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Home-produced eggs: An important human exposure pathway of perfluoroalkylated substances (PFAS)

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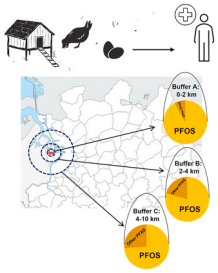
HIGHLIGHTS

- Eight PFAS were detected in home-grown eggs of free-ranging laying hens.
- PFOS was the dominant compound and concentrations decreased from the fluorochemical plant.
- Diet and age of laying hens were related to PFOS and PFOA egg concentrations.
- Homegrown eggs can be an important exposure pathway of PFAS to humans.
- Based on exposure estimation via egg intake, health guidelines were often exceeded.

GRAPHICAL ABSTRACT



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ABSTRACT

Humans are generally exposed to per- and polyfluoroalkyl substances (PFAS) through their diet. Whilst plenty of data are available on commercial food products, little information exists on the contribution of self-cultivated food, such as home-produced eggs (HPE), to the dietary PFAS intake in humans. The prevalence of 17 legacy and emerging PFAS in HPE ($N = 70$) from free-ranging laying hens was examined at 35 private gardens, situated within a 10 km radius from a fluorochemical plant in Antwerp (Belgium). Potential influences from housing conditions (feed type and number of individuals) and age of the chickens on the egg concentrations was examined, and possible human health risks were evaluated. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) were detected in all samples. PFOS was the dominant compound and concentrations (range: 0.13–241 ng/g wet weight) steeply decreased with distance from the fluorochemical plant, while there was no clear distance trend for other PFAS. Laying hens receiving an obligate diet of kitchen leftovers, exhibited higher PFOS and PFOA concentrations in their eggs than hens feeding only on commercial food, suggesting that garden produce may be a relevant exposure pathway to both chickens and humans. The age of laying hens affected egg PFAS concentrations, with younger hens exhibiting significantly higher egg PFOA concentrations. Based on a modest human consumption scenario of two eggs per week, the European health guideline was

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exceeded in ≥67% of the locations for all age classes, both nearby and further away (till 10 km) from the plant site. These results indicate that PFAS exposure via HPE causes potential human health risks. Extensive analysis in other self-cultivated food items on a larger spatial scale is highly recommended, taking into account potential factors that may affect PFAS bioavailability to garden produce.

1. Introduction

The human population will reach over 9 billion people by 2050 and projections estimate that 70% of humans will then live in urban areas (Galhena et al., 2013; Zipperer and Pickett, 2012). In parallel, food production will have to increase by 70% to meet the daily calorie intake demands of this growing population (Galhena et al., 2013). Consequently, novel food cultivation strategies will be required as available resources for food production, most importantly land surface, are limited. Hereby, self-cultivation of food, by means of crop production and farm animals, has been promoted and has become an increasing trend in private gardens from rural, urban and even industrial areas (Church et al., 2015; Van der Jagt et al., 2017).

Particularly, the housing of free-ranging chickens (*Gallus gallus domesticus* L.) has gained worldwide popularity over recent years (Capoccia et al., 2018; Padhi, 2016; Sioen et al., 2008). Chickens provide environmental and economic assets by means of kitchen waste disposal, egg production and low-cost maintenance (Waegeneers et al., 2009). Furthermore, home-produced eggs (HPE) are often perceived by the general public to have high nutritional value (Van Overmeire et al., 2006; Waegeneers et al., 2009). For instance, HPE accounted in 2017 for 17% of the egg consumption in Belgium and this number has been steadily increasing (VLAM, 2017). In this regard, free-ranging chickens offer unique opportunities for monitoring human exposure, as they are the most prevalent birds on earth in terms of biomass and usually live in close contact with humans (Bar-On et al., 2018; Scaramozzino et al., 2019). HPE have also been associated with higher concentrations of organic pollutants (Sioen et al., 2008; Waegeneers et al., 2009), including per- and polyfluoroalkyl substances (PFAS) (D'Hollander et al., 2011; Gazzotti et al., 2021; Zafeiraki et al., 2016).

PFAS are synthetic and organic compounds that have been produced for more than 70 years (Post, 2021). The combination of their amphiphilic properties and strong C–F bond makes them useful for a diverse range of commercial applications, such as soil- and water repellent clothing, cleaning products, food-packaging, paper coating and fire-fighting foams (Buck et al., 2011). On the other hand, these distinctive chemical properties make PFAS highly persistent in the environment and bioaccumulative in biota (Death et al., 2021; Giesy and Kannan, 2002). For instance, the serum half-lives in humans of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), which are the most widely studied PFAS to date, can reach approximately 5 and 3 years, respectively (Goodrum et al., 2021). Both experimental studies on laboratory animals and human epidemiological studies have identified PFAS with various health effects including liver damage, altered immune functioning, neurotoxicity and cancer (Briels et al., 2018; Fenton et al., 2021; Lilienthal et al., 2017; Sunderland et al., 2019).

Generally, the most important human exposure pathway of PFAS our diet (Cornelis et al., 2012; Roth et al., 2020). Numerous studies have reported PFAS concentrations in commercial food, notably those within the European PERFOOD project (https://ibed.fnwi.uva.nl/perfood/), in which fish and offal food were identified as the main dietary sources of PFAS (Cornelis et al., 2012; Klenow et al., 2013). Based on intake modelling, dietary PFAS exposure was estimated to be of no concern with respect to the former health guideline values for PFOS and PFOA set in 2008 (Klenow et al., 2013). However, PFAS intake exposures were mostly compared to outdated health guidelines derived from critical toxic endpoints, such as liver toxicity (Zafeiraki et al., 2016; Su et al., 2017), while recently established health guidelines point out that PFAS effects on more sensitive toxic endpoints, for instance immune toxicity, can occur at much lower intake levels (EFSA CONTAM Panel, 2020). These sensitive endpoints have rarely been evaluated and the additional contribution of home-produced food to the PFAS intake has only been considered to a limited extent in human health risk assessments (Gazzotti et al., 2021).

Therefore, self-cultivated food can be a major source of PFAS exposure to humans, especially in the neighbourhood of PFAS hot-spots, and should be taken into account for PFAS risk assessments (Death et al., 2021; Xu et al., 2021b). Recent human biomonitoring research across Flanders has consistently linked internal serum PFOS concentrations with the consumption of HPE (Buekers et al., 2021; Colles et al., 2020). HPE are often produced in less controlled housing and feeding conditions than commercial eggs, which have been shown to contain much lower PFAS concentrations (Zafeiraki et al., 2016; Su et al., 2017). In contrast to commercial laying hens, free-ranging laying hens in private gardens have continuous access to an outdoor enclosure. As such, they may be exposed to PFAS via ingestion of contaminated soil and dust particles, intake of rain water, soil invertebrates (eg. worms and insects) and kitchen waste products (Waegeneers et al., 2009; Wang et al., 2010). These intake media may be directly contaminated with PFAS through transfer from primary sources, such as direct emissions from fluorochemical industry via air and surface water into ground water and soil (Schroeder et al., 2021; Xu et al., 2021a). Additionally, secondary sources including precursor degradation and domestic emissions from consumer products and application products may also contribute to local contamination of the private garden (Liu et al., 2019).

Human intake assessments of PFAS are mostly restricted to the level of the general population, while very little is known about the potential exposure routes and scenarios for inhabitants living near PFAS point sources. Zafeiraki et al. (2016) measured relatively low PFAS concentrations in yolk of HPE from the Netherlands and Greece, with median sum PFAS concentrations of 3.1 and 1.1 ng/g wet weight (ww), respectively. However, these data were not reported in relation to any fluorochemical point source, that may explain variation across the samples. Recently, a few studies in China have reported mean sum PFAS concentrations of 122 ng/g egg yolk nearby PFAS industry, but only a limited spatial scale was considered (Wang et al., 2019) and sample sizes were too small (Su et al., 2017) to make any claims about representativity or potential health risks. Moreover, the impact of different feeding regimes (e.g. kitchen waste versus commercial feed) and local housing conditions of the laying hens on egg PFAS concentrations has, to the best of our knowledge, never been addressed.

The main objective of this study was therefore to examine the PFAS profile and concentrations in HPE in relation to the distance towards a known PFAS point source in Antwerp, Belgium. Secondly, we aimed to investigate the potential influence of housing conditions (feed type and number of individuals) and age of the laying hens on the egg PFAS concentrations, based on survey data. Lastly, possible human health risks of PFAS intake through consumption of HPE were assessed with respect to currently available health guidelines, by means of both critical (liver toxicity) and sensitive (immune toxicity) endpoints.

Given that eggs of several free-living bird species breeding near the fluorochemical plant site in Antwerp contained among the highest PFAS concentrations ever reported in bird eggs (Groffen et al., 2017, 2019a, 2019b; Lasters et al., 2021) and that egg PFAS concentrations in wild birds decreased from 3 km onwards of the plant site (Groffen et al., 2017), we hypothesize that the most diverse PFAS profile and highest concentrations in HPE are present within a 3 km radius from the plant

site. As a consequence, the potential risk for public health through HPE consumption is expected to be highest within this 3 km radius. Regarding the potential influences of housing and feeding conditions, the following hypotheses were tested: (i) higher egg PFAS concentrations may be related with a higher number of laying hens as increased scratching behaviour would result in less vegetation coverage and increased exposure with contaminated soil particles and invertebrates; (ii) eggs of younger hens contain higher egg PFAS concentrations due to less elimination time and fewer sequestration possibilities compared to older laying hens; and (iii) higher PFAS concentrations are detected in eggs from hens that are primarily fed with kitchen waste products, which may contain potentially contaminated garden produce that is cultivated in a less-controlled way compared to commercial feed.

2. Materials and method

2.1. Study area and sample collection

During the period July–September 2018, HPE (N = 70) were collected from 35 volunteers that kept free-ranging laying hens. Two eggs from each location were sampled at the same day to ensure that the eggs originated from different individual hens. These samples were collected within a 10 km radius from a known PFAS point source in Antwerp, Belgium (Groffen et al., 2019a; Lopez-Antia et al., 2019), as displayed in Fig. 1. The study area was divided into three concentric buffer zones (A: 0–2 km, N = 18; B: 2–4 km, N = 30; C: 4–10 km, N = 22) with increasing distances from this point source. The buffer zone categories were based on the typical spatial decrease of PFAS observed in earlier studies on terrestrial bird eggs in the studied area (Groffen et al., 2017, 2019a).

2.2. Volunteer selection and survey data

Volunteers that housed at least two free-ranging laying hens in their gardens were recruited via existing social networks and regular call-ups on social media. Moreover, only volunteers were selected that kept free-ranging laying hens of at least six months of age and which had continuous access to an uncovered outdoor enclosure.

After the eggs were collected, each volunteer completed a self-reporting survey in which information on the age and flock size of the laying hens was given (Table S1). Additionally, categorical data were obtained on the feed origin of the laying hens, consisting of the following subcategories: kitchen leftovers (LF; mainly vegetable scraps and/or garden produce), commercial feed (CF; commercial layer feed) or a mix of both (M). The age dataset of the laying hens was merged into three age classes, based on the age classification system of Joyner et al. (1987): young layers (<1 year old), older layers (1–2 years old) and old layers (>2 years old). Moreover, the distance (Euclidean) of each sampling location to the PFAS point source was assessed and each location was assigned to its associated buffer zone (0–2, 2–4, 4–10 km).

The personal data of all volunteers were treated confidentially, according to the current privacy regulations (GDPR). Data management was approved by the privacy policy of the University of Antwerp. Every volunteer gave explicit approval for the processing of their data within the context of the specific research goals of this study via an informed consent. The personal results were communicated to each volunteer via a short report containing background information on PFAS, a consumption advice based on their individual results and general strategies that may lower overall PFAS exposure. The researchers were available for tackling questions of the participating volunteers.

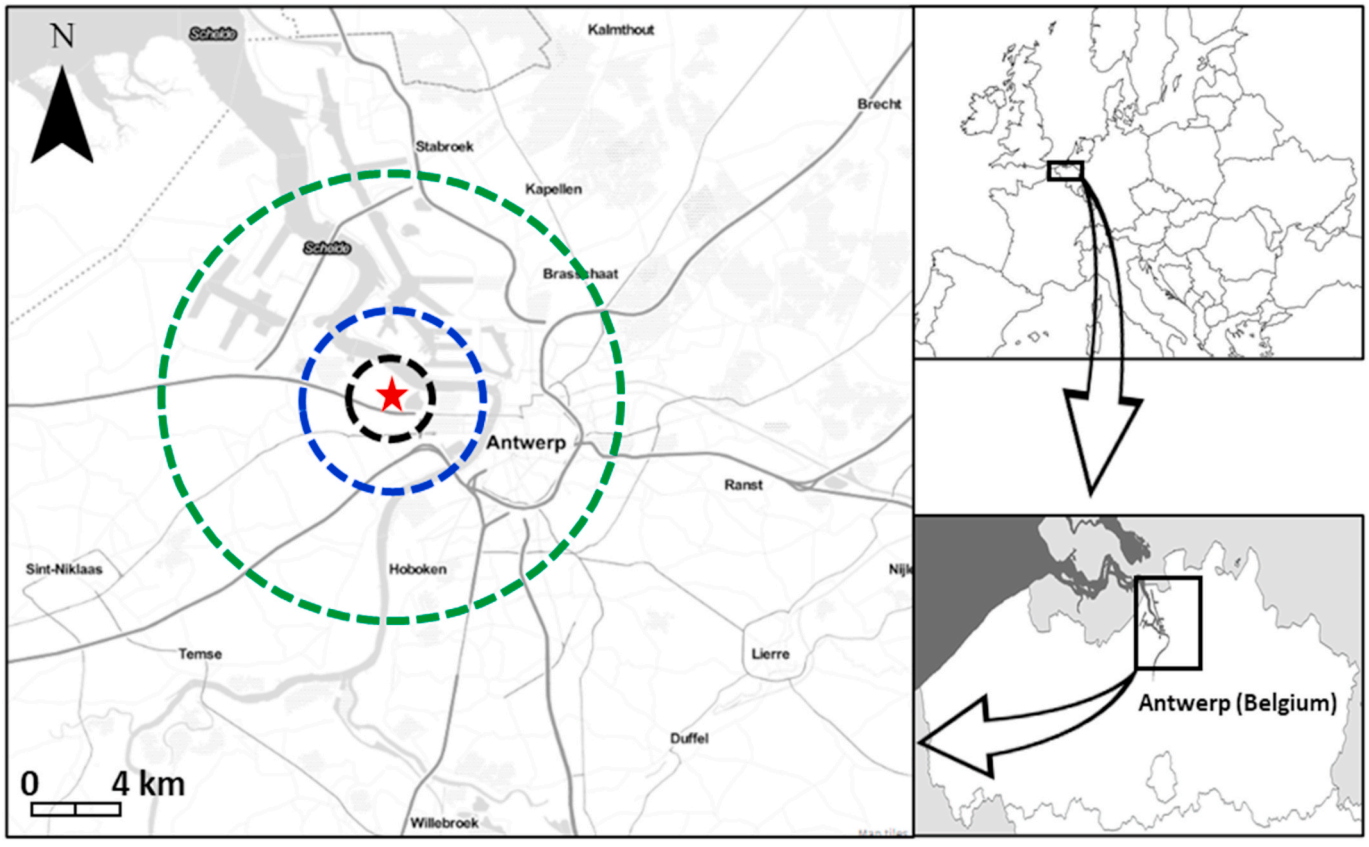

Fig. 1. Overview of the study area in which the home-produced eggs were sampled in 2018 in three concentric distance buffers located within a radius of 2 km (buffer A, N = 18), 4 km (buffer B, N = 30) and 10 km (buffer C, N = 22) from the fluorochemical plant site (red asterisk) in Antwerp, Belgium, respectively.

2.3. Chemical analysis

All used abbreviations of PFAS are based on Buck et al. (2011). Four target perfluoroalkyl sulfonic acids (PFSAs) (PFBS, PFHxS, PFOS and PFDS), 11 target pefluoroalkyl carboxylic acids (PFCAs) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and PFTeDA) and two emerging fluoroether PFAS (sodium dodecafluoro-3H-4,8-dioxanonanoate (NaDONA) and 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid (HFPO-DA or GenX) were analysed in the samples. The following isotopically mass-labelled internal standards (ISTDs) were used in the analysis: $^{18}O_2$-PFHxS, [1,2,3,4–$^{13}C_4$]PFOS, $^{13}C_4$-PFBA, [1,2–$^{13}C_2$]PFHxA, [1,2,3,4–$^{13}C_4$]PFOA, [1,2,3,4,5–$^{13}C_5$]PFNA, [1,2–$^{13}C_2$]PFDA, [1,2–$^{13}C_2$]PFUnDA and [1,2–$^{13}C_2$]PFDoDA (Wellington Laboratories, Guelph, Canada). The stock ISTD solution was diluted in a mixture of 50:50 (v:v) of HPLC grade acetonitrile (ACN) and Milli-Q water (VWR International, Leuven, Belgium) to a concentration of 125 pg μL^{-1} to spike the samples.

2.4. Chemical extraction

Prior to the extraction of the egg samples, three analytical methods were tested on a spiked blank matrix sample (= commercial eggs low in PFAS contamination, Table S2) in order to select a relatively robust, accurate and sensitive extraction procedure (see supplementary information: optimization extraction method). The clean-up extraction using graphitized Envicarb carbon powder (adopted from Powley et al., 2005) was selected for extraction of the samples, as the extraction recoveries of PFSAs were low when using the other two procedures (weak anion exchange solid-phase extraction (XAW method), detailed in Groffen et al., 2019c, and a combination of clean-up extraction with Envicarb powder followed by the XAW method) and would imply that PFHxS cannot be quantified (Fig. S1).

The egg content was transferred into a polypropylene (PP) tube and homogenized by repeatedly sonicating and vortex-mixing. The homogenized samples were weighed and around 0.3 g of homogenized sample was used (±0.01 mg, Mettler Toledo, Zaventem, Belgium) for the extraction. Homogenates were spiked with 80 μL of 125 pg μL^{-1} ISTD solution. After adding 10 mL of acetonitrile (ACN), the samples were sonicated three times (with vortex-mixing in between periods) and left overnight on a shaking plate (135 rpm, room temperature, 20 °C, GFL 3020, VWR International, Leuven, Belgium). Afterwards, the samples were centrifuged (4 °C, 10 min, 2400 rpm, 1037 g, Eppendorf centrifuge 5804R, rotor A-4-44) and the supernatant was stored in a 15 mL PP tube. Then, the supernatant was vacuum-dried to approximately 0.5 mL using a rotational vacuum concentrator (30 °C, type 5301, Hamburg, Germany). The extract was transferred to a PP Eppendorf tube which was filled with 50 mg of graphitized carbon powder (Supelclean ENVI-Carb, Sigma-Aldrich, Overijse, Belgium) and 35 μL of glacial acetic acid to remove chemical impurities. The 15 mL tube was rinsed twice with 250 μL of ACN, which was transferred to the Eppendorf tube. After thoroughly vortex-mixing the tube, the extracts were centrifuged (4 °C, 10 min, 10,000 rpm, 1037 g, Eppendorf centrifuge 5415R, rotor F 45-24-11). Then, the supernatant was transferred to a new Eppendorf tube and vacuum-dried until it was nearly completely dry. The dried extract was reconstituted in 100 μL of a 2% ammonium hydroxide solution diluted in ACN and filtered through a 13 mm Acrodisc Ion Chromatography Syringe Filter with 0.2 μm Supor (PES) membrane (VWR International, Leuven, Belgium) into a PP injector vial prior to instrumental analysis.

2.5. UPLC-TQD analysis

The target analytes were analysed using an ACQUITY Ultrahigh Performance Liquid Chromatography (ACQUITY, TQD, Waters, Milford, MA, USA) coupled to a tandem quadrupole (TQD) mass spectrometer (UPLC-MS/MS) with negative electrospray ionisation. To separate the different target analytes, an ACQUITY UPLC BEH C18 VanGuard Pre-column (2.1 × 50 mm; 1.7 μm, Waters, USA) was used. The mobile phase solvents consisted of ACN and HPLC grade water, which were both dissolved in 0.1% HPLC grade formic acid. The solvent gradient started at 65% of water to 0% of water in 3.4 min and back to 65% water at 4.7 min. The flow rate was set to 450 μL/min and the injection volume was 6 μL. PFAS contamination that might originate from the LC-system was retained by insertion of an ACQUITY BEH C18 pre-column (2.1 × 30 mm; 1.7 μm, Waters, USA) between the solvent mixer and the injector. The target PFAS analytes were identified and quantified based on multiple reaction monitoring (MRM) of the diagnostic transitions that are displayed in Table S3.

2.6. Quality control and assurance

Per batch of ten samples, one procedural blank (= 10 mL ACN spiked with ISTD) was included to detect any contamination during the extraction. To prevent cross-over contamination among samples during detection in the UPLC-MS/MS, ACN was regularly injected to rinse the columns. Limits of quantification (LOQs) were calculated for each analyte, in matrix, as the concentration corresponding to a signal-to-noise ratio of 10. Calibration curves were prepared by adding a constant amount of the ISTD to varying concentrations of an unlabelled PFAS mixture. The serial dilution of this mixture was performed in ACN. A linear regression function with highly significant linear fit (all $R^2 > 0.98$; all $P < 0.001$) described the ratio between concentrations of unlabelled and labelled PFAS. Individual PFAS were quantified using their corresponding ISTD with exception of PFPeA, PFHpA, PFTrDA, PFTeDA, PFBS, PFDS, HFPO-DA and NaDONA for which no ISTD were present. These analytes were all quantified using the ISTD of the compound closest in terms of functional group and size (Table S3), which was validated by Groffen et al. (2019c, 2021).

2.7. Health risk indications

The potential risk of PFAS intake via HPE consumption was estimated for each of the three buffer zones. The consumption scenario was based on the intake of two HPE per week, which is the general Flemish governmental health guideline for HPE and approximately corresponds to the average weekly egg consumption for a modal Belgian citizen (Lebacq, 2015; Sioen et al., 2008). The calculation of the PFAS intake values via eggs was conducted per age category, as younger people will have a higher relative PFAS intake per kg bodyweight (bw) compared to adults. To this end, mean body weight values were adopted from the latest food consumption datasets of the Belgian population (De Hoge Gezondheidsraad, 2003; Van der Heyden et al., 2018) for the following age intervals: 3–5, 6–9, 10–13, 14–17, 18–64 years old (Table S4). For the two latter age intervals, data were provided for both males and females as considerable weight differences exist between sexes within these age intervals. Finally, the estimated weekly intake (EWI) of PFAS was calculated by the following formula, according to Su et al. (2017):

$$EWI\ (ng\ /\ kg\ bw\ /\ week) = egg\ consumption\ (g\ /\ week)\ x\ egg\ PFAS\ concentration\ (ng\ /\ g\ ww\ of\ whole\ egg\ content)\ /\ body\ weight\ (kg) \quad (1)$$

The EWI was compared with two frequently used health guideline criteria with respect to the maximum tolerable intake of PFAS via food: the tolerable weekly intake value (TWI: 4.4 ng/kg bw per week) which considers the sum of PFHxS, PFOS, PFOA and PFNA (EFSA CONTAM Panel, 2020) and the maximum tolerable risk values (MTR: 43.8 ng/kg bw per week for PFOS and 87.5 ng/kg bw per week for PFOA) which are derived for PFOS and PFOA (Zeilmaker et al., 2016). These two criteria are based on a relatively sensitive toxic endpoint (= reduced antibody response to vaccination in infants) and a more critical endpoint (= liver hypertrophy in rats), respectively, in order to obtain a comprehensive risk estimate.

2.8. Statistical analysis

Statistical analyses were performed in the statistical software R (version 3.5.2) and in GraphPad Prism (version 9). The significance level for model testing was set at $P \leq 0.05$. The model assumptions were evaluated with the Shapiro-Wilk test for normality and data were log (x+1) transformed to comply with normality assumptions. For PFAS concentrations that were <LOQ, replacement concentration values were assigned following a maximum likelihood estimation method (Villanueava, 2005; De Solla et al., 2012).

For each distance buffer zone (A = 0–2 km; B = 2–4 km and C = 4–10 km), the PFAS profile and concentrations in the HPE ($N = 70$) were calculated using descriptive statistical parameters. The composition profile of the PFAS was given as the contribution of the concentrations from single compounds to the sum of PFAS concentrations in the eggs.

Potential relationships among the PFAS concentrations and the variables from the survey data were tested on location level ($N = 35$) for the following reasons: (i) due to practical constraints, some of the survey data (e.g. age) could not be derived for each individual egg and (ii) each egg cannot be considered as an independent replicate due to the hierarchical structure of the dataset (i.e. two eggs originated from different chickens which share one common environment and thus are nested within the same location). Therefore, the individual PFAS concentrations for the two eggs at each location were aggregated, resulting in independent mean values for each location ($N = 35$). Moreover, PFAS with an overall detection frequency <50% were omitted from the analyses to minimize left-skewness of the respective data distribution. A one-way ANOVA was used to test for potential differences in egg PFAS concentrations among the considered buffer zones at varying distance from the fluorochemical plant site in Antwerp. A general linear model, containing the number and average age of the laying hens as explanatory variables, was used to test their potential association with PFAS concentrations. Finally, the potential effect of feed origin on the egg concentrations was examined with a one-way ANOVA. For these two latter analyses, the data were tested independently from the buffer zones to increase the statistical power of the models that were fit.

3. Results

3.1. PFAS profile and concentrations in the buffer zones

The detection frequencies of all the detected PFAS in the eggs are given in Table 1 and displayed in Fig. 2. In total, eight out of 17 target PFAS were detected in the eggs of each buffer zone, except for PFHxS. This latter compound was not detected in buffer B, although the detection of PFHxS in buffer C originated from one location that was situated on the edge of buffer B and C. Only PFOA, PFDA and PFOS were detected in >50% of the eggs in each buffer zone. PFOS and PFOA were the most frequently detected compounds and were found in all the eggs

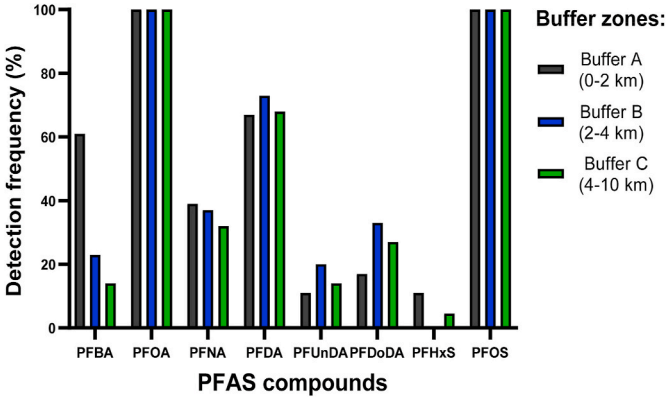

Fig. 2. Overview of the detection frequencies (%) of all the target PFAS in home-produced eggs of free-ranging laying hens within a radius of 2 km (buffer A, $N = 18$), 4 km (buffer B, $N = 30$) and 10 km (buffer C, $N = 22$) from the fluorochemical plant site in Antwerp, Belgium. PFHxS values for buffer A and buffer C are based on one datapoint.

Table 1
Limits of quantification (LOQs; ng/g ww, determined as 10x the S/N ratio), median and mean concentrations (ng/g ww), ranges (min. - max. in ng/g ww) and detection frequencies (Freq. (%) of the target PFAS analytes in the individual home-produced eggs of free-ranging laying hens within each buffer zone (range 0–10 km) from the fluorochemical plant site in Antwerp, Belgium.

LOQ		PFCAs (ng/g ww)						PFSAs (ng/g ww)	
		PFBA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFHxS[a]	PFOS
		0.10	0.13	0.16	0.21	0.14	0.080	2.5	0.13
Buffer A: 0–2 km ($N = 18$)	Median	1.8	0.64	0.29	0.55	0.70	0.52	3.4	11
	Mean	2.8	0.78	0.30	0.53	0.70	0.55	3.4	39
	Range (min. - max.)	0.44–9.1	0.26–2.4	<LOQ – 0.73	<LOQ – 0.78	0.49–0.91	0.48–0.65	3.3–3.5	<LOQ – 241
	Freq. (%)	61	100	39	67	11	17	11	100
	Contribution to ∑PFAS (%)	4.1	1.8	0.3	0.8	0.2	0.2	0.9	91.7
Buffer B: 2–4 km ($N = 30$)	Median	0.75	0.54	0.21	0.51	0.66	0.40	ND[b]	3.5
	Mean	0.75	0.57	0.27	0.66	0.78	0.57	ND	6.5
	Range (min. - max.)	0.54–0.96	0.21–1.0	<LOQ – 0.68	0.22–1.6	0.33–1.4	0.21–1.6	ND	0.54–44
	Freq. (%)	23	100	37	73	20	33	0	100
	Contribution to ∑PFAS (%)	2.2	7.1	1.2	6.0	1.9	2.3	0	79.3
Buffer C: 4–10 km ($N = 22$)	Median	0.50	0.53	0.28	0.48	0.87	0.47	3.6	3.3
	Mean	0.81	0.57	0.27	0.52	0.77	0.57	3.6	4.4
	Range (min. - max.)	0.40–1.5	0.13–1.0	<LOQ – 0.44	<LOQ – 0.99	0.54–0.90	0.23–1.3	3.6	0.78–13
	Freq. (%)	14	100	32	68	14	27	4.5	100
	Contribution to ∑PFAS (%)	1.8	9.6	1.4	5.9	1.8	2.6	2.7	74.1

[a] PFHxS values for buffer A and buffer C are based on one datapoint.
[b] ND = compound not detected.

from every buffer zone (Fig. 2). The highest detection frequency for PFBA and PFHxS was observed in buffer A, respectively in 61% and 11% of the eggs, compared to the other buffer zones. On the other hand, three long-chain PFCAs (PFDA, PFUnDA and PFDoDA) were all most frequently detected in buffer B (Fig. 2). None of the target emerging compounds (GenX and NaDONA) were detected in any of the eggs.

The descriptive statistics (min. – max., median and mean concentrations) of all the detected PFAS in the eggs are provided in Table 1. The mean PFOS concentrations in the eggs were significantly higher in buffer A (39 ng/g ww) compared to those from buffer B and C (both $P < 0.05$, $F_{2,32} = 4.0$), for which mean concentrations of, respectively, 6.5 ng/g ww and 4.4 ng/g ww were measured (Table 1, Fig. 3). The mean PFBA concentrations tended to decrease from buffer A to B ($P = 0.06$, Fig. S3), while there were no significant differences among the buffer zones for all the other PFCAs (all $P > 0.05$, Fig. S3). PFOS and PFOA concentrations in the eggs were positively correlated within buffer zone A (Fig. S4; $P < 0.001$; $R^2 = 0.81$), while this was not the case within other buffer zones.

Overall, PFOS was the dominant compound in all buffer zones, contributing for 91%, 79% and 74% to the ∑PFAS in respectively buffer A, buffer B and C (Fig. 4). For the ∑PFCAs, PFBA was the major compound in buffer A (55% contribution), whereas PFOA contributed most to the ∑PFCAs in buffer B and C (34% and 41% contribution, respectively). The contribution of the short-chain PFBA to the ∑PFCAs decreased from buffer A to buffer B, while the reverse was true for all the detected long-chain PFCAs (Fig. 4).

3.2. PFAS relationships with survey data

Eggs that originated from young laying hens were associated with higher PFOA concentrations compared to old laying hens ($P < 0.01$; Fig. 5), while there was no clear relationship with age and PFOS concentrations in the eggs ($P = 0.10$, $F_{2,28} = 5.9$; Fig. 5). Laying hens that were fed an obligate diet of kitchen leftovers tended to contain higher egg PFOS concentrations ($P = 0.08$, $F_{2,31} = 2.8$) and PFOA concentrations ($P = 0.07$, $F_{2,31} = 2.9$) compared to laying hens that were provided with commercial feed only. The number of chickens in the enclosure was not associated with PFAS concentrations in the eggs (all $P > 0.05$).

3.3. Human health risk

The intake estimations for the sum of four PFAS (PFHxS, PFOS, PFOA and PFNA) in different age intervals are provided in Table 2, based on a weekly egg consumption scenario of two HPE. In addition, the percentage exceedance of both the EFSA threshold (TWI; intake sum of four PFAS) and the RIVM threshold (MTR; intake of PFOS and PFOA separately) is given (Table 2). Overall, the EFSA health guideline was exceeded in the majority of the locations for all the age intervals (≥67%) within 10 km from the fluorochemical plant site. The median intake values for the sum of four PFAS were highest in buffer A, ranging from 75 ng/kg bw per week to 18 ng/kg bw per week in the average infant (3–5 years old) and average male adult (18–64 years old), respectively (Table 2). The intake values for the sum of four PFAS were on average 2.5 times higher in buffer A compared to both buffer B and C, while intake was only slightly higher in buffer B compared to buffer C.

The RIVM health guideline for PFOS was exceeded in 22–56% of the locations from buffer A (Table 2), while only infants (3–5 years old) and children (6–9 years old) exceeded this health guideline in ≤22% of the locations in the other buffer zones (Table 2). With respect to PFOA, the RIVM health guideline was never exceeded in any of the buffer zones.

4. Discussion

4.1. PFAS profile and concentrations in the distance buffer zones

Table 3 shows an overview of available literature data reporting PFAS concentrations (min. – max. range) in HPE from Europe and China. In Belgium, D'Hollander et al. (2011) measured among the highest PFOS concentrations ever reported in HPE within a similar distance from the fluorochemical plant in Antwerp. However, PFAS compounds other than PFOS and PFOA were not examined and it was not clear how spatial variation in PFAS concentrations related to the fluorochemical plant site as 29 samples were collected across Flanders, with only three samples being obtained close to the fluorochemical plant site in Antwerp. Nevertheless, maximum PFOS concentrations (up to 3473 ng/g ww) were more than 14 times higher compared to those reported in the present study (Table 3). This apparent decrease may be explained by the phase-out of PFOS, PFOA and related compounds since 2002 at this production facility (3 M, 2000). However, subsequent and more extensive monitoring campaigns are necessary to evaluate whether there is indeed a decrease over time.

Furthermore, the PFAS detection profile in HPE largely overlaps with those in eggs of wild great tits that were sampled within similar distance from the plant site in Antwerp (Groffen et al., 2017, 2019a). Nevertheless, much higher concentrations of PFAS were measured in great tit eggs, along with the detection of additional long-chain PFCAs (>C13), which were not present in HPE. This suggests that wild birds are being exposed to PFAS to a larger degree than domestic chickens through frequent consumption of highly exposed prey items. Compared to laying hens, wild birds may consume more highly contaminated animal prey items, as they are not confined to an enclosure and hence have access to a broader foraging area. In addition, domestic chickens are given more non-contaminated vegetable feed and may also be able to deposit PFAS into a larger amount of eggs than wild birds, as their egg laying cycle is longer and not restricted to a breeding season. Fewer target compounds could be detected in wild great tit eggs than in HPE, within similar range (4–10 km) from the plant site (Groffen et al., 2019a; Lasters et al., 2019).

For PFOS, a significantly exponential decrease was observed in egg concentrations with increasing distance from the fluorochemical plant

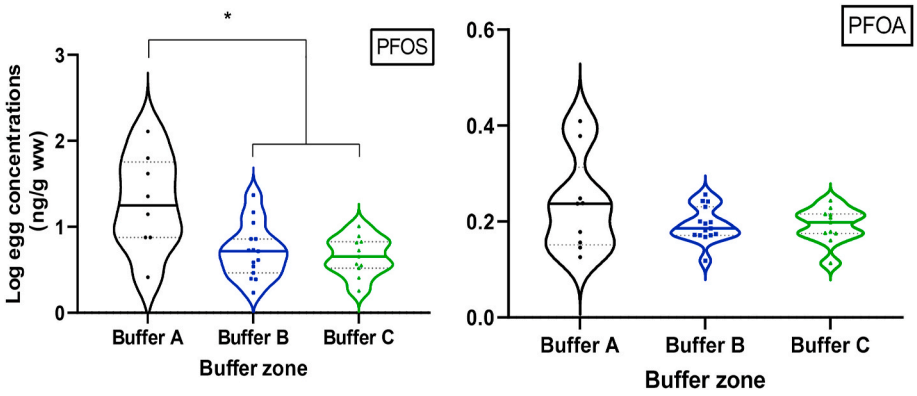

Fig. 3. Log PFOS and PFOA concentrations (ng/g ww) in home-produced eggs of free-ranging laying hens within each buffer zone (buffer A = 0–2 km, $N = 18$; buffer B = 2–4 km, $N = 30$; buffer C = 4–10 km, $N = 22$) from the fluorochemical plant site in Antwerp, Belgium. The asterisk indicates significantly higher PFOS concentrations in eggs of buffer zone A compared to those in eggs from both buffer zone B and buffer zone C (left graph; $P < 0.05$), while no significant differences were found among the buffer zones for PFOA (right graph; $P > 0.05$). Thick horizontal line in the violin plot represents the mean.

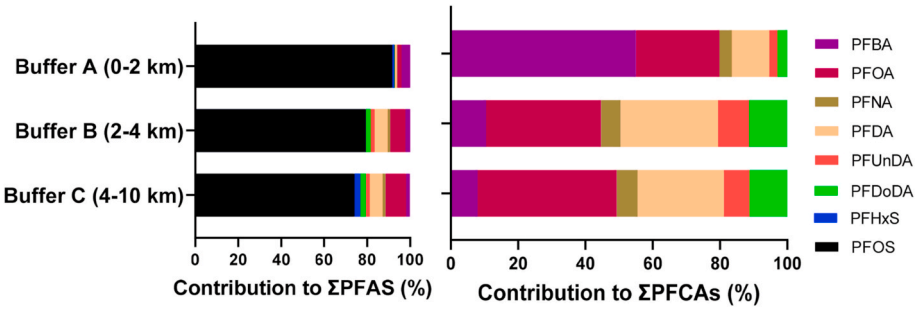

Fig. 4. Composition profile of the ∑PFAS (left graph) and ∑PFCAs (right graph) in home-produced eggs of free-ranging laying hens within a radius of 2 km (buffer A, $N = 18$), 4 km (buffer B, $N = 30$) and 10 km (buffer C, $N = 22$) from the fluorochemical plant site in Antwerp, Belgium. PFHxS values for buffer A and buffer C are based on one datapoint.

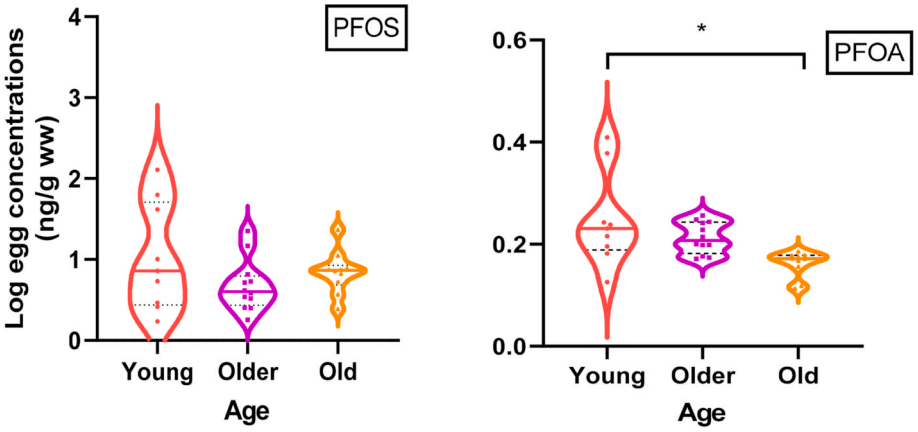

Fig. 5. Comparison of the log PFOS and PFOA concentrations (ng/g ww) in home-produced eggs among young, older and old laying hens (young: <1 year old, older: 1–2 years old, old: >2 years old). Young laying hens laid eggs with significantly higher PFOA concentrations ($P < 0.01$) compared to old laying hens, while no significant difference ($P = 0.10$) was found in egg PFOS concentrations among the age groups.

site (Fig. S2), while there was a declining trend for PFBA (Fig. S3). Until 2002, PFOS was the main product of 3 M at their production sites (3 M, 2000). The spatial variability of PFOS suggests that most of its accumulation in HPE within vicinity of the plant site is originating from historical industrial emissions. Previous studies on wildlife around this area also described this rapidly declining trend for PFOS (Dauwe et al., 2007; D'Hollander et al., 2014; Groffen et al., 2019a). Interestingly, the concentrations in HPE from buffer B and C were similar to those in other European studies, in which HPE were randomly collected without considering a distance gradient from a PFAS point source (Gazzotti et al., 2021; Zafeiraki et al., 2016). Although PFOA and PFOS concentrations in HPE from buffer A were correlated, this was not the case for eggs in buffer B and C (Fig. S4). Together, these findings indicate that PFOS and PFOA contamination in HPE within ± 2 km from a fluorochemical point site (0–2 km) is largely influenced by this primary source, whereas exposure in laying hens at more remote locations is more diffuse and complex.

In agreement with other European studies on HPE, PFOS was the dominant compound and contributed for at least 75% to the total PFAS profile in the eggs, followed by long-chain PFCAs (C ≥ 8). Furthermore, this finding was in accordance with previous monitoring studies of HPE in Europe (the Netherlands and Greece: Zafeiraki et al. (2016) and Italy: Gazzotti et al. (2021)). Moreover, PFOS is an extremely persistent compound and can be firmly retained in the subsurface soil layer for years, due to its very strong adsorption capacity with soil particles (Groffen et al., 2019d; Liu et al., 2020). The total organic carbon (TOC) content in the soil plays a central role in the adsorption capacity of PFAS to soil particles (Lu et al., 2018). Soil in chicken enclosures usually contains enriched amounts of TOC, due to the build-up of feed waste and manure (Ravindran et al., 2017). Consequently, it is hypothesized that subsurface soil in chicken enclosures from private gardens may be an important sink of PFAS, especially for those PFAS that have large soil adsorption capacity, such as PFOS and long-chain PFCAs (Lu et al., 2018). Hence, free-ranging laying hens may be directly exposed to these PFAS via digestion of contaminated soil particles and indirectly through intake of invertebrates, such as earthworms, which live in close contact with the soil. Furthermore, these long-chain PFAS show strong binding affinity towards egg (lipo)proteins, which may also explain the relatively large accumulation in eggs (Fedorenko et al., 2021).

Table 3 shows that, in contrast to studies in Europe, monitoring studies on HPE in north (Su et al., 2017) and central (Wang et al., 2019) China reported that PFBA and PFOA were the largest contributors to the total PFAS profile, instead of PFOS. Furthermore, the egg concentrations of these two formerly mentioned compounds were several orders of magnitude higher in China compared to those in Europe, both nearby and remotely from a PFAS point source. This discrepancy between both regions is most likely due to different historical and ongoing PFAS emission quantities and product output. In Europe, PFOS and PFOA have been gradually phased out from 2002 by its main manufacturers (Lau et al., 2007). Since then, China has become one of the largest global producers of PFOA (Land et al., 2018; Liu et al., 2021). In parallel with the phase out of long-chain PFAS, such as PFOA and PFOS, the short-chain PFBA has become one of the major substitute compounds in fluorochemical industry, resulting in frequent detection and increased concentrations in the environment and biota over recent years (Liu et al., 2021). This is also reflected in the present study, as the detection frequency and concentrations of PFBA in HPE tend to increase at locations closer to the plant site.

Table 2
Overview of the total PFAS intake values (min., median, mean and max. ng/kg bodyweight (bw) per week) for the sum of four PFAS (PFHxS, PFOS, PFOA and PFNA) in different age intervals per distance buffer zone.

BUFFER A (0–2 km, *N* = 18)	Intake parameters (ng/kg bw per week)				Percentage locations above health guideline (%) [a]		
Age interval (years)	Min.	Median	Mean	Max.	EFSA threshold	RIVM threshold	
						PFOA	PFOS
3–5	2.3	75	208	726	89	0	56
6–9	1.7	56	154	538	89	0	55
10–13	1.1	36	100	348	89	0	44
14-17 Male	0.68	23	63	220	78	0	33
Female	0.77	26	71	247	78	0	33
18-64 Male	0.53	18	49	172	78	0	22
Female	0.64	22	59	207	78	0	33
BUFFER B (2–4 km, *N* = 30)	Intake parameters (ng/kg bw per week)				Percentage locations above health guideline (%)		
Age interval (years)	Min.	Median	Mean	Max.	EFSA threshold	RIVM threshold	
						PFOA	PFOS
3–5	6.8	29	34	90	100	0	22
6–9	5.0	21	25	66	100	0	11
10–13	3.3	14	16	43	80	0	0
14-17 Male	2.1	8.7	10	27	73	0	0
Female	2.3	9.7	11	31	80	0	0
18-64 Male	1.6	6.8	8.0	21	67	0	0
Female	1.9	8.2	9.7	26	73	0	0
BUFFER C (4–10 km, *N* = 22)	Intake parameters (ng/kg bw per week)				Percentage locations above health guideline (%)		
Age interval (years)	Min.	Median	Mean	Max.	EFSA threshold	RIVM threshold	
						PFOA	PFOS
3–5	7.0	24	25	52	100	0	9
6–9	5.2	18	18	38	100	0	0
10–13	3.4	12	12	25	91	0	0
14-17 Male	2.1	7.4	7.4	16	91	0	0
Female	2.4	8.3	8.4	18	91	0	0
18-64 Male	1.7	5.8	5.8	12	73	0	0
Female	2.0	7.0	7.0	15	73	0	0

[a] The percentage of sampling locations exceeding the EFSA health guideline (4.4 ng/kg bw per week) and the RIVM health guideline (PFOS: 43.8 ng/kg bw per week and PFOA: 87.5 ng/kg bw per week) are provided for each age interval. The consumption scenario was based on the intake of two home-produced eggs per week of free-ranging laying hens.

4.2. PFAS relationships with survey data

To the best of our knowledge, our study is the first to investigate whether housing conditions (feed type and flock size) and age of the laying hens affect PFAS concentrations in HPE. The survey results indicated that young laying hens contained on average higher egg PFOA concentrations compared to relatively old laying hens. This age difference has also been observed in other studies on both terrestrial birds (Park et al., 2021) and waterfowl (*Uria aalge*; Holmström and Berger, 2008), and can be explained by both maternal transfer and fewer elimination possibilities of young birds compared to older individuals (Holmström and Berger, 2008).

Eggs are an important elimination route for pollutants in birds and laying order effects of PFAS have been demonstrated in laying hens, with the first laid eggs containing higher PFAS concentrations (Kowalczyk et al., 2020; Wilson et al., 2020). On average, laying hens start their first egg laying cycle around the age of 18–24 weeks (Colin et al., 2020). Therefore, young laying hens (<1 year old) might depurate larger amounts of PFAS in their eggs than older individuals (>2 years old), as they have only had their first egg laying cycle and relatively high PFAS body burdens due to the maternal transfer. Furthermore, older

Table 3
Concentration range (in ng/g ww; min. – max.) of frequently detected PFAS in home-produced eggs from various locations, based on the present study and available literature data.

Reference	Location	Egg matrix	Year	Range from fluorochemical plant (km)	PFBA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFHxS	PFOS
Present study	Antwerp (Belgium)	Whole egg	2018	0–2 km	0.44–9.1	0.26–2.4	<LOQ[a] - 0.73	<LOQ - 0.78	0.49–0.91	0.48–0.65	3.3–3.5	<LOQ - 241
	Antwerp (Belgium)	Whole egg	2018	2–4 km	0.54–0.96	0.21–1.0	<LOQ - 0.68	0.22–1.6	0.33–1.4	0.21–1.6	ND[b]	0.54–44
	Antwerp (Belgium)	Whole egg	2018	4–10 km	0.40–1.5	0.13–1.0	<LOQ - 0.44	<LOQ - 0.99	0.54–0.90	0.23–1.3	3.6 *	0.78–13
D'Hollander et al. (2011)	Antwerp (Belgium)	Whole egg	2010	0–1 km	NA	0.12–5.86	NA	NA	NA	NA	NA	53–3473
Wang et al. (2010)	Wuhan (central China)	NA[c]	NA	0–2 km	NA	ND - 1.91	NA	NA	NA	NA	ND - 2.24	0.80–283
	Wuhan (central China)	NA	NA	>3 km	NA	ND - 0.53	NA	NA	NA	NA	ND - 3.18	2.7–18.1
Wang et al. (2019)	Wuhan (central China)	Yolk	NA	0.5–3.65 km	ND - 1698	ND – 69.7	ND – 6.2	ND – 4.0	ND – 4.3	ND – 7.7	ND – 85	ND - 1062
Su et al. (2017)	Shandong (north China)	Whole egg	2015	0–20 km	0.54–22.5	2.5–125	0.14–0.33	0.17–0.40	<0.04–0.13	<0.02–0.12	<0.02	0.32–0.86
Zafeiraki et al. (2016)	Netherlands	Yolk	2013–2014	NA	NA	<0.5–2.7	<0.5–2.0	<0.5–3.0	<0.5–2.3	ND	<0.5–5.2	<0.5–24.8
	Greece	Yolk	2013–2014	NA	NA	<0.5	<0.5–1.0	<0.5–8.0	<0.5–4.5	ND	<0.5	<0.5–8.9
Gazzotti et al. (2021)	Italy	Yok	2018–2019	NA	NA	ND - 0.62	0.25–1.2	NA	NA	NA	0.25–0.50	0.25–3.47

[a] LOQ = limit of quantification.
[b] ND = compound not detected.
[c] NA = data not available or specified.

individuals have experienced multiple moulting periods by which they can sequestrate more PFAS into feathers, which is an important sequestration tissue of pollutants, including PFAS, in birds (Jaspers et al., 2009; Groffen et al., 2020). The relationship between age and egg PFOS concentrations was less clear, which may indicate that the intake of PFOS throughout the lifespan of the laying hen remains higher than the elimination rate.

Notably, backyard chickens in private gardens can become old and often keep laying eggs until the age of 8 years, whereas commercial laying hens are usually restrained for egg laying until 1.5 years of age (Ali et al., 2020). Moreover, the egg production of the average laying hen starts decreasing around the age of 16 months (Joyner et al., 1987), while the absolute yolk weight continuously increases with age (Suk and Park, 2001). The yolk is the main target tissue within the egg compartments, as approximately 90% and 99% of the deposited PFOA and PFOS egg concentrations, respectively, are transferred to the yolk (Su et al., 2017). Consequently, one would expect that laying hens build up again higher PFAS body burdens and lower elimination capacities from around 16 months of age onwards, with larger quantities of PFAS that can be transferred to a fewer number of eggs. Unfortunately, the age of the laying hens in the category "old" was still relatively young (33 ± 12 (SD) months of age) and the sample size was too low ($N = 10$) to properly test this hypothesis in the present study.

Laying hens that were fed an obligate diet of kitchen leftovers tended to contain higher egg PFOS and PFOA concentrations. Crop uptake of PFAS from contaminated soil has been shown to be an important entrance pathway to the terrestrial food chain (Lechner and Knapp, 2011; Liu et al., 2019). Contrary to other organic pollutants, PFAS accumulate both in vegetative and root parts of plants, which are dominated by short-chain PFAS and long-chain PFAS, respectively (Ghisi et al., 2019). Both plant tissues are frequently provided as leftovers to laying hens of private owners. This was also supported by the fact that these compounds were frequently detected in the chicken eggs. Moreover, many volunteers simultaneously cultivated their own plant crops besides the housing of chickens, which can contain relatively high PFAS concentrations compared to commercial feed as they are grown in less controlled conditions (Liu et al., 2019; Önel et al., 2018). Additionally, numerous carboxylates that were detected in the eggs are also typically found in rain water, which may be a contributing PFAS source as drinking water to the laying hens (Lu et al., 2018). Nevertheless, soil has also been identified as a major exposure source of organic pollutants to laying hens (Sioen et al., 2008; Waegeneers et al., 2009), including PFAS (Death et al., 2021). Besides self-cultivated crops, other potential food sources can be a significant source of contamination to domestic chickens (e.g. fat leftovers of meat and cheese crusts), which should be considered in future studies.

4.3. Human health risk indications

Overall, consumption of HPE may contribute to a large extent to the intake of PFAS in humans. For all age groups, the TWI of 4.4 ng/kg bw per week (for the sum of PFHxS, PFOS, PFOA and PFNA) was exceeded ($\geq$67% of the locations) in every buffer zone up till 10 km from the plant site (Table 2) at a consumption rate of two eggs per week. Similarly, the MTR of PFOS (43.8 ng/kg bw per week) was frequently exceeded within 4 km from the plant site, in particular for young children up to 9 years old.

The present study indicates that PFAS exposure in the Flemish population, both nearby (<2 km) large fluorochemical industry and in a 10 km radius from this point source, should be of high concern. Both health criteria (TWI and MTR) were frequently exceeded both closely and more remotely from the fluorochemical plant, and often to a great extent in the case of the TWI. Besides HPEs, the potential intake of PFAS via other sources, including commercial food (eg. fish, meat and offal food), self-cultivated vegetables, atmospheric dust and water, can be important additional pathways of human PFAS exposure (Herzke et al., 2013; Liu et al., 2019; Pasecnaja et al., 2022; Xu et al., 2021b). Likely, the total PFAS intake via multiple exposure pathways will be higher than the estimations made in the present study. Therefore, health effects due to PFAS intake via HPE cannot be excluded, especially on the immune system, for which human epidemiological evidence exists to date (EFSA CONTAM Panel, 2020; Grandjean et al., 2020; Sunderland et al., 2019). Although the underlying mode of action is still largely unknown, epidemiological studies have found strong indications that the immune system, on which the TWI criterion is based, is a major toxic endpoint of PFAS in humans (EFSA CONTAM Panel, 2020; Grandjean et al., 2020; Sunderland et al., 2019). In light of the SARS CoV 2 pandemic, for which increased severity of COVID-19 disease outcome has been associated with elevated PFBA plasma concentrations (Grandjean et al., 2020), it remains extremely important to further biomonitor PFAS and assess human exposure risks.

4.4. Future research perspectives

Our study, which aimed at examining the PFAS distribution in HPE, has several limitations which give rise to new research directions/questions that need to be tackled. Firstly, PFAS have the potential for air dispersion (Galloway et al., 2020) and knowing that the prevailing wind in most areas in Flanders is either northwest (0–90°) or southwest (180–270°) (Toparlar et al., 2018), higher egg PFAS concentrations are expected in gardens oriented towards these particular directions. Therefore, additional locations in missing wind directions will be sampled in successive monitoring campaigns to elaborate on this hypothesis. Secondly, our results demonstrate for the first time that housing conditions and biological factors can play a significant role in the exposure of PFAS to free-ranging laying hens. Future studies should consider relevant factors that may affect the PFAS exposure in laying hens. For instance, soil characteristics, scratching area and density (number of hens/m^2), vegetation coverage and shape of the chicken enclosure can (in)directly influence the bioavailability and exposure of organic pollutants to laying hens (Sioen et al., 2008; Waegeneers et al., 2009). Ultimately, this may result in remedial measures for inhabitants to reduce exposure to PFAS via self-cultivated food consumption. Finally, extensive research considering multiple self-cultivated food items other than HPE (vegetables and fruit), as well as relevant exposure sources to laying hens (soil, rain water and key prey items, such as earthworms) should be considered in future PFAS monitoring campaigns.

5. Conclusion

The present study detected numerous PFAS in HPE, both nearby (<2 km) and up to 10 km from a major known point source. PFOS was the dominant compound and present in relatively high concentrations, compared to other European studies on PFAS in food. PFOS concentrations steeply declined with increasing distance from the fluorochemical plant in Antwerp. By comparing our results to previous studies in the same study area, maximum PFOS concentrations seem to have declined over the years, probably resulting from the phase-out. Nevertheless, the present findings indicate that human exposure to PFAS via consumption of HPE can be relatively high, even for compounds that have been phased-out decades ago in Europe. Potential health risks with respect to currently established health guidelines cannot be excluded, as the tolerable weekly intake threshold was often exceeded in every examined buffer zone.

Author contributions statement

Robin Lasters: Conceptualization, Investigation, Methodology & Data Curation, Formal analysis, Visualization, Writing – Original Draft **Thimo Groffen:** Conceptualization, Investigation, Validation, Writing – Review & Editing **Marcel Eens:** Supervision, Funding Acquisition,

Conceptualization, Validation, Writing – Review & Editing **Dries Coertjens:** Methodology – Communication Strategy, Writing – Review & Editing **Wouter A. Gebbink:** Conceptualization, Writing – Review & Editing **Jelle Hofman:** Writing – Review & Editing **Lieven Bervoets:** Supervision, Funding Acquisition, Conceptualization, Validation, 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.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.136283.

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