

# Chickens' eggs and the livers of farm animals as sources of perfluoroalkyl substances

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Received: March 21, 2024 Accepted: June 12, 2024

## Abstract

**Introduction:** This study focuses on perfluoroalkyl substance (PFAS) content in chickens' eggs and the livers of farm animals. **Material and Methods:** Chickens' eggs (n = 25) and the livers of cows (n = 10), chickens (n = 7) and horses (n = 3) were collected from various regions of Poland. Samples were analysed using the isotope dilution technique with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). **Results:** The mean lower bound (LB) sum of four PFAS ( $\sum 4$  PFAS) concentrations (perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexanesulfonic acid (PFHXS)) were the highest in cows' livers (0.52 µg/kg) and much lower in chickens' (0.17 µg/kg) and horses' livers (0.13 µg/kg) and chickens' eggs (0.096 µg/kg). The ratio of  $\sum 4$  PFASs to the limits set by Commission Regulation (EU) 2023/915 was <7% for liver and <6% for eggs. Linear PFOS was the compound with the highest detection frequency (8% in eggs and 48% in all livers). In cows' livers it was detected in 80% of samples. The estimated exposure to LB  $\sum 4$  PFASs *via* consumption of liver tissue from farm animals (assuming 50 g and 100 g portions) was <52% of the tolerable weekly intake (TWI) for children and <17% of the TWI for adults. Dietary intake *via* the average portion of three eggs led to low exposure of <15% for children and <5% for adults. **Conclusion:** Neither eggs nor the livers of chickens or horses as analysed in this study are significant sources of PFASs, while cows' livers might contribute significantly to a child's overall dietary intake. Further investigation of PFOS in farm animal livers should be conducted.

Keywords: PFASs, eggs, bovine liver, chicken liver, horse liver, risk assessment.

### Introduction

Perfluoroalkyl substances (PFASs) are a group of man-made chemicals manufactured since 1950. Their chemical and thermal oxidation stability, resistance to degradation and unique water- and oil-repelling properties make them perfect surfactants in broad application as additives in food contact materials, clothes and firefighting foams (17, 28, 46). As very persistent and bioaccumulative chemicals they were listed in the Stockholm Convention on Persistent Organic Pollutants (POPs). Their negative impact on living organisms has been proved with their hepatotoxicity, immunotoxicity, neurotoxicity and causing of reproductive and developmental disorders (6, 20). Food and water as well as dust ingestion are the main sources of PFASs for humans (8, 9, 15, 16). Therefore maximum levels for PFASs in certain foodstuffs were established in 2022

(2022/2388/EU) and amended in 2023 (2023/915/EU) (12, 13). Based on the opinion of the European Food Safety Authority (EFSA), four compounds (perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexanesulfonic acid (PFHxS) are responsible for half of the PFAS exposure of the European population (15) despite the list of these substances being long. Therefore in 2020, a tolerable weekly intake (TWI) for the sum of these four PFASs ( $\Sigma$ 4 PFASs) was established at the level of 4.4 ng/kg body weight (b.w.) (15).

Farm animals might be exposed to PFASs through contact with contaminated environments or *via* contaminated plants or feed materials (3, 23, 27, 33, 39, 44). Worldwide studies investigate PFASs in foods of animal origin (5, 7, 45). There are few papers regarding PFASs in eggs and animal liver in Poland (31, 41), while much associated research has been carried out around the world (18, 22, 32, 34, 36, 42, 50). Previous preliminary research on PFASs in eggs from different types of husbandry in Poland revealed that regardless of the husbandry system, Polish eggs do not contribute significantly to consumers' PFAS intake (31). Polish data on liver contamination, especially that of chicken liver, indicate that its consumption may pose a health risk to humans (41).

The aim of the present study was expanding the knowledge on PFASs in the livers of farm animals and confirming the preliminary finding regarding the low contribution of egg consumption to PFAS intake. The detected concentrations were used to assess the exposure of Polish consumers in relation to the TWI.

#### **Material and Methods**

Sampling and sample collection. Sampling of chickens' eggs (n = 25), cows' livers (n = 10), chickens' livers (n = 7) and horses' livers (n = 3) was carried out according to the Commission Implementing Regulation 2022/1428 of 24 August 2022 laying down methods of sampling and analysis for the control of perfluoroalkyl substances in certain foodstuffs (10). Eggs were collected from both free-range and cage production. Samples were collected by the Veterinary Inspectorate in various regions of Poland. The liver samples were taken from the Podlaskie, Świętokrzyskie, Mazowieckie, Wielkopolskie and Pomorskie voivodeships. Eggs were sampled from free-range production (n = 7) in the Lubuskie, Opolskie, Wielkopolskie and Lubelskie voivodeships and from cage production (n = 18) in the Małopolskie, Podkarpackie, Wielkopolskie, Dolnośląskie, Śląskie and Kujawsko-Pomorskie voivodeships.

Analytes of interest. The compounds which were investigated and their limits of quantification (LOQ) and measurement uncertainty (MU) are listed in Table 1. Commission Recommendation (EU) 2022/1431 sets requirements regarding the LOQ which are 0.10  $\mu$ g/kg for PFS, PFOA, PFNA and PFHxS in the meat of terrestrial animals and 0.30  $\mu$ g/kg for these substances in eggs (11). The LOQ requirements were met for all compounds for both matrices.

The isotope dilution technique which was applied called for the use of the following isotopically labelled analogues: sodium perfluoro-1-(2,3,4- $^{13}C_{12}$ )butanesulphonate, sodium perfluoro-1-hexane( $^{18}O_2$ )sulphonate, sodium perfluoro-1-(1,2,3,4, $^{-13}C_{12}$ )octanesulphonate, perfluoro-n-(1,2,3,4, $^{-13}C_{12}$ )hexanoic acid, perfluoro-n-(1,2,3,4, $^{-13}C_{12}$ )heptanoic acid, perfluoro-n-(1,2,3,4, $^{-13}C_{12}$ )octanoic acid, perfluoro-n-(1,2,3,4, $^{-13}C_{12}$ )decanoic acid, perfluoro-n-(1,2,3,4,5,6,7, $^{-13}C_{12}$ )decanoic acid and perfluoro-n-(1,2,3,4,5,6,7, $^{-13}C_{12}$ )dodecanoic acid. Perfluoro-n-(1,2,3,4,5,6,7, $^{-13}C_{12}$ )dodecanoic acid. Perfluoro-n-(1,2,3,4,5,6,7, $^{-13}C_{12}$ )dodecanoic acid. Perfluoro-n-(1,2,3,4,5,6,7, $^{-13}C_{12}$ )octanesulphonate was used as a recovery standard. All standards were supplied by Wellington Laboratories Inc. (Guelph, ON, Canada).

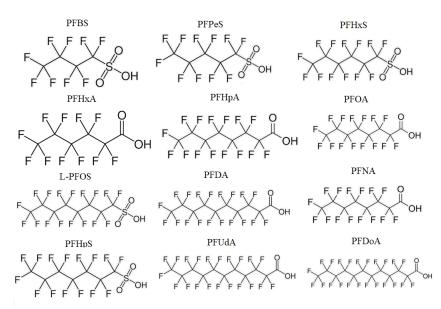
Sample preparation, extraction, purification and detection. Samples were freeze dried and a 2 g mass of lyophilised sample was spiked with an isotopically labelled standard. Extraction was carried out using 10 mL of 0.01M methanol (LGC Standards, Wesel, Germany) and potassium hydroxide (Honeywell Fluka, Seelze, Germany). After being shaken for 1 min, samples were left for 20 h. Next, two steps of purification were performed. In the first cleanup step, Oasis WAX solid-phase extraction cartridges (6 mL, 150 mg) were used (Waters Corp., Milford, MA, USA), and in the second step ENVI-Carb Solid Phase sorbent (6 mL, 500 mg) (Supelco, Bellefonte, PA, USA) was applied. Before detection, recovery standard was added. Liquid chromatography-tandem mass spectrometry was selected for detection (Triple Quad 7500 system; Sciex, Framingham, MA, USA). A Gemini C18 column (3 µm,  $50 \times 2.0$  mm) equipped with a guard column (Phenomenex, Torrance, CA, USA) was used for chromatographic separation. Methanol and 20 mM of ammonium acetate aqueous solution (Merck, Darmstadt, Germany) were used as a mobile phase.

Quality assurance and quality control. A blank sample and the certified reference material IRMM-427 Pike-perch, supplied from the European Commission Joint Research Centre Institute for Reference Materials and Measurements (JRC, Geel, Belgium), were analysed with each batch of samples. All reagents and chemicals used were verified to be PFAS free before routine analysis. The method was accredited in accordance with the ISO 17025:2018-02 standard. Performance of the method meeting the necessary standard was confirmed by successful participation in proficiency testing organised by the European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food (EURL, Freiburg, Germany).

**Statistical analysis.** The normality of the distribution of the data was checked by the Shapiro–Wilk test. Differences between individual experimental groups were checked using the Mann–Whitney and Kruskal–Wallis tests.

**Result presentation.** Concentrations are expressed as  $\mu g/kg$  wet weight. Sum concentrations are given as lower-bound (LB) concentration (concentrations below the LOQ were replaced with the value of 0).

**Dietary intake.** There is no data on average liver consumption in Poland, which is why one portion of 50 g and one of 100 g consumed per week were used for PFAS intake calculation. In terms of eggs, the average Polish weekly consumption of three eggs (38) and double this average were applied. Each egg was taken to weigh 50 g. The consumer's body weight was assumed to be 23.1 kg for a 3–10-year-old child and 70 kg for an adult, according to EFSA guidelines (14). To characterise the potential health risk associated with  $\Sigma$ 4 PFAS intake, doses ingested with milk and liver were compared to the TWI (4.4 ng/kg b.w. per week).



**Fig 1.** Chemical structure of the compounds analysed in chickens' eggs and farm animal livers. PFBS – perfluorobutanesulfonic acid; PFPeS – perfluoropentanesulfonic acid; PFHxS – perfluorohexanesulfonic acid; PFHxA – perfluorohexanoic acid; PFHpA – perfluoroheptanoic acid; PFOA – perfluorooctanoic acid; L-PFOS – linear perfluorooctanesulfonic acid; PFDA – perfluorodecanoic acid; PFNA – perfluorononanoic acid; PFHpS – perfluoroheptanesulfonic acid; PFUdA – perfluoroundecanoic acid; PFDoA – perfluorododecanoic acid

Table 1. Analytes of interest in chickens' eggs and farm animals' livers and the limit of quantification (LOQ) and measurement uncertainty (MU) validation parameters

Compound	Acronym	LOQ in meat (µg/kg w.w.)	LOQ in eggs (µg/kg w.w.)	MU in meat (%)	MU in eggs (%)
perfluorobutanesulfonic acid	PFBS	0.001	0.003	12	18
perfluorohexanoic acid	PFHxA	0.008	0.033	14	14
perfluoroheptanoic acid	PFHpA	0.013	0.048	18	11
perfluorohexanesulfonic acid	PFHxS	0.005	0.015	13	21
perfluorooctanoic acid	PFOA	0.016	0.032	16	26
perfluorononanoic acid	PFNA	0.010	0.020	10	19
linear perfluorooctanesulfonic acid	L-PFOS	0.009	0.017	27	25
branched perfluorooctanesulfonic acid	Br-PFOS	0.009	0.017	27	25
perfluorodecanoic acid	PFDA	0.014	0.027	15	11
perfluoropentanesulfonic acid	PFPeS	0.005	0.009	13	16
perfluoroheptanesulfonic acid	PFHpS	0.003	0.016	31	32
perfluoroundecanoic acid	PFUdA	0.013	0.024	24	32
perfluorododecanoic acid	PFDoA	0.011	0.028	31	25

## Results

Occurrence of the four PFASs with defined TWIs. The mean LB  $\geq$ 4 PFAS concentration was the highest in cows' livers (0.52 µg/kg), the next highest in chicken' livers (0.17 µg/kg), lower in horses' livers (0.13 µg/kg) and the lowest in chickens' eggs (0.096 µg/kg). None of the samples exceeded the maximum levels for  $\geq$ 4 PFASs set by Commission Regulation (EU) 2023/915 (Table 2). The concentration-to-limit ratio for  $\geq$ 4 PFASs was <7% for liver and <6% for eggs. A 64% proportion of all analysed compounds was  $\geq$ 4 PFASs in cows' and chickens' livers, a 68% proportion of all compounds was  $\geq$ 4 PFASs in horses' livers and 100% was  $\geq$ 4 PFASs in eggs. Livers were significantly more contaminated than eggs (P-value < 0.03). None of the compounds were detected in eggs from cage production.

The compound detected most frequently was linear PFOS (L-PFOS) (8% in eggs and 48% in all livers). In cows' livers it was detected in 80% of samples. Besides having the highest detection frequency, this compound also reached the highest concentration in cows' and chickens' livers and in eggs (Table 2). The highest level of L-PFOS was in cows' livers (0.24 µg/kg), where it accounted for 46% of the  $\sum$ 4 PFASs; together with branched PFOS (br-PFOS) the share of  $\Sigma$ 4 PFASs increased to 63%. Branched PFOS was much more frequently detected in cows' (90%) and horses' (80%) livers than in chickens' livers (14%), while no samples of eggs contained this contaminant. The ratio of br-PFOS to  $\Sigma$ PFOSs was the highest in cows' livers (mean 28%) and markedly lower in horses' and chickens' livers (15–18%). The total PFOS concentration-to-limit ratio was <8% for eggs, 6% for cows' livers and <2% for chickens' and horses' livers.

Perfluorononanoic acid was the compound reaching the second highest levels (0.21  $\mu$ g/kg in cows' livers). Perfluorooctanoic acid was measured at the highest concentration in chickens' livers but was found only in one sample, where compared to its concentration in other analysed liver samples and eggs it was at a high concentration of 0.17  $\mu$ g/kg; however, in relation to the maximum level this contaminant's concentration was <25% (Table 2). For PFNA the mean concentration-to-limit ratio was <53% for cows' livers, and was lower for chickens' livers at 25%, for horses' livers at <7% and for eggs at <5%. One sample of cow's liver exceeded the maximum level for PFNA (0.40 µg/kg) set by Commission Regulation (EU) 2023/915 (Table 2). Perfluorohexanesulphonic acid was only detected in one chicken liver sample (0.047 µg/kg) and had a concentration of around 10% of the maximum level.

 Table 2. Levels of total perfluorooctanesulphonic acid ( $\Sigma$ PFOS), branched (Br-) PFOS, linear (L-) PFOS, perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulphonic acid (PFHxS) determined in chickens' eggs and cows', chickens' and horses' livers

Compound			Concentration in liver (µg	Concentration in chickens' ML in Commission			
		Cows'	Chickens'	Horses'	eggs (µg/kg)	Regulation 2023/915	
	mean	0.33	0.090	0.11	0.081		
∑PFOS	median	0.22	0.090	0.13	0.081	liver 6.0 eggs 1.0	
ran	range	<loq-0.90< td=""><td><loq-0.15< td=""><td><loq-0.15< td=""><td><loq-0095< td=""><td>Cgg5 1.0</td></loq-0095<></td></loq-0.15<></td></loq-0.15<></td></loq-0.90<>	<loq-0.15< td=""><td><loq-0.15< td=""><td><loq-0095< td=""><td>Cgg5 1.0</td></loq-0095<></td></loq-0.15<></td></loq-0.15<>	<loq-0.15< td=""><td><loq-0095< td=""><td>Cgg5 1.0</td></loq-0095<></td></loq-0.15<>	<loq-0095< td=""><td>Cgg5 1.0</td></loq-0095<>	Cgg5 1.0	
	mean	0.11	0.029	0.021	<loq< td=""><td></td></loq<>		
Br-PFOS	median	0.046	0.029	0.023	-	-	
	range	<loq-0.32< td=""><td><loq-0.29< td=""><td><loq-0.29< td=""><td>-</td><td></td></loq-0.29<></td></loq-0.29<></td></loq-0.32<>	<loq-0.29< td=""><td><loq-0.29< td=""><td>-</td><td></td></loq-0.29<></td></loq-0.29<>	<loq-0.29< td=""><td>-</td><td></td></loq-0.29<>	-		
	mean	0.24	0.073	0.098	0.081		
L-PFOS	median	0.17	0.076	0.10	0.081	-	
	range	<loq-0.73< td=""><td><loq-0.13< td=""><td><loq-0.13< td=""><td><loq-0095< td=""><td></td></loq-0095<></td></loq-0.13<></td></loq-0.13<></td></loq-0.73<>	<loq-0.13< td=""><td><loq-0.13< td=""><td><loq-0095< td=""><td></td></loq-0095<></td></loq-0.13<></td></loq-0.13<>	<loq-0.13< td=""><td><loq-0095< td=""><td></td></loq-0095<></td></loq-0.13<>	<loq-0095< td=""><td></td></loq-0095<>		
	mean	0.032	0.17	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>		
PFOA	median	0.032	0.17	-	-	liver 0.70 eggs 0.30	
	range	<loq-0.032< td=""><td><loq-0.17< td=""><td>-</td><td>-</td><td>eggs 0.50</td></loq-0.17<></td></loq-0.032<>	<loq-0.17< td=""><td>-</td><td>-</td><td>eggs 0.50</td></loq-0.17<>	-	-	eggs 0.50	
	mean	0.21	0.10	0.028	0.031	1. 0.40	
PFNA	median	0.086	0.10	0.028	0.031	liver 0.40 eggs 0.70	
	range	<loq-0.40< td=""><td><loq-0.10< td=""><td><loq-0.033< td=""><td><loq-0.031< td=""><td>eggs 0.70</td></loq-0.031<></td></loq-0.033<></td></loq-0.10<></td></loq-0.40<>	<loq-0.10< td=""><td><loq-0.033< td=""><td><loq-0.031< td=""><td>eggs 0.70</td></loq-0.031<></td></loq-0.033<></td></loq-0.10<>	<loq-0.033< td=""><td><loq-0.031< td=""><td>eggs 0.70</td></loq-0.031<></td></loq-0.033<>	<loq-0.031< td=""><td>eggs 0.70</td></loq-0.031<>	eggs 0.70	
	mean	<loq< td=""><td>0.047</td><td><loq< td=""><td><loq< td=""><td></td></loq<></td></loq<></td></loq<>	0.047	<loq< td=""><td><loq< td=""><td></td></loq<></td></loq<>	<loq< td=""><td></td></loq<>		
PFHxS	median	-	0.0047	-	-	liver 0.50 eggs 0.30	
	range	-	<loq-0.047< td=""><td>-</td><td>-</td><td>eggs 0.50</td></loq-0.047<>	-	-	eggs 0.50	
	mean	0.52	0.17	0.13	0.096		
LB∑4 PFAS	median	0.26	0.17	0.14	0.096	liver 8.0 eggs 1.70	
_	range	0.11-1.8	0.000-0.26	0.04-0.18	0.094-0.097	eggs 1.70	

ML - maximum level

Table 3. Levels of the remaining PFASs determined in chickens' eggs and cows', chickens' and horses' livers ( $\mu$ g/kg)

		Concentrati	Concentration		
Compound		Cows	Chickens	Horses	(µg/kg) in chickens' eggs
PFHxA n	mean	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	median	-	-	-	-
	range	-	-	-	-
PFHpA me	mean	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	median	-	-	-	-
	range	-	-	-	-
PFDA median range	mean	0.18	0.12	0.032	<loq< td=""></loq<>
	median	0.084	0.12	0.028	-
	range	<loq-0.62< td=""><td><loq-0.12< td=""><td><loq-0.045< td=""><td>-</td></loq-0.045<></td></loq-0.12<></td></loq-0.62<>	<loq-0.12< td=""><td><loq-0.045< td=""><td>-</td></loq-0.045<></td></loq-0.12<>	<loq-0.045< td=""><td>-</td></loq-0.045<>	-
PFUnDA median range	mean	0.083	0.031	0.032	0.030
	median	0.032	0.031	0.029	0.030
	range	LOQ-0.35	<loq-0.040< td=""><td><loq-0.041< td=""><td><loq-0.030< td=""></loq-0.030<></td></loq-0.041<></td></loq-0.040<>	<loq-0.041< td=""><td><loq-0.030< td=""></loq-0.030<></td></loq-0.041<>	<loq-0.030< td=""></loq-0.030<>
r	mean	0.031	0.064	0.017	<loq< td=""></loq<>
PFDoA	median	0.022	0.064	0.017	-
range	range	<loq-0.066< td=""><td><loq-0.064< td=""><td><loq-0.020< td=""><td>-</td></loq-0.020<></td></loq-0.064<></td></loq-0.066<>	<loq-0.064< td=""><td><loq-0.020< td=""><td>-</td></loq-0.020<></td></loq-0.064<>	<loq-0.020< td=""><td>-</td></loq-0.020<>	-
PFBS media range	mean	0.0037	0.0014	0.0019	<loq< td=""></loq<>
	median	0.0037	0.0014	0.0019	-
	range	<loq-0.0011< td=""><td><loq-0.0011< td=""><td><loq-0.0019< td=""><td>-</td></loq-0.0019<></td></loq-0.0011<></td></loq-0.0011<>	<loq-0.0011< td=""><td><loq-0.0019< td=""><td>-</td></loq-0.0019<></td></loq-0.0011<>	<loq-0.0019< td=""><td>-</td></loq-0.0019<>	-
PFPeS median range	mean	<loq< td=""><td><loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	median	-	-	-	-
	range	-	-	-	-
PFHpS	mean	0.0057	0.0076	0.0034	<loq< td=""></loq<>
	median	0.0054	0.0076	0.0034	-
	range	<loq-0.0073< td=""><td><loq-0.010< td=""><td><loq-0.0036< td=""><td>-</td></loq-0.0036<></td></loq-0.010<></td></loq-0.0073<>	<loq-0.010< td=""><td><loq-0.0036< td=""><td>-</td></loq-0.0036<></td></loq-0.010<>	<loq-0.0036< td=""><td>-</td></loq-0.0036<>	-

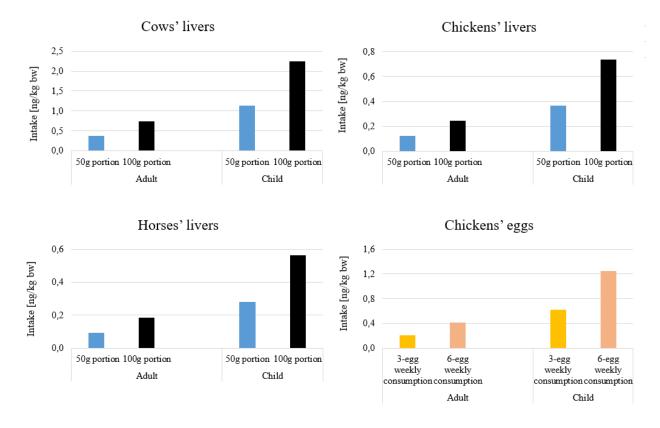


Fig 2. Estimated intakes (based on mean lower-bound concentrations) of the four PFASs (PFOS, PFOA, PFNA and PFHxS) with defined tolerable weekly intakes. Consumed amounts are 3 and 6 eggs and 50 g and 100 g of the livers of farm animals weekly

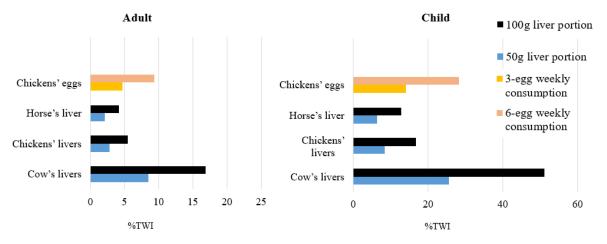


Fig. 3. Relationship to the tolerable weekly intake (TWI) of dietary intake of 3 and 6 eggs and portions of 50 g and 100 g of liver based on lowerbound concentrations

Occurrence of the remaining PFASs. None of the analysed samples contained quantifiable concentrations of PFHxA, PFHpA or PFPeS. Chickens' eggs contained only one of the remaining analysed PFASs (PFUnDA), which was found only in one sample of free range eggs in a concentration slightly over the LOQ ( $0.030 \mu g/kg$ ). Perfluorodecanoic acid and PFUnDA were found in all cow's liver samples, PFDoA in over 40% of them and PFHpS in 33%, but PFBS was seen in only one sample. The following compounds were detected in the livers of horses: PFDA, PFuDA, PFDoA, PFBS and PFHpS.

Intake of PFASs via egg and liver consumption. The estimated intakes of  $\sum 4$  PFASs were the highest when cows' livers were eaten and were 0.37 ng/kg b.w. for a 50 g portion and 0.74 ng/kg b.w. for a 100 g portion for adults and 1.13 ng/kg b.w. and 2.25 ng/kg b.w. for the same portions for children (Fig. 2). This corresponds to exposure of up to 17% of the TWI for adults and over 50% of it for children. A much lower intake was calculated for consumption of chickens' and horses' livers by adults (0.09–0.24 ng/kg b.w. from the smaller portion of the less contaminated of the two to the larger portion of the more contaminated) and children (0.28–0.74 ng/kg b.w. analogously) (Fig 2.). Referring these values to the TWI, exposure would be <6% for adults and <17% for children (Fig. 3). Dietary intake for

children *via* the average portion of three eggs would be 0.62 ng/kg b.w., which corresponds to exposure of <15% of the TWI, and intake for adults would be 0.21 ng/kg b.w., amounting to <5% of the TWI. A six-egg portion would double the exposure but still leave it far below 100% of the TWI (Fig. 3).

To reach the TWI dose based on mean LB concentrations, a child would have to consume around 200 g of cow's liver, 600 g of chickens' livers, almost 800 g of a horse's liver, or 22 chickens' eggs. The same simulation for adults indicates that they would have to consume 600 g of cow's liver, 1.8 kg of chickens' livers, almost 2.4 kg of a horse's liver, or 64 chickens' eggs.

### Discussion

According to the EFSA report, eggs are one of the largest contributors to dietary PFAS exposure for the European population (15). Chickens that are reared on open paddocks might be more exposed to environmental contamination by persistent organic pollutants because of their greater contact with soil (34, 40, 49). The properties of PFASs that facilitate their transfer from the soil to plants have made plants a potential additional source of these toxic compounds for chickens. Plant contamination is particularly likely with short-chain PFASs because their transfer rates from the soil are higher (2, 19). The main sources of PFASs for hens reared in cages are water and commercial feed, primarily feeds for the production of which fish were used (21). A positive correlation has been found between PFASs in drinking water for hens and the levels of those PFASs in eggs (48).

Toxicokinetic studies showed that after 25 days of exposure of hens to PFASs, PFOS levels were comparable in liver, kidney and plasma, an order of magnitude lower in muscle, but tenfold higher in chicken egg yolks compared to liver (25). In contrast, PFOA behaved differently and accumulated most in the kidneys, next most heavily in yolks, then in plasma and liver tissue and least in muscles (25). These compounds showed a similarity: after a period of depuration (67 days), they were not detected in eggs, plasma or muscle, liver or kidney tissue.

The results of the present study are consistent with those of a previous one that indicated low contamination of caged hens with  $\Sigma$ 4 PFASs (not all four compounds were detected) (31) and comparably low levels in freerange and organic eggs (mean LB  $\Sigma$ 4 PFASs equal to 0.096 µg/kg in this study and 0.10 µg/kg in the foregoing study for organic chickens' eggs) (31). In both studies, L-PFOS was the dominant compound. The sum of PFOS concentration in the current research (0.081 µg/kg) was at a comparable level with previous data (0.13 µg/kg in organic and 0.11 µg/kg in free-range eggs) (31). There is also a 2023 publication on PFAS levels in eggs in Poland in which PFOS was determined at much higher concentrations (mean 2.4 µg/kg) (41). Exposure related to  $\Sigma$ PFOSs was three-fold lower than the European average (0.27  $\mu$ g/kg) (15). Higher concentrations of PFOSs were determined in other European countries (18, 50). However the highest contamination of eggs was reported from places where hens were in contact with a polluted environment (plastic disposal and recycling sites) (32, 36) and where nearby industry used fluorochemicals (1, 4).

The present data regarding the egg-derived proportion of  $\sum 4$  PFAS intake by Polish consumers confirmed previously reported results indicating low exposure of <15% of TWI and <5% of TWI for children and adults, respectively (31).

Cow's liver is considered to be a source of vitamins, minerals and proteins (26), but because of its detoxifying role it may contain toxic contaminants (30, 42). Interactions with proteins (liver fatty acid binding proteins, serum albumin and organic anion transporters) change the toxicokinetics of PFASs (15). The data indicate variety in the behaviour of individual PFASs and their distribution in the cow's body. The final destination of PFOS in exposed cows is the muscles, where about 43% of this contaminant is deposited, while 18% reaches the liver and 14% is excreted in the milk (24). Around 11% is excreted in faeces while only 0.5%is eliminated in urine (30). Different toxicokinetic behaviour of PFOA was observed in dairy cows: immediately after exposure it is excreted in the urine (29). This might explain the levels of PFOA an order of magnitude lower than those of PFOS in the cows' livers found in this research. Cows' livers analysed in China contained 60 times more PFASs than muscle (43). Animals reared outdoors showed higher liver PFAS levels than animals reared indoors (50). Plants consumed by grazing animals are a potential source of these compounds because of their ability to accumulate them (37). In addition, grazing animals also take up small amounts of soil with plant material. The amount of soil taken up by the animals may be influenced by their grazing behaviours and the state of the pasture (47).

Our preliminary study indicates low contamination of the analysed horses' and chickens' livers and slightly elevated concentrations of PFASs in cows' livers. Perfluorooctanesulphonic acid concentration in the latter tissue type tested by us was four times lower than those reported in a study published in 2023 (41). Higher contamination with PFOS was reported in China of up to 2.54  $\mu$ g/kg.

## Conclusion

The low content of the compounds tested and the simulation results indicating the large portions that would have to be consumed to exceed the TWI allow us to conclude that the tested eggs and livers excluding those of cows are not significant sources of PFASs. Further research to monitor toxic levels of PFASs, especially in the livers of farm animals, is necessary to ensure the safety of Polish consumers.

**Conflict of Interests Statement:** The authors declare that there is no conflict of interests regarding the publication of this article.

**Financial Disclosure Statement:** This study was financed by the National Veterinary Research Institute, Puławy, Poland, as part of research project no. S/522.

## Animal Rights Statement: None required.

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