



Determination of contamination levels for multiple endocrine disruptors in hair from a non-occupationally exposed population living in Liege (Belgium)

Julien Claessens^{a,b,*}, Catherine Pirard^{a,b}, Corinne Charlier^{a,b}

^a Laboratory of Clinical, Forensic, Industrial and Environmental Toxicology, University Hospital of Liege, CHU (B35), 4000, Liege, Belgium

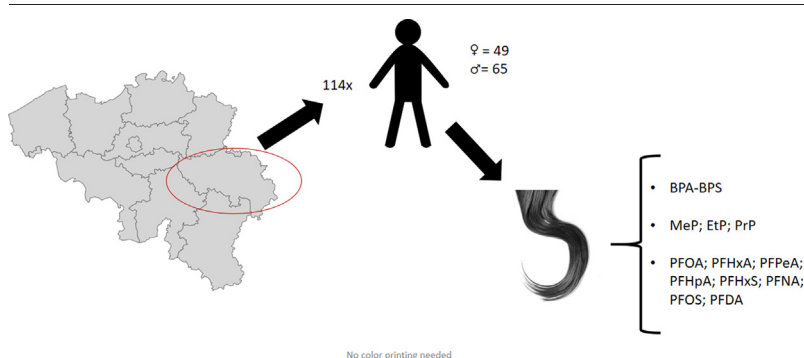
^b Center for Interdisciplinary Research on Medicines (C.I.R.M), University of Liège, CHU (B35), 4000, Liege, Belgium



HIGHLIGHTS

- 2 bisphenols, 3 parabens and 8 PFCs were measured in the hair from 114 volunteers.
- MeP, BPS, BPA, PrP, ETP and PFOA were quantified in minimum 46.4 of samples.
- Levels were not statistically correlated with gender, hair length or treatment.

GRAPHICAL ABSTRACT



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ABSTRACT

Today, the interest in hair as alternative matrix for human biomonitoring of environmental pollutants has increased, but available data on chemical levels in hair remain scarce. In this study, the measurement of 2 bisphenols (A and S), 3 parabens (methyl-, ethyl- and propylparabens) and 8 perfluoralkyl compounds (PFCs) namely perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroheptanoic acid (PFHpA), perfluoropentanoic acid (PFPeA) and perfluorohexanoic acid (PFHxA) was carried out, using a thoroughly validated UPLC-MS/MS method, in the hair from 114 adults living in Liege (Belgium) and surrounding areas. The most frequently quantified compounds in the population were: bisphenol S (97.4%, median = 31.9 pg·mg⁻¹), methylparaben (94.7%, median = 28.9 pg·mg⁻¹), bisphenol A (93.9%, median = 46.6 pg·mg⁻¹), ethylparaben (66.7%, median = 5.2 pg·mg⁻¹), propylparaben (54.8%, median = 16.4 pg·mg⁻¹) and PFOA (46.4%, median < 0.2 pg·mg⁻¹). The other PFCs were detected only in few samples although current exposure of the Belgian population to PFCs was previously demonstrated using blood analyses. Nonparametric statistical analyses were performed to evaluate the influence of gender, hair treatments and hair length, but no significant difference was observed. Only age was positively correlated with the propylparaben contamination. Although blood seems to remain more suitable for PFCs exposure assessment, the results of this study suggest that hair can be an appropriate matrix for biomonitoring of organic pollutants such as parabens or bisphenols.

1. Introduction

Since the last decades, humans have been exposed to a growing number of chemical substances present in the industrial and domestic environment.

Some of these chemicals are reported to disrupt hormonal systems of living organisms. Nowadays, more than 800 man-made chemicals are known or suspected to act as endocrine disrupting chemicals (EDC) including a wide variety of products such as pesticides, plasticizers, product additives,

* Corresponding author at: University of Liege, CHU (B35), avenue de l'Hôpital, 1, 4000, Liege, Belgium.

E-mail address: julien.claessens@uliege.be (J. Claessens).

personal care product ingredients, pharmaceuticals, etc. (Bergman et al., 2012; Gore et al., 2015).

These last fifty years, perfluororalkyl compounds (PFCs) have been widely used in different types of products, for industrial and domestic uses. The chemical structure of PFCs confers them particular properties including water and oil repellence, thermal stability, and surfactant properties (Gomis et al., 2018; Ritscher et al., 2018). Since the early 2000's PFCs have become a great cause of concern in term of toxicity and health as they are largely distributed in the environment. Significant concentrations of PFCs have been found in human serum ($\text{ng}\cdot\text{mL}^{-1}$) demonstrating the wide human exposure (Bartolomé et al., 2017; CDC, 2019; Coakley et al., 2018; Colles et al., 2020; Ledda et al., 2018; Norén et al., 2021; Pirard et al., 2020; Polachova et al., 2021). Number of toxic effects (altered hepatic function, influence on body weight, ...) have been input to PFCs (Lindstrom et al., 2011). They are categorized as persistent organic pollutants (POPs) and are under regulations in several countries. In Europe perfluorooctanoic acid (PFOA) production is banned since 2020 and European Food Safety Authority (EFSA) sets the tolerable weekly intake rates at 13 and 6 ng/kg body weight for perfluorooctanesulfonate (PFOS) and PFOA respectively (Agency for Toxic Substances and Disease Registry, 2018; EU, 2016a; Knutsen et al., 2018). Food intakes is the principal route of exposure to PFCs although human are also exposed by inhalation or ingestion of house dusts (Pirard et al., 2020).

Bisphenol A (BPA) is principally used for its plasticizing properties and is present in polycarbonate food containers, epoxy resins which coat conserve cans, dental implants and other plastic devices such as toys or compact discs (Ma et al., 2019; Ye et al., 2015). Humans are mainly exposed to BPA by ingestion of contaminated food but also by inhalation of house dusts and by dermal contamination (Ma et al., 2019; Rochester, 2013). BPA is an estrogenic compound (Vandenbergh et al., 2014; Viñas et al., 2012) and its reported health effects are various and targeted different endocrine systems (Andújar et al., 2019; Heindel et al., 2020; Liu et al., 2021; Ma et al., 2019; Rochester, 2013). Because of wide exposure and associated health effects, BPA is now submitted to different regulations. BPA is banned from the composition of feeding bottle or drinking cup intended for infant and since 2011, has been phased out from thermal paper. In other devices, authorization of BPA is subject to the specific migration limit of 0.6 mg of BPA per kg of food (EU, 2011, 2016b, 2018).

Due to these regulations, in some applications, industrials tend to replace BPA by its sulfonyl analogue: bisphenol S (BPS) (Banaderakhshan et al., 2022; Moon, 2019). BPS is largely present in the environment and people are exposed on a daily basis although safety profile of BPS hasn't been fully assessed yet (Eladak et al., 2015; Wang et al., 2019; Wu et al., 2018). First toxicological studies on BPS effects seem to suggest similar-to-worth harmful health impacts than those observed for BPA (Debost-Legrand et al., 2016; Rochester and Bolden, 2015; Rosenmai et al., 2014; Žalmanová et al., 2016). BPS is reported to disrupt endocrine systems, to promote oxidative stress and obesity. Exposure to BPS may thus be potentially harmful for health (Eladak et al., 2015; Wu et al., 2018).

Parabens are frequently used chemicals especially for their antimicrobial conservative properties and are largely present in cosmetics and personal care products. Parabens are also found in food or medical drugs (Cherian et al., 2020; Karpuzoglu et al., 2013). The main exposure route is through dermal application of parabens-containing products, but inhalation and ingestion are other possible routes of exposure (Hong et al., 2021; Wei et al., 2021). Parabens have been reported as weak endocrine disruptors with effects on estrogen signaling pathways and action on hormones (adipsine, leptin) or receptors involved in metabolic and energy balance suggesting the obesogenic potential of these compounds (Boberg et al., 2010; Darbre, 2017; Lincho et al., 2021; Šramkova et al., 2019). Parabens are restricted in cosmetic products at a concentrations of 0.4% for single use and of 0.8% as mixture (EU, 2014).

Today, human biomonitoring is generally performed using blood or urine. Due to its contact with the whole organism, blood is generally considered as the reference matrix and to reflect cumulative exposure especially

for persistent pollutants such as PFCs (Alves et al., 2014; WHO, 2015). On the other hand, urine is largely used for the biomonitoring of rapidly eliminated substances such as bisphenols or parabens because of the higher concentration of the compound or its metabolites and the easiness of collection (Alves et al., 2014; WHO, 2015). Besides these two main matrices, over the last ten years, hair has gained attention as a novel matrix for biomonitoring of organic pollutants (Appenzeller, 2015). Hair offers many advantages such as the easiness of sample collection, the stability of sample storage and an extended detection window from month to years depending on hair length (Appenzeller and Tsatsakis, 2012; Schramm, 2008). This latter property is particularly interesting for the biomonitoring of rapidly eliminated substances such as bisphenols and parabens for which conventional biological fluids only provide information on recent exposure and are subject to a high temporal variability (Dewalque et al., 2014; Faÿs et al., 2020; LaKind et al., 2019). Indeed hair has been reported to be less sensible to intraindividual temporal variability of non-persistent organic pollutants and suitable to assess their short or long term exposure (Béranger et al., 2018; Faÿs et al., 2021).

Analyses of environmental pollutants in hair include a wide variety organic compounds among which polychlorinated biphenyls, polybrominated diphenyl ethers, polycyclic aromatic hydrocarbons, pesticides, bisphenols, parabens or PFCs (Alves et al., 2015; Iglesias-González et al., 2020; Karzi et al., 2019, 2018; Katsikantami et al., 2020; Martín et al., 2019; Palazzi et al., 2018). Even though this last decade the number of studies on hair contamination has increased, some pollutants are still poorly studied in hair and number of regions of the world remain uncovered. Moreover, due to the frequent small-scale of the studies, few reference values have been established in general populations. Therefore, there is a need of performing biomonitoring studies in different regions and by different laboratories to enhance available data and allow the comparison of contamination levels between different populations and subpopulations.

The aims of this work were thus to provide data of contamination levels of 13 endocrine disrupting chemicals by measuring 8 PFCs, 2 bisphenols and 3 parabens in the hair of non-occupationally exposed adults living in Liege (Wallonia, Belgium), and to evaluate the suitability of using hair matrix for exposure assessment of these contaminants for further large-scale biomonitoring studies.

2. Materials and method

2.1. Chemicals and reagents

Solutions of perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroheptanoic acid (PFHpA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA) and their corresponding C_{13} mass labelled isotopes were purchased from Wellington laboratories, Inc. (Ontario, Canada). Bisphenol S (BPS), ethylparaben (EtP) and n-propylparaben (PrP) were purchased from Sigma Aldrich® (St Louis, MO, USA), BPS- D_8 was bought from Chiron AS® (NO-7041, Trondheim, Norway) and Bisphenol A (BPA) and Bisphenol A- D_{14} were obtained from Dr. Ehrenstorfer GmbH (Ausburg, Germany). Methylparaben (MeP) and $^{13}\text{C}_6$ -methylparaben were supplied by Cambridge Isotope Laboratories, while deuterated n-propylparaben (2,3,5,6- d_4) and ethylparaben (2,3,5,6- d_4) were obtained from C/D/N Isotopes Inc. (Quebec, Canada). Methylparaben standard, mass-labelled methyl-, ethyl-, propyl-parabens isotopes and all PFCs standards were obtained as solutions while other standards were purchased as crystalline powder.

Analytical grade acetone and acetic acid (HAc) were bought from Macron Fine Chemicals (Gliwice, Poland). LC-MS Grade methanol (MeOH) and water were bought from JT Baker (Deventer, The Netherlands), sodium dodecyl sulphate (SDS) (for analysis) was obtained from Sigma Aldrich (Germany) and Emsure grade ammonium acetate from Merck (Armstadt, Germany).

2.2. Volunteers and sample collection

Hair samples were collected between November 2020 and January 2021 from 114 volunteers aged from 17 to 64 years old and residing in Liege (Wallonia). The information collected from participants was gender, smoking habits, hair length and hair treatments. Samples (0.5–1 g) were cut at the occipital vertex and directly stored in paper envelope, in dark room at ambient temperature to avoid contamination and degradation until the analysis.

Prior to analysis, samples were washed using four different solvents according to Martín et al.'s (2016) procedure: firstly, with water, secondly by a SDS 0.1% bath and then two times again with water. At each step of the washing process, samples were sonicated for 5 min. After the washing procedure, samples were dried at room temperature and then cut into 2–3 mm pieces using stain steel scissors.

2.3. Extraction and analysis

The analytical procedure was strongly inspired from previously reported method by Martín and associates and adapted to our equipment (Martín et al., 2016). Briefly, 50 mg of each sample were weighted and put into glass extraction tubes. Ten μL of the internal standard solution (5 $\mu\text{g}/\text{mL}$ for $^{13}\text{C}_6\text{-MeP}$, PrP- d_4 , EtP- d_4 , BPS- d_8 , BPA- d_{14} and 0.1 $\mu\text{g}/\text{mL}$ for ^{13}C -PFCs) were added and tubes were let at ambient temperature for equilibration for 1 h. Then, 2 mL of a methanol/HAC mixture (85/15% v/v) were added and samples were incubated at 38 °C overnight in static position to allow the release of compounds from the matrix. After the incubation, samples were sonicated for 15 min with 2 \times 2 mL of acetone, centrifuged for 10 min (at 3000 rpm), and the supernatants were transferred into clean tubes before being evaporated to dryness at 30 °C under nitrogen flow. Extracts were reconstituted with 100 μL of ammonium acetate (2 mM) and methanol (70/30% v/v) mixture and transferred into vials for analysis.

The final determination was performed using an Acquity UPLC system coupled to a Xevo TQ-S triple quadrupole mass spectrometer (Waters, Milford, MA, US). The compounds were separated on an Acquity UPLC BEH C18 column (2.1 \times 100 mm, 1.7 μm) from Waters maintained at 50 °C, and using a constant flow rate of 0.35 mL \cdot min $^{-1}$. The mobile phase consisted of ammonium acetate 2 mM in water (A) and MeOH (B). The gradient started at 70% A held for 1 min, reached 60% A in 5 min, decreased to 20% in 10 min, then decreased again to 5% A in 0.2 min and held for 1.5 min. The return to the initial conditions (70% A) was reached in 0.2 min and maintained for 2.5 min.

The mass spectrometer operated in Multiple Reaction Monitoring (MRM), using negative electrospray ionization mode. The capillary voltage was set at 0.75 kV, the source and desolvation temperatures were set at respectively 150 °C and 450 °C, while nitrogen was used at a flow of 800 L/h as desolvation gas, at 150 L/h as cone gas, and under 7.0 bar for nebulizer gas. The precursor and daughter ions monitored, and the collision energy for each MRM transitions are gathered in Table S1 (Supplementary materials).

2.4. Method validation

The analytical procedure was validated according to the total error approach, in order to meet ISO 17025 and French Society of Pharmaceutical Sciences and Technique guidelines (Dubois et al., 2012; Hubert et al., 2007). The calibration curves and validation standards were respectively analyzed in duplicate and triplicate in a single day. Procedure was repeated on three different days to determine intra and inter-day precisions. The calibration curve consisted in reagent blank samples spiked at 8 different concentrations corresponding to respectively 10–25–50–100–500–1000–2000–4000 $\text{pg}\cdot\text{mg}^{-1}$ for BPA, PrP, MeP and PFOS; 2–5–10–20–100–200–400–800 $\text{pg}\cdot\text{mg}^{-1}$ for BPS and EtP; and 0.2–0.5–1–2–10–20–40–80 $\text{pg}\cdot\text{mg}^{-1}$ for the other PFCs. The validation standards were prepared by spiking at 9 different concentrations (10–15–25–35–50–400–2000–3000–4000 $\text{pg}\cdot\text{mg}^{-1}$ for BPA, PFOS, MeP, PrP; 2–3–5–7–10–80–400–600–800 $\text{pg}\cdot\text{mg}^{-1}$ for BPS, EtP

and 0.2–0.3–0.5–0.7–1–8–40–60–80 $\text{pg}\cdot\text{mg}^{-1}$ for other PFCs) dog hair as human hair substitute (because we didn't found human hair showing negligible levels for all targeted chemicals) previously checked for initial low contamination.

The validation parameters including limits of detection (LOD) and limits of quantification (LOQ) were calculated with Enoval Software v4.1 (Arlenda, Belgium), and are reported in Table 1. LOQ was defined as the smallest concentration with a maximal uncertainty not exceeding 40% and LOD was calculated as one-third of LOQ. Further details on the determination of the LOQ and other validation parameters were already described (Dubois et al., 2012).

The matrix effect was tested using the post extraction addition method (Matuszewski et al., 2003) by comparing 4 points-calibration curves performed using reference solutions on one hand, and 6 different hair samples extracted as real samples on the other hand. For each compound, the variation coefficient of the slopes of the calibration curves did not exceed 6.6%, demonstrating that potential matrix effects if present are corrected by the use of isotope labelled internal standards, and thus are under control.

2.5. Quality assurance and control

Each sequence of unknown samples included a calibration curve (blank samples spiked at 8 concentrations), 1 reagent blank, and 5 home-made quality controls (3 blank samples spiked at 50, 100 and 1000 $\text{pg}\cdot\text{mg}^{-1}$ for BPA, PrP, MeP and PFOS, at 10, 20 and 200 $\text{pg}\cdot\text{mg}^{-1}$ for BPS and EtP, and at 1, 2 and 20 $\text{pg}\cdot\text{mg}^{-1}$ for the other PFCs; and 2 dog hair samples spiked at 100 and 1000 $\text{pg}\cdot\text{mg}^{-1}$ for BPA, PrP, MeP and PFOS, 20 and 200 $\text{pg}\cdot\text{mg}^{-1}$ for BPS and EtP, and 2 and 20 $\text{pg}\cdot\text{mg}^{-1}$ for the other PFCs), all extracted and treated similarly than real samples.

2.6. Statistical analysis

The statistical analyses were performed using Rcmdr V4.0.3 and excel 2013 (Microsoft Office). Measured concentrations below LOQ were replaced by LOQ \times detection frequency because this provides a more realistic mean missing value than LOQ/2, LOQ/ $\sqrt{2}$ or zero (Ali et al., 2013; Dirtu et al., 2010; Gys et al., 2020; James et al., 2002). The inference statistics were performed only for BPA, BPS, MeP, EtP, PrP and PFOA because of the low quantification frequencies observed for the other compounds. Since the distributions were highly skewed for all pollutants, nonparametric tests were used: Spearman rank was used to assess the correlations between concentrations and age (as continuous variable), and Mann-Whitney to test contamination differences between men and women. For the other multiple category variables as the influence of hair length or hair treatments, the associations with the pollutant concentrations were assessed using Kruskal-Wallis test. No statistic was performed on smoking status because of the low percentage of population reported as smokers (3%). Statistical relevance was set at $p < 0.05$.

3. Results

3.1. Study population

The studied population consisted in 114 volunteers living in the Liege Region, aged from 17 to 64 years old, and distributed between 43% males and 57% females. The participants were asked to report their hair length: 45% have short hair (0–7 cm), 21% medium hair (7–15 cm) and 34% long hair (>15 cm). Hair treatments were categorized in: none (75%), dyeing (7%) and discoloration (18%). The population characteristics by gender are gathered in Table 2.

3.2. Concentration of targeted compounds

The Table 3 presents the descriptive statistics: geometric mean (GM), percentile 25 (P25), 50 (P50), 75 (P75), minimum and maximum concentrations measured, the standard deviations (SD), the interquartile range

Table 1

Validation parameters including trueness bias (%), repeatability (RSD%), reproducibility (RSD%), uncertainty (%), limit of detection (LOD), and limit of quantification (LOQ).

	Target conc. $\text{pg}\cdot\text{mg}^{-1}$	BPA	MeP	PrP	PFOS	Target conc. $\text{pg}\cdot\text{mg}^{-1}$	BPS	EtP	Target conc. $\text{pg}\cdot\text{mg}^{-1}$	PFOA	PFPeA	PFHxA	PFHpA	PFHxS	PFNA	PFDA
Trueness relative bias (%)	10	-9.5	7.5	-11.7	42.3	2	4.4	22.7	0.2	2	-26.6	5.5	-6.2	0.5	-1.7	-17.4
	15	-2.5	0.6	-0.6	21.3	3	-7.3	13.9	0.3	-0.3	-15.4	-0.6	-0.9	0.8	-0.2	1.9
	25	-0.3	3.6	-0.8	4.3	5	-3.5	12	0.5	-0.6	0.1	1.3	0.1	2.5	1.8	-0.6
	35	2.5	2.8	7.7	-0.5	7	0.03	14.2	0.7	1.1	15.4	1.5	-0.5	2.4	1.8	-0.5
	50	-2.8	-8.2	8.8	-1.3	10	-3.5	12.7	1	13.2	6.9	10.9	8.6	12.4	10	9.7
	400	-1.2	3.2	5.4	-8.8	80	-0.6	5.1	8	4.9	5.8	1.3	0.4	2.3	4.9	-0.1
	2000	-11.3	-0.1	-6	-1.2	400	0.5	-7.4	40	-3.1	2.9	-2.9	-0.2	-3.2	-2.1	-6.4
	3000	-0.3	0.4	-4.4	-7.4	600	-6.6	-4.6	60	-8.1	-0.7	-1.4	0.02	-1.5	1.8	0.1
	4000	-7	1.1	-	11.6	800	-2	-1.6	80	2.8	3.6	1.9	3.3	1.9	5.1	-0.5
	Intra-assay precision repeatability (%)	10	2.4	2.4	15	5.6	2	7	3.3	0.2	7.9	12.7	8.9	6.8	9.2	5.1
15		5.8	1.4	7.6	1.3	3	6.2	4.3	0.3	6.3	9	9.1	2.5	2.8	5.6	11.1
25		2.2	2.4	9.9	1	5	7.2	3.5	0.5	3.8	6.8	2.8	2.7	3.9	4.5	4.8
35		3.1	1.6	5.4	3	7	5.1	5.7	0.7	2.1	3.7	2.6	2.3	3.8	3.7	5
50		1.4	9	6.7	7	10	15.5	2.3	1	2.3	5.8	4.7	2.4	6.1	6.4	9.8
400		1.7	2.2	2.1	6.5	80	2.1	1.6	8	4.8	3.1	2.7	2.6	1	2.2	3.4
2000		2.6	1.7	2.1	4.8	400	2.6	2	40	2.7	3.8	3.2	3.8	3.3	2.1	2.6
3000		0.7	1.7	2	7.7	600	3.1	4.9	60	3.8	3.2	3.1	1.9	2.9	3.2	6.1
4000		2.4	1.3	-	4.4	800	1.3	2.2	80	1.9	0.7	1.7	2.1	3	1.7	3.4
Inter-assay precision intermediate precision (rsd %)		10	9.3	2.7	15	6.6	2	11.8	5.6	0.2	8.7	21.6	15.2	6.8	9.2	5.8
	15	8.2	16	10.6	3.8	3	6.2	10.4	0.3	6.3	13.5	8.1	2.5	2.8	6.2	11.5
	25	3.1	3.5	12.2	2.9	5	7.2	8.6	0.5	3.8	6.8	2.8	2.7	3.9	4.6	10.5
	35	5.1	2.7	9.5	3	7	5.1	9.7	0.7	2.1	5.6	3.3	2.3	3.8	3.9	6.3
	50	5.5	12.9	7.3	7.6	10	16.4	6.8	1	4.1	11.5	5.6	3.9	6.1	7.2	9.8
	400	8.3	6	6.7	6.7	80	2.1	6.3	8	5.1	7.2	6	2.6	1.5	6.4	3.5
	2000	3.8	2.6	3	4.8	400	4.2	2.3	40	4	4.8	4	4	4.2	2.3	4.8
	3000	3.5	1.7	4.9	9.6	600	9.4	6.3	60	4	3.2	3.9	2.3	3.6	3.1	6.5
	4000	8.5	1.8	-	8.1	800	3.1	6.7	80	2.2	1.8	3.2	2	3.1	1.9	4.6
	Uncertainty relative expanded uncertainty (%)	10	2.4	5.9	31.7	14.3	2	26.4	12.5	0.2	19.1	48.4	34.1	14.4	19.3	12
15		18.1	36.9	23.4	8.7	3	13.2	23.7	0.3	13.3	30.6	17.1	5.4	5.9	13	24
25		6.9	7.8	18	6.7	5	15.3	19.5	0.5	8.1	14.4	5.9	5.8	8.2	9.6	23.9
35		11.6	6.1	21.3	6.3	7	10.9	21.8	0.7	4.6	12.6	7.3	5	8	8.2	13.9
50		12.7	28.6	15.6	16.2	10	35.1	15.6	1	9.4	26	12.2	8.7	12.8	15.5	20.7
400		19.2	13.7	15.4	14.1	80	4.5	14.5	8	11	16.5	13.7	5.5	3.4	14.7	7.5
2000		8.5	6	6.6	10.2	400	9.5	5.1	40	8.9	10.5	8.7	8.5	9.2	4.9	10.8
3000		8.2	3.7	11.3	20.9	600	21.6	14.4	60	8.7	6.9	68.6	5	7.9	6.7	13.9
4000		19.7	4	-	18.1	800	7.1	15.3	80	4.7	4.2	7.4	4.3	6.7	4.1	10.2
LOD ($\text{pg}\cdot\text{mg}^{-1}$)			3.03	3.03	3.03	3.03		0.61	0.61		0.061	0.096	0.061	0.061	0.061	0.061
LLOQ ($\text{pg}\cdot\text{mg}^{-1}$)		10	10	10	10		2	2		0.2	0.317	0.2	0.2	0.2	0.2	0.271
ULOQ ($\text{pg}\cdot\text{mg}^{-1}$)		4000	4000	3000	4000		800	800		80	80	80	80	80	80	80
R ²		0.992	0.99	0.997	0.991		0.995	1		0.998	0.998	0.998	0.998	0.998	0.998	0.995
Model		Weighted	Weighted	Weighted	Weighted		Weighted	Weighted		Weighted	Weighted	Weighted	Weighted	Weighted	Weighted	Weighted
		1/X quad.	1/X Linear	1/X Linear	1/X Linear		1/X Linear	1/X Linear		1/X Linear	1/X Linear	1/X Linear	1/X Linear	1/X Linear	1/X Linear	1/X Linear
		Regression	Regression	Regression	Regression		Regression	Regression		Regression	Regression	Regression	Regression	Regression	Regression	Regression

Bold character indicates target concentration for different compounds on the right of the column.

Table 2
Population characteristics by gender.

	Total N (%)	Men N (%)	Women N (%)
All	114 (100)	49 (43)	65 (57)
Age			
Median	26.5	26.0	26.5
P25-P75	22.3–40.8	23.3–33.8	21.0–43.0
Smoking status			
Smoker	3 (3)	1 (2)	2 (3)
Non-smoker	111 (97)	48 (98)	63 (97)
Hair length			
Short	51 (45)	48 (98)	3 (5)
Medium	24 (21)	1(2)	23 (35)
Long	39 (34)	0 (0)	39 (60)
Hair treatment			
None	85 (75)	49 (100)	36 (56)
Dyeing	8 (7)	0 (0)	8 (12)
Discoloration	21 (18)	0 (0)	21 (32)

(IQR), and the detection and quantification frequencies for all target compounds. The most frequently detected chemical was MeP (100%), closely followed by BPS and BPA (99.1 and 97.4% respectively). The large majority of PFCs were detected and quantified only in few samples except for PFOA for which detection and quantification frequencies were 86.8 and 46.4% respectively. BPA and BPS were the compounds with the highest median concentration measured in the hair of our population (median: 46.6 and 31.9 pg·mg⁻¹ respectively). The measured median concentrations of parabens ranged between 5.2 and 28.9 pg·mg⁻¹, methylparaben showing the highest concentrations. Very few samples exhibited particularly high contamination levels of methylparaben and ethylparaben (maximum levels of several tens of thousands pg·mg⁻¹).

A comparison between concentrations measured in our subpopulation and those reported from other studies related to more than 30 volunteers is presented in Table 4 for PFCs and Table 5 for parabens and bisphenols.

3.3. Correlations and influence of gender, age and hair characteristics on contamination levels

Spearman's rank correlations between the contamination levels of the different pollutants are reported in Table 6. Significant correlations were observed between BPA and BPS ($p < 0.001, r = 0.55$), MeP and EtP ($p < 0.001, r = 0.36$), MeP and PrP ($p < 0.001, r = 0.61$), MeP and BPS

($p < 0.001, r = 0.26$), MeP and BPA ($p < 0.001, r = 0.38$), EtP and PrP ($p < 0.001, r = 0.37$), EtP and BPA ($p = 0.023, r = 0.21$), EtP and PFOA ($p = 0.021, r = 0.21$), PrP and BPS ($p = 0.038, r = 0.19$), PrP and BPA ($p < 0.001, r = 0.41$), PrP and PFOA ($p = 0.033$ and $r = 0.19$). Other correlations were highlighted between BPS and PFOA ($p < 0.001, r = 0.39$) and between BPA and PFOA ($p < 0.001, r = 0.40$). Weak but significant association with age was only highlighted for PrP in the whole studied population ($p = 0.044, r = 0.18$). This correlation was strengthened in women ($p = 0.038, r = 0.26$) but non-significant in men ($p = 0.774, r = 0.04$). Table 7 reports the median concentrations of parabens, bisphenols and PFOA according to gender, hair length or treatments. Higher levels were observed in the hair of women for all targets, but the differences were not statistically significant. No influence of hair length or treatments on the contamination burden was highlighted.

4. Discussion

In the present study, we measured the hair concentration of two bisphenols, three parabens and 8 PFCs. The concentrations and contamination tendencies observed in our population are discussed regarding the backdrop of literature in hair and other matrices.

In our study, the majority of PFCs were lowly detected except PFOA which was quantified in 46.5% of samples. These low quantification rates for the other PFCs could be explained by the LOQ (10 pg·mg⁻¹ for PFOS, between 0.2 pg·mg⁻¹ and 0.32 pg·mg⁻¹ for all other PFCs) higher than detectable concentrations in hair of our population. However, as shown in Table 4, these LOQs seem suitable with concentrations reported in the literature in the hair from Korean, Chinese or Greek populations (Kim et al., 2019; Liu et al., 2020; Martín et al., 2019). Lower concentrations were observed in China (Wang et al., 2018) or in Belgium (Alves et al., 2015). These differences in contamination levels could result from differences in contamination of the environment, from differences in alimentation and living habits or from differences in the analytical procedure. The very low detection rates observed were not expected since PFHxA, PFOA and PFOS were detected in 100% and PFNA, PFDA in respectively 99.6 and 85.6% of serum samples from a similar subpopulation living in Liege, demonstrating that human exposure to PFCs occurs in Wallonia (Pirard et al., 2020). This would argue that blood remains the most suitable matrix for the assessment of PFCs exposure.

In our study, no statistical difference in PFOA contamination was observed between men and women consistently with results reported in the hair of populations from South Korea and Spain (Kim et al., 2019; Martín et al., 2019). However these results are different from the ones obtained

Table 3

Summary of the pollutant concentrations measured in hair of our Belgian studied population: detection (N > LOD) and quantification (N > LOQ) frequencies (%), geometric mean (GM), standard deviations (SD), interquartile range (IQR), percentiles 25 (P25), 50 (P50). and 75 (P75), minimum and maximum concentrations measured in (pg·mg⁻¹).

	N > LOD (%)	N > LOQ (%)	GM pg·mg ⁻¹	SD pg·mg ⁻¹	IQR pg·mg ⁻¹	Min pg·mg ⁻¹	P25 pg·mg ⁻¹	P50 pg·mg ⁻¹	P75 pg·mg ⁻¹	Max pg·mg ⁻¹
Bisphenols										
BPA	97.4	93.9	46.1	82.7	67.1	<LOQ	21.7	46.6	88.8	587.1
BPS	99.1	97.4	32.8	266.7	47.0	<LOQ	15.4	31.9	62.4	2298.0
Parabens										
MeP	100.0	94.7	104.2	8437.1	180.6	<LOQ	24.1	28.9	204.8	53,356.0
EtP	87.7	66.7	8.7	2740.8	15.3	<LOQ	<LOQ	5.2	16.7	26,967.7
PrP	78.1	54.8	22.9	211.7	55.0	<LOQ	<LOQ	16.4	61.6	1517.1
Perfluoroalkyl compounds (PFCs)										
PFPeA	6.1	<LOQ	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHxA	18.4	5.3	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	2.4
PFHpA	4.4	1.8	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	1.4
PFOA	86.8	46.4	<LOQ	0.4	0.2	<LOQ	<LOQ	<LOQ	0.3	3.4
PFNA	43.9	19.3	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	1.7
PFDA	0.0	0.0	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
PFHxS	11.4	1.8	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	18.8
PFOS	0.9	0.9	<LOQ	-	-	<LOQ	<LOQ	<LOQ	<LOQ	11.3

Table 5
comparison of parabens and bisphenol contamination levels in hair observed in different studies (in $\text{pg}\cdot\text{mg}^{-1}$).

Reference	N	Country	Concentrations ($\text{pg}\cdot\text{mg}^{-1}$)									
			BPA		BPS		MeP		EtP		PrP	
			Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Current study	114	Belgium	46.6	<LOQ-587.1	31.9	<LOQ-2298.0	28.9	<LOQ-51307	5.2	<LOQ-26967.7	16.4	<LOQ-1517.1
(Martín et al., 2019)	42	Spain		24.4–1427	–	–	–	68.3–14,187	–	2.9–6565	–	12.5–9009
(Karzi et al., 2019)	95	Greece	–	–	–	–	1437.1	17.6–27.4	167.3	11–4224.5	–	–
(Katsikantami et al., 2020)	100	Greece	69.9	9.6–650.3	3.5	0.8–147.3	–	–	–	–	–	–
(Peng et al., 2020)	204	China	34.9	5.47–596	2.84	0.5–817	–	–	–	–	–	–
(Nehring et al., 2017)	311	France	118	17.1–1398	8.32	0.07–1246	–	–	–	–	–	–
	42	Poland	337.5	26.1–1498.6	–	–	–	–	–	–	–	–

Table 6
Spearman’s rank correlations (*p*-value) between levels of pollutants in hair.

	MeP	EtP	PrP	BPS	BPA	PFOA
MeP	–					
EtP	0.36 (<0.001)	–				
PrP	0.61 (<0.001)	0.37 (<0.001)	–			
BPS	0.26 (<0.001)	0.16 (0.077)	0.19 (0.038)	–		
BPA	0.38 (<0.001)	0.21 (0.023)	0.41 (<0.001)	0.55 (<0.001)	–	
PFOA	0.15 (0.109)	0.21 (0.021)	0.19 (0.033)	0.39 (<0.001)	0.40 (<0.001)	–

the matrix (Ruan et al., 2019). However, this reduction of observed contamination could also be attributed to a reduction of the external deposition rather than in incorporation. The influence of hair characteristics or cosmetic treatments remains unknown for number of chemicals and further studies are needed to take these parameters into account.

Some samples were highly contaminated by parabens, by 3 orders of magnitude higher than the present upper LOQ, and 5 times higher than median concentration of samples. After investigations, it appeared that all the

volunteers showing these outliers reported using hair wax or hair gels containing parabens. This suggests that gel or wax applied on hair could represent a bias for hair biomonitoring of compounds entering in the composition of such products. Very high concentration values for these compounds would likely indicate that the washing steps did not fully remove this kind of external contamination (gel or wax use to be quite sticky or greasy). On another hand, using such products could also increase the individual exposure by skin contact for instance, which will result in higher individual contamination level. This latter hypothesis could be confirmed by analyzing the contamination markers in other matrix such as urine or blood. Today external contamination remains a highly controversial issue in hair analysis and there is no consensus on hair decontamination procedure removing entirely but exclusively the external contamination (Appenzeller, 2015; Kucharska et al., 2015). External decontaminations are generally performed using water and/or water with shampoo (Appenzeller and Tsatsakis, 2012; Duca et al., 2014), or using organic solvents like methanol or acetone (Duca et al., 2014) but these latter need to be used carefully to avoid removing internal incorporation of pollutants (Kucharska et al., 2015). The use new original techniques to distinguish external contamination and incorporation from blood in hair structure would likely help to develop more efficient decontamination procedures remaining safe for the sample integrity (Erne et al., 2019).

Table 7
Median concentrations of parabens, bisphenols and PFOA according to gender, hair length or treatment (in $\text{pg}\cdot\text{mg}^{-1}$).

	NN	MeP			EtP			PrP		
		P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value	P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value	P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value
Gender				0.213			0.395			0.287
Women	65	64.9	<LOQ-53356.0		6.4	<LOQ-26967.7		65.0	<LOQ-1147.5	
Men	49	44.4	<LOQ-51370.0		3.9	<LOQ-11847.7		52.6	<LOQ-1517.1	
Hair length				0.741			0.720			0.596
Short	51	55.5	<LOQ-51370.0		4.7	<LOQ-11847.6		13.7	<LOQ-1517.1	
Medium	23	56.2	<LOQ-15447.2		2.3	<LOQ-26967.7		31.8	<LOQ-1100.4	
Long	43	62.9	14.2–53,356.0		5.8	<LOQ-2061.3		15.9	<LOQ-1147.5	
Hair treatment				0.447			0.427			0.429
None	77	48.1	<LOQ-53356.0		4.7	<LOQ-26967.7		15.9	<LOQ-1517.1	
Discoloration	7	97.4	17.33–845		8.1	<LOQ-82.4		19.2	<LOQ-164.9	
Dyeing	16	44.9	14.8–5139.3		5.1	<LOQ-2061.3		27.1	<LOQ-1147.5	
	NN	BPS			BPA			PFOA		
		P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value	P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value	P50 ($\text{pg}\cdot\text{mg}^{-1}$)	Range ($\text{pg}\cdot\text{mg}^{-1}$)	<i>p</i> -value
Gender				0.141			0.133			0.242
Women	65	37.1	<LOQ-2298.0		54.3	<LOQ-587.1		<LOQ	<LOQ-1.6	
Men	49	26.4	<LOQ-1416.8		35.4	<LOQ-292.8		0.2	<LOQ-3.4	
Hair length				0.318			0.092			0.359
Short	51	26.9	<LOQ-1416.8		35.9	<LOQ-287.1		0.2	<LOQ-3.4	
Medium	23	40.3	12.04–395.8		63.3	<LOQ-587.1		<LOQ	<LOQ-0.6	
Long	43	31.8	<LOQ-2298.0		47.0	<LOQ-299.2		<LOQ	<LOQ-1.6	
Hair treatment				0.159			0.354			0.687
None	77	30.8	<LOQ-2298.0		41.5	<LOQ-299.2		<LOQ	<LOQ-3.4	
Discoloration	7	15.8	3.0–122.4		35.5	<LOQ-213.2		<LOQ	<LOQ-1.4	
Dyeing	16	51.9	<LOQ-395.8		59.4	<LOQ-587.7		<LOQ	<LOQ-1.6	

The present study suffers from several limitations. Firstly, the small-scale sized population recruited is not representative of the Belgian population in terms of geographical area, socio-economic status, nor age. Secondly, the used external decontamination process didn't seem to be efficient enough to remove wax or gel when applied on the hair. The lack of washing standardization but also as the lack of hair reference materials (commercial quality control, blank matrix) for environmental pollutant analysis could lead to a variability between lab's results and thus could explain at least partially the differences in hair contamination levels reported in different studies.

Finally, the absence of significant difference between males and females in the present study and more generally all statistical results, should be considered in the view of the limited number of subjects included in this study, which probably doesn't allow to reach high statistical power. Moreover, the wide range of concentration is probably a confounding factor which could hide the real influence of tested parameters.

5. Conclusion

This manuscript presents the first study of contamination levels of three parabens, two bisphenols and 8 PFCs using hair as matrix from a subpopulation living in the city of Liege (Wallonia, Belgium). Our results demonstrate that the hair samples of the 114 individuals were widely contaminated by bisphenols, parabens and PFOA. On the other hand, other PFCs were lowly detected. No significant difference in hair contamination was observed according to the gender, hair length or hair treatment. However, the statistical power of our study needs to be interpreted considering the limited number of subjects included in this study.

Although our population was not representative and large enough to produce reference values as strictly speaking or to represent trends of a general population, the present study provides additional concentration values in hair of a sub-group of a non-occupationally exposed population and thus grows the available data on hair contamination levels for the target chemicals. If today blood seems to remain the most suitable matrix for PFCs exposure assessment, hair would be a promising approach for future exposure assessments of environmental pollutants such like bisphenols or parabens. Nevertheless, further studies are still needed to enlarge the number of chemical substances measurable in hair, characterize different populations by pollutants hair levels, and define standardized procedure for external decontamination steps.

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CRedit authorship contribution statement

Julien Claessens: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Investigation. **Catherine Pirard:** Methodology, Writing – review & editing, Conceptualization. **Corinne Charlier:** Supervision, Project administration, Writing – review & editing.

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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