SEAFOOD AND FISH PERFLUOROALKYL ACID CONTAMINATION, DIETARY EXPOSURE OF FRENCH GENERAL POPULATION, HIGH SEAFOOD CONSUMERS AND FRESHWATER FISH CONSUMERS

Yamada A[1], Bemrah N[1], Sirot V[1,], Desvignes V[1], Héraud F[1,a], Merlo M[1], Veyrand B[2], Cariou R[2], Marchand P[2], Antignac JP[2], Le Bizec B[2], Leblanc JC[1]*

[1]Risk assessment department – French agency for food, environmental and occupational health and safety (ANSES), 27-31 avenue du Général Leclerc, Maisons-Alfort, F-94701, France;[2]LUNAM Université, Oniris, Laboratoired'Etude des Résidus et Contaminants dans les Aliments (LABERCA), USC 1329 INRA, Nantes, F-44307, France;[a]Present address : Dietary and chemical monitoring unit (DCM), European food safety authority, Via Carlo Magno 1A, 43100 Parma, Italy.

Introduction

Perfluoroalkyl acids (PFAAs) are anthropogenic fully fluorinated amphiphilicsubstances with a high chemical and thermal stability[1]. Due to their physicochemical properties, these compounds were widely used in various industrial applications and consumer products since the late 1950's such as fire-fighting foams, cleaning agents, inks, nonstick cookware, metal painting, stain,as well assoil repellents for leather, textiles and paper[2]. However, the consequence of this stability and these uses is a global environmental contamination at a ng.g-1 level and a high persistence in water, sediment, and soil[3], as they could be also spread by atmospheric transport[2]. Health concerns have been raised with the detection of PFAAs at low levels in the serum of general population[4], and experimental animal studies have pointed out health hazards such as hepatotoxicity[5], immunotoxicity[6], developmental toxicity[7], effects on thyroid hormones[8] and carcinogenicity[9]. Among PFAAs, the half-life of perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexanesulfonate (PFHxS) in humans were respectively estimated to 5.4, 3.8 and 8.5 years[10]. PFAAs are not lipophilic like most of other persistent organic pollutants (POPs)including dioxins or polychlorinated biphenyls (PCB), but exhibit affinity with proteins. Food intake is thought to be the major exposure pathway for the general population, accounting for up to 90% of the total exposure[11]. Fishes are especially considered as an important contributor, regarding their level of contamination and consumption[12]. The aim of this studywas to investigate food PFAA levels in foodswith a specific focus on fish and seafood products,and to compare dietary exposure between different French population groups, namely general population, high seafood consumers and freshwater fish consumers.

Materials and methods

Food consumption data

Three sets of food consumption data were studied: the 2009 French national individual food consumption survey INCA2 for the general population, the 2006 CALIPSO study (Fish and seafood consumption study and exposure to trace elements, pollutants and omega 3) for the high seafood consumers, and the 2011 ICAR-PCB study (National PCBs impregnation study of freshwater fishes consumers) for the freshwater fish consumers. The recruitment methodologies have already been described elsewhere[13-15]. Briefly, the INCA2 study included 1918 adults between 18 and 79 years representative of the French population through stratification, who reportedtheir dietary habits through a 7-day diary record. The CALIPSO study included 993 adult high seafood consumers (seafood consumption frequency ≥ twice a week) in 4 French coastal areas: Gironde-South Charente Maritime, Normandy-Baie de Seine, South Brittany and Mediterranean-Var. The ICAR-PCB study included 606 adults, anglers of members of their family, representing 21180 angler households in 6 areas corresponding to the 6 major French rivers. The CALIPSO and ICAR-PCB subjects completed a food frequency questionnaire including portion sizes.

Food sampling

The methodology of food collection has already been described elsewhere[16-18]. Briefly, the most consumed products, representing about 90% of the total diet of French general population, were collected by the way of the second French total diet study (TDS 2). Composite samples (n=599) were prepared as consumedby the general

population and analyzed. For high seafood consumers, 40 fresh or frozen marine fish species, 29 fresh or frozen crustaceans, mollusks and shellfishes, 6 canned fish-based products, 4 smocked fish-based products and 4 other seafood-basedproducts were collected during the CALIPSO study, representing 88 to 100% of total seafood consumption rate. The edible parts of the composite samples (n=159) were analyzed. For freshwater fish consumers, 16 freshwater fish species, representing almost 100% of total freshwater fish consumption rate, were collected from the ICAR-PCB study. The composite samples of fillets (n=387) were analyzed.

Sample analysis:
The used methodology targeted 15 PFAAs: perfluorobutanoic acid (PFBA), perfluorobutanesulfonate (PFBS), perfluorodecanoic acid (PFDA), pefluorodecanesulfonate (PFDS), perfluoroheptanoic acid (PFHpA), perfluoroheptanesulfonate (PFHpS), perfluorohexanoic acid (PFHxA), PFHxS, perfluorononanoic acid (PFNA), PFOA, PFOS, perfluoropentanoic acid (PFPA), perfluoroundecanoic acid (PFUnA), perfluorotetradecanoic acid (PFTeDA) and perfluorotridecanoic acid (PFTrDA). Solid food samples were freeze-driedand extracted with methanol, while milk and dairy products samples were extracted using acetone[22]. For fish samples a dispersive SPE based on charcoal particles was then applied, while other food extracts werepurified onto two consecutive SPE columns (copolymeric reversed phase and charcoal). Water samples (100 mL), were directly deposited on a reversed-phase SPE column. Final purified extracts were analyzed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with negativeelectrospray ionization[23].Two diagnostic signals (MRM transitions) were recorded per analyte. Quantification was performed according to the isotope dilution principles:each sample was supplemented by 11 ^{13}C-labelled internal standards ($^{13}C_4$-PFOA, $^{13}C_4$-PFOS, $^{13}C_4$-PFBA, $^{13}C_5$-PFHxA, $^{13}C_4$-PFHpA, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_7$-PFUdA, $^{13}C_2$-PFDoA, $^{18}O_2$-PFHxS, and $^{13}C_4$-PFOSi). Quality controls as well as proceduralblank sampleswere includedineach batch of analyses. The limits of detection and quantification, depending on the considered matrix and compound, ranged from 0.2 pg/g fresh weight (fw) to 3.73 ng/g fw.

Dietary exposure assessment
Individual dietary exposure was calculated by combining individual food consumption data, food contamination and individual body weight, according to the following formula:

$$E_{i,j} = \frac{\sum_{k=1}^{n} C_{i,k} L_{k,j}}{BW_i}$$

where $\mathbf{E_{i,j}}$is the daily exposure of the subject *i* to the PFAA *j* (ng.kg^{-1} bw.d^{-1}), $\mathbf{C_{i,k}}$is the daily intake of the food item *k* by the subject *i* (g.d^{-1}), $L_{k,j}$is the mean concentration of the PFAA *j* in the food item *k* (ng.g^{-1} wet weight), *n* is the number of foods in the diet of the subject *i*, and $\mathbf{BW_i}$is the body weight of the subject *i* (kg).The exposure for general population was assessed by crossing INCA2 consumption data and French TDS2 data, for high seafood consumers by crossing CALIPSO consumption data, CALIPSO contamination data for seafood and French TDS2 for the other food items, and for freshwater fish consumers by crossing ICAR-PCB consumption data, ICAR-PCB contamination data for freshwater fish, CALIPSO contamination data for seafood, and French TDS2 for the other food items.As the rates of the left-censored-data i.e. non-detected contamination are high in the 3 studies (99.3% for TDS 2, 69.9% for CALIPSO, 64.3% for ICAR-PCB), the WHO GEMS/Food-EURO workshop recommendations[19]which defined twoscenarii for the treatment of left-censored data were applied: the lower-bound one (LB) in which all the data below the limit of detection (LOD) are considered as null and the upper-bound one (UB) in which the same data are considered as equal to the LOD. As a result, 2 exposure scenarii, LB and UB, taken into account uncertainty arising from analyses are presented.

Results and discussion

Food contamination
The food contamination distributions for foods analyzed from the three studies are presented in table 1. Data shows that PFAA contaminations detected in the TDS 2 food items are in average around 20 times lower (under LB scenario) compared to those in seafood and freshwater fishes. Our results are consistent with published literature[12,20].Swordfish (*Xiphiasgladius*) for marine fish and brown trout (*Salmotruttafario*) for freshwater fish were speciesshowing the lowest PFAA contamination levels, while seabass(*Dicentrarchuslabrax*) and gudgeon (*Gobiogobio*) were species associated with the highest one. PFOS was found to be the prevailing PFAA in freshwater fish contamination (74% of the total contamination under LB) whereas marine fish contamination is

shared between PFOA (24%), PFOS (20%), PFHxA (15%), PFHpA (11%) and PFBA (11%). Reasons or origin for such different contamination profiles between marine and freshwater fishes could be due to differences in bioaccumulation potency and/or in environmental contamination patterns.

Dietary exposure

The mean dietary exposure to PFAAs is presented for each population group in Table 2 for LB and UB scenarii, except for general population (LB) for whichdietary exposure was close to 0 due to high level of censored data (about 90%) and low fish consumption. The dietary exposure for PFTrDA and PFTeDAwas not assesseddue to the lack of data in several food items.

Table 1: Food contamination distribution from TDS 2, CALIPSO and ICAR-PCB studies, sum of the 15 PFAAs (ng.g^{-1}) on food groups (TDS2) or on species (CALIPSO and ICAR-PCB)

			LB			*UB*		
Study	Food items		Mean	Std	Min-Max	Mean	Std	Min-Max
TDS 2	Whole diet*	Σ PFAA	0.0	0.0	0-0.2	1.7	0.9	0.1-3.6
CALIPSO	Marine fish	PFOS	0.6	0.6	0-2.6	0.6	0.6	0.0-2.6
		PFOA	0.7	0.5	0-1.7	0.7	0.4	0.0-1.7
		Σ PFAA	2.9	1.6	0.3-6.8	3.3	1.5	1.1-7.1
	Mollusk, crustacean, shellfish	Σ PFAA	1.7	2.1	0.0-7.0	2.2	2.1	0.2-7.5
	Canned, smocked, seafood-based product	Σ PFAA	0.3	0.5	0-1.9	1.1	0.6	0.3-2.2
ICAR-PCB	Freshwater fish	PFOS	42.0	38.0	7.3-138.8	42.0	38.0	7.3-138.8
		PFOA	0.2	0.5	0-1.8	0.3	0.5	0.0-1.8
		Σ PFAA	56.3	49.0	9.4-168.4	57.6	49.2	10.3-169.9

* Fish and seafood products were excluded

Table 2: Mean dietary exposure to PFAAs for metropolitan French general adult population (TDS 2), high seafood consumers (CALIPSO study) and freshwater fish consumers (ICAR-PCB study) including its upper quartile (Q4, n=152) (ng.kg^{-1} bw.d^{-1})

	LB: all foods (% from fish and seafood)			*UB: all foods (% from fish and seafood)*			
PFAA	High seafood consumers	Freshwater fish consumers	Q4 freshwater fish consumers	General population	High seafood consumers	Freshwater fish consumers	Q4 freshwater fish consumers
PFBA	0.43 (100)	0.05 (100)	0.07 (100)	2.57 (1.1)	4.3 (12.6)	4.49 (1.7)	4.41 (3.2)
PFBS	0.02 (0.06)	0.01 (0.1)	0.01 (0.4)	1.16 (1)	1.74 (3.2)	1.88 (0.6)	1.82 (1.7)
PFDA	0.16 (97.2)	0.08 (89.2)	0.42 (97.4)	0.34 (2.3)	0.73 (3.05)	0.64 (13.3)	0.96 (45.8)
PFDS	0.02 (100)	0 (100)	0.03 (100)	0.4 (2)	0.74 (10.8)	0.67 (2.4)	0.69 (8.5)
PFHpA	0.45 (91.1)	0.08 (61)	0.1 (69.7)	0.76 (2.4)	2.73 (16.9)	1.2 (5)	1.18 (8.7)
PFHpS	0 (100)	0 (100)	0.03 (100)	0.7 (1.1)	0.85 (7.8)	1.06 (1.5)	1.06 (5.7)
PFHxA	0.64 (86.5)	0.09 (76.6)	0.16 (87.4)	0.86 (1.6)	1.87 (32.1)	1.41 (5.8)	1.45 (13.3)
PFHxS	0.06 (31.9)	0.02 (24.5)	0.05 (68.3)	0.38 (1.4)	0.67 (11.8)	0.66 (2.9)	0.69 (11.1)
PFNA	0.18 (98.9)	0.03 (89.7)	0.07 (94.5)	0.49 (1.9)	1 (23.9)	0.86 (4.3)	0.86 (10.1)
PFOA	1.16 (97.1)	0.15 (92.7)	0.23 (94.9)	0.74 (1)	2.06 (58.2)	1.23 (12.8)	1.27 (19.4)
PFOS	1.53 (98.2)	1.17 (98.8)	7.51 (99.8)	0.66 (4)	2.45 (61.9)	2.13 (54.6)	8.42 (89.1)
PFPA	0.21 (100)	0.02 (100)	0.03 (100)	1.5 (1.5)	2.39 (12)	2.2 (1.9)	2.12 (4.4)
PFUnA	0.43 (99.9)	0.19 (99.5)	1.28 (99.9)	3.23 (1.6)	5.27 (10.1)	5.06 (4.3)	5.8 (22.7)

Results show that in the three studies, fish and seafood appear to be the major food contributors under LB scenario for all PFAA congeners except PFBS for which the major contributor is water. The contribution of fish and seafood exposure rangesaround 32% and 100% for high seafood consumers, 25% and 100% for freshwater fish consumers and 68% and 100% for the upper quartile of freshwater fish consumers, when PFBS is excluded. Under UB scenario, other food items can be pointed out as major contributors, reflecting the impact of the analytical limits. As expected, high seafood consumers (seafood consumption frequency twice a week or more) and freshwater fish consumers, especially those in the upper quartile (freshwater fish consumption frequency 45 times per year or more), are more exposed to the 13 PFAA compounds presented above than the general population. Whatever the considered scenario, dietary exposure to the different PFAAs appears quite similar between all 606 anglers or member of their family and those in the upper quartile, except for PFOS, due to the high levels of freshwater fish PFOS contamination. The same applies to the high seafood consumers who are

more exposed to PFOA due to the high level of PFOA contamination among the marine fishes. Among seafood, the major contributors of overall dietary exposure were eel (*Anguilla Anguilla*), ray (*Raja clavata, Raja naevus, Raja circularis*), and coalfish (*Pollachuisvirens*). Among the freshwater fishes, the major contributors were brown trout, gudgeon and common roach (*Rutilusrutilus*).The PFAA dietary exposures from our studies are consistent with those found in the literature for other European countries[21].In further study, a look for the source of PFAA contamination will be attempted, especially for freshwater fishes. Freshwater fish contamination data and river contamination data could be analysed at the same time to eventually point out an association between water, sediment, and fish contamination and define environmental indicator for monitoring health if needs to be set in the future.

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