such as fish, seafood, organs (liver) and meat products are described in the literature (D'Hollander et al., 2010; EFSA, 2012). Likewise, data in water and/or drinking water are relatively abundant (D'Hollander et al., 2010; Eschauzier et al., 2011). On the other hand, sufficient data on other food items such as cereals, fruits, vegetables, fats, sugar-rich products and processed food are occasionally described even though they are consumed in high amounts (Ericson et al., 2008; Noorlander et al., 2011; Vestergren et al., 2012b). This could be explained by the lack of reliable sensitive analytical methods which are required to detect PFAAs at the pg/g levels. Furthermore, the complexity of the food matrices plays a major role in the extraction and analysis of PFAAs (van Leeuwen et al., 2009). Recently, suitable sensitive methods to achieve these goals have been published (Ballesteros-Gómez et al., 2010; Ullah et al., 2012; Vestergren et al.,

2012a). Dietary intake assessments have been published earlier but the uncertainties of most of these studies are rather high due to the high limits of quantification (LOQs) and/or to the limited amount of analysed food items.

The calculation of the dietary intake of PFAAs is not straightforward and apart from the direct intake of PFAAs, involves the intake of precursor compounds that potentially can degrade to PFAAs contributing to the total dietary intake (Ullah et al., 2014; Vestergren et al., 2008). In this study, the additional intake of precursor compounds is not taken into account.

The contamination of fruit, cereals, sweets and salt can occur via atmospheric deposition, uptake from crops via soil or water but also via processing, packaging or food preparation (Domingo, 2012; Gebbink et al., 2013, Xu et al., 2013). The current literature on dietary exposure is difficult to interpret and hard to compare as the studies differ in sampling design (food basket, duplicate diet, 24h recall) and in analytical performance. If the analytical methods applied are not sensitive enough, most of the PFAAs analysed are reported as below LOQ which results in great variety between upper and lower bound (UB and LB, respectively) approaches for the exposure estimation.

In order to enable a direct comparison between European data on perfluorinated alkylated substances the European Union project PERFluorinated Organics in Our Diet (PERFOOD, FP7-KBBE, grant agreement no. 227525, www.perfood.eu) adopted a harmonised integrated approach in designing the sampling plan and applied high analytical quality and low method detection limits.

Within the PERFOOD project the present study focused on four out of fourteen collected categories, i.e. cereals, sweets (sugar and honey), salt and fruits. The PFAA concentrations and the dietary intake estimation for such food items are presented in this work. Studies by Herzke et al. (2013) and Hlouskova et al. (2013) report the results for vegetables and food items of animal origin, respectively, and, together with a detailed exposure estimation study by Klenow et al. (2013) describe the overall aims, sampling procedures and handlings within the PERFOOD project.

Materials and methods

Sampling

The sampling plan for the PERFOOD project was based on the different food consumption data from European countries whereby the regional differences in dietary items are taken into account in order to estimate the exposure of the European population to PFAAs. Therefore four countries were selected for collecting the food items i.e. Norway, the Czech Republic, Italy and Belgium representing northern, eastern, southern and western Europe according to the Country Assignments to GEMS/Food program (WHO, 2003). Sampling took place between spring and summer 2010.

The selection of food items was based on several equally important criteria described by Herzke et al. (2013): (1) relevance of the intakes for the selected European area, (2) lack of data on PFAA concentrations in food , and (3) practicability of the sampling strategy according to human, instrumental and budgetary resources available within the PERFOOD project. For the PERFOOD project, food items representing 14 categories suggested by EFSA (EFSA, 2011a) were sampled. The current paper focuses on four categories i.e. cereals, sweets (sugar and honey), salt and fruits. An overview of the sampled items in each region is given in Table 1. Items were collected and registered according to the guidance document provided by EFSA (EFSA, 2010). The individual items were randomly selected in three national retail stores covering different brands or countries of origin per item. Of each item, three to ten single lots were sampled for the preparation of the pool, depending on the availability present in the stores. If 1-3, 4-10 or more than 10 lots or brands are present in the stores, the number of lots to compose a pool should be 3, 5, 10 or >15 respectively. For the cereals and sweets at least 200 g of each selected brand was sampled. For fruits 2-4 individual items of each lot were sampled to represent one lot. For grapes and strawberries respectively, 200 and 250 g were taken. To simplify a harmonised sampling and sampling handling in all four regions, a detailed sampling manual was applied by the four partners involved in the sampling (NILU, Tromsø, Norway; Institute of Chemical Technology, Prague, Czech Republic; Istituto Superiore di Sanità, Rome, Italy; University of Antwerp, Belgium). This sampling manual was based on the Annex I to Commission

Regulation (EC) No 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs (The Annex I to Commission Regulation (EC) No 1883/2006 of 19 December 2006) but is modified according to the needs of the PFAAs analysis in food. Sampling, pooling and homogenization of the samples analysed within the PERFOOD project are also described elsewhere (Herzke et al., 2013; Hlouskova et al., 2013).

Homogenization and pooling of the samples

The purchased items were stored maximum 3 days at 4 °C before further handling. All the material used was rinsed with methanol/acetonitrile (depending on the laboratory) prior to use. The fruit items were rinsed with water (reversed osmosis water) and non-edible parts were removed with a stainless steel knife. Each lot was homogenised individually in a blender and subsamples of 100 g were taken, pooled and homogenized again to construct the pooled sample. Of this pooled sample, 2 subsamples of 100 g were taken and stored in polypropylene containers at -20 °C until shipment or extraction. The remaining of the individual lots were preserved individually for later analysis if required.

Chemicals The target analytes included 8 perfluoroalkyl carboxylic acids (PFCAs: C₆-C₁₄) and 4 perfluorosulfonic acids (PFSAs: C₄, C₆, C₈ (linear, branched and total PFOS) and C₁₀). The isotopically labelled internal standards (¹³C₂-perfluorohexanoic acid (PFHxA), ¹³C₈-perfluorooctanoic acid (PFOA), ¹³C₉-perfluorononanoic acid (PFNA), ¹³C₆-perfluorodecanoic acid (PFDA), ¹³C₇-perfluoroundecanoic acid (PFUnDA), ¹³C₂-perfluordodecanoic acid (PFDsDA), ¹⁸O₂-perfluorohexane sulfonic acid (PFHxS) and ¹³C₄- perfluorooctane sulfonic acid (PFOS)) were provided by Wellington Laboratories (Guelph, Canada). Acetonitrile and water were of HPLC-grade (Acros Organics, New Jersey, USA).

Extraction and analysis All the food items discussed in this paper were extracted and analysed in the laboratory of SPHERE (University of Antwerp). The analytical method applied to the samples was based on a modification of the method described by Young and Tran (2006). Briefly, 10 g homogenate was spiked with internal standard mixture before adding 10 mL of 10 mM methanolic KOH (Merck, Darmstadt, Germany). After sonication (10 min), shaking (16 hrs) and centrifugation, the supernatant was transferred and diluted to 20 mL with water. The pH was adjusted to 4-5 with 1/1 (v/v) formic acid/water. This solution was then loaded on an Oasis Wax cartridge (3 cc, 60 mg, Waters). Elution was performed with 2 mL 1% NH₄OH in acetonitrile. Before injection, 70 µL of the eluent was mixed with 130 µL of HPLC grade water (more details see SI). Analysis was performed using an ACQUITY UPLC coupled to a tandem quadrupole mass spectrometer (ACQUITY, TQD Waters, USA) with an electrospray interface operating in negative ion mode (ESI-MS/MS). Separation was performed on an ACQUITY BEH C18 column (2.1 x 50 mm; 1.7 µm, Waters, USA) kept at 60 °C fitted with a VanGuard Pre-column (2.1 mm x 5 mm, Waters). A pre-column (ACQUITY BEH C18 column 2.1 x 30 mm; 1.7 µm, Waters, USA) was inserted between the solvent mixer and the injector to delay any PFAs originating from the UPLC system in their retention time. For details regarding the mobile phases, conditions and run time see SI. Acquisition was performed in the multiple reaction monitoring (MRM). Transitions (precursor ion (m/z) → product ion (m/z)) used for determination are given in Table SI-1.

Calibration Non-labelled standards of all the target analytes were used to construct ten-level calibration curves ($r^2 > 0.99$) encompassing the entire linear range (0.0125 till 16 ng/mL). Internal standards were added to the samples prior to extraction and the same amount of these internal standards was added to each calibration point. Each PFAA was quantified using the corresponding internal standard with the exception of PFBS, PFDS, PFTrDA and PFTeDA of which no labelled standards were available. PFBS and PFDS were quantified using ¹⁸O₂PFHxS and ¹³C₄-PFOS

respectively, whereas for, both PFTrDA and PFTeDA, $^{13}\text{C}_2$ -PFDoDA was used. Results are corrected for matrix effect and recovery was based on the response (area) of the internal standards. The concentration of the sulfonates are based on the ion, not on the salt. The sum of the branched isomers of PFOS and the linear PFOS were quantified separately by using a technical PFOS mixture as an external calibration standard.

Quality Control. The quality control was performed by regular analysis of procedural blanks (one in each batch of 10 samples) in which minor contamination (< 0.2 pg/ μL) of PFHxA, PFHxS, PFOA and PFOS was observed. These levels were subtracted from the correspondent concentrations found in the samples. Furthermore, in each batch a spiked mixture of banana, apple and orange as reference material was included, since standard reference materials for PFAAs were not available commercially. The accuracy of the applied method, based on 6 replicates of the spiked fruit mixture, varied between 79-124% and the precision between 2-15% for all PFAAs. LOQs were calculated as 10 times signal to noise ratio and ranged from 1 pg/g to 40 pg/g. The mean recoveries were 80% for PFHxS, 68% for PFOS and ranged from 67 to 106% for the PFCAs for all the samples analysed. Within the PERFOOD project, three rounds of intercalibration exercises were arranged in order to monitor the performance of the laboratories involved (n=7). Furthermore the analytical quality of the laboratory has been approved through regular participation with satisfactory results in interlaboratory studies on PFAAs in environmental samples (van Leeuwen et al., 2009; Weiss et al., 2013).

Dietary intake

The dietary intake of PFAAs through the consumption of cereals, sweets and fruit was estimated by multiplying the individual PFOA and PFOS concentrations by the consumed amount per day for adults divided by mean bodyweights of 75, 66, 73 and 71kg for the Czech Republic, Italy, Norway and Belgium respectively (EFSA, 2009; 2011b). No intake assessment was evaluated for the other PFAAs, since the detection frequency was below or equal to 20%. As the authors expected a low

contribution of these food items to the dietary exposure, the 95th percentile of the consumption data were used to include possible risks for high consumers. Details on the consumption data are provided in Table SI 3. When possible, the respective food items of each country were used for the assessment. For exotic- and citrus fruits which are (mostly) imported from southern European countries or non-European countries the overall mean of the concentrations from the four countries was taken for each region (same PFAAs levels for each of the four regions). The UB approach has been used whereby concentrations below LOQ are replaced by the LOQ itself. It is important to highlight that the UB approach in combination with the 95th consumption data, the almost highest possible exposure estimation via dietary intake of fruit/sweets/cereals has been estimated. Consequently, the dietary intake of PFOA and PFOS through the consumption of these food groups the general population will be lower.

Results and Discussion

Thirteen different PFAAs were determined in fruits and other food groups (other FG: cereals, sweets and salts). In general, the PFAA concentrations in all analysed food items were low and ranged from <LOQ to 539 pg/g. An overview of the range, median, mean and detection frequency of the individual analytes and the Σ PFCAs (sum of PFCAs (C_6-C_{14})), Σ PFSAs (sum of PFSAs (C_{6-10})) and Σ PFAAs (Σ PFCAs and PFSAs) is given in Table 2. The concentrations in the individual items are displayed in Table SI 2 of the supporting information. The detection frequency of PFCAs (65%) was almost twice as high as that of PFSAs (35%). The main contributors to Σ PFCAs and Σ PFSAs were PFOA and PFOS, respectively. Overall, PFOA was also the most abundant compound (detection frequency of 49%) followed by PFOS (29%). PFHxA and PFNA were detected in 20% of the samples. PFHpA, PFDoDA, PFTeDA and PFDS were only sporadically detected and PFUnDA and PFTrDA were not found in any of the samples. The detection rates in the fruits were in general higher compared to the other FG with the exception of PFDA (4%), PFHpA (0%) and PFDS (0%). The highest concentrations were found for PFOS (539 pg/g) followed by PFNA, PFHxS, PFHpA, PFHxA and PFOA which all showed similar maximum

concentrations of approximately 200 pg/g. The maximum concentrations of PFDA, PFBS and PFDS were all below 100 pg/g and for PFDaDA and PFTeDA even below 10 pg/g.

Comparison of the detection frequencies of PFCAs and PFSAs between the different countries (Table 2) revealed an equally detection in the Czech Republic (62 and 31%), Italy (62 and 31%) and Norway (60 and 30 %) but a higher in Belgium (70 and 46%).

The mean Σ PFAA concentrations per food category for the four sampling countries are displayed in Figure 1. It is important to keep in mind that the composition of each food category differs between the different countries. For example, the category cereals for Czech Republic consists of wheat and rye samples, whereas for Belgium this category consists of rice, wheat and oats (Table 1). Nevertheless, the highest levels were found in Belgium, followed by the Czech Republic ~ Italy and finally Norway. Overall, the levels in the fruit items were higher compared to the other FG with the exception of two wheat samples from Italy and Belgium and the sweets collected in Belgium (Table SI 2). Within the fruit category pipe and stone fruit showed higher concentrations compared to the other analysed fruits and will be discussed further on. Exotic fruits such as bananas, melons and grapes were not sampled in each country as the origin of these fruits is similar throughout Europe. The concentrations shown for exotic fruits in Figure 1 are thus representative for Europe in general. In Italy, where only bananas and grapes were sampled, PFAA concentrations were <LOQ, except for PFNA, PFHxS and PFOS in bananas (3,8 and 7 pg/g, respectively, Table SI 2). On the contrary, in the grapes collected in Belgium PFOA, PFNA and PFOS were found (7, 7 and 37 pg/g respectively, Table SI 2). Overall, PFOS showed the highest concentrations in fruit samples: 539 pg/g in pears from the Czech Republic and 320 pg/g in apples from Belgium. Only apples and pears were sampled in the four countries allowing a direct comparison of the concentrations. From Figure 2 it is clear that apples collected in Belgium showed the highest concentrations of Σ PFAAs, followed by Italy, Norway and the Czech Republic. For pears, the order of PFAA concentrations per region was different whereby the ones from the Czech Republic >> Belgium ~ Italy > Norway. For the interpretation of the results it

is important to note that the fruit items were purchased in local retail stores and therefore not necessarily originated from the sampling countries. However, the Italian apples only contained samples cultured in Italy itself and thus are also representative for the levels of PFAAs in Italy. The difference observed between apples and pears sampled in the same country could be explained by the difference in origin of the samples as within a country similar ways of contamination of apples and pears could be expected.

In Figure 3 the differences in PFAA profiles in apples and pears from the sampled countries are shown. A similar profile in the four countries related to PFCAs in apples was observed (PFHxA ≈PFOA>PFNA) with the exception of Norway where no PFNA was detected. The Italian and Belgium apples had also a similar profile related to PFSAs (PFOS>PFHxS>PFBS) which indicate similar sources for PFCAs and PFSAs. Norwegian apples showed a slightly different profile as no PFNA, PFBS or PFHxS were detected. This could probably be explained by the fact that the levels of the compounds detected were lower compared to the other countries and therefore only the most dominant compounds were detected above the LOQ (PFHxA, PFOA and PFOS). In pears, no similarities in the profiles could be observed.

Although in recent years the literature on PFAAs in food increased, data on PFAAS in fruit, cereals, and other food items that are consumed in low quantities (such as sweets and salt) remain scarce (Cornelis et al., 2012; Ericson-Jogsten et al., 2009; Noorlander et al., 2011; Vestergren et al., 2012b). Vestergren et al. (2012b) estimated the dietary exposure to PFAAs in different food items purchased in Sweden. The individual items were pooled into different food categories and included fruit, sugar and sweets and cereal products. PFOA and PFOS concentrations in fruits from the Swedish study ranged from 7.8 to 15 pg/g and 1.9 to 4.4 pg/g respectively. The mean PFOA level found in the fruit samples of the present study (12.2 pg/g) is in good agreement with the correspondent levels in fruits from the Swedish study. On the contrary the mean PFOS concentration (50.6 pg/g) is higher and this is due to the contribution of some fruit items from the Czech Republic and Belgium. For PFHxA and

PFNA, the concentrations of the Swedish study were five times lower compared to the mean concentrations found in the current study. In the cereal products (flour, grain, corn flakes, pasta and bread) Vestergren et al. (2012b) detected PFHxA, PFHpA, PFOA and PFOS concentrations ranging from 2.2 to 62 pg/g. but only for PFOA concentrations > 11 pg/g were found. In the current study PFHpA and PFOA are found in similar concentrations (mean 16.6 and 4 pg/g respectively). It should be noted that in our study PFHpA was detected in only one wheat sample (183 pg/g). In the sweets of the Swedish study quantifiable concentrations for PFOS (3.6-4.2 pg/g) and PFOA (13-47 pg/g) were reported. The mean PFOS concentration in the sweets analysed in this study is similar (6.8 pg/g) but PFOS was found in only one sample (honey collected in Belgium). The mean PFOA concentration in the EU sweets is lower (2.3 pg/g) compared to what was found in Sweden. The higher levels of PFOA observed in the Swedish study could possibly be explained by the fact that the sweets from the Swedish study also contained processed food (such as chocolate, sauces and sugar sweets) while the sweets of the current study only contained white and brown sugar as well as honey. It is known that food processing can contaminate food via the use of materials that contain polytetrafluoroethylene (PTFE), better known as Teflon®. Also food packaging material (wraps, papers, containers) could be a potential source as PFAAs have the potential to migrate from the package material into food (Begley et al., 2008). Cornelis et al. (2012) determined the total human exposure to PFOS and PFOA in Belgium by using, inter alia, PFAAs levels in fruit and cereals. The concentrations used for the assessment were based on two studies: the study itself in which the analyzed food items were all locally cultivated in Belgium and the results of the study of Ericson et al. (2008) who analyzed food items randomly purchased in Spain. For PFOS and PFOA, average concentrations of 350 and 430 pg/g were used for fruit and 52 and 55 pg/g for the cereal products. These levels are approximately an order of magnitude higher compared to the levels measured in the current study but are in good agreement with some of the levels detected in the samples collected in Belgium and the Czech Republic. Finally, Noorlander et al. (2011) estimated the dietary intake of PFAAs for the Dutch population. To this PFAAs were analyzed in 15 food categories which were purchased in retail stores

in the Netherlands. In the category 'flour' eight out of 14 PFAAs were detected (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFHxS) with concentrations ranging from 4 to 17 pg/g. For some compounds and/or flour samples, current LOQs were high (20 or 40 pg/g) which can explain the higher detection frequency in the Dutch study. However, current mean PFOA and PFDA concentrations of the flour samples ($n=8$) of 5.5 and 5.4 pg/g are in the same line as the levels found by Noorlander et al. (17 and 9 pg/g). In the flour sample collected in Italy a concentration of 183 pg/g for PFHpA was detected but the mean of all the wheat samples (23 pg/g) in the present study is comparable to the concentrations of 14 pg/g found in the study of the Netherlands. Unfortunately, Noorlander et al. (2011) did not distinguish between fruit and vegetables as these were pooled within one category. It consisted of 39% fruit (apple, orange, grape and banana) but the majority of the pooled sample were vegetables which makes it difficult to compare with the fruits in our study. Nevertheless, 4 PFAAs were detected including PFBA, which was not included in the present study, PFOA, PFNA and PFDA with concentrations of 130, 5, 1 and 2 pg/g respectively. The levels of PFOA, PFNA and PFDA are 3-10 times higher compared to these in the present study (mean 12.2, 10.5 and 0.8 pg/g respectively). To the best of our knowledge, only one study analyzed PFAAs in salt but none of the 11 analyzed PFAAs could be detected in the common salt acquired in local stores in Tarragona Country, Spain (Ericson-Jogsten et al., 2009).

Dietary intake

The mean dietary intake from the four countries of the three food categories for high consumers (95th percentile consumption data) equaled 0.35 ng/kg bw/day and 1.00 ng/kg bw/day for PFOA and PFOS respectively. For PFOA the highest intake was observed in Belgium (0.65 ng/kg bw/day) which was almost twice as high as in Italy (0.39 ng/kg b/day) and three times higher than in the Czech Republic (0.23 ng/kg bw/day) and Norway (0.15 ng/kg bw/day). For PFOS the highest intake was again observed in Belgium (1.75 ng/kg bw/day), followed by the Czech Republic (1.12 ng/kg bw/day), Italy (0.86 ng/kg bw/day) and Norway (0.27 ng/kg bw/day). These values remain far below the

established tolerable daily intakes (TDIs) suggested by EFSA of 1500 and 150 ng/kg bw/day for PFOA and PFOS respectively (EFSA, 2008). In the present study, the highest intake of both PFOA and PFOS was observed in Belgium but these intakes equal only 0.04 % and 1.16% for the respective TDIs. This indicates that the consumption of fruit, cereals and sweets will not cause immediate health effects although they do contribute to the total amount of PFAAs in our diet. Several studies and EFSA reported that the most contaminated matrices in the human diet were fish, seafood and pig/bovine liver (EFSA, 2012; Haug et al., 2010; Hlouskova et al., 2013; Noorlander et al., 2011).

Within the PERFOOD project, Klenow et al. (2013) assessed the total dietary exposure in the four countries for seven PFAAs (PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS) and for two age classes (adults and children), including the data of the present study. Fruits and vegetables dominated the dietary intake of PFAAs in almost all four countries but differences with respect to the food groups and PFAAs could be found. Klenow et al. (2013) described which food categories contribute the highest to the total dietary intake of PFAAs and fruits had a high impact on the total dietary intake of PFAAs. For PFHxA, fruits contributed for 39, 58, 60 and 20% in the Czech Republic, Italy, Norway and Belgium respectively. For PFOA, the contribution of fruits equaled 66% to the total dietary intake in the Czech Republic (12% in Italy and 26% in Belgium). For the dietary intake of PFNA, fruit contributed 46, 30 and 68% in Italy, Norway and Belgium, respectively. For PFHxS, the contribution in Italy was as high as 93% and 68 and 76% in Norway and Belgium respectively. For PFOS, fish and seafood contributed more to the total dietary intake compared to fruit in the Czech Republic, Italy and Norway. However, for Belgian children the contribution of fish and seafood was less compared to the fruit (46%). All these findings were based on the LB approach in the study of Klenow et al. (2013). For the UB estimates, the consumption of foods of plant origin (fruits and vegetables) contributed significantly to the dietary exposure to all PFAAs in all four regions (46-76% and 55-87% for adults and children respectively). Vestergren et al. (2012b) determined a contribution of approximately 17% for fruit to the total dietary intake for PFHxA, and 7-8% for PFOA and PFDA in Sweden, whereas the contribution of the cereals to the total dietary intake accounted for more than

25% for PFHxA and PFOA and approximately for 55% for PFDA. The study of Vestergren et al. (2012b) clearly showed that although the levels in cereals and fruits are relatively low, the contribution of these items to the total dietary intake of PFAAs can be substantial. Unfortunately, many other studies on dietary intake did not include fruits and/or cereals. Probably this exclusion can be explained by the lack of appropriate analytical methods until recently to detect the low levels present in the respective food categories. In the last five years, several extraction procedures were described in literature (Ballesteros-Gómez et al., 2010; Ullah et al., 2012; Vestergren et al., 2012a) that allow detection of ultra-low levels in food items and therefore it is strongly recommended to include these items in future dietary intake assessments for PFAAs.

Conclusion

Overall, the consumption of cereals, sweets and fruit will not cause any direct health risk associated with PFAAs for the general European population. However, the contribution of fruit to the total dietary intake of PFAAs can play a major role depending on the region and the studied PFAA. For PFOA and PFOS, fruit contributes about 12-66 % to the total dietary intake whereas for other compounds the contribution of fruit can even be higher (up to 93% for PFHxS, Klenow et al., 2013). Given the variety between the countries and the detected levels in combination with the high consumption of these items it is important to include these food categories in dietary intake assessments. To reveal the origin of the contamination of the studied food items, more research on the PFAAs uptake of plants and the origin of these items is necessary and underway (Felizeter et al., 2013).

Acknowledgments

This work was supported by the FP7 European Union project PERFOOD (PERFluorinated Organics in Our Diet) under Grant number KBBE-227525 and by the Federal Public Service of Health, Food Chain Safety and Environment (contract RF6181)..

References

- Ballesteros-Gómez A., Rubio, S., van Leeuwen, S., 2010. Tetrahydrofuran–water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low pictogram per gram level. *J Chromatogr A* 1217:5913–21.
- Begley, T.H., Hsu, W., Noonan, G., Diachenko, G., 2008. Migration of fluoroochemical paper additives from food-contact paper into foods and food simulants. *Food Addit Contam A* 25: 384–390.
- Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P.J., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess Manag* 7:513-541.
- Cornelis, C., D'Hollander, W., Roosens, L., Covaci, A., Smolders, R., van den Heuvel, R., Govarts, E., van Campenhout, K., Reynders, H., Bervoets, L., 2012. First assessment of population exposure to Perfluorinated compounds in Flanders, Belgium. *Chemosphere* 86: 308–314.
- Domingo, J.L., 2012. Health risks of dietary exposure to perfluorinated compounds. *Environ Int* 40: 187-195.

D'Hollander, W., de Voogt, P., de Coen, W., Bervoets, L., 2010. Perfluorinated substances in human food and other sources of human exposure. *Rev Environ Contam Toxicol* 208: 179–215.

EFSA (European Food Safety Authority), 2008. Opinion of the scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. *EFSA J* 653: 1–131.

EFSA (European Food Safety Authority), 2009. EFSA concise European food consumption database: general principles for the collection of national food consumption data in the view of a pan-European dietary survey. *The EFSA J* 7: 14325.

EFSA (European Food Safety Authority), 2010. Standard sample description for food and feed. *EFSA J*: 8: 1457-1511. doi:10.2903/j.efsa.2010.1457. Available online: www.efsa.europa.eu

EFSA (European Food Safety Authority), 2011a. The EFSA Comprehensive European Food Consumption Database [Internet]. [cited 2013 May 15]. Available from: <http://www.efsa.europa.eu/en/datexfoodcldb/datexfooddb.htm>

EFSA (European Food Safety Authority), 2011b. Use of the EFSA comprehensive European food consumption database in exposure assessment. *EFSA J*: 9: 2097-2030.

EFSA (European Food Safety Authority), 2012. Perfluoroalkylated substances in food: occurrence and dietary exposure. *EFSA J* 10:2743.

Ericson, I., Martí-Cid, R., Nadal, M., Van Bavel, B., Lindström, G., Domingo, J.L., 2008. Human exposure to perfluorinated chemicals through the diet: intake of perfluorinated compounds in foods from the Catalan (Spain) market. *J. Agric. Food Chem* 56: 1787–1794.

Ericson-Jogsten, I., Perelló, G., Llebaria, X., Bigas, E., Martí-Cid, R., Kärrman, A., Domingo, J.L., 2009. Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. *Food Chem Toxicol* 47: 1577–83.

Eschauzier, C., de Voogt, P., Brauch, H.-J., Lange, F.T., 2011. Polyfluorinated chemicals in European surface waters, ground- and drinking waters. *Handbook of Environ Chem*, 73-102.

Felizeter, S., McLachlan, M.S., de Voogt, P., 2013. Uptake of Perfluorinated Alkyl Acids by Hydroponically Grown Lettuce (*Lactuca sativa*). *Environ Sci Technol* 46: 11735–11743.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds: exposure assessment for the general population in Western countries. *Int J Hyg Environ Health* 212: 239–70.

Gebbink, W.A., Ullah, S., Sandblom, O., Berger, U., 2013. Polyfluoroalkyl phosphate esters and perfluoroalkyl carboxylic acids in target food samples and packaging-method development and screening. *Environ.Sci. Pollut. Res* 20: 7949-7958.

Haug, L.S., Salihovic, S., Ericson-Jogsten, I., Thomsen, C., van Bavel, B., Lindström G., Becher, G., 2010. Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere* 80: 1137-1143.

Herzke, D., Huber, S., Bervoets, L., D'Hollander, W., Hajlova, J., Pulkrabova, J., Brambilla, G., De Filippis, S. P., Klenow, S., Heinemeyer, G., de Voogt P., 2013. Perfluorinated alkylated substances in vegetables collected in four European countries; occurrence and human exposure estimations. *Environ Sci Poll Res* 20: 7930-7939.

Hlouskova, V., Hradkova, P., Poustka, J., Brambilla, G., De Filippis, S.P., D'Hollander, W., Bervoets, L., Herzke, D., Huber, S., de Voogt, P., Pulkrabova, J., 2013. Occurrence of perfluoroalkyl substances (PFASs) in various food items of animal origin collected in four European Countries. *Food Add Contam A* 30: 1918-1932.

Kissa, E., 2001. Fluorinated surfactants and repellents, vol 97, 2nd edition. Marcel Dekker, New York

Klenow, S., Heinemeyer, G., Brambilla, G., Dellatte, E., Herzke, D., de Voogt, P., 2013. Dietary exposure to selected Perfluoroalkyl acids (PFAAs) in four European regions. *Food Addit Contam A* 30: 2141-2151.

Noorlander, C.W., van Leeuwen, S.P.J., Biesebeek, J.D.T., Mengelers, M.J.B., Zeilmaker, M.J., 2011. Levels of perfluorinated compounds in food and dietary intake of PFOS and PFOA in The Netherlands. *J Agric Food Chem* 59: 7496–7505.

Ullah, S., Alsberg, T., Vestergren, R., Berger, U., 2012. Determination of perfluoroalkyl carboxylic, sulfonic, and phosphonic acids in food. *Anal Bioanaly Chem* 404: 2193-2201.

Ullah, S., Huber, S., Bignert, A., Berger, U., 2014. Temporal trends of perfluoroalkane sulfonic acids and their sulfonamide-based precursors in herring from the Swedish west coast 1991-2011 including isomer specific considerations. *Environ Int* 65:63-72.

van Leeuwen, S.P.J., Swart, K., van der Veen, I., de Boer, J., 2009. Significant improvements in the analysis of perfluorinated compounds in water and fish: results from an interlaboratory method evaluation study. *J. Chromatogr A* 1216: 401–409.

Vestergren, R., Cousins, I.T., Trudel, D., Wormuth, M., Scheringer, M., 2008. Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. *Chemosphere* 73: 1617–24.

Vestergren, R., Cousins, I.T., 2009. Tracking the pathways of human exposure to perfluorocarboxylates. *Environ. Sci Technol* 43: 5565–5575.

Vestergren, R., Ullah, S., Cousins, I.T., Berger, U., 2012a. A matrix effect free method for reliable quantification of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids at low parts per trillion levels in dietary samples. *J Chromatogr A* 1237:64–71.

Vestergren, R., Berger, U., Glynn, A, Cousins, I.T., 2012b. Dietary exposure to perfluoroalkyl acids for the Swedish population in 1999, 2005 and 2010. Environ Int 49: 120-127.

Weiss, J.M., van der Veen, I., van Leeuwen, S.P.J. , Cofino, W., Crum, S., de Boer, J. ,2013. Analytical improvements shown over four interlaboratory studies of perfluoroalkyl substances in environmental and food samples. Trends Anal Chem 43: 204-216.

WHO (World Health Organization), 2003. GEMS/FOOD - Regional per capita consumption of raw and semi-processed agricultural commodities. [Internet]. [cited 2014 Jul 28]. Available from:
http://www.who.int/foodsafety/chem/en/gemsRegional_diet.pdf?ua=1

Xu, Y., Noonan, G. O., Begley, T. H., 2013. Migration of perfluoroalkyl acids from food packaging to food simulants. Food. Addit. Contam.A 30: 899-908.

Young, M.S., Van Tran, K., 2006. Oasis Wax® sorbent for UPLC™/MS for determination of PFOS and related compounds in water and tissue. Application note waters, available on
<http://www.waters.com/webassets/cms/library/docs/720001817en.pdf>

Figures and Tables

Table 1: Overview of the number of individual items collected to compose a pooled sample in the Czech Republic, Belgium, Norway and Italy for cereals, sweets and fruit and the number of pooled samples (n) that were analyzed for the representative items.

Representative part Sampling Country	EAST EU Czech Rep.	WEST EU Belgium	NORTH EU Norway	SOUTH EU Italy	n
Cereals					
Rice	-	10	-	4	2
Wheat (white)	3	5	13	5	4
Oats	-	3	-	9	2
Rye	9	-	-	-	1
Sweets					
Sugar (beet)	3	5	-	3	3
Sugar (cane)	-	-	5	-	1
Honey	15	10	17	3	4
Fruits					
<i>Berries</i>					
Strawberries	3	3	2	-	3
<i>Citrus fruit</i>					
Oranges	3	10	5	-	3
Tangerines	3	-	-	-	1

Lemons	-	3	6	-	2
Grapefruits	-	-	-	2	1
<i>Pipe and stone fruit</i>					
Apples	15	5	10	6	4
Pears	3	3	2	4	4
Peaches	3	-	-	9	2
Plumes	-	5	-	9	2
<i>Others and exotic fruit</i>					
Melons	3	-	9	-	2
Grape	-	5	-	5	2
Bananas	-	-	-	6	1
<u>Misscellaneous</u>					
"Rock" salt	9	-	13	-	2
"Marine" salt	-	-	-	4	1

-; Not sampled