J Vet Res 67, 593-602, 2023
DOI: 10.2478/jvetres-2023-0058

Occurrence of perfluoroalkyl substances in cow's, goat's and sheep's milk – dietary intake and risk assessment

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Received: June 1, 2023 *Accepted: October 10, 2023*

Abstract

Introduction: Milk from cows, goats and sheep was analysed in terms of content of fourteen perfluoroalkyl substances (PFASs). **Material and Methods:** Altogether, 73 milk samples from cows (n = 38), goats (n = 20) and sheep (n = 15) were collected from various regions of Poland. Concentrations of analytes were determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS). **Results:** The lower-bound sum of four PFAS (∑4 PFASs) concentrations (perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid, perfluorononanoic acid and perfluorohexanesulfonic acid) were highest in sheep's (0.0055 µg/kg), lower in goat's (0.0046 µg/kg), and lowest in cow's milk (0.0008 µg/kg). Goat's and sheep's milk was statistically significantly more contaminated than cow's milk. None of the samples exceeded the indicative values set by Commission Recommendation (EU) 2022/1431, and even the maximum detected concentrations were an order of magnitude lower. The most frequently detected was linear PFOS, which was found in 33%, 76% and 93% of cow's, goat's and sheep's milk samples, respectively. Based on mean upper-bound ∑4 PFAS concentrations and average milk consumption, the estimated intake of ∑4 PFASs ranged from 0.153 to 0.266 ng/kg body weight (b.w.) for children and from 0.050 to 0.88 ng/kg b.w. for adults, which indicates that exposure is very low and is merely <7% of the tolerable weekly intake (TWI) for children and <2% of the TWI for adults. **Conclusion:** Regardless of the milk type, the intake of PFASs *via* consumption of Polish milk does not contribute significantly to the overall PFAS intake of either adults or children.

Keywords: PFAS, cow's milk, small-ruminant milk, POPs, risk assessment.

Introduction

Perfluoroalkyl substances are a group of chemicals which have been manufactured since 1950. Environmental contamination with PFASs is not only with those which were manufactured, because their precursors may also become PFASs through transformation and add to the burden (3). The water- and oil-repelling properties of PFASs make them substances with wide application: in fire-fighting foams, food contact materials, clothes, and emulsifiers (12, 28, 49). As persistent organic pollutants (POPs) they are degradation resistant, bioaccumulative and able to be transported over long distances. Perfluoroalkyl substances are responsible for a number of toxic effects on humans and wildlife, among which a decreased response of the immune system to vaccination seems the most critical detrimental effect on humans (8, 40). Food, drinking water, inhalation and dust ingestion are the main sources of PFASs for humans (6, 7, 8, 11). A European Food Safety Authority risk assessment performed in 2020 established a tolerable weekly intake (TWI) for the sum of four PFASs (∑4 PFASs), namely perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluoro-hexanesulfonic acid (PFHxS), at the level of 4.4 ng/kg body weight (b.w.) (8). These four compounds are responsible for half of the exposure to PFASs of the European population. In 2022 the European Commission established maximum levels for PFASs in certain foodstuffs, but milk was not included (2022/2388/EU). Milk was included, however, in Commission Recommendation (EU) 2022/1431 of 24 August 2022 on the monitoring of perfluoroalkyl substances in food: it established indicative levels (IL) for milk of 0.020 μg/kg for PFOS, 0.010 μg/kg for PFOA, 0.050 μg/kg for PFNA and 0.060 μg/kg for PFHxS and required further investigation of the causes of contamination when the ILs are exceeded.

It was proved that the major binding protein for PFOS and PFOA is serum albumin (4), and the binding of PFASs to protein is the most probable mechanism for the transfer of these compounds to milk during lactation and the potential exposure of consumers (29). Cow's milk might be a source of various PFASs, since their transfer to milk has been proved (20, 23, 45, 48).

Poland ranks 12th in the world and 5th in Europe for the volume of cow's milk produced (14,457 million litres annually). The dominant region is the Podlaskie voivodeship which produces over 3,000 million litres (43). Poland's annual export of milk is approximately one million tonnes (43). The substantial volume of dairy farming output in Poland implies that contamination of Polish milk is a subject worthy of attention. Nevertheless, data on the occurrence of PFASs in milk from the country are scarce, with only one publication (45). Addressing the lack of data and illustrating the state of Polish milk contamination in the light of the legal regulations introduced and the TWI established, this study aimed to investigate the levels of 14 PFASs in cow's, goat's and sheep's milk. It compares the levels found and assesses the risk to consumers in relation to the TWI of 4.4 ng/kg b.w.

Material and Methods

Sampling and sample collection. Altogether, 73 milk samples from cows (n = 38), goats (n = 20) and sheep (n = 15) were collected from various regions of Poland by the Veterinary Inspectorate (Fig. 1). Sampling was carried out according to 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. Samples were analysed by the National Reference Laboratory for halogenated POPs at the Radiobiology Department of the National Veterinary Research Institute, Puławy, Poland.

Analytes of interest. Fourteen PFASs were investigated: perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), PFHxS, perfluoroheptanesulfonic acid (PFHpS), linear and branched PFOS (l-PFOS and br-PFOS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), perfluoro-tridecanoic acid (PFTrDA), and perfluorotetradecanoic acid (PFTeDA).

Fig. 1. Map of Poland showing sampling points in different voivodeships

Isotopically labelled analogues were used: sodium perfluoro-1-(2,3,4-$^{13}C_3$) butanesulfonate, sodium perfluoro-1-hexane($^{18}O_2$)sulfonate, sodium perfluoro-1-(1,2,3,4-$^{13}C_4$) octanesulfonate, perfluoro-n-(1,2,3,4,6-$^{13}C_5$) hexanoic acid, perfluoro-n-(1,2,3,4-$^{13}C_4$) heptanoic acid, perfluoro-n-(1,2,3,4-$^{13}C_8$) octanoic acid, perfluoro-n-(1,2,3,4,5-$^{13}C_5$) nonanoic acid, perfluoro-n-(1,2-$^{13}C_2$) decanoic acid, perfluoro-n-(1,2,3,4,5,6,7-$^{13}C_7$) undecanoic acid, perfluoro-n-(1,2-$^{13}C_2$) dodecanoic acid, and perfluoro-n-(1,2-$^{13}C_2$) tetradecanoic acid. Perfluoro-(1,2,3,4,5,6,7,8-$^{13}C_{12}$) octanesulfonate was used as a recovery standard. All standards were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada).

Reagents and chemicals. Oasis WAX solid-phase extraction (SPE) cartridges (150 mg, 6 mL) were used from Waters Corp. (Milford, MA, USA) and ENVI-Carb SPE sorbent (500 mg, 6 mL) was selected from Supelco (Bellefonte, PA, USA). Methanol was obtained from LGC Standards (Wesel, Germany), sodium hydroxide pellets (>98%) were purchased from Honeywell Fluka (Seelze, Germany), ammonium acetate and ammonium solution 32% were ordered from Merck (Darmstadt, Germany), ultrapure water (18.2 MΩ/cm) was sourced from Supelco and acetic acid was procured from J.T. Baker (Phillipsburg, NJ, USA).

Sample preparation, extraction, purification and detection. Raw milk samples (250 mL) were freeze dried and 2 g masses of lyophilised sample were fortified with isotopically labelled standards. Extraction was carried out using 10 mL of 0.01M methanol/ potassium hydroxide. Samples were shaken for 1 min and left for 20 h. Purification was performed on an Oasis WAX (150 mg, 6 mL) SPE cartridge and ENVI-Carb SPE sorbent (500 mg, 6 mL). Recovery standards were added before liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis.

Concentrations of analytes were determined using LC-MS/MS on a Triple Quad 7500 system (Sciex, Framingham, MA, USA) operated in negative electrospray ionisation (ESI−) mode. For chromatographic separation, a Gemini C18 column (3 µm, 50 × 2.0 mm) equipped with a guard column (Phenomenex, Torrance, CA, USA) was used. The mobile phase consisted of 20 mM of ammonium acetate aqueous solution and methanol. The limits of quantification (LOQ) were between 0.001 µg/kg and 0.004 µg/kg and met the criteria set down in Commission Recommendation (EU) 2022/1431 of 24 August 2022 on the monitoring of perfluoroalkyl substances in food. Based on this recommendation, the LOQ for milk analysis should be at or below 0.010 µg/kg for PFOS and PFOA, 0.020 µg/kg for PFNA and 0.040 µg/kg for PFHxS. Acquisition and processing were performed using the Sciex OS software.

Quality assurance and quality control. The SPE cartridges and all solvents were checked for purity before routine analysis. Blank samples and IRMM-427 Pike-perch certified reference material purchased from the European Commission Joint Research Centre Institute for Reference Materials and Measurements (JRC, Geel, Belgium) were analysed with each series of samples to achieve internal quality assurance and control (QA/QC). Successful participation in proficiency testing (PT) served as external QC organised by the European Union Reference Laboratory for Halogenated Persistent Organic Pollutants in Feed and Food (EURL, Freiburg, Germany).

Statistical analysis. The Shapiro–Wilk test was used to verify the normal distribution of the data. Differences between individual experimental groups were checked using the Mann–Whitney and Kruskal–Wallis tests (at $p \leq 0.05$). To find associations between occurrences of PFASs and fat content, Spearman's rank correlation coefficient was used.

Result presentation. Concentrations of individual PFASs and the sum concentration are expressed as µg/kg of milk. Sum concentrations are given as lower-bound (LB) concentration (concentrations below the LOQ were replaced with the value of 0) and as upper-bound (UB) concentration (concentrations below the LOQ were replaced with the value of the LOQ).

Dietary intake. Dietary intake was calculated for the statistically average monthly milk consumption in Poland of 3.5 L (43) and for the recommended daily milk consumption of 0.5 L. To characterise the potential health risk associated with ∑4 PFAS intake, doses ingested with milk were compared to the TWI (4.4 ng/kg b.w. per week). Body weight was assumed to be 70 kg for an adult and 23.1 kg for a 3–10-year-old child (9).

Results

Occurrence of the sum of four perfluoroalkyl substances and the sum of fourteen perfluoroalkyl substances. The LB ∑4 PFAS concentrations declined from their highest in sheep's milk to lower ones in goats' milk and the lowest in cow's milk (Fig. 2). Statistically significant differences were observed both between sheep's milk and cow's milk ($p = 0.002$) concentrations and between goat's milk and cow's milk ($p = 0.027$) concentrations, but the goat's milk and sheep's milk LB ∑4 PFAS concentrations did not differ significantly ($p = 0.401$). The LB ∑14 PFAS concentrations descended in order from sheep's milk to goat's milk and lastly to cow's milk (Fig. 2). The cow's milk LB ∑14 PFAS concentration did not differ significantly from that of goat's milk ($p = 0.057$) but was statistically significantly lower than that of sheep's milk ($p = 0.005$). No differences between voivodeships in LB ∑4 PFAS levels in cow's, goat's or sheep's milk ($p = 0.878$, $p = 0.984$ and $p = 0.771$, respectively) were found.

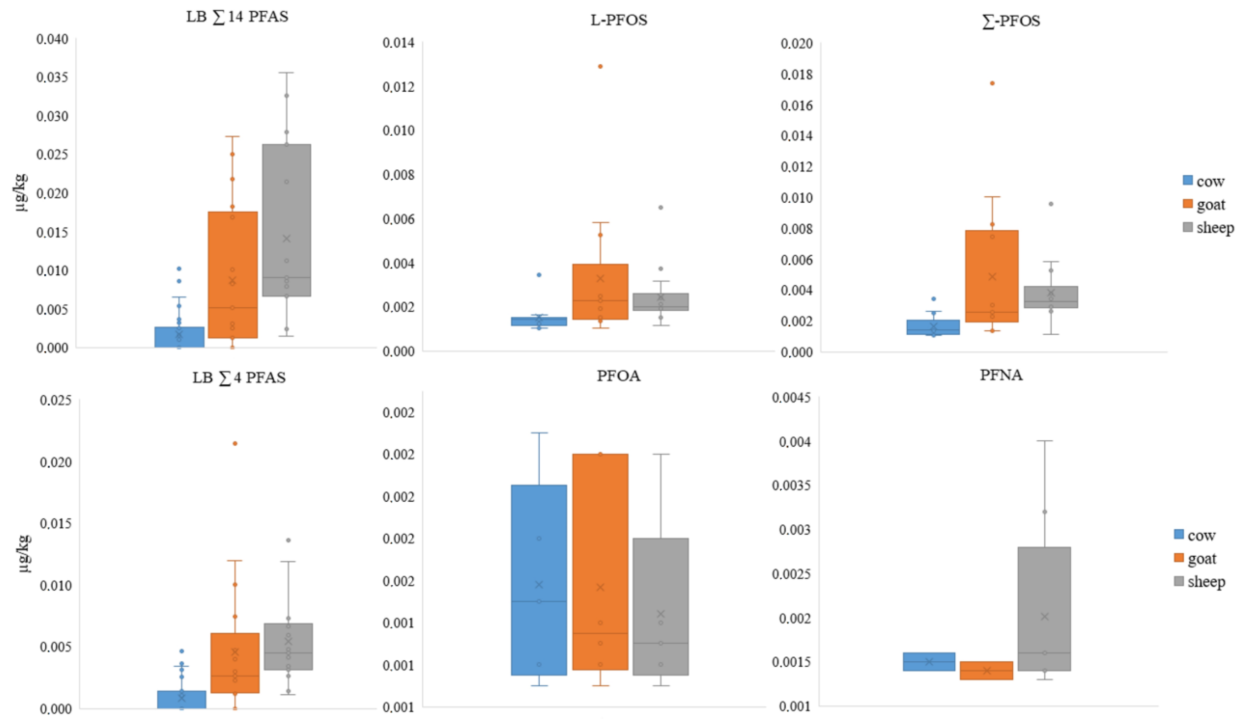

Fig. 2. Distribution of the results for concentrations of lower-bound sum of 4 and of 14 perfluoroalkyl substances (LB ∑4 PFAS and LB ∑14 PFAS), perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA)

The LB ∑14 PFAS concentrations also did not differ significantly between voivodeships (at $p = 0.982$ for cow's milk, $p = 0.912$ for goat's milk and $p = 0.999$ for sheep's milk). The Lubelskie and Świętokrzyskie voivodeships were not included in statistical analysis respectively for goat's and cow's milk because the number of samples was insufficient.

Occurrence of four perfluoroalkyl substances (perfluorooctane sulfonic acid, perfluorooctanoic acid, perfluorononanoic acid and perfluoro-hexanesulfonic acid). Summarised data on four PFAS concentrations in the analysed milk samples from the three species are presented in Table 1. None of the samples exceeded the indicative values established by Commission Recommendation (EU) 2022/1431, and even the maximum detected concentrations were an order of magnitude lower. Only in one, which was the most contaminated goat's milk sample, was the sum br-PFOS and l-PFOS (∑PFOS) concentration result (0.0174 µg/kg) close to the indicative value of 0.020 µg/kg, but the mean concentration was below 24% of the IL. The mean PFOA concentrations were almost equal in the three types of milk and were below 17% of the IL. For PFNA, this share in relation to the limit was smaller at <4%. In the only sample in which PFHxS was detected, the concentration-to-limit ratio was <5%.

The most frequently detected substance was l-PFOS, which was found in 33%, 76% and 93% of cow's, goat's and sheep's milk samples, respectively. Branched PFOS was much less frequently found in cow's (5%) and goat's milk (35%) but was detected in similar abundance to l-PFOS in sheep's milk samples (87%). The highest mean levels of l-PFOS and ∑PFOSs transpired to be in goat's milk at 0.0033 µg/kg and 0.0048 µg/kg, respectively, but no statistical significance ($p = 0.197$, $p = 0.414$) was found between species (Fig. 2) in these concentration differences. Perfluorooctanoic acid was the second most frequently occurring compound and was detected in 13% of cow's milk, 35% of goat's milk and 47% of sheep's milk samples. The mean concentrations (0.0016–0.0017 µg/kg) did not differ significantly between species ($p = 0.981$). The similarity in concentrations between PFOS and PFOA despite the greater transmission to milk of the former may suggest that cows were exposed to much higher levels of PFOA than PFOS.

Perfluorononanoic acid was detected to a lesser extent in cow's milk, found only in 5% of samples, and in goat's milk, noted in 12% of samples, than in sheep's milk. This perfluoroalkyl substance was identified in 53% of sheep's milk samples. The mean levels in all three milk types showed similarity (0.0014–0.0016 µg/kg).

Perfluorohexanesulfonic acid contaminated only a single sample of goat's milk, where its concentration was 0.003 µg/kg.

Excluding one PFHxS detection result in goat's milk, the percentage shares in the LB ∑4 PFASs indicate the dominant role of PFOS: 53% and 40% in goat's and sheep's milk, respectively. Contrastingly, in cow's milk the proportions of PFOS, PFOA and PFNA were almost equal.

Table 1. PFOS, PFOA, PFNA and PFHxS levels in cow's, goat's and sheep's milk (µg/kg)

Compound		Cow	Goat	Sheep	LOQ	Indicative level
	mean	0.0017	0.0048	0.0038		
∑PFOS	median	0.0014	0.0026	0.0033	0.001	0.020
	range	<LOQ–0.0034	<LOQ–0.0174	<LOQ–0.0096		
	mean	0.0015	0.0033	0.0025		
L-PFOS	median	0.0014	0.0023	0.0020	0.001	-
	range	<LOQ–0.0034	<LOQ–0.0129	<LOQ–0.0047		
	mean	0.0016	0.0016	0.0017		
PFOA	median	0.0015	0.0014	0.0014	0.001	0.010
	range	<LOQ–0.0023	<LOQ–0.0022	<LOQ–0.0026		
	mean	0.0015	0.0014	0.0020		
PFNA	median	0.0015	0.0014	0.0016	0.001	0.050
	range	<LOQ–0.0016	<LOQ–0.0015	<LOQ–0.0037		
	mean	<LOQ	0.0027	<LOQ		
PFHxS	median	-	0.0027	-	0.001	0.060
	range	-	<LOQ–0.0015	-		
	mean	0.0043	0.0073	0.0075		
UB∑4 PFAS	median	0.0040	0.0056	0.0065	0.0040	-
	range	0.0040–0.0064	0.0040–0.0224	0.0041–0.0156		
	mean	0.0008	0.0046	0.0055		
LB∑4 PFAS	median	0.0000	0.0026	0.0045	-	-
	range	0.0000–0.0035	0.0000–0.0214	0.0011–0.0136		

LOQ – limit of quantification; ∑PFOS – sum of linear and branched perfluorooctanesulfonic acid; L-PFOS – linear PFOS; PFOA – perfluorooctanoic acid; PFNA – perfluorononanoic acid; PFHxS – perfluorohexanesulfonic acid; UB∑4 PFAS – upper-bound sum of 4 perfluoroalkyl substances; LB∑4 PFAS – lower-bound sum of 4 perfluoroalkyl substances

Table 2. Levels of other perfluoroalkyl substances determined in cow's, goat's and sheep's milk (µg/kg)

Compound		Cow	Goat	Sheep	LOQ
	mean	<LOQ	<LOQ	0.0055	
PFHxA	median	-	-	0.0028	0.001
	range	-	-	<LOQ–0.0114	
	mean	<LOQ	0.0015	0.0016	
PFHpA	median	-	0.0015	0.0016	0.001
	range	-	<LOQ–0.0015	<LOQ–0.0019	
	mean	0.0021	0.0023	0.0024	
PFDA	median	0.0021	0.0024	0.0020	0.001
	range	<LOQ–0.0021	<LOQ–0.0032	<LOQ–0.0042	
	mean	0.0019	0.0017	0.0018	
PFUnDA	median	0.0019	0.0016	0.0013	0.001
	range	LOQ–0.0019	<LOQ–0.0026	<LOQ–0.0032	
	mean	0.0011	0.0014	0.0017	
PFDoA	median	0.0011	0.0014	0.0015	0.001
	range	<LOQ–0.0011	<LOQ–0.0015	<LOQ–0.0026	
	mean	<LOQ	0.0057	0.0058	
PFTrDA	median	-	0.0057	0.0050	0.004
	range	-	<LOQ–0.0070	<LOQ–0.0091	
	mean	0.0055	0.0063	0.0073	
PFTeDA	median	0.0057	0.0057	0.0061	0.004
	range	<LOQ–0.0065	<LOQ–0.0105	<LOQ–0.0129	
	mean	0.0012	0.0015	0.0015	
PFBS	median	0.0012	0.0015	0.0014	0.001
	range	<LOQ–0.0012	<LOQ–0.0020	<LOQ–0.0020	
	mean	<LOQ	<LOQ	<LOQ	
PFPeS	median	-	-	-	0.001
	range	-	-	-	
	mean	<LOQ	<LOQ	<LOQ	
PFHpS	median	-	-	-	0.001
	range	-	-	-	

LOQ – limit of quantification; PFHxA – perfluorohexanoic acid; PFHpA – perfluoroheptanesulfonic acid; PFDA – perfluorodecanoic acid; PFUnDA – perfluoroundecanoic acid; PFDoA – perfluorododecanoic acid; PFTrDA – perfluorotridecanoic acid; PFTeDA – perfluoeotetradecanoic acid; PFBS – perfluorobutanesulfonic acid; PFPeS – perfluoropentanesulfonic acid; PFHpS – perfluoroheptanesulfonic acid

Occurrence of the rest of the analysed perfluoroalkyl substances. The occurrence of the rest of the analysed PFASs in milk samples from the three species is presented in Table 2. Generally, the levels found for most of the analysed PFASs in milk were low and below or only slightly above the LOQ. This is in line with European data, in which most PFASs were not detected in milk, and the detected compounds were noted with a frequency of below 4% (8). Regardless of the species, PFPeS and PFHpS were not detected in any samples. Differences between species in some compounds' occurrences were noticed. Perfluorohexanoic acid was not found in any cow's or goat's milk, while it was detected in 20% of sheep's milk samples, concentrated at 0.0055 μg/kg (mean).

No sample of cow's milk contained PFHpA or PFTrDA, whilst goat's and sheep's milk samples yielded both compounds. The frequencies of PFHpA detection were 6% and 13% and for PFTrDA 12% and 27% in goat's and sheep's milk, respectively. The compound which transpired to be the most abundant was PFTeDA, being in 13% of cow's milk, 29% of goat's milk and 33% of sheep's milk samples. This compound also occurred in the highest concentrations of all 14 investigated PFASs, peaking in sheep's milk at a mean 0.0073 μg/kg. Interspecies differences in its concentration were not significant (p = 0.704).

Perfluoroalkyl substance intake *via* milk consumption. Based on mean UB ∑4 PFAS concentrations and average full milk consumption, the estimated intakes of PFASs were 0.153–0.266 ng/kg b.w. for children and 0.050–0.88 ng/kg b.w. for adults (Table 3), which indicates that exposure is very low and is merely <7% of the TWI for children and <2% of the TWI for adults (Fig. 3).

Table 3. Estimated intake (ng/kg body weight) of perfluoroalkyl substances (perfluorooctanesulfonic acid, perfluorooctanoic acid, perfluorononanoic acid and perfluorohexanesulfonic acid) with a statistically average weekly portion and recommended weekly portion of milk based on mean and 95th percentile upper-bound concentrations

	Perfluoroalkyl substance concentration			
	Children		Adults	
	Mean	95th Percentile	Mean	95th Percentile
	Intake ng/kg b.w			
Cow	0.153/0.652	0.199/0.848	0.050/0.215	0.066/0.280
Goat	0.259/1.106	0.529/2.258	0.086/0.365	0.175/0.745
Sheep	0.266/1.136	0.486/2.076	0.088/0.375	0.160/0.685

b.w. – body weight

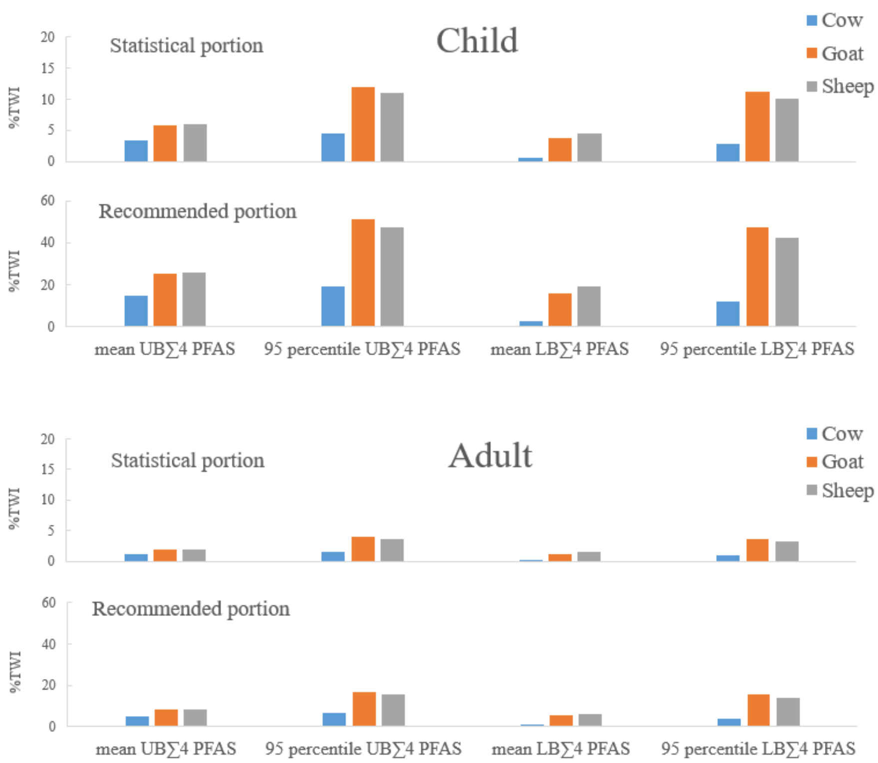

Fig. 3. Relationship to the tolerable weekly intake (TWI) of dietary intake with a statistically average weekly portion and recommended weekly portion of milk based on lower- and upper-bound mean and 95th percentile concentrations related to the TWI

When 95th percentile concentrations are considered, exposure increases by approximately 30% from cow's milk consumption and approximately 80–100% from goat's and sheep's milk. However, relating these values to the TWI raises the fraction of the intake to <12%. Calculating exposure based on LB concentrations, the intake would be over 80% lower for cow's milk, 27% lower for sheep's milk and 37% lower for goat's milk.

Since the recommended portion of milk is over four times larger than the average portion, exposure based on mean UB ∑4 PFAS concentration from ingestion of the recommended portion would increase by a factor of four. Intake would be <26% and <9% of the TWI for children and adults, respectively, and based on the 95th percentile concentration would be <48% and <16% of the TWI (Fig. 3). Goat's and sheep's milk are less popular and consumed in lesser amounts, especially by children, but cow's milk should not be of concern despite being consumed more often. Cow's milk is safe, since the estimated intake *via* the recommended weekly consumption of 3.5 L was <20% of the TWI for children and <7% for adults (based on the 95th percentile UB concentration). The calculation based on the upper-bound concept is an overestimation, and exposure based on the LB 95th percentile concentration would be <12% of the TWI for children and <4% for adults.

To reach the TWI dose, an adult would have to drink 70 L and a child over 23 L of cow's milk contaminated at the average UB level in a week. This suggests that neither the statistical average nor the recommended consumption of cow's, goat's or sheep's milk analysed in this study contributes significantly to the overall PFAS intake, even when the milk is the most contaminated.

Discussion

The levels of PFOS in cow's milk from this study were an order and two orders of magnitude lower than previously reported results from Poland (45) and other parts of the world (20, 29, 31, 37, 52) but similar to the European data reported by the European Food Safety Authority (mean LB concentration 0.001 μg/kg) (8). The high detection frequency of PFOS is consistent with the highest secretion into cow's milk among PFASs having been indicated for these compounds (15%), surpassing by several times that of PFHxS (2.5%) and PFOA (0.1%) (23). The concentrations of PFOA in our study were an order and orders of magnitude lower than those reported previously in cow's milk from Poland (45) and from other countries (20, 29, 31, 37) but higher than the European mean concentration (LB 0.000 μg/kg) (8).

The differences between PFOS and PFOA concentrations were more pronounced in sheep's and goat's milk than in cow's milk (Table 1). This is in line with the result of research by Kowalczyk *et al.* (22), which indicated that PFOS was excreted in sheep's milk to a greater extent than PFOA but that excretion was low at <2%, and lower than in cow's milk (22, 23). In their research on sheep, they indicated that overall excretion of PFOS (6%, 4–5% of which was *via* faeces) was significantly lower than excretion of PFOA (53–56%, of which 51–55% was cleared in urine as the primary route) (22). Differences in the milk/serum concentration ratios for PFOA in sheep samples (0.05) (22) and cow samples (0.4) (48) indicated species-specific differences in clearance from serum to milk. Higher levels of PFOS in sheep's than in cow's milk despite toxicokinetic data demonstrating significantly lower transfer to sheep's milk indicate that sheep were exposed to higher levels of contamination. Apart from differences in excretion of PFAS to milk resulting from species toxicokinetic characteristics, the length of time spent on open pasture can also be assumed to drive differences in PFAS exposure between species and within them. Grazing time might play a significant role in PFAS contamination. The livers of animals reared indoors showed lower levels of PFAS than those of animals reared outdoors (53). Sheep and goats, and not cows, spend the majority of their time on pastures. Grazing animals might be contaminated by plants because the transfer of PFASs to plants has been proved (42). Along with plant food, animals also take up soil, which is a well-known reservoir for POPs (2, 21, 33, 38, 50). The amount of soil taken up depends on the amount of grass available and the quality of the meadow, but is >3% of grass mass ingested by cattle, while for sheep and goats the percentage of ingested mass which is soil is higher (up to 20%), due to their grazing being closer to the ground (51). A source of soil contamination might be irrigation with sewage and sludge (15, 39, 41). Perfluoroalkyl substances might then move from soil to ground and surface water, which can also be taken up by animals (17). Pesticides used in agriculture might be a source of PFASs for plants (25), and PFASs leaching out of high-density polyethylene containers in which pesticide products are stored could contaminate the plants on which those products are used (46). Additionally, contaminated commercial feed cannot be excluded as a potential source of PFASs (5, 27, 47).

The PFNA cow's milk concentration in this study of 0.0015 is higher than the European mean LB concentration (0.000 μg/kg) but several times lower than those previously reported from Poland (45) and worldwide (31, 37, 44).

Perfluorohexanoic acid was not found in any cow's or goat's milk, while it was detected in 20% of sheep's milk samples, concentrated at 0.0055 μg/kg (mean). This compound was rarely detected or not detected at all in cow's milk from China (29), Germany (44) and Spain (7), but was detected in milk from South Africa at a concentration of 0.01 ng/mL (31).

Perfluorotetradecanoic acid was also found to be dominant in infant formula from Poland and Germany, mainly in products derived from cow's milk (34). According to Kowalczyk *et al*. (23), the longer the chain, the lower the excretion *via* milk from dairy cows, but our study revealed that it was long-chain PFASs such as PFTeDA which reached the highest levels in milk across all species.

Long-chain PFASs may be more available to animals that spend more time on pasture and take soil up with grass, because long-chain congeners tend to remain in the top layer of surface soil, while short-chain congeners generally penetrate to greater depths (13). However, short-chain PFASs, which are transferred more readily from soil to plants (1, 15), might be ingested through leaves of plants, in which they are preferentially distributed to the greatest extent (10, 18). There are no data on PFAS levels in soil from Poland, but in other research, long-chain PFASs were detected with varying frequencies: PFTrDA was frequently detected in soil samples from Sweden (55%), but PFTeDA was found rarely (21), and contrastingly, PFTeDA was found in all soil samples in North America, Europe, Asia, Africa, Australia, South America and Antarctica (39).

In addition to protein transfer as mentioned before, lipid PFAS transfer is also possible from the animal to its product (16). Since the PFASs with the C11–C14 long chains are more lipophilic (19), more of them might transfer to sheep's milk, because on average it has almost double the fat content of cow's and goat's milk. Our results do not confirm this relationship because no significant differences were found between species (Table 2). However, LB ∑4 PFAS concentrations positively correlated with fat content in all three species' milk (cow's ($r = 0.51$, $p = 0.0002$), goat's ($r = 0.82$, $p = 0.00001$) and sheep's ($r = 0.55$, $p = 0.0014$)), as did LB ∑14 PFAS concentrations (cow's ($r = 0.54$, $p = 0.00008$), goat's ($r = 0.71$, $p = 0.00005$) and sheep's ($r = 0.58$, $p = 0.0006$)).

In this work, only PFAS intake *via* milk is calculated, but it should be remembered that other consumed food or water might contain PFASs, and therefore the total intake might exceed the TWI. This is especially likely if contaminated food like Baltic Sea fish or eggs from free-range production are consumed (24, 32, 34). Moreover, in this study raw milk sampled directly from the farm was analysed and further contamination of milk with PFASs downstream of the farm during production or *via* packaging materials cannot be excluded (14, 26, 44).

According to the recent EFSA opinion on the risk to human health related to the presence of PFASs in food, cow's milk is not a significant contributor to the overall exposure (8). Our conclusion is in line with the EFSA position, but some research indicates that milk might nevertheless be a significant contributor (20, 36).

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 research project number S/522.

Animal Right Statement: None required.

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