2. PERFOOD Scientific and Technological Results

2.1 WP1 Development of tools for reliable analysis of PFASs in diet

Introduction

WP1 focused on the development of analytical tools to improve the performance of PFAS determination in food and drinking water, i.e. to lower detection limits and improve precision and accuracy of reported data. Trace analysis of PFASs in complex samples has proven to be challenging, and the quality of reported data is still a major issue of concern [5]. Challenges include the lack of high quality standards (e.g. mass labeled, linear or branched), background contamination, chromatographic interferences, and poor recoveries. Fully validated, sensitive analytical methods and regular interlaboratory comparison studies are important tools to identify problems and to stimulate improvements within the analytical field.

Prior to the PERFOOD project, PFAS analysis in food mostly focused on PFOA and PFOS, and did not take any other homologues or precursors to PFAAs into account. The main objective of WP 1 was therefore to develop robust, easy-to-use, yet very sensitive methods for quantification of a suite of fluorinated organics including non-persistent precursors in a wide variety of food and beverage items. Pre-PERFOOD, method detection limits in analysis of PFASs in food or biota were typically in the range of 0.1 to 1 ng/g wet weight (ww). This was by far not low enough for reliable quantification of a suite of PFASs in different food items. Typical levels of PFASs in common high-consumption food items are in the low pg/g ww or even fg/g ww range [6]. Therefore, WP1 targeted at developing methods with detection limits that were two to three orders of magnitude lower than what could be achieved before. In addition to sensitivity, the development was also focused on the suitability of the methods for routine analysis.
Due to the protein binding capacity and fairly good water solubility of several of the PFAAs, both solid foods and beverages may be sources of human exposure. Consequently, the target matrices for method development included protein- and lipid-rich food items (e.g. fish, meat, dairy), fiber- and water-rich food items (e.g. vegetables) and food composites/food duplicates, as well as drinking water (tap water).

Method development

Seven different sample preparation methods have been developed and validated in the laboratory of the Department of Applied Environmental Science (ITM), Stockholm University, to cover the broad range of target analytes (both PFAAs and their precursors) as well as virtually all types of food and beverage samples. The method protocols are given in Annex 1. Additionally, four different instrumental methods were developed for separation, detection and quantification of the PFASs in the sample extracts (also given in Annex 1). The instrumental methods can be freely combined with the sample preparation methods for corresponding analytes. Furthermore, a range of additional methods were developed by other PERFOOD partners [7-10]. However, these methods are not discussed here, as their application ranges greatly overlap with the methods developed at ITM.
Sample preparation methods for PFAAs – The main objective of method development for PFAAs was to achieve a highly sensitive and accurate analytical method for the compounds of most concern, i.e. the PFCAs and PFSAs. To achieve method quantification limits in the single digit to sub pg/g range, a high sample-to-extract concentration factor was necessary. Such a high concentration factor requested a rigorous extract clean-up, in order to eliminate matrix effects and in order to guarantee broad applicability of the method to a vast variety of food items (including beverages). This approach led to method 1 (Annex 1), which is unique in terms of applicability range, sensitivity and accuracy [11]. Figure 2.1.1 shows the chromatograms obtained using method 1 and instrumental method A (Annex 1) for the internal standards spiked at 1 pg/g to a duplicate diet sample. The drawbacks of method 1 are that it is time consuming and does not include PFPAs. Therefore, two multi-chemical methods including PFCAs, PFSAs and PFPAs were developed that can be routinely applied to food items (method 3, Annex 1) or drinking water (method 4, Annex 1), respectively. The methods are easy to use, robust, sensitive and accurate. The breakthrough for successful inclusion of PFPAs in the methods was the use of a mixed-mode SPE column containing a quaternary amine function in combination with the introduction of 1-methyl piperidine as modifier in sample preparation as well as in the instrumental method. Detailed discussions of methods 3 and 4 are published elsewhere [12, 13]. For analysis of PFCAs and PFSAs in samples of animal origin (eggs, meat and fish), for which ultra-trace sensitivity is not required, a simple and quick method has further been developed (method 2, Annex 1) [14]. The only food matrix encountered so far that could not be analyzed satisfactorily with any of the methods 1-4 is cow's milk. Therefore, method 5 (Annex 1) for PFCA and PFSA analysis in milk was developed. It is a modification of a method originally developed for analysis of human serum [15]. The method allows for detection limits in the single digit pg/g range.

In summary, the developed analytical methods for PFAAs represent the following significant improvements compared to methods published pre-PERFOOD:

- Significantly lower detection limits
- Matrix-effect free analysis, resulting in highly accurate results
- Multi-chemical methods including PFPAs and a large range of PFAA homologues
- Broader applicability to a variety of food items and food composites (excluding or including beverages)

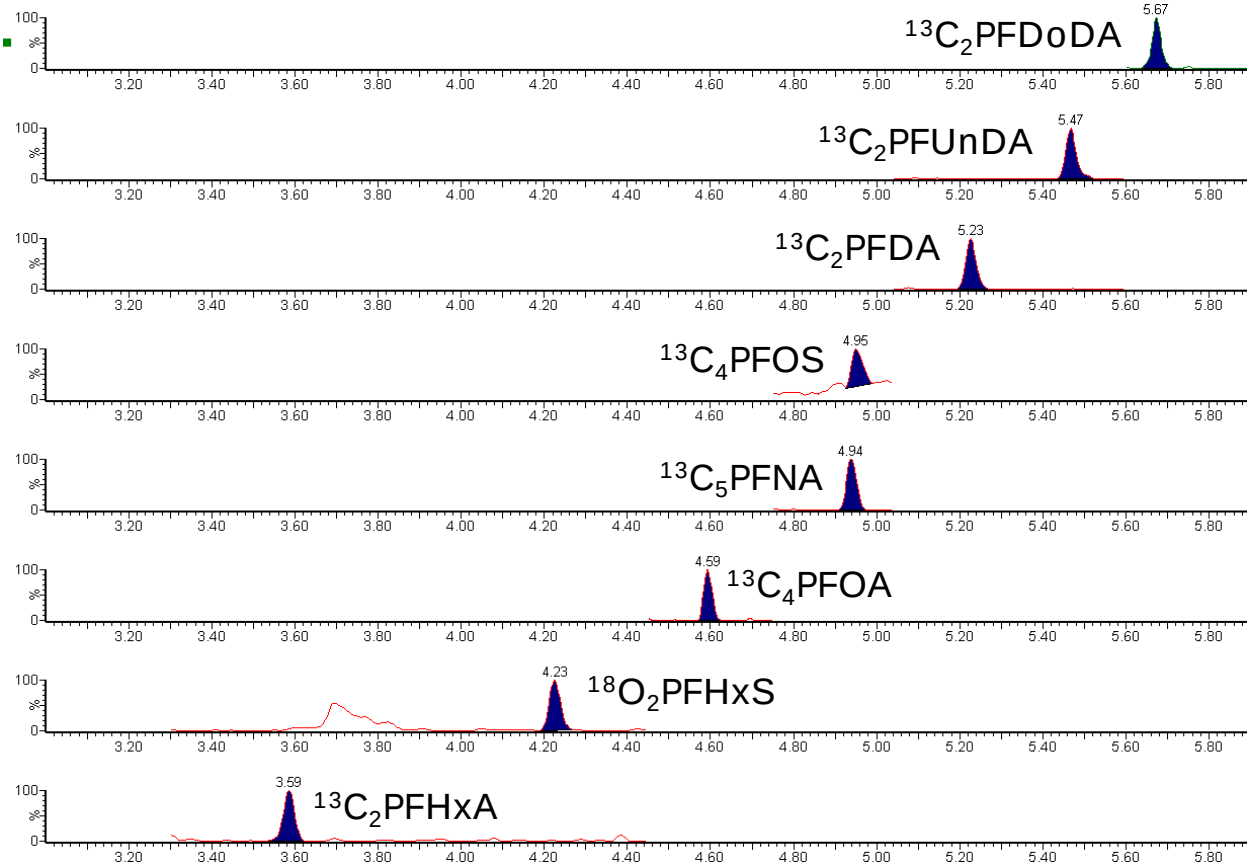

Figure 2.1.1. Extracted MS/MS chromatograms of the internal standards spiked at 1 pg/g to a duplicate diet sample. For more details see [11].

Quality assurance for short-chain PFCAs – During the first round of food sample monitoring within the PERFOOD project it became apparent that the quality assurance in the analysis of short-chain PFCAs, especially PFBA, was not sufficient using conventional analytical methods based on LC/MS/MS. This was due to the poor retention of the short-chain PFCAs eluting shortly after the dead-time, the consequently poor chromatographic resolution, the relatively low and thus unspecific m/z values monitored and the unspecific fragmentation used in the MS/MS transitions (loss of CO_2). This all led to a relatively high risk of false positives or overestimations due to interferences. Therefore, different MS techniques (MS/MS and HRMS) as well as different chromatographic systems were tested in order to improve the reliability of results for short-chain PFCAs at trace levels. Several food samples were identified in which an unknown interference would be misinterpreted as PFBA using a conventional reversed phase column and low resolution MS/MS detection. However, both the use of HRMS as well as the use of a chromatographic system with greater retention of short-chain PFCAs proved to be suitable to discriminate between PFBA and the interference. These techniques are recommended to use in order to qualify positive detections of short-chain PFCAs in food matrices.

Sample preparation methods for PFAA precursors – The main objective of method development for PFAA precursors was to achieve highly sensitive, semi-quantitative methods for a first screening of PFAA precursors in food. Two methods were developed. Method 6 (Annex 1) allows for simultaneous analysis of PFSAs and a multitude of PFSA precursor compounds in fish. It is a modification of a method described elsewhere [16]. The modification comprises the inclusion of FASAAs and a significant improvement in sensitivity for most analytes. Method 7 (Annex 1) describes the analysis of FTOH-based polyfluoroalkyl phosphate mono-, di- and tri-esters (PAPs, PFCA precursors) in food items and in drinking water. The method represents a big step forward in PAPs analysis in terms of sensitivity and reliability, as it is the first analytical method for PAPs including a clean-up step for the crude extract [17]. In summary, the two

methods for PFAA precursor analysis allow for screening of a variety of the most important precursor compounds at trace levels (single digit to sub pg/g) in food items and drinking water (only PAPs).
Instrumental methods – Method A for instrumental analysis of PFCAs and PFSAs by UPLC/tandem MS (Annex 1) is the method of choice for routine analysis of these compounds in sample extracts [11]. It combines high sample throughput (total run time 10 min) with excellent chromatographic performance (e.g. sharp peak for PFBA and baseline separation of the sum of branched PFOS isomers from linear PFOS) and unexcelled sensitivity (instrumental detection limits in the absence of background contamination between 5 and 50 fg on column). For multi-chemical analysis of PFCAs, PFSAs and PFPAs, Method B (Annex 1) based on HPLC/HRMS was developed [12]. This is the first method that overcomes the main challenges in instrumental PFPA analysis (poor chromatographic resolution and poor detector sensitivity) through introduction of 1-methyl piperidine as modifier in the HPLC mobile phase. Method C (Annex 1) is a modification of method A that allows the inclusion of a number of PFSA precursors in UPLC/tandem MS analysis. The drawback of method C is the relatively poor sensitivity of ESI neg-MS for FASEs. Alternatively, these compounds can be analyzed by GC/PCI-MS with significantly lower instrumental detection limits. Finally, method D (Annex 1) was developed for simultaneous determination of mono, di and triPAPs by UPLC/MS/MS. This method shows excellent sensitivity, reaching instrumental detection limits as low as 5 fg on column for 6:2 diPAP [17].
Application of DART-MS techniques in analysis of PFASs in food contact materials – The potential of DART–HRMS was tested for the analysis of a standard mixture of PFCAs and PFSAs. Detection limits were 1000 times higher than what is typically achievable by HPLC/MS/MS, thus DART is not suitable for targeted trace analysis of PFASs. However, it can be used for the rapid screening of PFASs in treated products. In the negative ion mode (DART(–)) mass spectra of ions with mass difference of 99.99 Da were recorded for PFAS blends, which relate to CF_2CF_2 units. Direct examination of paper samples used in food packaging and baking using DART(–)–HRMS revealed that 16 out of 31 examined samples contained PFASs. The positive samples were wraps and boxes from fast food chains, paper muffin baking cups, and boxes for popcorns from cinemas and bags for microwave popcorn. DART–HRMS is thus a new technique with a high potential for rapid, direct screening of compounds present on a sample surface of treated products.

2.2 WP2 Development of tools II: Quality assurance/Quality control

Introduction

The second work-package within the PERFOOD project focused on validation of the analytical determination in food products. There is no legislative framework available for the validation of PFAS methods specifically. Moreover, there are no set requirements that methods for PFASs in food should meet. Nevertheless, there are several documents available on which the validation of the analytical methods was based {Currie, 1999 #14; Eurachem, 1998 #15; ISO5725-1, 1994 #17; ISO11843-2, 2000 #16; Vessman, 2001 #18}. The poor quality of PFAS data obtained was reflected in the unsatisfactory results obtained in the 1st interlaboratory study (ILS) conducted in 2004/2005 on human and environmental matrices {Van Leeuwen, 2006 #10}. As a consequence of this initial indication of the status regarding analytical performance for PFAS analysis in the environment, several ILSs were organized with different design and goals of the studies {Van Leeuwen, 2011 #3; Van Leeuwen, 2009 #12}. In general, due to an increase of available high quality and mass labeled standards and improved awareness of the behavior of the PFASs, an improvement could be seen over the years the ILSs were arranged. However, no ILSs had been performed at the low PFAS levels encountered in food.

Interlaboratory exercises

The validation of the analytical performance of the participating laboratories within PERFOOD was performed in 2 phases. Phase 1 primarily focused on the calibration of the instrument, precision and selectivity. Phase 2 focused on the analysis of real samples in order to assess accuracy, precision and robustness. Phase 1 consisted of a first interlaboratory exercise where two extracts (herring and spinach) and one standard solution with PFASs in undisclosed concentration were distributed for analysis. The samples were analyzed in replicates to evaluate the intra-laboratory performance and the ISO 5725 “Accuracy (trueness and precision) of measurement result” was used as guidance to evaluate the accuracy of the chemical analytical methods {ISO5725-1, 1994 #17}. Phase 2 was performed in collaboration with an international QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) ILS.
The objectives of the ILS were; 1) to assess the intercomparability of the data produced by food and drinking water analysis laboratories, which are matrices with low concentrations of PFAS; 2) to follow-up on earlier ILSs for

environmental matrices. In total 31 laboratories participated in the international ILS (including 9 PERFOOD members) and the results have been reported elsewhere {Van der Veen, 2012 #4; Weiss, 2012 #19}. The results reported from Phase 1 and 2 of WP 2, *i.e.* the evaluation of the analytical performance and methods used by the PERFOOD members has been reported separately {Weiss, 2012 #21}.

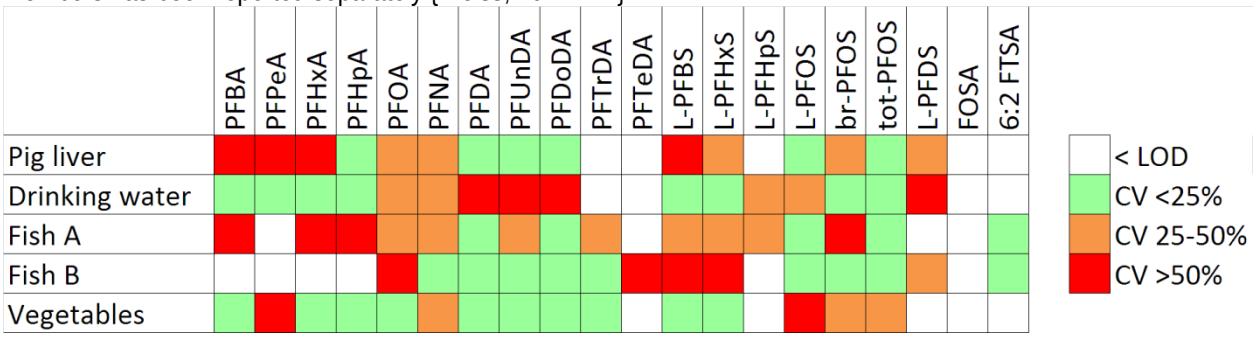

Figure 2.2.1. *The coefficient of variation (CV) between laboratory participating in the ILS (2011) is illustrated in a color scheme where lightest cells (green) corresponds to satisfactory CV's (<25%), medium to CV's between 25% and 50%, and dark cells (red) to non-satisfactory CVs above 50%.*

The experience from Phase 1 was taken into Phase 2 ILS and considering the low concentrations of the target compounds in the samples – improvements can be reported in the analytical performance of the reported PFASs in food and environmental samples. The ILS resulted in satisfactory in between laboratory CV's (<25%) for the major PFAS determinants in the matrices analyzed (Figure 2.2.1).

As one fish sample was identical to the one used in a previous ILS {Van Leeuwen, 2009 #12}, direct comparison between the results could be made. Participants in this ILS reaching satisfactory z-scores were obtained by a higher percentage model CVs are lower for fish than in a previous study and (Figure YA and B, respectively). Comparison of the average of absolute z-scores per matrix obtained by PERFOOD partners with the average of absolute z-scores obtained by all participants in the international ILS showed that PERFOOD partners performed within the same range as other participants.

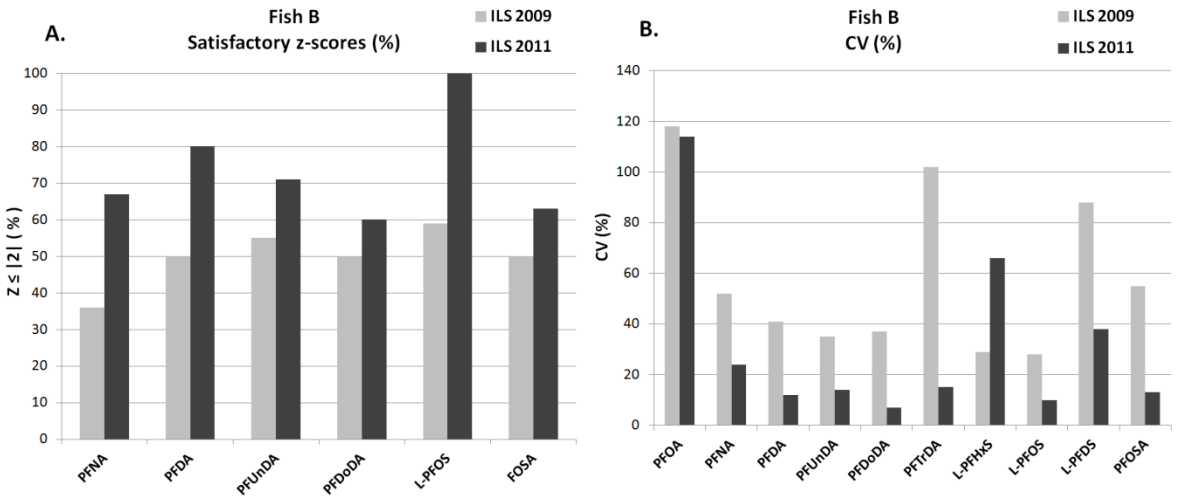

Figure 2.2.2 A and B. Comparison of group performance of ILS 2009 {Van Leeuwen, 2011 #631} and ILS 2011 for the analysis of PFASs in the identical fish muscle tissue expressed as *A. percent satisfactory z-scores (i.e. Z≤|2|) and B. in between laboratory coefficient of variation (CV, %)*.

Despite the improved analytical performance, there are sources of variance still to be considered. The participants were encouraged to report the linear isomer values instead of the sum for PFBS, PFHxS, PFHpS and PFOS, and in addition the branched and sum of the PFOS isomers. More than one third of the participants (37%) still reported the sum of the isomers and this contributed to the variation in the reported values. In addition, the reported PFOS concentrations in fish may be overestimated by some participants due to the presence of taurodeoxycholic acid

(TDCA). TDCA needs special attention to be correctly chromatographically separated from PFOS, or by MS, which was not taken care of by 24% of the participants.

The production of Certified Reference Material for PFASs in fish and water

Within-laboratory validation characteristics such as precision, accuracy, and robustness need to be determined. One important tool for evaluating accuracy is the use of certified reference materials (CRMs); however, the availability of CRMs for organohalogen micropollutants is limited. Recent feasibility studies evaluated the production and certification of CRMs for brominated flame retardants in fish (EU-BROC) and sterilized milk, pork, fish, fish oil and feed (EU-DIFFERENCE). These studies showed that it is possible to successfully produce matrix-type CRMs while keeping the physical state of the sample matrix intact. This is very important as the CRM should closely approximate real sample matrices. In line with these past experiences, and the lack of CRMs for PFASs, the PERFOOD consortium decided to create two CRMs. The two matrices selected were water and fish. The Joint Research Centre (JRC) Institute for Reference Material and Measurements (IRMM) has been collaborating with PERFOOD in the production line of the two CRMs. IRMM is the responsible party for the storage and sales of the materials. Due to their expertise in the field they have been giving valuable advice in the production and representatives from IRMM have been present to control and assist the fish and water matrix production performed at IVM and KWR respectively.
The production of such large volume of material demands thorough preparation and follows a well-defined protocol to ensure homogeneity and stability of the material. All equipment used during the production was thoroughly cleaned and material used for storage was tested regarding its stability after freezing/unfreezing and absorption of PFASs to the material. In total 80 kg of frozen pike-perch fillet and 650 L of tap water was used to prepare more than 1200 jars of reference material. The jars were transported to IRMM who will store the material at room temperature and will keep jars apart for stability tests at different temperatures. The water material will be certified for the PFOA and PFOS content and the fish for the PFDA and PFOS content. The other homologues will be reported as indicative together with the material. The homogeneity of the materials has been tested. The concentrations will be certified and the long-term stability evaluated for the final product. The certification of the material is planned to be finished in 2013. The material will be available for purchase via IRMM.

2.3 WP3 Providing data on PFASs in the diet

Introduction

Recent data on PFAS levels in human show a relatively homogeneity worldwide, indicating that dietary habits may be a minor issue, but that a general source common to most of the population contributes to the PFAS burden. Possible exposure pathways include beverages, food in general and migration from food packing or cookware (Fromme et al., 2007, Lau et al., 2007).
Most European countries carry out national monitoring programs (food basket studies) in order to assess the daily intake of persistent organic pollutants. PFAS have not yet been included in these studies in most countries. In addition, since food basket studies mainly are carried out be national authorities, no coordinated approach is used, making comparison between different countries difficult.

WP3 was tasked to develop a standardized selection of food items, sampling procedures and apply validated ultra-sensitive analytical methods, enabling a unique assessment of the occurrence of PFAS in European food as well as the identification of major sources of PFAS exposure via food.

Inventory food consumption data and design of food sampling campaign

After the literature survey delivered by WP 6 (Exposure assessment) we were able to establish the most relevant and recent food preferences in four key-region of Europe. Consumption data from 8 European countries were used to assess the relative importance of food categories. Based on this food preference information, a sampling design was established for four selected geographical regions with different dietary habits representing the northern, eastern, central and southern parts of Europe: i) Norway, ii) Czech Republic, iii) Belgium/The Netherlands and iv) Italy. An overview over the mainly consumed food groups in the four countries is presented in table 2.6.2. of WP6.
In close cooperation with WP6, contacts with EFSA and national food authorities of member states a European relevant coverage of food items was selected. The sampling design included two sampling campaigns in the selected key-regions: i) a first sampling campaign in spring 2011, collecting single raw food items representing all 14 EFSA food categories and ii) a second campaign focussing on food items with increasing influence of industrial processing as well

as raw food items collected close to point sources to cover worst case risk evaluations and follow up samples of the first campaign to assess elevated levels:

- Follow up samples from 1st round (raw food items with elevated levels)
- Point source samples (raw food items; worst case scenario)
- Composite food (Ready to eat/cook food items) sampled in Belgium and Italy
- Cauldrons (whole meals and mixed duplicates)

The cauldron sampling was designed to sample different eating habits in at least two key-regions along with school meals and a variety of fast food as well as mixed duplicates for a whole week and cauldrons of warm dishes representing a whole week. In addition, several of the selected composite food items were analysed non-cooked as well as cooked to evaluate change of PFAS content due to water uptake or loss, heating, contact with backing paper etc.
Sampling manuals were developed to cover all sample categories defined in campaign 1 and 2. The manuals and a list food items collected in campaign 1 and campaign 2/ category I) – IV) can be found in the Annex.

The samples were collected in the selected regions and analysed in four dedicated laboratories:

- Fruits, cereals, sweets, salt, coffee, tea → UoA
- Meat, fish, seafood, eggs, milk, dairy products, beer/wine → ICT Prague
- Vegetables, Fruits, cereals, sweets, salt, coffee, tea → NILU
- Water and beverages → KWR

The approach of dedicated laboratories in favour of local labs doing all samples from the countries were the samples were collected in, was chosen to improve data quality and ensure the comparability of results within the food item categories. After collection, the selected single food items where pooled together within their defined subcategory and country (Annex I). All sample pools were stored at -20 C prior shipment.

Data sets for PFC concentrations in food collected in different European countries

All samples were treated and homogenised according to the developed manuals. The PERFOOD consortium has by now collected about 800 single food samples and more than 300 composite and cauldron samples which will be available for future investigations as well. A number of 250 pooled samples were prepared which represent all 14 food categories and 70 pooled samples representing composites and cauldrons. These samples were shipped to the assigned laboratories and analysed for PFAS.

Chemical trace analyses was carried out using especially adapted methods for the food sub groups i) animal origin, ii) plant origin, iii) beverages, iv) dairy products. The datasets for all analysed samples were delivered to EFSA and can be found in Annex I and II.
The results of the first round of screening were used to identify those food categories that are the main sources for dietary intake of PFAS. According to the results of the first campaign, some food categories seem to be more contaminated with PFAS than other items. The food groups containing elevated levels were seafood, fish, bovine liver, butter, pork and bovine meat, spinach and hen eggs.

Table 2.3.1 lists PFAS levels determined in animal samples collected for PERFOOD, 1st campaign, in comparison with recent studies of PFAS in food. Fish and seafood data are not included in the list due to the broad variety of fish and seafood findings globally. Table 2.3.2 shows the PFAS levels in vegetables reported in PERFOOD compared with other studies. Not many studies are available at the time of reporting, however the most recent studies show a good agreement with PERFOOD data for most of the food groups investigated (Haug et al, 2010, Noorlander et al, 2010, Vestergren et al., 2012). However, results with a much higher levels are reported as well (Cornelis, et al., 2012) in addition to reported non-detects stating elevated detection limits of 1000 pg/g (Clarke, et al., 2009). Limit of detections within the PERFOOD consortium ranged between 10- 100 pg/g ww for all PFAS analysed.

Table 2.3.1: PFAS levels in animal samples

		PFOA	***PFNA***	***PFDA***	***PFUdA***	***PFDoA***	**PFHxS**	**Tot-PFOS**
Cheese								
	Czech Republic	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Belgium	5.0	20.4	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Italy	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Norway	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Haug et al., 2010 Norway	13	16	6.6	4.1	<15	<0.65	12
	Vestergren et al 2012 ; Sweden 20	28.5					1.0	5.6
	Noorlander et al 2010, Netherland		7.0	8.0				
	Cornelis et al., 201 Belgium	120						250
Pork								
	Czech Republic	9.2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.5
	Belgium	9.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	27.6
	Italy	7.5	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.3
	Norway	7.8	< LOQ	< LOQ	8.1	< LOQ	< LOQ	15.1
	Haug et al., 2010 Norway	15	5.5	16			1.2	17
	Vestergren et al 2012 ; Sweden 20	12.0	5.8	6.3	2.5	1.1	4.5	25.3
	Noorlander et al 2010, Netherland	15.0	2.0	2.0				14.0
	Cornelis et al., 201 Belgium	55.0						170
Liver								
	Czech Republic	< LOQ	5.0	24.5	11.6	< LOQ	< LOQ	204
	Belgium	< LOQ	39.3	272	32.0	< LOQ	< LOQ	2600
	Italy	< LOQ	33.6	159	46.4	< LOQ	< LOQ	418
	Norway	< LOQ	47.0	89.7	105	40.4	< LOQ	380
	Clarke et al, 2009 UK	1100						2500
Hen eggs								
	Czech Republic	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	3.1
	Belgium	6.1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	61.4
	Italy	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	2.3
	Norway	< LOQ	< LOQ	< LOQ	24.5	< LOQ	4.9	120
	Haug et al., 2010 Norway	30		12	9.9		3.5	39
	Vestergren et al 2012 ; Sweden 20	38.7		3.3			2.5	39.2
	Noorlander et al 2010, Netherland		6.0	11.0				29.0
	Cornelis et al., 201 Belgium	860						6860
Preserved		PFOA	PFNA	PFDA	PFUdA	PFDoA	PFHxS	Tot-PFOS

skimmed milk								
	Czech Republic	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Belgium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	8.4
	Italy	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	4.9
	Norway	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Haug et al., 2010 Norway	4.7		4				7
	Clarke et al, 2009 UK							
	Noorlander et al 2010, Netherlands	1		1				10
	Cornelis et al., 201 Belgium	120						250
Butter								
	Czech Republic	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Belgium	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	32.9
	Italy	23.0	< LOQ	< LOQ	< LOQ	< LOQ	9.9	57.9
	Norway	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
	Vestergren et al 2012 ; Sweden 20				5.8			13.4
	Noorlander et al 2010, Netherlands	16	2	6		2	16	33
	Cornelis et al., 201 Belgium	120						250

Table 2.3.2: PFAS levels determined in vegetable samples collected for PERFOOD, 1st campaign, in comparison with recent studies of PFAS in food.

Vegetables	*Reference*	*PFOA*	*PFNA*	*PFDCA*	*PFUNA*	*PFDOA*	*PFHXS*	*tot-PFOS*
Norway	PERFOOD	1	2.					
Italy	PERFOOD	2		0.		2.		5.
Belgium	PERFOOD	1		0.			0.	3.
CzR	PERFOOD		2.	0.				0.
Norway Lettuce	Haug et al., 2010	1.		0.		1.		0.
Norway Carrot	Haug et al., 2010	2.						0.
Norway Potatoes	Haug et al., 2010	5.		3.	2.			1.
Sweden vegetables 2010	Vestergren et 2012	2		2.			1.	
Sweden potatoes 2010	Vestergren et al, 2012	5		2.				
UK vegetables	Clarke et al., 2009	< 10						< 10
NL vegetables/fruit	Noorlander e al, 2010,	5.	1.	2.				
B/ESP vegetables	Cornelis et al 2012	6						6
B/ESP potatoes	Cornelis et al 2012	6						61

On the basis of the results of the 1st sampling campaign several food items were identified showing higher PFAS levels than the average and follow-up analyses were undertaken to investigate the elevated findings in more detail. The data for the follow up results can be found in the Annex.

Impact on food processing on PFAS content

The second sampling campaign included composite samples in the form of ready to eat/cook products representing food sources from animals, plants and beverages which are among other aspects characterised by paper food packaging. Food packaging collected during the first sampling campaign was delivered to WP 5 for further analyses. Cauldrons representing different food habits and age groups were also sampled in all four key-regions.

Composite food items have undergone a variety of industrialised processing steps which include partitioning, heating/ cooling, mixing, blending applying potentially PFAS containing equipment. The results show that raw food items contain higher concentrations of PFAS than the processed or cooked food products in most cases. One major reason is the dilution effect due to the addition of a broad variety of ingredients to the respective composite product. So is in case of liver pate the content of liver in general is less than 30% and not always originates from one species. However, composites consisting mainly of fish, liver and the other mentioned PFAS-containing food groups, showed higher PFAS levels than other composite products but on a lower scale. WP 6 calculated similar PFAS levels in raw and cooked food items when considering only single parts of the food (for example raw fish and cooked fish). The same picture can be found when comparing fish-, meat- or egg-based cauldrons of mixed duplicates and whole meals with the PFAS content in the raw main ingredients (see appendix for data).

Cooking experiments resulted in a similar picture. Boiling pasta diluted the PFAS levels by uptake of water. Mass balance calculations revealed that there is a small transfer of PFAS from water into pasta; however dilution turned out to be the dominating process. Heating of spinach in the microwave did not significantly change levels. Grilling hamburgers seems to reduce levels to a small extent. However, this result is probably affected by difference in LOQs of raw and prepared food.

In general, the levels in raw food, unprepared composites samples, warm meal cauldrons, composite food prepared with non-stick cookware are not a matter of concern. Exposure estimates are far below the Tolerable Daily Intake (TDI) of 150 ng/kg_{bw}/day for PFOS and 1.5µg/kg_{bw}/day for PFOA as long as no food produced at PFAS point sources (Hot

Spots) is consumed. If that is the case, local food safety regulators have to advise the population on safe daily intake amounts for different age groups (see WP 6 for more information).

PFAS levels from hot spot locations
Samples from source near (hot-spots) locations were collected in a number of countries. We focused on locations located close to industrial PFAS manufactures, airports and other industrial activity.

- Germany/ Bavaria: eggs, fish, bovine and pork meat/offal
- Italy/ Ferrara: tapwater, vegetables
- Netherlands/ Twente kanal: fish
- Norway/ Tromsø airport and other locations: Fish and mussels from effluent areas with AFFF use
- Belgium/ Antwerp: eggs, milk, fruit, vegetables
- Czech Republic/ Elbe, Bilina River: fish and offal from chicken, rabbit, pork
- Sweden/ Arlanda airport Lake Mälaren: fish

Elevated PFAS levels could be found in almost all selected hotspots. The data were applied by WP6 in risk evaluations for worst case evaluations.

2.4 WP4 Tracking the sources of PFCs in food, beverages and drinking water

Introduction
Presence, sources and removal of PFAA from the drinking water cycle.

PFAA exposure from water
Nowadays many surface waters are found to contain (polyfluoralkyl acids) PFAA at a base level of contamination due to pollution from diffuse sources and global/continental distribution occurs . The background level in many European rivers has been known for some years and discussed in several scientific reports. An overview has been compiled for the PERFOOD project, see ref (Eschauzier et al., 2012). The presence of PFAA in the aquatic environment can usually be traced to a discharging factory, accidental spill or wastewater treatment plant. The contamination of surface waters by PFAA may act as a source of the presence of PFAA in drinking water. Contamination of groundwater and its relation to drinking water contamination is a subject poorly explored in the scientific literature until now.
Regarding PFAA distribution throughout Europe: PFAA concentrations in the Central and Southern European rivers, such as in Italy, Germany, The Netherlands, and UK, generally seem to be higher than in Northern Europe. This is well illustrated when levels reported for the Scandinavian countries and Northern Poland are compared. The rivers Po, Rhine and Seine appear to be the major rivers in Europe discharging PFAAs into the oceans (McLachlan et al., 2007). The reports generally focus on the presence of PFOA and PFOS in the environment. However, as a result of substitution of C8 chemistry by C4 and C6 perfluorinated and polyfluorinated telomer compounds, respectively, it is expected that concentrations of the substitutes or their metabolites will increase in the environment. Unfortunately, PFHxA, PFBA and PFBS have been monitored only scarcely thus far.
Concentrations in (European) drinking waters remain on average fairly low such as shown in the PERFOOD sampling campaigns and available published peer-reviewed literature. Drinking water produced from raw water extracted in the vicinity of a PFAAs spill tends to be contaminated. The same is valid for contaminated groundwater. Although drinking water produced from groundwater tends to have low contamination levels, when a source to local aquifers is present, the marginal groundwater treatment (often only aeration and rapid sand filtration) will not remove PFAA from the water.

As for the overall removal of PFAAs during drinking water preparation several conclusions can be drawn from the experimental work conducted in PERFOOD. In practice, two technologies are known to remove PFAA and these are also used in the drinking water production chain: membranes (reversed osmosis and membrane filtration) and activated carbon filtration. The difference in PFAA baseline concentrations in drinking water will depend on the technologies used in different treatment plants. Drinking water prepared by a treatment which does not include GAC filtration or reverse osmosis will generally contain higher PFAA levels when the raw source water is contaminated, such as is often the case in Central Europe where river water is being used for that purpose.

PFAA in drinking water production chain

The actual behavior of PFAAs in the field from intake (raw source water) to finished drinking water was assessed in depth at one of the largest drinking water production plants in the Netherlands. Samples were taken from influent and effluent of the several treatment steps used in a drinking water production chain. These consisted of intake, coagulation, rapid sand filtration, dune infiltration, aeration, rapid sand filtration, ozonation, pellet softening, granular activated carbon (GAC) filtration, slow sand filtration, and finished drinking water. In the intake water taken from the Lek canal (a tributary of the river Rhine), the most abundant PFAA were PFBA (perfluorobutanoic acid), PFBS (perfluorobutane sulfonate), PFOS (perfluorooctane sulfonate), and PFOA (perfluorooctanoic acid). During treatment, longer chain PFAA such as PFNA (perfluorononanoic acid) and PFOS were readily removed by the GAC treatment step and their GAC effluent concentrations were reduced to levels below the limits of quantitation (LOQ). However, more hydrophilic shorter chain PFAA (especially PFBA and PFBS) were not removed by GAC and their concentrations remained constant throughout the treatment chain. A reduced removal capacity of the GAC was observed with increasing carbon loading and with decreasing carbon chain length of the PFAAs. This study shows that none of the treatment steps studied, including softening processes, dune passage of pre-cleaned river water (Eschauzier et al., 2010) are effective for PFAA removal, except for GAC filtration. The same is valid for UV/H2O2 treatment and riverbank filtration of raw river water (unpublished data). GAC can effectively remove longer chain PFAA from the drinking water cycle, whereas for shorter chain analogues the renewal of the active carbon beds is more critical and common practice reveals that C4 and C5 compounds often break through and show up in the finished water. In addition, the enrichment of branched PFOS and PFOA isomers relative to non branched isomers during GAC filtration was observed during treatment.

In drinking waters prepared from surface waters concentrations of C4-PFAA can amount to several tens of ng/L. Other PFAAs were generally present at levels below 4 ng/L. Although reversed osmosis and nano filtration were shown to be able to remove PFAA from water, few treatment plants actually use these technologies because of the high investments associated.

PFAA in groundwater

Sources to groundwater have been shown to be equally diverse as to surface water. An elaborate sampling campaign showed that point sources (such as landfills and airforce bases) as well as diffuse sources (infiltrated rain water) can play an important role in areas including former landfills which were leaching in the groundwater (Eschauzier et al. submitted). After breakthrough of the landfill leachate, the sum concentration of PFAA in the drinking water produced at the substraction well was calculated to amount to some 13 $ng.L^{-1}$. This is in the same range as current average concentrations of PFAA observed in drinking water in the Netherlands and elsewhere in Europe.

Beverages and tap water

The behavior of PFAA during beverage preparation processes, such as coffee, and cola from automats was assessed in detail in a study recently published, see (Eschauzier et al.,2012). Tap water based beverages contribute to the overall dietary exposure as a result of the PFAS present in the tap water from which the beverages are prepared.

Uptake by plants

To assess the potential uptake of PFAAs by plants, greenhouse experiments and a field experiment were conducted. Four different crop species were tested for their uptake behavior when grown hydroponically; Lettuce and Cabbage as leafy vegetables and Tomato and Zucchini as fruit bearing vegetables. The plants were grown in spiked test solutions with 6 replicates per concentration. In the field experiment Corn, Pea, Radish and Lettuce plants were grown on soil spiked with a mixture of PFAA at four different concentrations. For detailed experimental set-up see appendix.

The extraction method for plant samples was developed in work package 1. The methods for soil and water analysis include solid phase extraction. Analysis of the extracts was done with HPLC-MS/MS. For detailed information see appendix.

Results from the greenhouse experiments showed high concentrations in the roots of the plants especially of long-chained PFAAs, which is likely due to sorption of the compounds to the root surface. Lipophilic absorption would be a negligible uptake mechanism for anions, but due to the strong electron-withdrawing effect of fluorine atoms and thus the dislocation of the negative charge, PFAAs may behave more like neutral compounds.

Root uptake factors were calculated from the greenhouse experiments as well as from the field experiment. Uptake factors derived from the field experiments showed lower factors than from the greenhouse experiment, but a similar pattern over chain length. The reason for lower uptake factor in the field experiment might be the bioavailability of the compounds. In the greenhouse the PFAAs are available at constant concentrations, while in the field experiment the

compounds, especially the long-chained PFAAs, are sorbed to the soil and become only available for uptake when enough water is present in the soil. Analyses of the drainage water showed, that the short-chained PFAAs are also washed out of the system quite quickly, which leaves less for uptake.
Even though the short-chained PFAAs were washed out with the drainage water in the field experiment, concentrations of PFBA, PFPeA and PFBS were highest in all vegetative parts of all plants. Interestingly, PFHxA showed lower concentrations than PFPeA and PFHpA in roots, stems and leaves of the plants, meaning that the chain length is not the sole determinant for uptake of PFCAs.
The leaves of all plants contained the highest concentrations of all PFAAs in the vegetative parts, with the short-chained compounds (PFBA, PFPeA, PFBS) in the highest concentrations. The concentrations in the roots were dominated by the long-chained compounds. This proves that the transfer from roots to the vegetative parts is inhibited for the long-chained compounds with increasing inhibition from PFNA to PFTeA.
All plants in both experiments show the same accumulation pattern as a function of PFAA chain length. PFCA concentrations decrease first with increasing chain length, finding a minimum in PFHxA, increase afterwards up to PFOA-PFDA and finally decrease for the rest of the compounds (C13-14). PFSA concentrations generally increase with increasing chain length. In Peas, Pea pods and maize corns, however, the PFSA pattern is switched and decreases with increasing chain length.
Lettuces and radishes grown in the highest spiked soil showed a significant inhibition of growth. It is not possible to state with this experimental set-up, if a single compound or the mixture of them was responsible for the inhibited growth. Further studies are needed to elucidate this phenomenon.
Analysis of the Leaves of a lettuce plant showed higher concentrations in the outer, older leaves, constantly decreasing towards the inner, younger leaves. This proves that PFAAs are indeed accumulating in the leaves with time.
The greenhouse and field experiments show, that PFAAs are not following existing uptake models for either ionic or neutral compounds. A new uptake model has to be developed to describe the uptake mechanism of PFAAs.
The experiments have shown that vegetables, if grown on contaminated soil, take up PFAAs. The short-chained PFAAs are of particular importance, since they get transferred best to the vegetative parts of plant (which usually constitute the edible parts), even though they don't remain in the soil for a long time. Leafy vegetables pose the highest risk for human exposure due to the accumulation of PFAAs especially in the leaves. Even the lowest spiked soil concentration of 100µg/kg, which is considered a threshold value in some countries, yielded considerable concentrations in the edible part of lettuce and peas and also in the vegetative parts of Maize plants, which is used for cattle fodder.

Biotransfer of perfluoroalkyl acids in dairy cows in a naturally contaminated environment

Introduction

Dietary monitoring studies (Vestergren et al., 2012) have demonstrated that consumption of meat and/or dairy products are significant sources of exposure to long-chain (more than 7 perfluoroalkyl carbons) perfluoroalkyl carboxylates (PFCAs) and perfluorooctane sulfonic acid (PFOS) (Vestergren et al., 2012). However, the transfer mechanisms of PFCAs and PFOS to food remain poorly understood. In this report we present a steady-state mass balance study of PFSAs and PFCAs in mature dairy cows receiving a naturally contaminated diet. The overarching aim is to achieve a quantitative description of the transfer of PFCAs and PFSAs to meat and dairy products.

Materials and methods

In order to estimate the transfer of chemicals at steady-state the mass balance was conducted at the scale of a whole farm which would average out individual physiological differences and temporal variation in milk production between the animals. All dairy cows in the barn were above 24 months of age (fully grown adults) and calving dates were randomly distributed around the year. Feed (silage, barley and supplement concentrates) and water were considered as possible intake routes of PFCAs and PFSAs. In line with earlier feeding studies of PFOA performed on beef cattle and sheep (Lupton et al., 2012), the absorption efficiency after oral intake was assumed to be 100% in this study. The fluxes of chemical (ng day^{-1}) were calculated from the fluxes of intake and excretion media (kg day^{-1}or L day^{-1}) multiplied by the measured concentration of chemical (ng kg^{-1} or ng L^{-1}) in the media (Figure 2.4.1). Biotransfer factors (day g^{-1}) were defined as the concentration of chemical in cow tissues (ng kg^{-1}) over the total daily dietary intake of chemical (ng day^{-1}) from drinking water and feed.

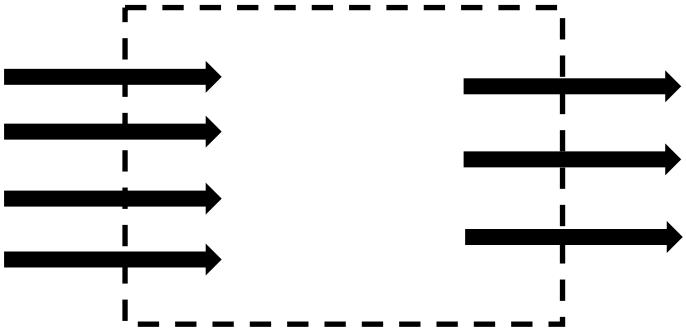

Figure 2.4.1. Schematic picture of the input and output flux parameters of the mass balance. Sampling and flux parameters

Results and discussion

Perfluorooctanoic acid (PFOA), perfluorononaoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perflhbergma1uorododecanoic acid (PFDoDA) and PFOS were quantified in cow tissues and one or several of the intake and excretion media. The results from the mass balance demonstrate that the intake of silage dominated the cow's external exposure for all of the study compounds. Milk and urine were both important routes of excretion whereas fecal excretion was found to be negligible. Biotransfer factors of PFCAs and PFOS in meat and milk and liver are displayed in Figure 2.4.2 Overall, the highest BTFs were observed for PFOS, PFNA and PFDA and PFUnDA in liver. BTFs in milk and meat were approximately an order of magnitude lower than those in liver for all compounds except PFOA.

Biotransfer factors provide a useful tool to risk assessors to estimate the human exposure via consumption of milk, meat and offal food. This study demonstrates relatively low biotransfer factors of PFCAs and PFOS in meat and milk and from dairy cows. However, the increased enrichment of PFOS and long-chain PFCAs in liver indicate that consumption of offal food from dairy cows will result in a higher human exposure.
The study was conducted under typical Swedish conditions for cow management. The feeding regimes, milk yields etc are, however, similar to other European countries (Thomas et al. 1999). Thus, the biotransfer factors estimated here could be used to estimate the resulting human exposure from milk, beef and offal food and support exposure and risk assessment of PFCAs and PFOS.

Transfer from feed to farmed fish

The presence of PFAAs in fish feed is mainly depending on the origin and the amount of the fish meals in the formulation. PFOS is considered the main contaminant, due to its toxicokinetic features in fish. However, the PFOS contamination described and modelled in fish feeds (in the range of few ng/g) do not give rise to an appreciable occurrence (< 50 pg/g) in the fillet of farmed sea bream and sea bass of commercial size (400 g body weight). FOSA and fluorotelomer alcohols, which are PFOS and PFOA precursors, appear to be poorly metabolised into PFOS and PFOA in fish, leading to a negligible bio-magnification of the latter. Carry-Over Rates from feed to fish have been estimated to be in the range of 0.06 to 0.6 % according to the minimum/maximum PFOS occurrence data reported for anchovies and sardines, which are the most frequently used species in fish meal production. The regular consumption of farmed fish instead of wild fish would lower the PFOS intake by about 10 times. A further risk mitigation could be the progressive replacement of fish meals with less contaminated plant proteins meals, together with more environmentally sustainable scenarios of fish farming practices.

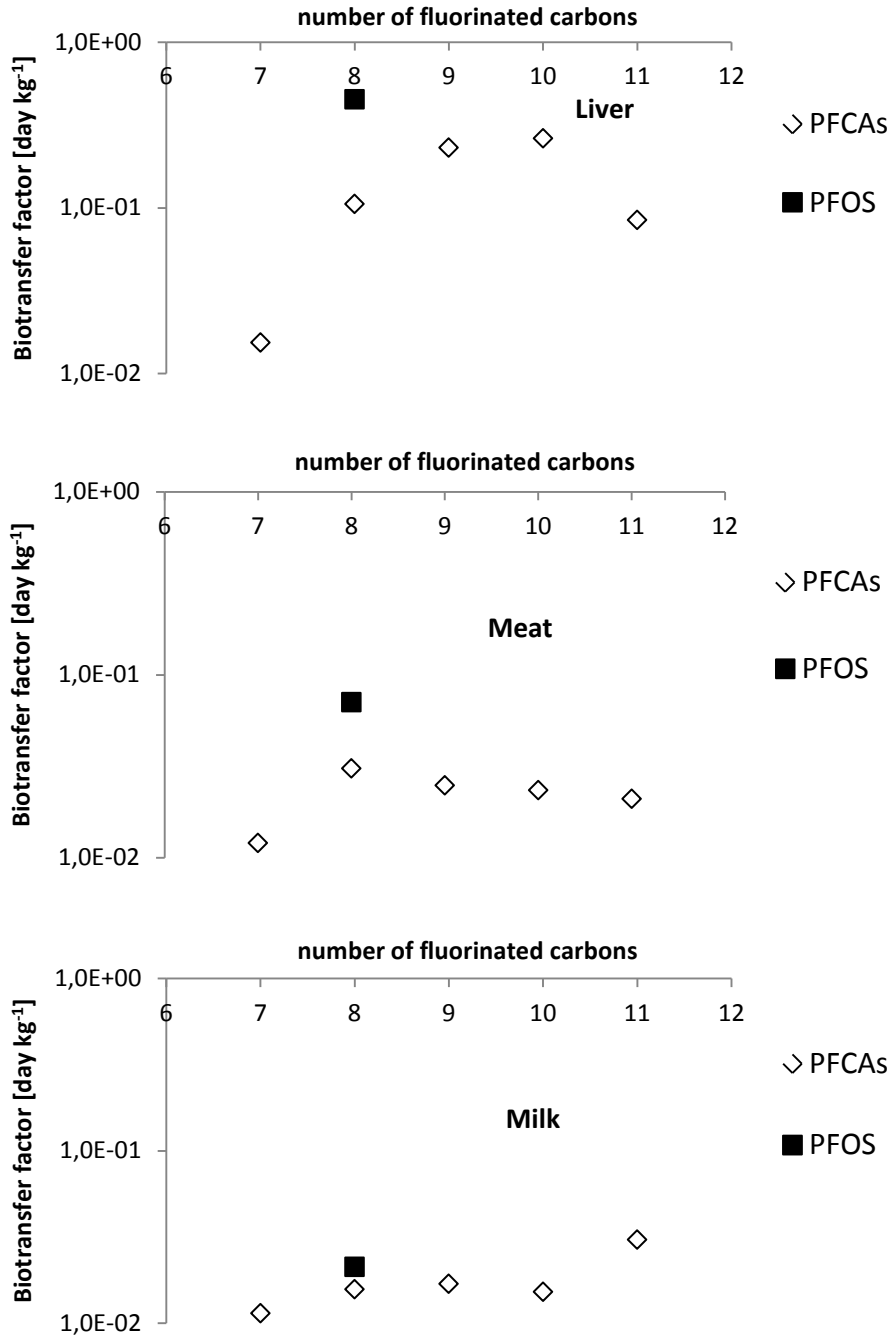

Figure 2.4.2. BTFs (day kg-1) for PFCAs and PFOS in meat, milk and liver.

2.5 WP5 Impact of food contact materials and process technologies

Introduction

Studies on the impact of FCM on PFAA levels in focused firstly on the migration potential of FCM, i.e. the presence of PFAA, direct precursors (like FTOH) and larger molecules containing polyfluorinated moieties in FCM. Secondly, migration into food items and food simulants was studied in depth applying such previously well-characterized FCM. Investigation of process technologies effecting PFAA concentrations in food started with laboratory trials applying spiked food items. Then home cooking procedures were studied and an industrial process was selected and mass balanced.

Migration potential

Oligo- or polymers of polyfluorinated compounds are used amongst others as coating for paper and cardboard to create a grease protective and waterproof finish. Examples for such PFCs are perfluoro alkyl phosphates (PAPs) or perfluoro acrylates. The molecular weight of PFCs ranges from 500 to 3000 Da (oligomers) as well as from 20,000 to 40,000 Da (polymers). For a selection of fluorine containing FCM from the market screening methods for fluorine (F) were developed and applied. All these investigations aimed at the understanding of the migration potential of a given FCM. The key findings are given below.

F-screening by spectrometric means: Spectrometric hand held instruments were the preferred screening methods aiming at the detection of F in paper-based packaging and other food contact materials (FCM). By comparison with wavelength dispersive X-ray fluorescence analysis and more sophisticated approaches (see below) sliding spark spectrometry (SSS, Iosys GmbH) turned out to be a quick and reasonable precise F-screening tool. A total of 430 European packaging and other food contact materials were analyzed [Fiedler et al. 2011]]. The samples were collected mainly in Germany (n=238), Italy (n=83) and Norway (n=56), a limited number of samples came from the Netherlands (n=13), Belgium (n=16), and Greece (n=10) (see Fig. 2.5.1.). The share of F-containing FCM in Germany, Norway, the Netherlands, Belgium, Italy, and Greece accounted for 27%, 0%, 23%, 19%, 12% and 0% of the samples of these countries. However, due to differences in sampling strategies and the number of delivered samples, conclusions on regional differences have to be drawn with caution. Therefore, further investigations focused on the whole data set. Samples were assigned to 13 FCM categories with respect to their function, i.e. the typical use as packaging material or baking aid for a special type of food. TEFLON® coated multiuse baking papers were allotted to FCM intended for baking. Figure 2.5.2. shows the relative contribution of different FCM categories. Miscellaneous paper packaging shows the highest share of all samples (33%), followed by FCM intended for baking (22%). The amount of F-positive and F-negative samples in the different FCM categories is presented in Fig. 2.5.3. The share of F-positive samples in these groups differed significantly. No positive samples were found in packaging of beverages and eggs, filters and popcorn bags. F-positive samples were accounted for 10 % in cheese/sausage packaging and miscellaneous packaging. In contrast, the occurrence of F-positive samples in butter wraps, fast food packaging, papers intended for baking, sandwich papers and take away food packaging accounted for 24 %, 23%, 56 %, 66 % and 43 %.

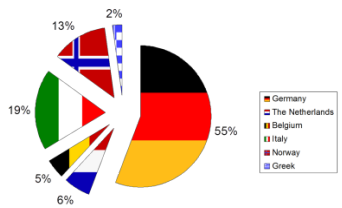

Fig. 2.5.1. Origin of the investigated packaging samples

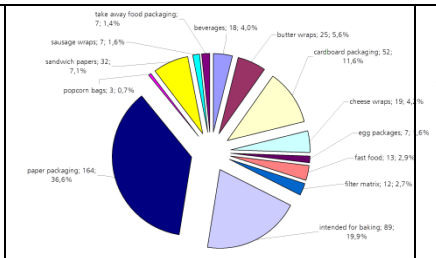

Fig. 2.5.2. Application related categories the investigated food contact materials

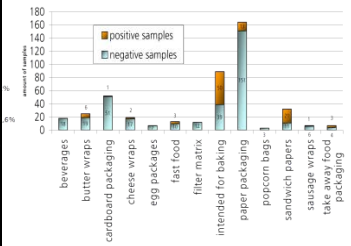

Fig. 2.5.3. Kinds and amount of investigated food contact materials

Fluorine screening by P&T EPED: A more sophisticated methodological approach was developed in order to detect volatile F (and sulphur) containing compounds, which are emitted from FCM at 80-200 °C. A purge&trap autosampler (PTA3000, IMT) was connected to a GC (AG6890, Agilent) and a recently developed Plasma Emission Detector with Echelle Spectrometer (EPED, IMT Innovative Messtechnik GmbH). The EPED detector combines a long term stable pulsing plasma cell with a high resolution Echelle spectrometer showing high sensitivity and selectivity for sulphur and the halogens chlorine, bromine, fluorine and iodine with detection limits for the above mentioned elements < 10 pg/s and a linearity about 3-4 decades. This instrument has been applied to textiles and produced F-screening results comparable to wave length dispersive XRF and SSS [Gruber et al. 2011]. The possibility of screening sulphur enables to distinguish between PAPs and S-PAPs.

LC-MS screening of FCM extracts. A set of oligo- or polymers of polyfluorinated compounds applied as coating materials in FCM were subjected to LC-MS screening and results were compared to MeOH extracts of nearly 60 FCM. They comprised a wide application area in the food sector. Characteristic mass profiles were obtained for the majority of the compounds in the range of m/z = 500 to 1500. The most intensive fragments were used for identification and quantification purposes. The results show that some paper grades contain not only one particular PAP, but mixtures of

several PAPs. The largest portion of papers containing PAPs is represented by baking papers followed by fast food wrappings. However, also disposable plates and cups were found PAP-coated. Grease resistant papers used as sandwich wrapping consist almost exclusively of perfluorinated compounds whereas the finishing's used for baking papers are comprised of more complex mixtures containing PFCs, silicone oils and other polymers. This screening method is capable of analyzing various complex mixtures of different paper coatings and is only limited by the molecular weight of the oligomers (~ 2000 Da). Although it was not always feasible to precisely monitor the whole oligomer, it is possible to assign it into the correct substance class (selected fragment ions according to Begley et al, 2008.

PFC and FTOH in FCM extracts: Perfluorinated carboxylic acids (PFCAs), perfluorinated sulfonic acids (PFSAs), and perfluorinated telomer alcohols (FTOH) were investigated in 47 paper-based F-positive FCM (detected by SSS previously). Extracts were analyzed for PFAA and FTOH by isotope dilution techniques. Results indicated significant amounts of FTOH (ppm range) and PFCA (ppb range) in special food packaging items, which both may serve as a source of PFAA in packed food. PFSA were not detected.

Volatile emission from FCM: In general, the migration of a compound from a FCM into food (or a food simulant) may take place at the interface food-FCM directly and/or by a gaseous transport via the intermediate gas phase. This is especially the case for FCM in baking and cooking applications. Therefore, gaseous emissions from FCM were tested in laboratory trials at temperatures of 150°C (paper based FCM) and 250°C - 350°C (PTFE pans). Whereas 6:2, 8:2 and 10:2 FTOH were the most abundant fluorinated compounds detected in the emission samples of paper based FCM, they were not found in PTFE based FCM. In 30 minute overheating tests of PTFE pans, however, an emission of perfluorinated C4- to C12 carboxyl acids was monitored in the range from 20-2500. This is surprising since only an emission of PFOA was expected due to its known usage in the production of PTFE. However, the supplier of the tested PTFE pans stated to use PFOA free PTFE. Therefore, the sources of the analyzed PFCA emissions require further investigations.

Conclusion on migration potential:

- Baking papers, sandwich papers and butter wraps have the highest share of F containing FCM
- Specific screening detected PAPs and S-PAPs as well as PTFE in these FCM. FTOH were detected in almost every F positive FCM, maximum PFCA levels were in the upper ppb range, however, they were not detectable in a series of FCM.
- At T > 120°C a significant gaseous emission of FTOH was analyzed.
- At T > 220°C PFCA evaporated from PTFE coated FCM

Migration tests

Specific migration testing is a tool to investigate the transfer of target compunds from FCM into food in a harmonized way. In order to prevent significant over- or underestimations the migration conditions address realistic application profile on the one hand and base on a worse case approach on the other hand. Target analytes of the migration trials performed in PERFOOD were FTOH based polyfluoroalkyl phosphate esters (PAPs), FTOH and PFAA, since for these compounds labeled internal standards were available and enable a reliable analysis.

PAPs are a group of anthropogenic highly fluorinated chemicals that are used in coatings for food contact paper and food packaging materials made from board or paper. PAPs have further been shown to be biotransformed into perfluoroalkyl carboxylic acids (PFCA) using a rat model (D'eon and Marbury 2007). In the PERFOOD project three studies investigated the migration of PAPs.

Firstly, migration of PAPs was explored from surface-treated FCM as well as FCM made from recycled cellulose fibers, both obtained from the German retail market (Küchler 2012). The selection of FCM was performed with respect to the use pattern of typically fluorine-containing FCM. Four application profiles were investigated, including long-term packaging at fridge temperatures (4°C), long-term packaging at ambient temperatures (20°C-40°C), short-term packaging at elevated temperatures (~80°) and baking applications (temperatures up to 220°C). For the migration testing real food items and food simulants were applied, including butter, cheese and Tenax®. Surface-treated sandwich paper was identified as an important source of exposure to PAP via food intake. The migration rates of PAPs into emulsified and fatty foods like butter and cheese increased considerably compared to dry foods and Tenax®, respectively. In case of longer contact times and higher storage temperatures an accelerated degradation of diPAP to monoPAP and FTOH was monitored, in addition to increased migration rates for PAP.

A second study observed the migration of PAPs from Swedish packaging materials into food (Sandblom 2012). 15 food packaging materials were randomly selected from a local supermarket and a fast food restaurant and analyzed for PAPs. Results showed the presence of PAPs in all of the analyzed samples, with a dominance of diPAPs. The highest amounts were found in a microwavable popcorn bag with up to 52 pg/cm2 packaging material for individual monoPAPs and up to 1500 pg/cm2 for individual diPAPs. Analysis of PAPs in the food items that were packed in the 15 selected packagings showed the presence of diPAPs in all food samples, both uncooked and after preparation. In some cases the concentrations of diPAPs in the food increased during food preparation. The concentrations found in the different food items where in the pg/g range with the highest level of an individual diPAP found in a hamburger at 62 pg/g. These results demonstrate that PAPs migrate from food contact materials into food and constitute a potential source of human internal exposure to PFCAs.

Results of both above mentioned studies were confirmed by a third study [Hajslova et al. 2011]. Considering the high number of positive packaging materials found in Czech fast food restaurant, migration tests were designed to simulate typical storage conditions (up to 60°C, storage time 1–72h). As food simulants, water, aqueous acetic acid (3%, w/w) and olive oil were used. In line with the earlier published data, the highest migration rate was into the olive oil. Interestingly, leaching for 1 hr at 60°C resulted in comparable amounts of diPAPSs that were found after 24 hrs at ambient temperature. No further increase of diPAPSs occurred at longer migration times (up to 72 hours). Migration of triPAPSs into olive oil was significantly lower in both experiments (1% of amount extracted by ethylacetate) and slightly increased with time of migration. While monoPAPSs were not detected in olive oil at all, the 6:2 monoPAPS was transferred into warm water (60°C) in the same extent as into the methanol, on the other hand, the migration intensity of 8:2 monoPAPS into water was only 20% of that content in methanolic extract, for 10:2 monoPAPS less than 1%. DiPAPSs migrated also into the water, but the amount was less than 0.1% of dichloromethane extract.

No migration of triPAPSs and diPAPSs was found into the acidic water. Acidification of water decreased migration of 6:2 and 8:2 monoPAPSs significantly, approx. 100 times.

FTOH an PFCA: Migration of PFCA and FTOH from FCM was examined with respect to four typical application profiles (Fengler 2011): a) long-term packaging at fridge temperatures, b) long-term packaging at ambient temperatures, c) short-term packaging at elevated temperatures (~80°C) and d) baking applications (short-term contact at high temperatures). Real food items and food simulants were applied, including polar and non-polar solvents as well as Tenax®. Before and after the migration contact, FCM, food items and food simulants were extracted and analyzed. Results of FCM were expressed in ng/dm² FCM. Levels identified in food (simulants) were reported in terms of ng/dm² of FCM, these food items were exposed to. The majority of the investigated papers show levels below LOD (0.06 to 0.89 ng/dm²) for all analyzed PFCA and PFSA. This is especially valid for materials like baking paper, cheese wrapper and FCM for frozen food. In contrast, sandwich and butter wraps, muffin papers and one investigated cardboard based packaging contained significant amounts of the fluorinated compounds (e.g. up to 246 ng/dm² for PFHxA). Low levels of PFOA (0.1-7.2 ng/g) were identified in Teflon based baking sheets. After the migration contact, those food items, which were exposed to FCM with levels above 10 ng/g showed a significant increase in concentrations, especially butter exposed to butter wraps and bread exposed to sandwich paper.

FTOHs were found in almost every FCM with concentrations ranging from 12 to 15532 ng/dm² (6:2 FTOH), 12 to 8844 ng/dm² (8:2 FTOH) and 2 to 5068 ng/dm² (10:2 FTOH), with lowest levels in a baking paper and highest in a cardboard sample. Migration of FTOH was observed in a series of food items. Percentage migration rates were computed by dividing the additional amounts of FTOH in the food item after the migration contact by FTOH amounts initially present in the FCM. At fridge and ambient temperatures these rates range normally between 0.4 and 70 %. FTOH migration rates increased 100% (range 130 to 2735 %), however, in a number of trials performed at baking temperatures (muffin paper →muffin) and moderate temperatures (sandwich paper → bread; butter wrap → butter). This clearly indicates at a release of FTOH from high molecular mother compounds. In addition, a significant migration of 8:2 and 10:2 FTOH from Teflon baking sheets was estimated (up to 64 ng 8:2 FTOH/dm²), although FTOH was not detected in baking sheet before the migration trial. This may be explained by a degradation of precursor compounds as proposed previously (Wolz et al 2010).

There is only little knowledge on the migration kinetic of PFAA or precursor compounds (Begley et al. 2008). This knowledge is relevant in order to decide, whether existing migration models can be applied to predict the migration behavior of PFAS. Therefore, we studied the migration of perfluorinated compounds (PFC) and fluorotelomer alcohols from paper based baking sheets into the food simulant Tenax® over 10 days (60°C), 6 h (120°C) and 2 h (200°C). For each setup 10 samples were prepared and placed into an oven at the given temperature. At 10 given sampling times

one sample were taken out of the oven and the Tenax was removed from the FCM immediately. FCM and Tenax were analyzed separately for PFAA by LC-MS/MS and for FTOH by GC-CI-MS.

FTOH results are well comparable for 6:2-, 8:2- and 10:2 FTOH. In the FCM there is a significant increase of concentrations, followed by a steep decrease. This is due to the release of FTOH from precursor compounds and a subsequent evaporation and/or migration from the FCM. Levels in Tenax start at low levels and increase shortly after the levels in the FCM begin to rise. PFAA levels were not detectable in Tenax®, however, in the FCM we observed the same time trend as described for FTOH: a slight increase of PFOA levels followed by decreasing levels. Both results indicate at the production of possibly migrating compounds in the FCM during the experiments as previously discussed.

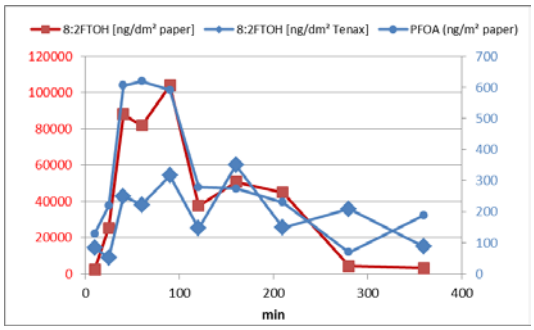

Fig 2.5.4: Paper –Tenax migration kinetic of PFOA and FTOHs

Whereas migration of chemicals from paper and board has been successfully described by migration models, a production of migrants during the migration trial has not dealt with in such models, yet. However, partition coefficients between paper and Tenax were calculated for all data points. This exercise revealed that in most cases concentrations in Tenax were below 2% of the corresponding level in the FCM. As long as no suitable model is available this relatively stable ratio may be used for risk assessment calculations.

Processes

Food processing exposes food items to increased temperatures, FCM, other food items (like drinking water), fermentation processes and mechanical forces (like separation of cream from raw milk). Each of these processes may increase or reduce levels of PFAA (Del Gobbo 2008). In addition, processing may lead to a degradation of precursor compounds into PFAA (Dinglasan 2004). Within the PERFOOD project three studied were performed to further elucidate effects of food technologies.

Laboratory trials with spiked food items (Sauer 2011): Carbohydrate rich food (potato dumplings, “Spätzle”, “Eierspätzle“, pasta) was cooked in salt water and either food or water was spiked with PFCs. Those were transferred from food to water as well as from water to food, but cooking times were too short to reach equilibrium between food and water. PFC migration increased with increasing food surface (potato dumplings < “Spätzle” < pasta). The transfer of long-chain PFCs from water into food increased by adding eggs to “Spätzle”. The reverse process was hardly affected. Additionally the polarity of PFCs influenced their distribution – apolar, long-chain PFCs were detected in food in higher concentrations than polar, short-chain PFCs. On the contrary the nature of the acidic group did not influence the behavior of PFCs.

The concentrations of PFCs in carbohydrate rich food were hardly reduced by cooking in water. Only for PFBS and PFHxA the transfer to water might be relevant in the case of a large food surface area and reached 22 % in the experiments. The cooking of carbohydrate rich food in PFC-containing water led to much higher PFC transfers. After cooking 80 % of PFOS was found in pasta, but only 7 % in potato dumplings. The PFC transfer from water to food increased with surface area of the food and with the amount of absorbed water during cooking.
The processing of milk products was examined by processes with a phase separation, for example the production of butter, cheese and cream, as well as the forming of milk skin after cooking. Spiked PFCs were present in both phases after phase separation. However, PFC concentrations in fatty phases were higher than in low-fat phases, even if the protein content in the fatty phase was low. In general the concentrations of PFCs increased with their chain length. Additionally the nature of the acidic group influenced the distribution in butter and cream. PFSAs were present in higher concentrations in the fatty phase than PFCAs with similar or higher chain lengths. Thus different kind of interactions between PFCs and constituents of milk products are taking place. From the results it can be concluded, that after processing of milk products concentrations of PFCs in fatty products are higher than in low-fat products.

Home cooking (Sölch 2012): The purpose of this study was to investigate the effect of the main home cooking processes on the concentration of per- and polyfluorinated chemicals in different food items. Six food items were selected and prepared with different techniques and FCM: fish fingers were fried in a PTFE coated frying pan, dough was baked on a baking sheet in the oven, French fries were prepared on a permanent PTFE coated baking sheet in the oven, pasta was boiled in a pot with salted tap water, spinach was heated in a microwave and hamburgers were grilled with an electric grill.

Food items, FCM and other additives (Salt, oil, etc.) were sampled before and after preparation and analyzed. Preparing fish fingers in a PTFE coated pan doubled the total PFC concentration in the breadcrumb of the fish finger (sum of PFC 0.3 ng/g). In the fish filet concentrations of PFC increased by 40 percent to levels of 0.45 ng/g.

The raw dough contained 0.15 ng/g PFC and almost tripled this value during the baking process. FTOH concentrations in dough increased from <5 to over 100 ng/g. The baking sheets reduced their PFC content from over 1 µg/g to 0.35 µg/g and FTOH levels doubled from 8000 to 16000 ng/g.

Baking fries on two investigated permanent baking sheets increased the PFC levels of the fries from 0.1 to 0.8 ng/g with sheet A and to 0.3 ng/g with sheet B. Both sheets reduced their concentrations during the process by 30 percent. Boiling pasta diluted the PFC levels by uptake of water. Mass balance calculations revealed that there is a small transfer of PFC from water into pasta; however dilution turned out to be the dominating process. Heating of spinach in the microwave did not significantly change levels. Grilling hamburgers seems to reduce levels to a small extent. However, this result is probably affected by difference in LOQs of raw and prepared food. However, the levels in food prepared with non-stick cookware are not a matter of concern. Exposure estimates are far below the Tolerable Daily Intake (TDI) of 150 ng/kgbw/day for PFOS and 1.5µg/kgbw/day for PFOA.

Industrial processes (Still 2012): There have been a number of screening studies on dietary exposure towards PFC (Ericson et al., 2008; Haug et al., 2010; Noorlander et al., 2011), which identified cow's milk as a food item with slightly increased PFC levels. The impact of milk processing on the concentrations of PFC in raw milk was therefore studied in industrial scale. Milk has been chosen as a preferred food item, because of the homogeneity of the input (raw milk) and the various kinds of milk processes that are likely to influence the concentration of PFCs (Dinglasan et al., 2004; Begley et al., 2005; del Gobbo et al., 2008). They include heating in pasteurized and UHT treated milk, microbial processes in ripened yogurt and cheese, phase separation in cream and butter as well as buttermilk and a whey drink and packaging processes in butter.

The study was performed in cooperation with a German dairy. In the first part of the study different kinds of milk and dairy products were selected from the portfolio of the dairy and screened for their PFAA content. In the second part of the study the process of butter production was selected and investigated in more detail. During the whole production process the PFAA content was monitored beginning with the raw milk. The raw milk has to undergo two steps of phase separation to be converted first into cream and then into butter. Byproducts with lesser fat content are skimmed milk and buttermilk. Finally, the step of packaging was included into the study as grease proof papers with fluorine containing coatings are applied. To examine the impact of migration during storage seven pieces of butter were packed and stored at 5°C. The PFC and FTOH content in the butter as well as the packaging material is measured after 7, 14, 21, 28, 35, 42 and 45 days.

Screening results indicated very low levels in most samples. However, if compared to raw milk, slightly increased levels of longer chain PFC were quantified in products with increased fat content. The sum of PPFBA, PFHxA, PFOA, PFNA and PFOS accounted for 15 pg/g in raw milk, 29 pg/g in yogurt (high fat content), 36 pg/g in cream and 49 pg/g in butter.

The mass balance sampling stated both, the very low levels in the milk products and an increase of levels of longer chain PFC from raw milk to butter. With respect to PFOA, PFNA and PFOS, levels in butter extended raw milk concentrations by a factor of 5. Packaging however had the highest influence on levels in the butter.

Processing drinking water

Whereas WP 4 dealt with the preparation of drinking water from raw water, WP 5 investigated hot and cold beverages prepared from drinking water, as exposure pathways studies have shown that low concentrations of PFAA in tap water already may pose a high contribution to daily human exposure. Therefore, the presence and sources of perfluorinated alkyl acids (PFAAs) in tap water and corresponding tap water based beverages collected in the city of Amsterdam was analyzed (Eschauzier et al 2012).

Tap water samples (n = 4) had higher concentrations of PFAAs than the corresponding post-mixed cola (n = 4). The lower PFAA levels in the cola were attributed to the pre-treatment of tap water in the mixing machines and dilution with cola syrup. In coffee samples from a coffee machine perfluorooctanoic acid (PFOA) at 4 ng L1 was the dominating analyte (n = 12). The concentrations of PFHpA, PFOA and non-branched PFOS were found to be significantly higher in manually (self) brewed coffee than in the corresponding tap water (n = 4). The contribution from short-chain PFAA analogs could not be quantified due to low recoveries. Leaching experiments at different temperatures were performed with fluoropolymers-containing tubes to investigate the potential of leaching from tubes used in beverage preparation (n = 16). Fluoropolymer tubes showed leaching of PFAAs at high (80 C) temperature but its relevance for contamination of beverages in practice is small. The specific contribution from perfluoropolymer tubing inside the beverage preparation machines could not be assessed since no information was available from the manufacturers.
The present study shows that although different beverage preparation processes possibly affect the concentrations of PFAAs encountered in the final consumed product, the water used for preparation remains the most important source of PFAAs. This in turn has implications for areas where drinking water is contaminated.

2.6 WP6 Quantifying the dietary intake in Europe

Introduction

This report summarizes the exposure intake estimates and risk characterization undertaken as part of WP6 in the PERFOOD project. The intake assessments have been focused on a selection of perfluoroalkyl acids(PFAAs) (PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS) in four selected regions, (Belgium, Italy, the Czech Republic, and Norway). Dietary exposures were estimated for the general, background exposed populations and the risk characterization undertaken accordingly for these chronically exposed populations in the selected EU and non-EU countries.

The main pillars for the food intake assessment

The following two main pillars were identified for undertaking a reliable food intake estimation:

The first pillar: occurrence data focused on those food items that with respect to the existing literature at the beginning of the project, seemed most relevant for a geo-referenced assessment.
In particular focus was given to the following:

- PFAA occurrence in drinking, bottled and mineral water, with particular reference to their site-specific collection (i.e. freshwater vs groundwater).
- PFAA occurrence in some food of vegetal origin poorly represented in the inventoried database, but of utmost relevance for the intakes, according to the inventoried national food consumption databases.
- Potential analytical bias in the occurrence data referred to potatoes and eggs, where large discrepancies in the PFAA levels were reported in the scientific literature.
- Differences in the PFAA levels in seafood from wild and farmed species, respectively.
- Potentially higher contamination in composite samples and fast food meals, due to PFAA contamination from packaging, cooking and serving.

To estimate worst-case exposure intakes, a dedicated sampling of single food items was planned from areas known to be highly contaminated with PFAAs (hot spots). Also whole meals/cauldrons were considered for sampling both to reduce the number of analytical determinations, and to cross-check the intake estimates with those estimated from analysis of single raw food items (occurrence in the single raw food items multiplied by its dietary consumption). The cauldron approach can also account for potential contamination from food processing/serving.

The second pillar: food consumption databases harmonized for the four European gegions considered: BE, CZ, IT, & NO.
At the end of January 2010, the national food consumption databases from the following countries were obtained: Belgium, Czech Republic, Germany, Italy, the Netherlands, and Norway. For Sweden, the United Kingdom and Europe (as reported by WHO) food consumption estimates were obtained from scientific publications. The food grouping has been re-arranged to match the categories and sub-categories in the “EFSA Concise European Food Consumption Database”. On the basis of the food consumption database it was possible to identify recommended upper limits for the

LOQs for the different food items which would result in dietary exposures less than 10% of the TDI. The lowest TDI proposed for PFOS (150 ng/kg bw per day) was considered as r a conservative approach. Proposed LOQs were in the range 0.005 – 0.020 ng/g for the different food items. All laboratories involved in the analytical work were required to meet these minimum requirements. For the final PFAA PERFOOD intake assessment, the more detailed comprehensive Food Consumption Database (cFCDB) was adopted as soon as it was made officially available by EFSA.
Because of the poor representativeness of the infants diets in the cFCDB, baby food and milk formulas additionally sampled and analyzed for inclusion in the PERFOOD intake assessment.

The PERFOOD dietary intake estimates

The PERFOOD dietary intake estimates based on the average concentration and the average food consumption figures from the respective regions (BE, CZ, IT, and NO) are reported in Table 2.6.1.

		MEAN							
		BE		CZ		IT		NO	
		Adults	Children	Adults	Children	Adults	Children	Adults	Children
PFHxA	LB	289	873	7	19	30	63	14	34
	UB	424	1137	130	233	96	195	66	128
PFOA	LB	186	280	16	43	133	250	80	152
	UB	231	389	188	326	197	378	107	195
PFNA	LB	91	299	30	66	30	66	20	22
	UB	145	402	142	254	97	200	70	115
PFDA	LB	43	57	24	50	26	51	17	13
	UB	99	165	135	237	107	209	104	179
PFUnDA	LB	20	16	15	32	129	269	47	36
	UB	92	174	153	295	326	651	107	157
PFHxS	LB	86	288	1	1	16	36	7	10
	UB	105	329	59	113	91	179	35	69
PFOS	LB	336	960	370	959	183	404	87	80
	UB	405	1113	449	1094	258	543	152	208

Table 2.6.1. Mean dietary exposure estimates in pg/kg bw per day for seven selected PFAAs. Results are presented for the lower bound (LB) and the upper bound (UB) approach in four European regions and two different age classes: adults (aged 18-64 years) and children (aged 3-9 years). Deterministic calculations are based on aggregation level 2.

Measurements below the limit of detection have been treated using a lower and upper bound approach, taking the zeros and the LOQs as surrogates for the measured values below, respectively.

It was possible to note distinct differences in the relative contribution of food categories for the exposure to PFAAs in different European regions, both as consequence of the different occurrence and of the different food items consumption. Most sensitive food items for PFOS intake were represented both from food of animal origin (fish and seafood, animal offal, eggs) and vegetables, with distinct regional differences at non-hotspot areas.

Country	Data Source	Infants		Children		Adults	
		mean	P95	mean	P95	mean	P95
BE	EFSA	1.2 – 9.1	na	1.2 – 7.4	5.5 – 14	0.8 – 2.6	3.6 – 6.3
	Perfood- s	na	na	1.0 – 1.1	2.7 – 2.9	0.3 – 0.4	1.3 – 1.4
	Perfood- c	na	na	na	na	0.1 - 0.1	na
CZ	EFSA	na	na	1.5 – 6.8	7.6 –15	0.70 – 2.9	3.6 – 6.8
	Perfood- s	na	na	1.0 – 1.1	3.0 – 3.1	0.4 – 0.4	1.4 – 1.5
	Perfood- c	na	na	na	na	0.4 – 0.5	na
IT	EFSA	0.9 – 1.1	na	2.7 – 7.4	8.5 – 16	1.4 – 3.2	4.1 – 6.8
	Perfood- s	0.4 – 0.6	na	0.4 – 0.5	2.2 – 2.3	0.2 – 0.3	0.9 – 1.0
	Perfood- c	0.2 – 0.8	na	na	na	0.2 – 0.2	na
NO	EFSA	na	na	na	na	na	na
	Perfood- s	na	na	0.1 – 0.2	0.3 – 0.5	0.1 – 0.2	0.3 – 0.4
	Perfood- c	na	na	na	na	0.1 – 0.2	na

Table 2.6.2. Comparison of the intake estimates for PFOS from EFSA opinion (2012) and PERFOOD project (s = systematic; c = cauldrons; na = not available).

For PFOA, the occurrence in vegetables was the main factor. A direct comparison of the food intake estimates from EU PERFOOD data, with those reported in the peer reviewed literature, can be made, if the following quality aspects are taken into account: a) the sampling strategy: in some cases, food intake has been calculated on composite meals or duplicate diets; in others, only fish and seafood and drinking water categories have been considered; b) the occurrence data: the analytical performances both in terms of sensitivity and the PFAAs investigated generated in the reported data that may not represent current exposures; c) the way the data have been expressed (lower bound, medium bound, and upper bound approaches); d) the target population and sensitive groups investigated, sometimes the estimates are referred to a specific town/district, or recognize some gender issues (women); e) the food consumption database used, when not harmonised with the comprehensive one proposed by EFSA. Table 2.6.2. presents a comparison of the intake estimates from PERFOOD with those from reported in a recent EFSA opinion Estimates of dietary intake made in PERFOOD are much lower than in the EFSA database.

Aggregated exposure: dust contribution to the intake

Because PFAA intake could be determined also for dust ingestion, part of the assessment was focused on the contribution of dust ingestion to the overall exposure intakes. Dust intake could be of potential relevance for infants, due to their mouthing behavior and the time spent in close contact with the floor and carpets). Because no study has yet been undertaken focusing specifically on ingestion of house dust, but rather on soil ingestion, all estimations of exposures by house dust ingestion are afflicted by an unknown degree of uncertainty. In the evaluation, the German median estimate (16 mg per day) (AUH, 1995) was considered for a lower average intake, US EPA value (60 mg per day for 1-6 y children) as central tendency, while the German 95th percentile (110 mg per day) for a conservative estimate of exposure. Similar estimates for house dust exposure estimates have been used in the opinion on risk assessment for PFOA and PFOS recently prepared by the EFSA (2008).

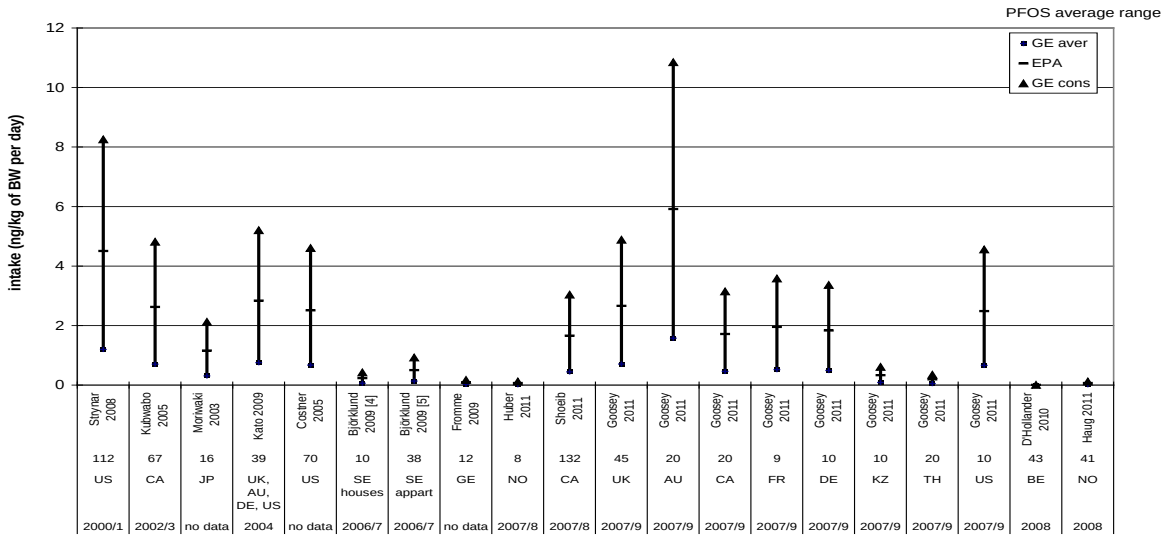

Figure 2.6.1. Estimation of PFOS exposure (ng/kg bw per day) by ingestion of house dust, given as the range of an average estimate. The estimates are arranged in order of the year of sampling, also indicating the number of samples and the country of evaluation. The estimations are based on mean concentrations as reported by the authors indicated at the x-axis and by recommendations for house dust intake made by the German AUH (1995), and the US EPA (2008).

The dust ingestion estimates for PFAAs used a georeferenced set of literature data (from Belgium, Netherlands, Norway, Sweden) and were in good agreement with other recent studies (Haug et al., 2010, Egeghy, 2011, Noorlander et al., 2010). Under the worst case scenario, the exposure to PFAAs via dust ingestion was the same order of magnitude (hundreds of pg/kg bw per day) as the dietary exposure estimate. However, it is worth noting that the huge variability reported in the dust contamination may suggest possible sampling and analytical bias that introduces a high degree of uncertainty in the dust ingestion estimates.
Inhalatory and dermal routes were considered to be not relevant for the general population, with estimated averaged contributions to the intake well below 1 pg/kg bw per day for PFOS and PFOA for non-occupationally exposed populations.

Matching the external dose estimates with internal doses from georeferenced biomonitoring studies.

Figure 2.6.2 shows a comparison of reconstructed total exposures from biomonitoring data with the average upper bound (UB) and lower bound (LB) dietary intakes estimated in the PERFOOD project (see previous section). For PFOS the median exposure calculated from biomonitoring data was 15 and 12 times higher compared to the estimated dietary intake for the Belgian and Norwegian population respectively. A somewhat better agreement was observed for the Italian population with calculated exposures within a factor of 3 of the dietary intake estimates. For PFOA (Figure 2) the median exposure calculated from biomonitoring data were within a factor of 2 of the estimated dietary intakes for the Belgian and Italian population. A slightly higher discrepancy between reconstructed exposures and dietary intake estimates (factor of 4) was observed for the Norwegian population.
Applied as a screening level tool, the steady state one-compartment TK model indicates that dietary intake contributes between 7 to 42% of the total exposure to PFOS for the different European countries included in this study. The TK modeling results therefore suggest that non-dietary dietary exposure pathways constitute a major part of the total exposure to PFOS. Nevertheless, it should be noted that modeled exposures from biomonitoring data reflect a past exposure whereas dietary intake estimates reflect the ongoing exposure. Because of the relatively long elimination half-life of PFOS in humans and the sampling dates of human serum samples, the modeled total exposures in Figure 2 reflect an exposure from the early 2000s. Biomonitoring studies have shown a decreasing temporal trend of PFOS and PFOA in human serum samples after the year 2000 when PFOS and related chemicals were phased out by the major manufacturer. It is therefore noteworthy that the best agreement between dietary intake estimates and modeled total exposure to PFOS was observed for the most recent set of biomonitoring samples from the Italian population. Based on estimated elimination half-lives for PFOS, the external exposure has decreased by approximately a factor of ten after the year 2000. Thus, the discrepancy between modeled exposures from biomonitoring data and dietary intake estimates for the Italian and Belgian population may be largely attributed to the decreasing exposure over time.

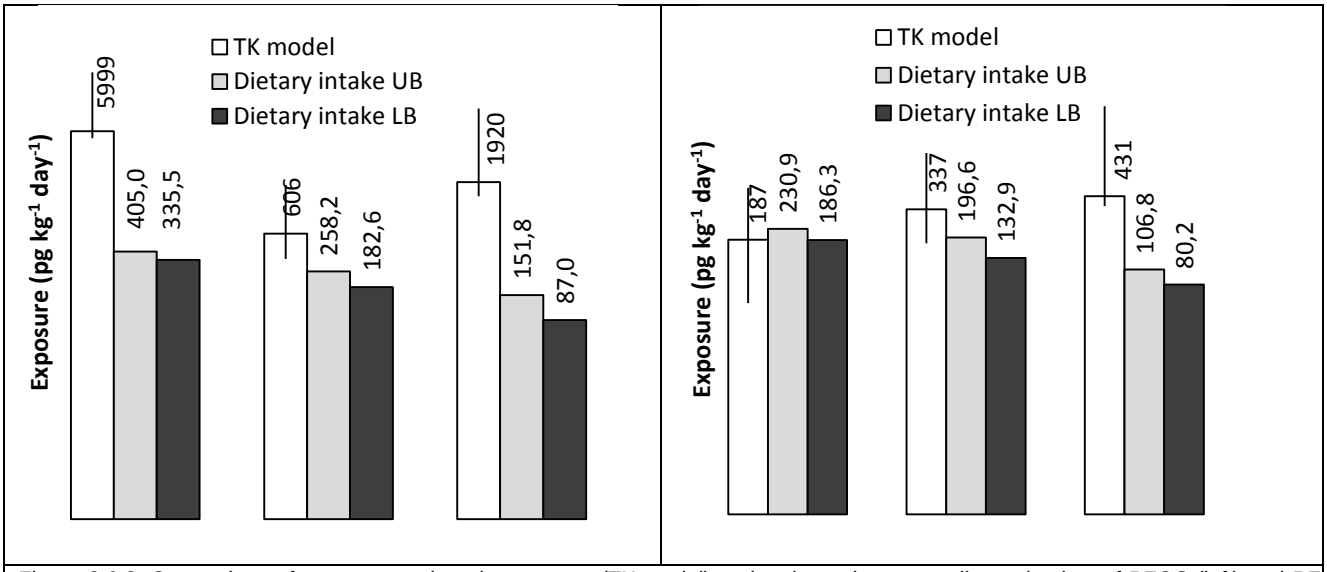

Figure 2.6.2. Comparison of reconstructed total exposures (TK model) and estimated average dietary intakes of PFOS (left) and PF (right) for the Belgian (BE) Italian (IT) and Norwegian (NO) population. Solid white bars represent reconstructed intermediate (medi exposure and the error bars depict the low and high exposure. Light grey and dark grey solid bars represent the upper bound (UB) a lower bound (LB) assumption for non-detects respectively.

The Risk Characterisation

Specific TDIs are available for PFOS and PFOA (150 and 1,500 ng/kg bw per day, respectively), according to the consolidated toxicological evidence. The toxicological data in experimental animals from chronic studies (oral route) do not highlight the same end-points, even if some similarities are present. Therefore, it was not possible to generalize about the toxicity of PFAAs or to treat them as a homologous class. It is not possible therefore to perform a cumulative exposure estimation nor the corresponding cumulative risk characterisation. In the spirit of a conservative risk characterization, we assumed the TDI reported for PFOS was the same for PFHxS, and that the TDI for PFOA was the same for all perfluoroalkyl carboxylic acids as potential reference guidance values for intakes, on the basis of the structural similarity of the molecules. It was then possible to derive a Margin of Safety (MOS, as ratio between the TDI and the intake estimate) for the most bioaccumulating PFAAs.

Within the PERFOOD project, the only reason for exceeding the existing TDIs is when food originates from a contamination "hot spot" area. A critical exposure (MOS from <5 to >1) was estimated only for PFOS in the considered PERFOOD hot spot areas: in Belgian adults and in egg consuming children, and in Czech children and in high consumer adults. A potential exceedance of the TDI forPFOS (MOS < 1) has also been traced in the same hot spot areas: in the Belgian adults consuming high amounts of eggs, in the Czech children consuming high amounts of fish, and in Norway, both in adults and children from the general population and from high fish consumers. Free range eggs and feral fish from contaminated area represented the major source of intakes. For the Italian region, the Ferrara hot spot considered for the PFOA occurrence in water did not reveal potential exceedance of the TDI. Also the feral fish intake scenario from the Mediterranean Sea indicated a MOS of 22 for PFOS intake in high consumer children, as a worst case.

Sensitivity analysis with drinking water exposure

The sensitivity analysis shows that, based on the average national water consumption data, it is extremely unlikely that the TDI for PFOA will be exceeded. The contamination levels required to exceed the TDI are only infrequently observed in extreme contamination "hot spots" (e.g. drinking water near fluoropolymer production sites). (Figure 2.6.3).

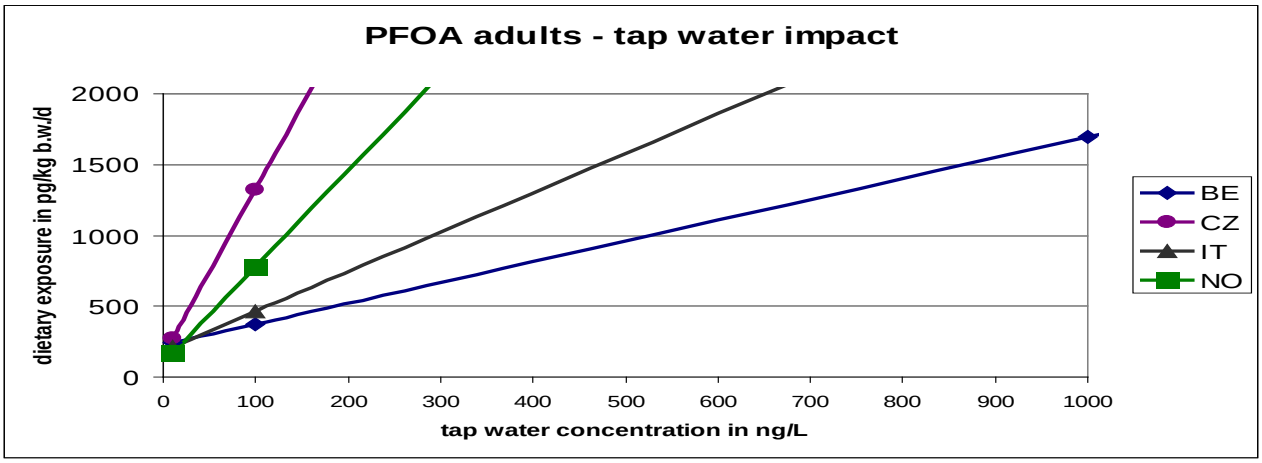

Figure 2.6.3. Sensitive analysis for the critical concentrations of PFOA in drinking water that would lead to an exceedance of the TDI of 1,500 ng/kg bw per day, based on the national food consumption habits in BE, CZ, IT and NO.

Conclusion

The following main conclusions can be drawn from the WP6 work in PERFOOD:

- The sampling program in PERFOOD was not designed to find the statistical distribution of PFAAs in each food item in the consumption database for each country studied. Therefore, the dietary exposures estimated cannot be considered representative of the general population
- Estimates of dietary intake made in PERFOOD are much lower than in EFSA database. It is our belief that this lower estimation is partly a reflection of improvements in analytical chemistry. Data in the EFSA database are from studies which used older instrumental equipment and methods. Consequently, these older studies had much higher limits of detection. Another problem with the EFSA database is that it does not separate data from "hot spot" areas from those from background contaminated areas as was done in the PERFOOD project.
- Due to the much lower reported dietary exposure, PFAA exposure from ingestion of dust is now often similar in magnitude to dietary exposure. However, there is a higher variability in dust concentrations either due to variability in sampling and analytical methods or variability in contamination in indoor environments,
- Baby food and milk products do not provide additional high exposure to PFAAs for infants.
- Levels of PFOA in blood can be explained quite reasonably well from dietary exposure according to results from a steady-steady toxicokinetic model. For PFOS, however, blood levels still appear to decreasing following the phase-out of PFOS-containing consumer products by the major manufacturer.
- There are different dietary intake patterns from region to region as a result of different food consumption and contamination patterns, but a more representative sampling strategy would be needed to study this more closely.
- Different food items are important for the various PFCA and PPSA homologues. For PFOS and long-chain PFCAs with greater than 8 perfluorinated carbons (PFNA and above), fish is the most important dietary item. For the short-chain PFCAs, vegetables and beverages are the most important dietary items.
- If it can be assumed that PFOS and PFOA TDIs can be transferred to other PFAAs then safe limits for dietary exposure are not exceeded. TDIs can be exceeded, however, if local foods are consumed in areas of "hot spot" contamination.

Within the PERFOOD Project, the WP6 activities have tried to cross-link different environmental and food safety aspects, to come to food intake estimates well characterised with respect to the sources of uncertainties. To this respect, a challenging boundary for PFAAs food intake risk assessment may be acknowledged in the investigation of the relationship between a good environmental status and the use local food resources, water included. A sound evaluation of aggregate human exposure (food, dust, soil, air) will be able to reveal time trends, to correlate body burdens and potential toxicological outcomes in the population (i.e. thyroid dysfunction, dismetabolisms, immuno-suppression, reproductive outcomes), and to predict new scenarios for PFAAs sourcing determined by the changes in the environmental, economic, social, and legislative dynamics.