ACCUMULATION OF PERFLUORINATED CHEMICALS IN BELGIAN HOME-PRODUCED CHICKEN EGGS

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Introduction

Perfluorinated compounds (PFCs) comprise a large group of chemicals that have been produced since the 1950s. Due to their unique physico-chemical properties they have been used in a broad range of applications in industrial processes and consumer products. PFCs are ubiquitous environmental contaminants, which persist and may bioaccumulate through the food chain [1,2,3]. In recent years, an increasing number of studies worldwide show that humans are exposed to these chemicals and high levels of PFCs have been detected in human blood, serum and breast milk[4,5]. Human exposure assessments have indicated that non-occupational exposure to PFCs can occur through a variety of exposure pathways including inhalation of indoor air and dust. However, dietary intake appears to be the major exposure pathway[6,7,8]. Nevertheless, data on levels of PFCs in the human diet are still rather scarce[8].
Chicken eggs are an important part of the human diet but also here information on PFC-levels is mainly lacking. Home-produced chicken eggs may contain higher levels of environmental pollutants[9,10] compared to commercially produced eggs due to historical pollution of the environment. Ingestion of contaminated soil and consumption of feedstuff have been reported as the principal contamination sources with persistent pollutants, such as dioxins and polychlorinated biphenyls for the chicken and further to the eggs[10].
The aim of the present study was to gather information on the PFC contamination in home-produced chicken eggs and investigate the contamination sources water and soil. Finally, the data will be used to assess the daily intake of PFOS through the consumption of eggs in Flanders, Belgium.

Materials and methods

Sampling and target analaytes
A total number of 29 private chicken owners volunteered to participate in this study. Sample locations were spread throughout Flanders in the 5 different provinces, although more locations were sampled in Antwerp where a perfluorochemical plant is located. All the chickens were free-foraging animals. At each location 3 eggs were sampled, preferably on the same day to be sure that the eggs were from different hens. Soil from the chicken run and the drinking water of the chickens were also sampled. From each location the origin of the drinking water, tap or rain water was noted. Each egg was homogenized individually with a PFC-free blender. Soil and egg samples were kept frozen in polypropylene tubes until analysis. The water bottles were stored at 4°C and analysis was carried out within a month.
Target analytes were 4 perfluorosulfonates (perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorodecane sulfonate (PFDS)) and 11 perfluorocarboxylates (perfluorobutanoate (PFBA), perfluoropentanoate (PFPeA), perfluorohexanoate (PFHxA), perfluorooheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorononaoate (PFNA), perfluorodecanoate (PFDA), perfluorodecanoate (PFDA), Perfluorododecanoate (PFDoA), perfluorotridecanoate (PFTrA) and perfluorotetradeaconoate (PFTeA)).

Sample preparation and clean up
The extraction method for the eggs and soil samples was based on a method described by Powley et al. [11] with some modifications. Internal standards (^{13}C-PFOS, ^{13}C-PFHxS, ^{13}C-PFBA, ^{13}C-PFHxA, ^{13}C-PFOA, ^{13}C-PFNA, and ^{13}C-PFDA) were added to 1g sample in a polypropylene tube and mixed thoroughly. After adding 10 mL

acetonitrile to the samples, the solution was sonicated and centrifuged. The supernatant was concentrated to 1 ml under a gentle nitrogen stream, followed by a clean-up with 50 mg Envi Carb® and 50 µl acetic acid. Egg and soil samples were extracted in duplicate.
Water samples (tap water 1L, rain water 0.25L) were extracted using a procedure based on Taniyasu et al.[12]and Kärrman et al.[5]. Rain water samples were filtered through a Whatman filter paper before adding the internal standards (^{13}C-PFOS, ^{13}C-PFHxS, ^{13}C-PFBA, ^{13}C-PFHxA, ^{13}C-PFOA, ^{13}C-PFNA, and ^{13}C-PFDA). The samples were extracted on Oasis WAX cartridges and analytes were eluted with ammonium hydroxide in acetonitrile. Analysis was performed using an ACQUITY UPLC coupled to a tandem quadrupole mass spectrometer (ACQUITY, TQD, Waters, USA) with an electrospray interface operating in negative ion mode (ES-MS/MS). Separation was performed on an ACQUITY BEH C18 column (2.1 X 50 mm; 1.7µm, Waters, USA). A pre-column was inserted between the solvent mixer and the injector to remove any PFC originating from the UPLC system. The injection volume was 10 µL and the flow rate was set to 450 µL/min. A gradient program delivering mobile phases consisted of acetonitrile and water, both with 0.1 % formic acid. The common interference of taurodeoxycholate with the PFOS transition 499-> 80 was excluded. Results are corrected for matrix effects and recovery was based on the response (area) of the internal standards.

Results and discussion:

PFC Levels
The highest detection frequency was observed for PFOS. The mean concentrations of PFOS in the eggs collected at each site ranged between 0.4 ng/g and 3473 ng/g ww with a median concentration of 6.8 ng/g. The extremely high concentrations, only observed in the vicinity (< 1km) of the perfluorochemical plant in Zwijndrecht (Antwerp), ranged from 53 ng/g to 3885 ng/g. A high variation among the 3 egg samples from most locations was observed (see table 1). As the volunteers were asked to sample 3 eggs at the same day, it is certain that the eggs originate from different hens. The observed difference could be possibly explained by an age difference of the hens. PFOS levels in soil samples varied between 0.1 ng/g and 33 ng/g (median 1 ng/g), in drinking water between <LOD and 120 ng/L (median 0.7 ng/L). In both soil and water samples the highest concentrations were also measured in Zwijndrecht. If these are excluded from the range, the highest levels for soil and water were 4 ng/g and 21 ng/L respectively. All highest levels were measured in samples from the province of Antwerp. A study carried out in China, investigated the distribution of PFOS in the environment around a manufacturing facility of PFCs through the analyses of soil and eggs of chicken eggs[13]. Current results in eggs are similar to the results of the Chinese study (eggs 0-283 ng/g) except for the location where extremely high levels where measured. The mean concentration in soil of the Chinese study (22.6 ng/g) was comparable with the maximum levels found in the present study. For water samples a difference between rain- and tap water was observed with the higher levels in rainwater. In tap water the PFOS concentrations ranged between <LOD and 8.7 ng/L (median 0.5 ng/L) whereas for rainwater levels between <LOD and 120 ng/L (median 2.5 ng/L) were observed. Rainwater sample collection sited included 3 locations nearby the perfluorochemical production unit, so this can give a biased impression on levels in rainwater in general. An overview of the levels detected in eggs, soil and water is shown in table 1.

Correlations
A non-parametric test (Spearman Rank) was used to check possible correlations between the mean PFOS levels in eggs, water and soil at each location. A significant positive correlation was found for PFOS concentrations in eggs and soil (Spearman's rank correlation; $r = 0.66$, $\alpha = 0.05$; fig. 1). For all the water samples, no correlation was observed but if the analysis were carried out for each type of water individually, positive correlations were found. The positive correlation between PFOS in eggs and rainwater ($r = 0.79$) was stronger compared to the correlation with tap water ($r = 0.59$). Logically, a positive correlation between soil and rainwater was observed ($r = 0.73$) while this correlation was lacking for soil and tap water.
These results suggest that both soil and drinking water are important sources of PFCs for home-produced eggs laid by free-foraging chickens. Rainwater as drinking water for chickens will result in a higher exposure to PFOS compared to tap water.

Table 1. Overview of the PFOS concentrations in eggs (mean, SD, n=3), soil and water samples at each location.

Sample location	eggs (ng/g)		soil (ng/g)	origin of	water (ng/L)
	mean	SD		water	
Gavere	52.8	30.9	1.5	Rain	0.1
Mechelen	5.3	3.5	2.4	Rain	1.7
Grimbergen	7.0	4.7	0.1	Tap	0.0
Zwijndrecht	386.1	307.9	33.7	Rain	61.4
Kessel	2.5	1.0	0.3	Tap	0.9
Westmalle	0.4	0.5	2.3	Tap	0.5
Nijlen	4.5	5.3	1.1	Rain	2.5
Hoevene	13.0	9.0	1.6	Tap	0.3
Arendonk	9.2	4.6	0.8	Tap	0.6
Olmen	3.1	1.8	0.6	Tap	0.6
Olmen	3.3	1.7	0.6	Rain	1.2
Lille	3.4	1.8	0.2	Tap	0.3
Zwijndrecht	109.9	59.2	21.3	Rain	66.6
Edegem	7.8	5.9	1.9	Rain	2.3
Liedekerke	3.0	2.9	0.2	Tap	2.1
Meerhout	0.4	0.7	0.3	Rain	0.0
Oelegem	4.7	3.1	0.7	Tap	0.1
Grembergen	8.4	3.0	2.3	Tap	0.7
Schoten	8.9	3.7	1.5	Tap	0.6
Grimbergen	2.6	1.9	0.7	n.a.	n.a.
Kappellen	18.1	6.8	3.8	Tap	0.3
Borgerhout	6.9	4.2	2.0	Rain	21.2
Zwijndrecht	3472.8	575.9	24.0	Rain	119.7
Voeren	6.3	1.5	0.3	Rain	1.2
Brecht	12.3	4.3	2.6	Tap	0.6
Sinaai	15.1	0.6	0.7	Tap	0.1
St. Job	22.3	3.6	4.3	Tap	0.2
Kortrijk	10.4	6.0	1.2	Rain	5.3
Neeroeteren	5.6	1.8	0.8	Tap	8.7

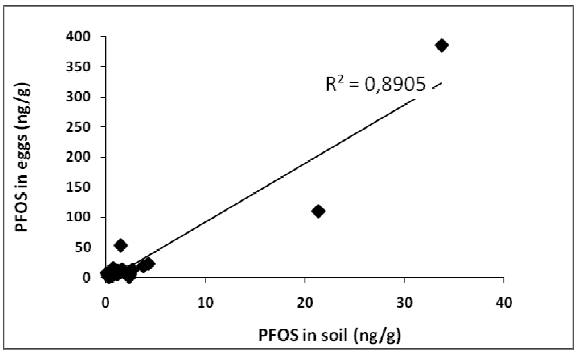

Figure 1. Correlation between PFOS concentrations in eggs and soil of each sample location with the exception of one sampling point in Zwijndrecht (eggs: 3473 ng/g, soil 24 ng/g).

Dietary intake of PFCs via consumption of eggs

The daily intake of PFCs via consumption of home-produced eggs was calculated from the daily intake of eggs and from the concentrations of PFOS expressed in ng/g ww. The average egg consumption by the Belgian population older than 15 years is 10.0 g/day[14]. However, the average egg consumption of Belgian people who own chicken can be twice as high i.e. 20.3 g/day as calculated in the study of Overmeire et al. [10]. Combining these intake data with current PFOS concentration in eggs this results in a median intake of 70 ng/day for the lower egg consumption. For the highest average PFOS concentration this results in an intake of nearly 35µg/day. Average intake equals 1.5 µg/day but this is most probably an overestimation for the Flemish population as 3 sample locations out of the 29 were nearby the perfluoorchemical production unit. If an average consumption of 20.3 g egg/day is used, this results in a median intake of 142 ng/day. The provisional tolerable daily intake (TDI) value proposed by the European Food Safety Authority [15] for PFOS is 1500 ng/kg bodyweight/day. If an average bodyweight of 70 kg is assumed for adults, this results in a TDI for PFOS of 10 500 ng/d. The calculated median daily intake through eggs, accounts for only 0.7 % of the TDI for the low egg consumption. Nonetheless for the 3 volunteers who live nearby the perfluorochemical plant, the daily intake through eggs accounts for 10.4, 36 and 330%. The lowest value for these individuals is derived from chicken eggs of hens that were held in a greenhouse during winter. So for some time of the year the soil where they foraged was not exposed to the open air. For one location the average intake of PFOS through consumption of home-produced eggs exceeds more than 3 times the provisional TDI.

Overall, the contamination of eggs with PFCs appears to be non harmful for the average Belgian population but there is a reason of public health concern for people who live nearby the perfluorochemical plant and consume their home produced chicken eggs.

Acknowledgements:

The authors gratefully acknowledge the volunteers who participate in this study. The study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RF6181).

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