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Background contamination of perfluoralkyl substances in a Belgian general population

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ABSTRACT

The few Belgian studies on the human exposure to perfluoroalkyl substances (PFASs) have until now concerned the Northern part of Belgium (Flanders), while data related to Wallonia (South region) are missing. To fill this gap, 8 perfluorinated carboxylic acids and 3 perfluorinated alkyl sulfonates were measured in the serum of 242 adults (> 18 years old) recruited in 2015 and living in the Province of Liege. Some multivariate regression models were also built with the PFAS levels and the participant's answers to a questionnaire about their diet and lifestyle habits in order to identify some predictors of exposure. The results obtained showed that although PFAS levels observed in our population seemed to be similar or lower than those reported in other countries, and especially lower than in the Northern part of Belgium, half of the population showed PFOS and PFOA serum levels above the health guidance values set by the German HBM Commission. As expected, age and gender were the main covariates explaining the different PFAS serum levels between participants, while breastfeeding (for women), consumption of fish and seafood, consumption of rice, and use of nail polish seemed also to impact the PFAS body burden of our population. Nevertheless, the statistical models were poorly predictive suggesting that the main sources of exposure were not taken into account.

1. Introduction

Perfluoroalkyl substances (PFASs) are man-made chemicals that have been widely used since the mid-20th century in many industrial applications as well as in everyday life consumer products, due to their unique oil and water repellant properties as well as their high thermal and chemical stabilities. They have been employed among others as surfactants in cleaning agents or in firefighting foams, as surface protectors in carpet, textiles, upholstery, food packaging, and non-stick cookware (ATSDR, 2018; Kissa, 2001; Lindstrom et al., 2011). They have also been useful in the automotive and aerospace industries, in building construction, in personal care products, etc (OECD, 2006; Wang et al., 2013). Due to their persistence, their ubiquity in the environment and their health concerns, a phase out has been initiated from 2000 by some American industries (Renner, 2008), and subsequent restrictions were implemented mainly in United States (US EPA, 2015) followed few years later by the European countries (Schröter-Kermani et al., 2013). The impact of these production and use reductions on the human exposure was confirmed by the decreasing temporal trend of the serum levels from worldwide general populations reported from 2000 to 2010 for some PFASs, mainly perfluorooctane sulfonate (PFOS) and to a lesser extend perfluorooctanoic acid (PFOA), (Berg et al., 2014; Glynn et al., 2012; Kato et al., 2011; Schoeters et al., 2017; Schröter-Kermani et al., 2013; Toms et al., 2014; Yeung et al., 2013). Nevertheless in some countries (i.e. China) where PFASs are still used and produced (Li et al., 2017; Xie et al., 2013), higher PFAS serum levels would be expected compared to other populations which might be associated with several health effects such like a decline renal function, hepatotoxicity or hypertension (Bao et al., 2017; Nian et al., 2019; Wang et al., 2019).

There are different reasons why actual human biomonitoring focused on PFAS contamination is needed so far. Firstly, decrease of PFOS exposure related to the implementation of last regulations still deserves to be confirmed (i.e. inclusion of PFOS in the Annex B of the Stockholm Convention, inclusion of PFOA and other perfluorinated carboxylic acids on the candidate list of substances of very high concern within the

Abbreviations: PFAS, Perfluoroalkyl substance; PFOS, perfluorooctane sulfonate; PFOA, perfluorooctanoic acid; PFBS, perfluorobutane sulfonate; HBM, human biomonitoring; PFPeA, perfluoropentanoic acid; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUnDA, perfluoroundecanoic acid; PFDoA, perfluorododecanoic acid; PFHxS, perfluorohexanesulfonate; LOQ, limit of quantification; BMI, body mass index

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European REACH program). Secondly, for other PFAS than PFOS and PFOA, the temporal trends of serum levels seemed to vary according to the perfluorinated chemicals, the time and the country where the study took place. For instance, upward trends have been reported for perfluorononanoic acid (PFNA), perfluorohexanesulfonate (PFHxS) and perfluorobutanesulfonate (PFBS) in Sweden between 1996 and 2010 (Glynn et al., 2012) or in United States between 1999 and 2008 (Kato et al., 2011), while no clear trend was observed for PFNA in Germany during the two last decades (Schröter-Kermani et al., 2013). Then, human contamination data concerning short chain PFAS such as PFBS, a recently used as an alternative of restricted PFASs (Wang et al., 2013) are still lacking. And finally, other fluorinated substances like fluorotelomers or functionalized perfluoropolyethers demonstrated to be potential precursor of PFOS and/or PFOA are until today not regulated (Wang et al., 2013).

In addition, if food intake and especially seafood product consumption was considered for a long time as the main contributor to the human exposure (Haug et al., 2011; Vestergren and Cousins, 2009), other food items and other routes of exposure have been demonstrated to substantially contribute to the total exposure (Bartolomé et al., 2017; Berg et al., 2014; de la Torre et al., 2019; D'Hollander et al., 2015; Fraser et al., 2012,2013; Klenow et al., 2013; Shoeib et al., 2011). For instance, the contribution of fruits to the total dietary intake could vary from 20 to 93 % depending on the PFAS considered, the country where the assessment was carried out, or the population targeted (adults or children) (D'Hollander et al., 2015). Inhalation or ingestion of house dust were estimated to represent from 1 to 13 % of the dietary exposure of PFAS, these percentages could even increase up to 50 % for toddlers (de la Torre et al., 2019; Shoeib et al., 2011). Definitely, the contributions of these currently known exposure routes have not been yet fully determined, would be country or region dependent, and would likely not account for the totality of the human exposure suggesting that other sources have not yet been clearly identified (Ericson et al., 2008; Ingelido et al., 2010; Jain, 2014; Kärrman et al., 2009; Kato et al., 2011). Thus further studies are needed to better characterize the current sources of exposure to PFASs and their contribution to the global exposure.

The PFAS exposure of the general population has been well documented since 2000's but only a few dozen recent data on blood concentration in Europe, produced after 2010, are up to now available. In Belgium, Flanders (Northern part of Belgium) was one of the European pioneers by developing and implementing HBM programs since 2002. Indeed the Flemish Environment and Health Studies (FLESH) have covered 3 distinct time periods from 2002 and 2015, and have included more than 5800 participants (Reynders et al., 2017; Schoeters et al., 2017). Among the fifty hazardous chemicals included, PFASs were measured in different population categories in the 2 last campaigns (2007–2011 and 2012–2015) thus producing recent trends for these persistent organic pollutants in the Northern half of Belgium. The opposite situation is occurring in Wallonia (Southern part of Belgium) where the Walloon policy-makers are only beginning to invest in HBM to assess environment health and support policy actions. Nevertheless, few small-scale HBM studies were already locally carried out in Wallonia (Hoet et al., 2013; Koppen et al., 2019; Pirard et al., 2012, 2014,2018) but none were so far focused on PFASs.

Thus, the aims of the present study were to assess the background PFAS contamination in the general Walloon population, and to explore the contribution of food consumption and lifestyle habits as well as some demographic characteristics on the current PFAS exposure in Wallonia. For these purposes, serum samples collected during a previous study from 242 people older than 18 years old and living in the Province of Liege (Pirard et al., 2018) were analyzed for 8 perfluorinated carboxylic acids and 3 perfluorinated alkyl sulfonates, including short- and long-chain PFASs. The answers to the questionnaire related to food habits, life styles and home environment were also included in statistical models to identify some predictors of PFAS exposure.

Table 1
Some characteristics of the studied population.

	N	%		N	%
Gender			*Usual consumption of rice*		
Men	122	50.4	Never	19	7.9
Women	120	49.6	< 1 ×/month	40	16.5
Residence place			1 to 3 ×/month	84	34.7
Urban	119	49.2	1 ×/week	74	30.6
Rural	123	50.8	Several ×/week	25	10.3
Smoker status			*Usual consumption of game*		
Smoker	55	22.7	Never	124	51.2
Non-smoker	187	77.3	Others	118	48.8
Usual consumption of shellfish and crustaceans			*Usual consumption of meat offal*		
Never	59	24.7	Never	195	81.6
< 1 ×/month	88	36.8	Others	44	18.4
1 to 3 ×/month	73	30.5	*Usual consumption of milk*		
1 ×/week	14	5.9	Several ×/week	185	76.8
Several ×/week	5	2.1	Others	56	23.2
Usual consumption of seafish			*Usual consumption of fast food*		
Never	24	10.0	Never	76	31.5
< 1 ×/month	55	22.8	< 1 ×/month	70	29.0
1 to 3 ×/month	75	31.1	1 to 3 ×/month	53	22.0
1 ×/week	62	25.7	1 ×/week	33	13.7
Several ×/week	25	10.4	Several ×/week	9	3.7

2. Materials and methods

2.1. Study participants

In 2015, 252 participants aged from 18 to 76 years and living in the Province of Liege were recruited through the Provincial High Schools and Provincial offices, to obtain a non-occupationally exposed population homogeneously distributed between gender, age classes (18–29, 30–39, 40–49, 50–59, > 60 years), and the rural or urban character of their residence place. They signed an informed consent, provided a blood sample in clot activator tubes (without gel), and answered a questionnaire about their food habits, life style and home environment. This questionnaire was initially designed for a HBM study focused on organochlorine pesticides, PCBs, metals (cadmium and mercury), phthalates and parabens. Some characteristics of the studied population are gathered in Table 1. Deeper details about the recruitment and the population characteristics were previously reported (Pirard et al., 2018). The protocol was approved by the Hospital Faculty Ethics Committee of the University of Liege. From the 252 serum samples initially collected, 242 had a sufficient remaining volume for the PFAS determination.

2.2. Chemical analyses

The serum samples were analyzed for the determination of perfluoropentanoic (PFPeA), perfluorohexanoic (PFHxA), perfluoroheptanoic (PFHpA), perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), perfluoroundecanoic (PFUnDA), perfluorododecanoic (PFDoA) acids, perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), and linear perfluorooctanesulfonate (PFOS), according to an analytical method already described elsewhere (Dufour et al., 2018). Briefly, 1 ml of serum previously acidified with formic acid was extracted by solid phase extraction using Oasis WAX cartridge eluted with 2 × 2 ml of ammonium hydroxide in methanol (2 %). After evaporation to dryness, the extract was reconstituted in 80 µl of a mixture of ammonium acetate in water (2 mM) and ammonium acetate in acetonitrile (2 mM) in proportion 8/2 (v/v) corresponding to the initial composition of the mobile phase. Samples were injected into an Acquity Ultra Performance UHPLC system (Waters, Milford, MA, USA) equipped with a Kinetex F5

Table 2
Parent and daughter ions monitored, cone voltages, and collision energies used in the MS method, as well as limits of quantification of the whole method.

PFASs	Parent ion (m/z)	Daughter ion (m/z)	Cone voltage (V)	Collision energy (eV)	LOQ (ng/ml)
PeFPA	262.8	**218.9**	10	10	0.1
		262.8	10	7	
PFBS	298.8	**80**	45	30	0.1
		99	45	30	
PFHxA	312.8	**118.9**	10	20	0.1
		268.9	10	10	
PFHpA	362.8	**168.9**	10	15	0.1
		318.9	10	10	
PFHxS	398.7	**80**	45	35	0.1
		99	45	30	
PFOA	412.7	**168.9**	14	20	0.5
		368.9	14	10	
PFNA	462.7	**219**	10	15	0.1
		418.9	10	10	
PFOS	498.7	**80**	50	50	0.5
		99	50	35	
PFDA	512.7	**219**	13	20	0.15
		468.8	13	10	
PFUndA	562.7	**268.9**	15	20	0.1
		518.8	15	10	
PFDoA	612.8	**568.9**	15	15	0.2

Core-Shell (2.1 × 100 mm, 1.7 µm) from Phenomenex (Torrance, CA, USA), and coupled to a Quattro Premier XE (Waters) mass spectrometer operating in negative electrospray ionization at 1 kV. The parent and daughter ions monitored in multiple reaction monitoring mode, the cone voltages, and the collision energies are gathered in Table 2.

2.3. Quality assurance

The quantification was performed using an 8-point matrix matched calibration curves built in fetal bovine serum spiked from 0.5–50 pg/ml serum for PFOS and PFOA, and 0.1–10 ng/ml serum for all other PFASs, and extracted as real samples. Each sequence included the calibration curve, 27 unknown samples, a reagent blank, a matrix blank (fetal bovine serum), a home-made Quality Control (fetal bovine serum spiked at 1.5 ng/ml for PFOS and PFOA, and 0.3 ng/ml for all other PFASs), and 2 reference materials (low and high levels) obtained during different German External Quality Assessment Schemes (G-EQUAS, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg), with levels for PFOS and PFOA ranging depending of the round analyzed from 0.5 to 3 ng/ml, and 3–50 ng/ml for low and high levels respectively. The analytical method was previously validated according to the total error approach (Dubois et al., 2012; Hubert et al., 2007) using E-noval software V4.0 (Arlenda, Liege, Belgium), and based on the standard addition method. Moreover, the lab successfully passed the ICI/EQUAS exercises organized within the frame of HBM4EU project (Göen et al., 2017) and was thus certified as HBM4EU qualified laboratory for the analysis of PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS and PFOS in human serum. The limits of quantification (LOQs) were defined as the smallest concentrations measurable in spiked fetal bovine samples with a total error not exceeding 30 %, and are reported in Table 2.

2.4. Statistical analyses

The statistical analyses were performed using Statistica 12 (Dell, Software, France) and Microsoft Excel (Redmond, WA). The levels measured below the LOQ were replaced for each PFAS by its LOQ × its detection frequency from the present studied population (Ali et al., 2013; Dirtu et al., 2010; Dufour et al., 2018). The normality of the distribution evaluated using the Shapiro-Wilk test was highly skewed for each biomarker, inducing the use of nonparametric tests applied on biomarkers showing detection frequency higher than 70 %. Univariate or bivariate linear regressions adjusted for age and gender were firstly carried out to compare biomarker concentrations according to each data collected through the questionnaire. Correlations between individual PFAS levels and continuous variables (age, body mass index, number of children and breastfeeding duration in month for women) were assessed using Spearman rank, whilst Mann-Whitney tests were used to highlight difference of PFAS level between gender, between smoker and non-smoker, between the urban or rural character of the residence place. Kruskal-Wallis tests were performed when using multiple categories variables such as household income, education level, fish consumption, etc (the categorization was detailed in Pirard et al., 2018). The natural log-transformed biomarker levels and the covariates associated with a p-value < 0.1 in the univariate analyses were included in a multivariate regression model. Statistical significance was set at $p < 0.05$. Moreover, parity and breastfeeding duration were tested for women only in a multivariate regression adjusted for age.

3. Results and discussion

3.1. Levels of exposure

Among the PFASs monitored in the serum of our participants, PFBS, PFPeA, PFHxA, and PFDoA were never detected in any of the 242 samples. Although these compounds have usually been rarely positively measured in the general population (Cariou et al., 2015; Ericson et al., 2007; Kato et al., 2011; Lee et al., 2017; Long et al., 2015; Schröter-Kermani et al., 2013), the human exposure to PFBS, PFPeA and PFHxA was expected because these shorter-chain are used to replace long-chain PFASs as alternatives in several applications such like the surface treatment of textile, leather and carpets, the metal plating, or as flame retardants for polycarbonate resins in electronics (Wang et al., 2013). Moreover, they could also result from impurities or from the degradation of fluoropolymer- or fluorotelomer-based alternatives (Wang et al., 2013). Their increasing presence in our indoor environment has been confirmed by de la Torre et al. (2019) who observed an increase of PFBS in Belgian house dust from 2008 and 2016. Despite its lower persistence and lower ability for bioaccumulation than longer-chain PFASs, the PFBS levels in the serum of Swedish primiparous women were demonstrated to increase from 2006 to 2010 (Glynn et al., 2012), but remained below our LOQ set at 0.1 ng/ml. Furthermore, for some short-chain PFASs such like PFHxA, whole blood has been suggested as the most suitable matrix for their determination compared to serum or plasma (Poothong et al., 2017).

The results for the 7 other PFASs for all participants and according to gender are presented in Table 3. All the participants showed measurable levels of PFOS, PFOA, PFHxS and PFNA (except one participant for PFNA), while positive levels of PFDA and PFUndA were observed in a large majority of them, respectively in 85 and 65 %. As expected, PFOS contributed for most of the median PFAS body burden with 52 %, followed by PFOA (23 %), PFHxS (13 %), PFNA (7 %), PFDA and PFUndA (3 and 2 % respectively), consistently with the serum PFAS patterns reported in other studies (Bartolomé et al., 2017; Bjermo et al., 2013; Ingelido et al., 2010; Kato et al., 2011; Schröter-Kermani et al., 2013). Because the serum levels of PFASs, mainly PFOS and PFOA tend to decline this past decades (Berg et al., 2014; Glynn et al., 2012; Jain, 2014; Kato et al., 2011; Schoeters et al., 2017; Schröter-Kermani et al., 2013; Toms et al., 2014; Wang et al., 2011; Yeung et al., 2013), only recent studies focused on the general adult populations (both men and women) were included in Table 4 for comparison. Not surprisingly, median PFOS, PFOA and PFNA levels measured in the present study were lower than those reported during HBM from Sweden, Spain, Greece, Italy or Korea carried out between 2008 and 2011 (Bartolomé et al., 2017; Bjermo et al., 2013; Ingelido et al., 2010; Lee et al., 2017; Vassiliadou et al., 2010). Focusing on the more recent studies, levels

Table 3
Detection frequencies (N > LOQ), percentiles, and minimal and maximal concentrations (in ng/ml) according to the gender.

	N > lOQ (%)	Min (ng/ml)	P25 (ng/ml)	P50 (ng/ml)	P75 (ng/ml)	P95 (ng/ml)	Max (ng/ml)
PFHpA							
All (N = 242)	20.7	< 0.05	< 0.05	< 0.05	< 0.05	0.11	0.65
Women (N = 120)	15.8	< 0.05	< 0.05	< 0.05	< 0.05	0.11	0.65
Men (N = 122)	25.4	< 0.05	< 0.05	< 0.05	0.04	0.11	0.31
PFHxS							
All (N = 242)	100.0	0.11	0.64	1.07	1.63	2.73	7.45
Women (N = 120)	100.0	0.11	0.49	0.75	1.22	2.14	7.45
Men (N = 122)	100.0	0.14	0.87	1.41	1.96	2.98	6.03
PFOA							
All (N = 242)	100.0	0.38	1.25	1.91	2.86	4.72	6.79
Women (N = 120)	100.0	0.38	1.13	1.61	2.35	4.23	6.56
Men (N = 122)	100.0	0.53	1.55	2.29	3.08	5.07	6.79
PFNA							
All (N = 242)	99.6	< 0.10	0.40	0.54	0.84	1.41	2.58
Women (N = 120)	100.0	0.11	0.37	0.53	0.84	1.38	1.96
Men (N = 122)	99.2	0.10	0.43	0.56	0.83	1.45	2.58
PFOS							
All (N = 242)	100.0	0.87	2.83	4.30	6.89	11.80	29.09
Women (N = 120)	100.0	0.89	2.35	3.80	5.92	10.45	12.32
Men (N = 122)	100.0	0.87	3.12	4.88	7.69	12.81	29.09
PFDA							
All (N = 242)	85.1	< 0.15	0.19	0.29	0.41	0.82	1.37
Women (N = 120)	87.5	< 0.15	0.21	0.29	0.44	0.82	1.15
Men (N = 122)	82.8	0.13	0.18	0.28	0.40	0.79	1.37
PFUndA							
All (N = 242)	64.9	< 0.10	< 0.10	0.13	0.20	0.38	0.73
Women (N = 120)	72.5	< 0.10	< 0.10	0.13	0.22	0.38	0.65
Men (N = 122)	58.2	< 0.10	< 0.10	0.11	0.18	0.39	0.73

were very close to those observed during the 2015–2016 cycle of the US NHANES (CDC, 2019), or those measured in Norway between 2013 and 2014 with PFOS and PFNA levels slightly higher in the latter study (Poothong et al., 2017). Compared to the results obtained in the Northern part of Belgium, PFAS levels were drastically lower than those measured on Flemish adults recruited between 2008 and 2009, and also nearly twice lower than more recent data collected in 2014 from an older population (50–65 years old). Although seafood consumption and gender are usually considered as the main predictors of serum levels for PFASs, age has also been suggested to influence the body burden of PFAS with an increase serum level with age (Bjermo et al., 2013; Ingelido et al., 2010; Kato et al., 2011; Lee et al., 2017). Thus to compare similar populations in terms of age, the median concentrations of PFOS, PFOA, PFHxS and PFNA were estimated considering only the Walloon participants aged from 50 to 65 years old, and were re-presented on Fig. 1 with the corresponding Flemish population. Although apparently less pronounced, the difference between population living in the North and in the South Belgium was still measurable. This regional difference in Belgium was also observed by Dufour et al.

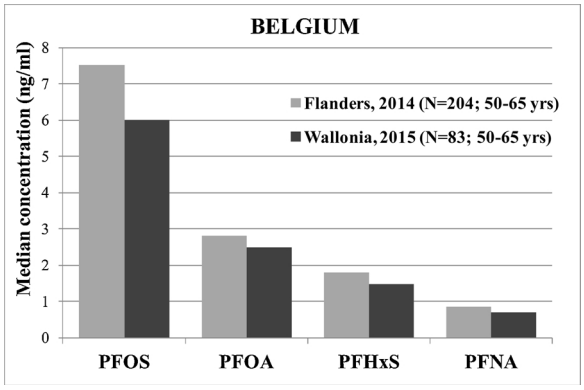

Fig. 1. Median serum concentration of PFOS, PFOA, PFHxS and PFNA measured in Flanders (in 2014) and in the present study (in 2015) when considering similar population (participants aged between 50 and 65 years old).

Table 4
PFAS levels measured recently in different general populations (in ng/ml).

Country	N participants	Age (yrs)	Years of the study	PFAS results (ng/ml)						References
				PFHxS	PFOS	PFOA	PFNA	PFDA	PFUndA	
Belgium, Wallonia	242	18-76	2015	1.07	4.30	1.91	0.54	0.29	0.13	Present study
Belgium, Flanders	204	50-65	2014	1.80	7.52	2.81	0.86	–	–	Het Steunpunt Milieu en Gezondheid, 2015
Belgium, Flanders	200	20-40	2008-2009	–	14.80	3.60	–	–	–	Cornelis et al., 2012
Norway	61	20-66	2013-2014	0.78	5.24	1.90	0.94	0.37	0.37	Poothong et al., 2017
Sweden	270	18-80	2010-2011	1.95	11.20	2.25	0.80	0.39	0.33	Bjermo et al., 2013
Spain	755	18-65	2009-2010	0.82	7.55	2.03	0.92	0.36		Bartolomé et al., 2017
Greece	142	15-88	2009	–	7.03-13.69	1.70-3.14	–	–	–	Vassiliadou et al., 2010
Italy	230	20-65	2008	–	6.31	3.59	–	–	–	Ingelido et al., 2010
Korea	1874	18-69	2009-2010	1.31	10.08	3.22	1.78	2.22	–	Lee et al., 2017
USA	2100	12- > 60	2007-2008	2.00	13.60	4.30	1.50	0.30	< LOD	Kato et al., 2011
USA	1993	> 12	2015–2016	1.20	4.80	1.57	0.60	0.10	< 0.1	CDC, 2019
New Zealand	734	19-64	2011-2013	1.00	3.40	2.40	0.66	–	–	Coakley et al., 2018

(2018) when assessing the PFAS contamination of cord blood collected at the University Hospital of Liege between 2013 and 2016. Some differences have likewise been reported between regions within the same country for instance in Greenland (Long et al., 2015), Sweden (Bjermo et al., 2013), Spain (Bartolomé et al., 2017) or Italy (de Felip et al., 2015). As explanation, these authors suggested that dietary habits, lifestyle or local contamination of drinking water could differ from one region to another within the same country, thus resulting in different PFAS serum levels in these populations. In Belgium, food consumption recorded in 2014 was reported to slightly differ between Flemish and Walloon (Belgian Food Consumption Survey, 2014), with among others a little higher amount of fish, dairy products, vegetables, fruits, and potatoes consumed by Flemish, all these items being demonstrated to mostly contribute to the PFAS exposure of the Belgian population (Cornelis et al., 2012; Klenow et al., 2013). Nevertheless, these difference were very low and would unlikely explain alone the exposure variability between the North and the South part of Belgium. On the other hand, a high PFAS contamination has been observed in soil, water and wildlife in the vicinity of a fluorochemical production unit located in Antwerp (Flanders) (Dauwe et al., 2007; Groffen et al., 2017; Hoff et al., 2005), substantially increasing the human exposure in this area. Obviously this particular situation concerned only a small part of Flanders, but included in the Flemish biomonitoring program.

To evaluate the individual health risk related to hazard chemical exposure, the biological level measured for each participant could be compared with health-based guideline values when existing. The most commonly used methods for determining these guidance values are the derivation based on epidemiological human data (i.e. reporting association between health effects and internal dose), the derivation based on defined tolerable intake values (i.e. EFSA's Tolerable Daily or Weekly Intake; EPA's Reference Dose, etc), and to a lesser extend the derivation based on a critical effect using data from studies with experimental animals (Angere et al., 2011: Apel et al., 2017). For PFOS and PFOA, the German HBM Commission recently developed HBM-I values which represent the concentration of a substance in a biological sample below which no adverse effect is expected (Schulz et al., 2007). These HBM-I values were set at respectively 5 and 2 µg/l for PFOS and PFOA based on human epidemiological studies reporting association with PFOS and PFOA exposure and fertility impairments, reducing weight of newborns at birth, lipid metabolism disorders, and immunity impairments after vaccination (Apel et al., 2017). These values would also be consistent with extrapolation from animal studies reporting among others hormonal development disturbance or thyroid metabolism disorders (Apel et al., 2017). On the other hand, no threshold above which exposure reduction would be needed (HBM-II) has been yet established, thus drawn conclusion about the actual risk related to the PFAS would be hazardous. Nevertheless, since 43 and 48 % of the participants exceeded the HBM-I values as illustrated in Fig. 2, it seemed essential to investigated more deeply and minimize their current sources of exposure, and to perform further HBM studies focused on PFAS.

The European Food Safety Authority (EFSA) recently revised and drastically decreased the Tolerable Weekly Intake (TWI) for PFOS and PFOA, now set at 13 and 6 ng/kg/week respectively. These values were based on an increase of serum cholesterol observed in several human epidemiological studies as critical effect (EFSA Scientific Opinion, 2018). Among our population, only one individual exceeded the benchmark doses used as point of departure by EFSA (21 – 25 ng/ml and 9.2–9.4 ng/ml for PFOS and PFOA respectively). However the German HBM-I values were more conservative and thus resulted in higher exceedance rate, they used to be well-accepted as reference values based on heath effects and were selected by the scientist community within the HBM4EU project (Buekers et al., 2018).

3.2. Predictors of exposure

The results of the final multivariate regression models built with the natural logarithm of the concentrations of PFHxS, PFOA, PFNA, PFOS, PFDA and the covariates roughly significantly associated in the univariate analyses ($p < 0.1$) are presented in Table 5. All PFAS concentrations included in the statistical models are positively associated with age, and all except PFDA levels were significantly higher in men than in women. The relation between PFAS serum levels and age reported in the literature seems to be inconsistent (Bjermo et al., 2013; Ericson et al., 2007; Góralczyk et al., 2015; Ingelido et al., 2010; Kato et al., 2011; Lee et al., 2017; Midasch et al., 2006; Schröter-Kermani et al., 2013; Vassiliadou et al., 2010; Wilhelm et al., 2009). This inconsistency could result from differences between study designs, sizes of the population recruited, types of samples analyzed (pooled or individual samples), etc (Vassiliadou et al., 2010). Another explanation could be the year of the sample collection. It seems that most of the studies carried out before the phase out of PFOS-related products by 3 M (completed in 2002) did not find any significant difference according to the age whereas a large majority of those carried out after the different restrictions implemented between 2006 and 2010 observed an increased PFAS levels with increasing age. This could suggest that the age dependency would rather be linked to a different exposure pattern over time, the youngest people being lower exposed compared to their elders at the same age, than a body accumulation. Lower PFAS levels observed in adult women compared to men have been reported in a majority of studies (Bartolomé et al., 2017; Bjermo et al., 2013; Coakley et al., 2018; Góralczyk et al., 2015; Ingelido et al., 2010; Jain, 2014; Kato et al., 2011; Lee et al., 2017; Midasch et al., 2006; Schröter-Kermani et al., 2013; Vassiliadou et al., 2010; Wilhelm et al., 2009). Breastfeeding and/or menstruation have been suggested as specific gender elimination route for women resulting in lower levels compared to men (Bjermo et al., 2013; Ingelido et al., 2010; Kato et al., 2011; Schröter-Kermani et al., 2013, Toms et al., 2014). In the present women population (Table 6), only PFOA levels were significantly negatively associated with breastfeeding duration (breastfeeding duration was set to zero for women who never breastfed). Moreover, inconsistently to Bartolome's results obtained from Spanish adults (Bartolomé et al.,

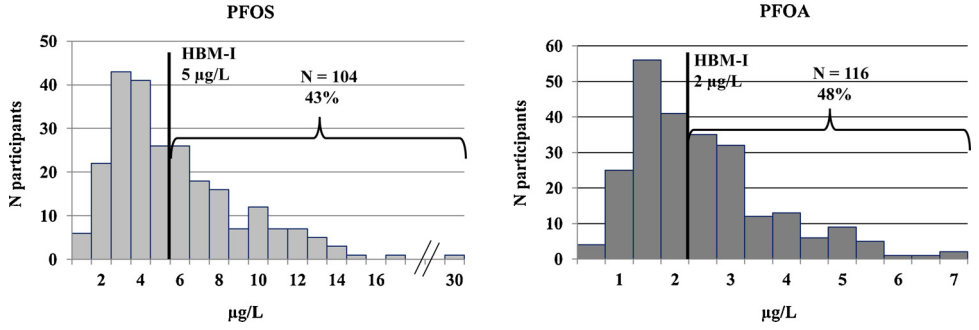

Fig. 2. Number (and percentage) of participants showing serum levels of PFOS and PFOA above the HBM-I values.

Table 5
Results of the multivariate linear regressions using the log-transformed concentration of biomarkers.

		PFHxS (R^2 = 0.324)		PFOA (R^2 = 0.242)		PFNA (R^2 = 0.281)		PFOS (R^2 = 0.301)		PFDA (R^2 = 0.297)	
		β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Intercept		***−0.548***	***0.016***	***−0.315***	***0.012***	***−1.362***	***< 0.001***	***1.069***	***0.001***	***−1.512***	***< 0.001***
Sociodemographic characteritics											
Age (year)		***0.016***	***< 0.001***	***0.014***	***< 0.001***	***0.014***	***< 0.001***	***0.018***	***< 0.001***	***0.014***	***< 0.001***
Sex (Male vs female)		***0.508***	***< 0.001***	***0.417***	***< 0.001***	***0.237***	***0.013***	***0.259***	***0.003***	0.078	0.374
Residence place (Urban vs rural)						***−0.141***	***0.042***	−0.117	0.131	***−0.153***	***0.024***
BMI (kg/m^2)								***−0.026***	***0.003***	***−0.022***	***0.003***
Smoker vs non-smoker										***−0.157***	***0.044***
Eduction level	Technical secondary education					0.066	0.640				
	Short cycle higher education					0.136	0.339				
	Long cycle higher education (except University)					−0.229	0.194				
	University education					0.138	0.388				
	Post-university education					0.359	0.201				
Diet habits											
Consumption of shellfish and crustaceans	≤ 1x/month vs never			0.107	0.198	0.019	0.834	0.112	0.278	0.060	0.477
	2-3x/month vs never			0.140	0.103	0.118	0.224	***0.246***	***0.030***	*0.164*	*0.079*
	1x/week vs never			0.120	0.424	−0.014	0.928	0.056	0.757	0.066	0.665
	> 1x/week vs never			***0.619***	***0.009***	*0.409*	*0.099*	0.350	0.244	***0.528***	***0.026***
Consumption of seafish	≤ 1x/month vs never					−0.109	0.401	0.008	0.959	−0.057	0.655
	2-3x/month vs never					−0.014	0.913	0.061	0.687	0.037	0.768
	1x/week vs never					0.128	0.343	*0.291*	*0.060*	***0.318***	***0.016***
	> 1x/week vs never					0.147	0.345	0.087	0.623	0.209	0.166
Consumption of meat offal	Never vs ≤ 1x/month to > 1x/week	*−0.185*	*0.065*					−0.069	0.502		
Consumption of game	Never vs ≤ 1x/month to > 1x/week					−0.073	0.302	−0.038	0.640		
Consumption of rice	≤ 1x/month vs never	−0.163	0.322			−0.161	0.267				
	2-3x/month vs never	0.015	0.918			0.020	0.881				
	1x/week vs never	−0.095	0.532			−0.046	0.733				
	> 1x/week vs never	***−0.410***	***0.023***			***−0.427***	***0.008***				
Consumption of fast food	< 1x/month vs ≥ 1x/month									0.107	0.133
Consumption of chocolate	> 1x/week vs never to 1x/week					*0.120*	*0.077*			***0.159***	***0.015***
Use of cosmetics and personal care products											
Use of shampoo (N/week)		***−0.045***	***0.005***								
Use of nail polish	Yes vs no			0.121	0.400	***0.270***	***0.009***			***0.298***	***0.003***
Use of nail polish remover	Yes vs no			0.162	0.231					−0.048	0.244
Use of shower gel (N/week)											
Number cosmetics daily used (except makeup)										−0.003	0.898

2017) or Bjermo's observations from Swedish adults (Bjermo et al., 2013), the significant gender difference still occurred for PFHxS, PFOA and PFOS when comparing men and women who never breastfed, as shown in Fig. 3. This demonstrates that although breastfeeding could decrease some PFAS women body burden, this route of excretion could not explain alone the difference between men and women. Because lower PFAS levels have been already observed in blood donors or individuals undergoing venesection (Lorber et al., 2015; Rotander et al., 2015), and because the different PFAS levels between girls and boys (aged from 3 to 18 years old) were reported to only occur for girls in puberty (Kang et al., 2018), menstruation could be a more plausible explanation for the difference of PFAS levels observed between men and women. Our results although not contradictory were not able to confirm this hypothesis since our population included only adults, and information about blood donation were not collected. Beside menstruation or breasfeeding, additional characteristics differing between male and women such like parity, lifestyle, diet habits or food quantities ingested should also contribute to difference in serum PFAS levels (Bartolomé et al., 2017; Berg et al., 2014; Lee et al., 2017; Midasch et al., 2006). In the present study, parity was only significantly negatively associated with PFOA level, whereas it was previously reported to be an important factor impacting the serum PFAS concentration (Berg

Table 6
Multivariate regression models built with the natural logarithm of the concentration of PFHxS, PFOA, PFNA, PFOS and PFDA and breastfeeding duration (month) and parity for women only.

	PFHxS (R^2 = 0.242)		PFOA (R^2 = 0.312)		PFNA (R^2 = 0.194)		PFOS (R^2 = 0.205)		PFDA (R^2 = 0.128)	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Intercept	***−1.109***	***< 0.001***	−0.278	0.058	***−1.403***	***< 0.001***	***0.505***	***0.002***	***−1.807***	***< 0.001***
Age	***0.024***	***< 0.001***	***0.022***	***< 0.001***	***0.018***	***< 0.001***	***0.019***	***< 0.001***	***0.014***	***< 0.001***
Breastfeeding duration (month)	−0.010	0.201	***−0.013***	***0.033***						
Parity (N)	−0.113	0.064	***−0.107***	***0.030***						

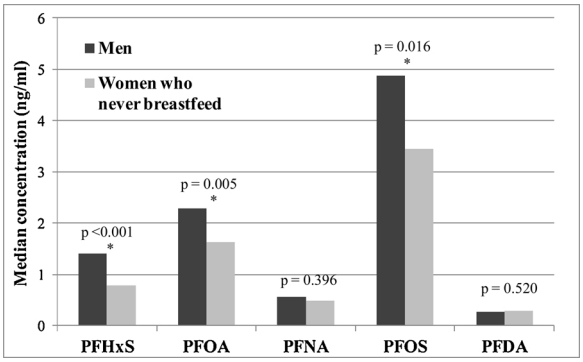

Fig. 3. Median levels of PFAS in all participating men, and participating women who never breastfed (in ng/ml). Asterisks showed significant difference between men and women ($p < 0.05$).

et al., 2014). Focusing on diet habits, Belgian men seem to consume significantly more potatoes, meat, fish, egg products, and alcohol than women according to the last Belgian Food Consumption Survey (2014). Because all these food items were demonstrated to potentially contribute to higher PFAS exposure levels (Cornelis et al., 2012; Klenow et al., 2013), this would also argue in favor of a potential influence of different diet habits between male and female on their PFAS body burden. Lower levels of PFNA and PFDA were observed for participants living in urban area, which would rather result from different lifestyle factors (i.e. presence of carpets or textile at home, time spent indoor, origin of tap water, dietary habits) than from a different environmental background contamination. Body mass index (BMI) was negatively associated with PFOS and PFDA levels while lower levels of PFDA were observed in smokers vs non smokers. The relation with BMI was not expected since these chemicals are not as lipophilic as the other Persistent Organic Pollutants. Nevertheless, decreased levels of some PFAS with increasing BMI have been previously reported (Berg et al., 2014; Hölzer et al., 2008). If smoking has been suggested to induce phase II metabolization enzymes and therefore to induce lower xenobiotic body burden (Zevin and Benowitz, 1999), most of the biomonitoring studies on PFASs did not find any association (Bjermo et al., 2013; Jain, 2014).

The dietary contribution to total PFAS exposure has been extensively studied through biomonitoring combined to questionnaire or through PFAS measurements in food. However the method, the observations were not always consistent and used to be region or country dependent (Bartolomé et al., 2017; Berg et al., 2014; Bjermo et al., 2013; D'Hollander et al., 2015; Ericson et al., 2008; Jain, 2014; Klenow et al., 2013; Lee et al., 2017; Rivière et al., 2014). Among them, the project PERFluorinated Organic Compounds in Our Diet (PERFOOD) was designed to provide reliable and harmonized European data on PFAS intake from food and drinking water. One of the aims was to identify and quantify PFAS in food and assess the dietary exposure in four representative European regions (D'Hollander et al., 2015; Klenow et al., 2013). The results demonstrated that fish and sea food were the main sources of exposure for Belgians only for PFOS, PFDA and PFUnDA, while for carboxylic PFAS with shorter chains or sulfonates, fruits, potatoes or alcoholic beverages would be the main contributors (Klenow et al., 2013). Similarly, in the present study, the only items within the diet habits being significantly associated with higher PFAS levels were the usual frequency of consumption of shellfish and crustaceans, and sea fish. Indeed consuming shellfish and crustaceans once a week compared to never, and 2 or 3 times per month versus never resulted respectively in significant higher levels for PFDA and PFOS, while eating sea fish once a week significantly increased the PFDA levels. Unfortunately, the questionnaire administrated to our participants did not include information about consumption of fruits, potatoes or alcohol. The lower PFNA and PFHxS levels for people who usually eat rice more than once a week would likely be due to the subsequently lower consumption of other starchy foods such like potatoes known to be a non-negligible source of some PFAS exposure for Belgian people (Cornelis et al., 2012; Klenow et al., 2013). The reason why the high usual consumption of chocolate would lead to higher level of PFDA remains unclear or may be due to chance. Note that many diet habits frequently suspected as significant predictors of exposure such like eating eggs or egg products, games, offal, cheese, milk and dairy products, fast food, cereals, fresh fruits and vegetables (Berg et al., 2014; Cariou et al., 2015; Cornelis et al., 2012; Jain, 2014; Klenow et al., 2013) were not pointed out by the statistical analyses. The use of cosmetics and personal care products was recently highlighted as some determinants of exposure for the Flemish population (Colles et al., 2019). In the present study, the questionnaire initially devoted to exposure of phthalates and parabens included issues about the frequency of use of mouthwash, hand soap, shower gel, shampoo, bath salts, deodorant, hair spray or gel, lip balm, makeup, makeup remover, nail polish, nail polish remover, shaving cream, after-shave, face and body lotions, false nails and sunscreen. Among all of them, only the use of nail polish was positively associated with PFNA and PFDA levels.

All the multivariate regression models were characterized by weak determination coefficients (R^2), meaning that the covariates included in the statistical models only explained a small part of the variance (between 24 and 32 % depending on the PFAS), with age and gender being the main or sometimes the only contributors (results not shown). Even if some relevant covariates for PFAS were not included in the statistical model due to the initial aims of the questionnaire administrated (consumption of alcoholic beverage or some diet items such like potatoes, use of non stick cooking tools, nature of food wrapping, indoor environment characteristics, etc), the miss of relevant predictors of exposure has already been risen by other authors (Jain, 2014; Lindh et al., 2012), suggesting that several current sources of exposure to PFAS are still unidentified.

4. Conclusion

The human background contamination of PFASs was measured for the first time in Wallonia (Southern part of Belgium) by determining the levels of 11 PFASs in 242 serum samples collected in 2015 from individuals living within the Province of Liege. For all participants, at least 4 PFASs were detected simultaneously, with levels close to those recently reported in other European or North American countries, but substantially lower than levels measured in the North of Belgium. However, only half of the population showed PFOS and PFOA levels below the peer-recognized health guidance values set by the German HBM Commission suggesting that for the other half, the sources of exposure should be investigated and minimized. If as expected age and gender were the main characteristics influencing the serum PFAS levels, the statistical models used were poorly predictive suggesting that the main sources of exposure (within food items or lifestyle characteristics) were not taken into account.

Ethical approval

This protocol was approved by the Hospital Faculty Ethics Committee of the University of Liege (B707201422894).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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