



Perfluorooctanesulfonate (PFOS) exposure in ringed seals (*Pusa saimensis*) in Lake Saimaa, Finland

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ABSTRACT

Perfluorooctanesulfonate (PFOS) is common in the environment, highly accumulative, toxic, and thus a good indicator for per- and polyfluorinated alkyl acid (PFAS) contamination. In this study, PFOS was analysed from the tissues of Saimaa ringed seal (*Pusa saimensis*) pups and adults to investigate the exposure to PFAS in Lake Saimaa. Additionally, a toxicity evaluation was performed for the seals based on the thresholds from the literature, PFOS concentration data from the national exposure database, and the current study. For all studied specimens, the highest PFOS concentrations were found in the liver, but interestingly, high concentrations were also observed in the kidneys of lanugo pups. The toxicity evaluation indicated that the PFOS concentrations in the prey fish are so high that the tolerable daily and weekly intake values from literature as well as the environmental quality standards calculated in this study were exceeded. Furthermore, a mixture of PFAS has been observed in the fish of Lake Saimaa, which means that these seals are exposed to multitudes of PFAS. This mixture of PFAS may be more harmful than PFOS alone and together with PFOS toxicity evaluation results, despite the confounding factors of the assessment, this raises concern that PFAS contamination in Lake Saimaa may cause detrimental effects in Saimaa ringed seals.

1. Introduction

Per- and polyfluorinated alkyl acids (PFAS) are a synthetic chemical group with thousands of compounds and their toxicity has been extensively studied in wildlife and humans (Liu et al., 2019; EFSA, 2020; Tarapore and Ouyang, 2021; Lohmann et al., 2024). They cause for example reproductive problems as they can interfere with spermatogenesis (Shi et al., 2024), reduce the weight of the offspring (Case et al., 2001; EFSA, 2020), cause abortions and stillbirths (Case et al., 2001; Aghaei et al., 2022; Lohmann et al., 2024), and give rise to placental dysfunction (Aghaei et al., 2022; Shi et al., 2024). In addition, they bioaccumulate and biomagnify in the environment (Androulakis et al., 2022; Boisvert et al., 2019; Taylor et al., 2021; Lohmann et al., 2024). They are also highly persistent in the environment (Liu et al., 2019; Jiao et al., 2021) and are thus often dubbed as ‘infinity chemicals’ (Shi et al., 2024). Despite the obvious problems that may stem from the use of such chemicals, PFAS have become a broadly used group of chemicals due to their properties as dirt, grease, and water repellents (Ahrens and Bundschuh, 2014; Brendel et al., 2018; Liu et al., 2019; Shi

et al., 2024). This has led to a widespread environmental PFAS contamination (Ahrens and Bundschuh, 2014; Boisvert et al., 2019; Liu et al., 2019; Lohmann et al., 2024). Therefore, efforts to restrict the use of long-chained PFAS have been established (UNEP, 2024a, 2024b) and this has led the industry to adapt the use of short-chain PFAS as they are considered to be safer than their long-chained counterparts (Brendel et al., 2018; Gomis et al., 2018; Zhang et al., 2019; Dickman and Aga, 2022). However, the short-chain PFAS have also been proven to be toxic and bioaccumulative (Brendel et al., 2018; Liu et al., 2019; Shi et al., 2024). Despite the legislative efforts, both long- and short-chain PFAS are still present in marine and freshwater environments (Kumar et al., 2022; Androulakis et al., 2022; Lee et al., 2023). Especially long-chained PFAS have been reported in the tissues of the ringed seals (*Pusa hispida botnica*), harbour seals (*Phoca vitulina*), and grey seals (*Halichoerus grypus*) from the Baltic Sea (Boisvert et al., 2019; Roos et al., 2019; HELCOM, 2021) and from the fish of Lake Saimaa (Kumar et al., 2022) in addition to their short-chained counterparts. This highlights that the long-chained PFAS are still a source for environmental contamination.

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The Saimaa ringed seal (*Pusa saimensis*) is an endemic species that resides only in Lake Saimaa, Finland (Löytynoja et al., 2025). Due to extensive conservation efforts (Kunnasranta et al., 2021) this endangered (Hyvärinen et al., 2019) seal now supports a population of roughly 500 individuals (Metsähallitus, Parks and Wildlife Finland, 2024). Nevertheless, this population still faces threats such as climate change, narrow gene pool, small population size, and anthropogenic threats such as by-catch mortality and chemical contamination (Kunnasranta et al., 2021). In addition, the population has relatively high pup mortality and stillbirth rate, which varies from year to year (Sipilä et al., 1990; Auttila et al., 2014; Kunnasranta et al., 2021). Some of this variation is caused by climate change and variation in annual environmental conditions,

but exposure to chemicals could also be a factor. As the area around Lake Saimaa is industrialised and populated, the anthropogenic chemical exposure the seals are faced with has been under investigation for decades (Helminen et al., 1968; Hyvärinen and Sipilä, 1984; Hyvärinen et al., 1998; Kostamo et al., 2002; Lyytikäinen et al., 2015; Simola et al., 2024). These studies have mainly concentrated on mercury due to its use in the pulp industry around Lake Saimaa and it has been suggested to be one of the causes of stillbirths in the 1980s (Hyvärinen and Sipilä, 1984), although a direct connection has not been proven. However, Simola et al. (2024) suggested that adverse effects from mercury exposure are still possible in this population even though the concentrations in adults have decreased since the 1960s (Helminen et al., 1968; Kari and

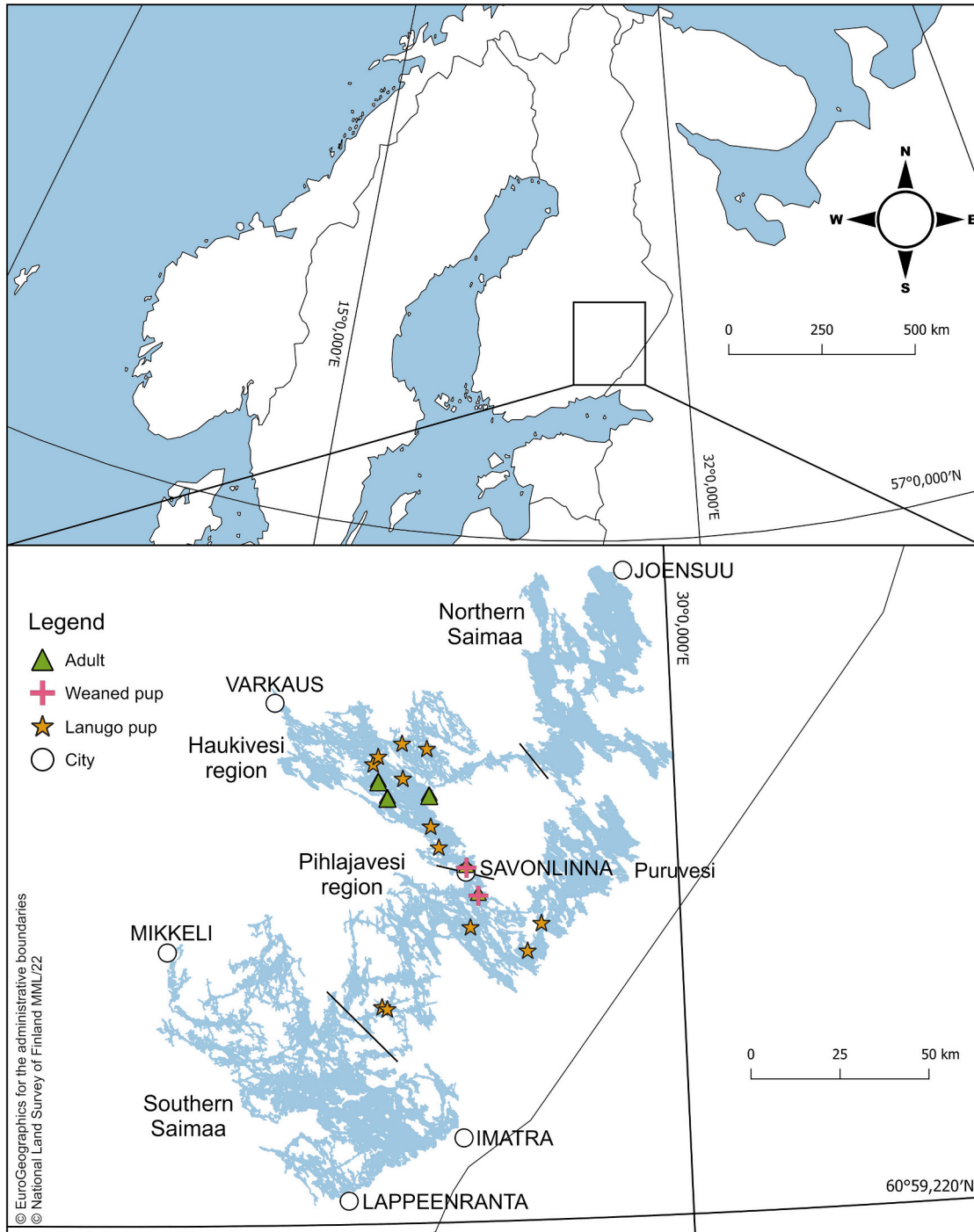


Fig. 1. Map of Lake Saimaa. Black lines separate the four defined study regions of Lake Saimaa.

Kauranen, 1978). Consequently, possible effects of chemicals on pup mortality and stillbirths in this population remain undisclosed. Therefore, PFAS are a rational course for the next investigation as these chemicals have already been observed in fish from Lake Saimaa (Kumar et al., 2022; Suomi et al., 2024) and because of their detrimental effects on reproduction and health (Case et al., 2001; Aghaei et al., 2022; Lohmann et al., 2024; Shi et al., 2024).

The aims of this study were 1) to study the PFAS exposure by analysing tissue concentrations of perfluorooctanesulfonate (PFOS) in the tissues of Saimaa ringed seals, 2) to perform toxicity evaluation for Saimaa ringed seals based on the regulatory values, thresholds, and concentration values found in the literature as well as the PFOS concentration data in this study and from a national exposure database.

2. Material and methods

2.1. Sampling

The seal samples were obtained from Lake Saimaa (61° 05' to 62° 36' N, 27° 15' to 30° 00' E) between 2014 and 2022 from various locations throughout the lake (Fig. 1). Seal placentas were collected during annual lair monitoring (see Auttila et al., 2014). Tissue samples from seals found dead were obtained during annual autopsies in the premises of Finnish Food Authority, except for brain tissue, which was collected as described in Simola et al. (2024). Placentas were divided into three categories of preservation (good, average, and poor) based on visual assessment (see Simola et al., 2024). All samples were stored at -20 °C. Sample collection was authorised by the Centre for Economic Development, Transport and the Environment (permit number VARELY/3480/2016).

Blubber, brain, kidney, liver, and muscle tissues were collected from 12 lanugo pups, two weaned pups, and three adult seals (SI 1 Table 1). Additionally, 12 placentas, which could be connected to these lanugo pups, were collected. Out of these twelve lanugo pups, three were stillborn, nine died shortly after birth in the lair. The weaned pups in this study were more than three months old, but less than a year. The adult seals were more than three years old.

2.2. Chemical analysis and quality control

The method used here was modified from the method developed by Verreault et al. (2007) and later utilised by Berger et al. (2009) and Roos et al. (2019). The modified method is presented here in short. First, 10 g of tissue sample was homogenised in sterile 50 ml falcon tube (QuEChERS Extract Tubes, 10 g by Agilent Technologies) with homogeniser (Janke & Kunkel Ika®-Labortechnik Ultra-Turrax T25). The blade was washed with dish soap and methanol (LiChrosolv® from Merck, CAS 67-56-1) between each sample. After homogenisation, 10 nanograms of isotope labelled internal standard was added to the sample (PFOS/PFOA [perfluorooctanoic acid] extraction standard mixture 13C8, 99 % 2000 ng/ml in methanol from Cambridge Isotope Laboratories, Inc.).

Two extractions were performed for each sample. For the first extraction, five millilitres of acetonitrile (HPLC gradient grade from VWR, CAS 75-05-8) was added to the sample. Salts from the extraction kit (10 g - sodium chloride and magnesium sulphate from the Original QuEChERS Extract Tubes kit) were added to the sample. These salts were added to further increase extraction. The sample was ultra sonicated (Bandelin Sonorex Super RK 106) for 15 min and centrifuged (Thermo Scientific Megafuge 40R) for 5 min (2000 rounds per minute). Supernatant was carefully removed from the falcon tube to a five-millilitre glass test-tube with Pasteur pipette. For second extraction, five millilitres of acetonitrile was added to the sample which was ultra sonicated and centrifuged as previously described. Supernatant from the second extraction was added to the test-tube with the supernatant from the first extraction.

The combined supernatant was evaporated into one-millilitre volume with nitrogen evaporator (Organomation Associates, Inc. OA-SYS N-EVAP™112). The condensed supernatants were transferred into two-millilitre Eppendorf-tubes, which had been cleaned with methanol. To further clean the condensed supernatants, 25 micrograms of graphitised carbon (Supelclean™ ENVI-Carb™ SPE Bulk) and 50 µl of acetic acid (99–100 % from J. T. Baker, CAS 64-19-7) were added into the tubes. The samples were shortly vortexed for 20 s and centrifuged for 10 min (10,000 rounds per minute). Half a millilitre of cleaned supernatant was carefully pipetted into a new, methanol washed Eppendorf-tube. A half millilitre of four millimolar ammonium-acetate (in milliQ-water, ammonium acetate from J. T. Baker, CAS 631-61-8) was added into the tubes. The samples were centrifuged for 10 min (10,000 rounds per minute with VWR MicroStar 12). Finally, the supernatant was diluted in relation of one to nine with acetonitrile into two-millilitre HPLC-vials. All the prepared samples were stored in a freezer for further analysis.

The sample preparation was performed in 11 batches. Preparation for one batch of samples was done on two consecutive days where the two extractions were performed during the first day, and the combined supernatant was stored in the fridge overnight. The sample preparation was finished during the next day, and the analysis ready supernatant sample was stored in the freezer. For the first three batches, two blanks were prepared containing only milliQ-water and no internal standard. From fourth batch onwards, only one blank was prepared and additional two samples with milliQ-water and 10 nanograms of internal standard were added into each sample. Additionally, in the first three batches, a freeze-dried non-fortified human serum reference sample for organic contaminants (NIST Standard Reference Material® 1957) was prepared for method validation in the same manner as the seal tissue samples.

A native unlabelled PFOS/PFOA (5000 ng/ml) standard mixture in methanol (Cambridge Isotope Laboratories, Inc.) was used to make an eight-point standard curve (2 ng, 10 ng, 20 ng, 55 ng, 100 ng, 165 ng, 495 ng, and 1485 ng of analyte). Internal standard (10 ng) was added to each of the standard point samples. The samples and standards were analysed with a method described by Zarei et al. (2024) with minor modifications. Shortly, the analytes were separated using reversed phase liquid chromatography and detected with high resolution mass spectrometry (Vanquish Flex UHPLC system coupled to Q Exactive Focus Orbitrap, Thermo Scientific, Bremen, Germany). The sample injection volume was 2 µl and the separation was performed using water (eluent A) and methanol (eluent B) gradient and a reversed phase column (Zorbax Eclipse XDBC18, 2.1 × 100 mm, 1.8 µm, Agilent Technologies, Palo Alto, CA, USA). Both eluents contained 0.1 % (v/v) of formic acid. The methanol gradient was: 0–10 min: 2 to 100 % B, 10–14.50 min: 100 % B, 14.50–14.51 min: 100 to 2 % B; 14.51–20 min: 2 % B. The column temperature was maintained at 40 °C, and the sample tray was kept at 10 °C. Negative electrospray ionization was used for analysis and following ESI source settings were employed: a spray voltage of 2.5 kV, sheath gas at a flow rate of 50 (arbitrary units), and auxiliary gas at a flow rate of 12.5 (arbitrary units). The capillary temperature was set to 300 °C and the probe heater was set to 425 °C. A full scan range from 120 to 1200 (*m/z*) was used with mass resolution of 70,000. The analytes were detected in the samples by creating extracted ion chromatograms using accurate masses of their (M-H)⁻ ions: *m/z* 498.9302 for PFOS, *m/z* 412.9664 for PFOA, and *m/z* 506.9573 for ¹³C8-PFOS. The initial chemical analysis results were further analysed with Thermo Trace Finder™ (version 5.1, Thermo Fisher Scientific Inc.) and Thermo FreeStyle™ (version 1.8.63.0, Thermo Fisher Scientific Inc.).

2.3. LC-MS analysis and quality control

With the LC-MS method used, PFOS and ¹³C8-PFOS give a good negative ion peak at *m/z* 498.9302 and *m/z* 506.9573, respectively. PFOS was well separated from its isomers and interfering substances. In the seal samples the straight chain isomer of PFOS was dominant, and it

was used for quantitation (SI 1 Fig. 1). The accuracy of the method was found adequate by running triplicate standard reference samples (87.7 %, SD 4.0 %). The PFOS levels were within the calibration range in all samples. All the blanks except for one with internal standard in batch seven were clear. However, new blanks prepared after the blank showing contamination in batch seven showed no contamination. Therefore, batch seven was included in the statistical analysis as is. The three analysed reference materials showed the PFOS recovery rate of 57.8 %, 66.0 %, and 62.1 % respectively.

On the contrary, PFOA did not show good chromatographic peaks. Thus, it was decided that this study would focus only on PFOS as it has been observed from the fish of Lake Saimaa (Kumar et al., 2022; Suomi et al., 2024) and is known to cause reproductive problems which makes it a good indicator for PFAS contamination (Case et al., 2001, Aghaei et al., 2022, Lohmann et al., 2024, Shi et al., 2024).

2.4. Statistical analysis

For the statistical analysis, the normality of residuals was tested with Shapiro-Wilk's test. When normality was not met, a base-ten logarithmic transformation was applied, and the homogeneity of variances for the residuals were tested with Levene's test. Rosner's test was used to check the residuals for possible outliers. Two-way ANOVA was applied to test whether the pup milk consumption, study region, sex of the seal, or age class of the seal affected the tissue PFOS concentrations. Furthermore, two-way ANOVA test was utilised to test whether sampling site and the degree of preservation of the placentas had significant effect on the found concentrations in the placentas. Correlation and regression analysis were performed to investigate connection between PFOS concentrations in placenta and lanugo pup tissues. The residuals were checked for normality with Shapiro-Wilk's test and for outliers with Rosner's test. Residuals were plotted to check for linearity. Breusch-Pagan test was used to check the residuals for homoscedasticity. Statistical analysis was performed with R Statistical Software (v4.4.3; R Core Team, 2025).

2.5. Toxicity evaluation

Environmental quality standards (EQS) from the European Commission (EC) and thresholds found in the literature were used for toxicity evaluation (Table 1). EC's environmental quality standards for humans as well as environmental quality standards calculated for the Saimaa ringed seal were used for the evaluation. The latter were calculated by applying average seal weight and food consumption to the equation used to calculate the human quality standards.

The quality standard for human health determined in edible fish ($QS_{biota, hh}$) (Eq. (1)) (European Commission, 2011a) was used as a basis to derive a quality standard for Saimaa ringed seal prey fish ($QS_{biota, srs}$). The equation was used as it was presented in the Common Implementation Strategy for the Water Framework Directive (2000/60/EC), Technical Guide for Deriving Environmental Quality Standards (European Commission, 2011a, 2011b). The environmental quality standard by EC (European Commission, 2011a) has been used for example as a threshold value in PFOS evaluation in the Baltic Sea by HELCOM (2023). The equation reads as follows:

$$QS_{biota, hh} = \frac{0.1 \times TL \times 70}{0.115} \quad (1)$$

where 0.1 is the percentage of consumed fishery products that exceed the threshold level (European Commission, 2011a). TL refers to a threshold level used to determine the effect of a substance in question ($\mu\text{g}/\text{kg}$ bw/d). The value 70 refers to the average body weight of a human being, and 0.115 is the mean amount of fish products consumed daily in kilograms.

In this study the equation was used to determine the quality standard for PFOS concentrations in Saimaa ringed seal prey fish (Eq. (2)). The

Table 1

Environmental quality standards and threshold values used in the literature-based toxicity evaluation.

	B/ W	Value	Unit	Effect	Source
TDI	B	0.15	$\mu\text{g}/\text{kg}$ bw/d	Based on lowest no-observed-adverse-effect level in Cynomolgus monkeys' (<i>Macaca fascicularis</i>) lipids and thyroid hormones.	EFSA, 2008
TWI	B	13	ng/kg bw/ w	Adverse effect on vaccination response and reduced birth weight.	EFSA, 2018
TDI	B	0.1	mg/kg bw/d	Developmental neurotoxicity on rodents	EFSA, 2020
EQS	B	33	$\mu\text{g}/\text{kg}$ ww.	Secondary poisoning for top predators.	European Commission, 2011a
EQS	W	0.002	$\mu\text{g}/\text{l}$	Secondary poisoning for top predators.	European Commission, 2011a
LOEC	B	63	$\mu\text{g}/\text{g}$	Elevated PPAR α -mediated transcriptional activity in Baikal Seals (<i>Pusa sibirica</i>).	Ishibashi et al., 2008b
IC50	B	42	$\mu\text{g}/\text{g}$	Displacement of Fluormone Pan-PPAR Green for Baikal Seals.	Ishibashi et al., 2019
SSD	W	2.52	$\mu\text{g}/\text{l}$	Endocrine disruptive effects	Zhang et al., 2024
SSWD	W	3.02	$\mu\text{g}/\text{l}$	Endocrine disruptive effects	Zhang et al., 2024

TDI stands for tolerable daily intake, TWI stands for tolerable weekly intake, EQS stands for environmental quality standard, LOEC stands for lowest observable effect concentration, IC50 stands for inhibitory concentration 50 %, NOEC stands for no-observable effect concentration, SSD stands for species sensitivity distribution, SSWD stands for species sensitivity weighted distribution, and ww. stands for wet weight. B/W denotes whether the threshold is for biota (B) or for water (W). PPAR stands for peroxisome proliferator-activated receptors.

equation was modified to fit Saimaa ringed seals as follows:

$$QS_{biota, srs} = \frac{0.1 \times TL \times X}{Y} \quad (2)$$

where 0.1 also refers to the percentage of consumed fish that exceeds the threshold level (European Commission, 2011a). The same threshold level was used for PFOS as the European Food Safety Authority (EFSA) used for humans (0.15 $\mu\text{g}/\text{kg}$ bw/d) (EFSA, 2008). X was the mean seal body weight and Y the mean amount of fish that seals consume daily.

Quality standards were calculated for two different age classes of seals: weaned pups and adults. For weaned pups, the body weight of 20 kg was used in calculations (Kunnasranta et al., 2021). In the case of adults, 59 kg was used as the average body weight. To our knowledge, daily fish consumption has not been studied for weaned ringed seals. Therefore, average daily fish consumption of 3.5 kg (Kunnasranta et al., 1999) was used for weaned pups and adult seals as the calorie intake of pups is roughly the same as it is for adults (Lowry et al., 1980). For simplicity, the average for both body weight and daily fish consumption is used, even though these metrics may vary throughout the year. The use of averages increases the uncertainty of the derived quality standards to a degree, which is inherently the case in all quality standards and thresholds. Additionally, Eqs. (1) and (2) accept only one value for weight variable and hence the use of the average is justifiable. This also produces a singular quality standard for both age classes, which is then easier to apply to the toxicity evaluation. This equation was not used for lanugo pups since their only source of nourishment is mothers' milk and the equation is calibrated for fish consumption.

In addition to the environmental quality standards calculated in this study, environmental quality standards from EC (2011a), tolerable weekly and daily intake (TWI and TDI, respectively) by European Food Safety Authority (EFSA) (2008, 2018, 2020), lowest observed effective concentration (LOEC) and inhibitory concentration 50 (IC50) by Ishibashi et al. (2008b, 2019), and thresholds by Zhang et al. (2024), were used to evaluate the possibility for adverse effects by PFOS to the Saimaa ringed seal population (Table 1).

The environmental quality standards and thresholds were compared to the observed PFOS concentrations from this study, in typical seal prey fish (Kunnasranta et al., 1999; Auttila et al., 2015) (SI 1 Tables 2 and 3), and in water samples (SI 1 Table 4). PFOS concentration data were available for perch (*Perca fluviatilis*, $n = 22$ pooled samples), roach (*Rutilus rutilus*, $n = 15$), vendace (*Coregonus albula*, $n = 60$), and for water samples ($n = 3$) from Lake Saimaa. This data were used to evaluate the possibility for adverse effects of PFOS to this seal population. Perch data are from the environmental burden database, KERTY (Finnish Environment Institute, 2024) and from study by Suomi et al. (2024). Roach and vendace data are from study by Suomi et al. (2024). PFOS data for freshwater were collected from the KERTY database (Finnish Environmental Institute, 2024). The KERTY database is an open database managed by the Finnish Environment Institute.

3. Results

3.1. PFOS in seals

PFOS was observed in all analysed tissues (SI 2). The highest PFOS concentrations were observed in kidney and liver samples of all age classes (Fig. 2). This difference was statistically significant in lanugo pups and adults (Table 2), although not between kidney and liver. In lanugo pups, the mean kidney and liver PFOS concentrations were 19.8 ($\sigma 12.0$) ng/g ww. ($n = 12$) and 26.8 ($\sigma 12.8$) ng/g ww. ($n = 12$), respectively (Table 3). In weaned pups, the mean kidney and liver concentrations were 9.3 ($\sigma 7.1$) ng/g ww. ($n = 2$) and 26.7 ($\sigma 8.9$) ng/g

ww. ($n = 2$), respectively (Table 3). For adult Saimaa ringed seals, the mean kidney and liver concentrations were 9.0 ($\sigma 2.7$) ng/g ww. ($n = 3$) and 20.4 ($\sigma 14.3$) ng/g ww. ($n = 3$), respectively (Table 3).

There were no statistically significant differences in tissue PFOS concentrations between the lanugo pups that had consumed milk and those that had not ($n = 52$, F-statistic = 0.82, $p = 0.37$). Therefore, they could be included in the subsequent statistical analyses. Additionally, there was no difference in tissue PFOS concentrations between sexes ($n = 52$, F-statistic = 1.34, $p = 0.25$). However, they had significantly higher PFOS tissue concentrations than the older seals ($n = 64$, ANOVA F-statistic = 7.3, Post hoc $p \leq 0.01$ between lanugo pups and adults, $p = 0.04$ between lanugo and weaned pups). Placentas were omitted from the analysis between age classes. There was no statistical difference in tissue PFOS concentrations between the weaned pups and adult seals. Furthermore, lanugo pups from Pihlajavesi (Fig. 1) showed higher tissue PFOS concentrations than from Haukivesi ($n = 52$, F-statistic = 10.37, $p \leq 0.01$). In placentas however, neither degree of preservation nor region had effect on PFOS concentrations ($n = 12$, F-statistic = 0.92, $p = 0.45$, F-statistic = 0.98, $p = 0.36$, respectively). Additionally, a correlation between PFOS concentrations in placenta and lanugo pup livers was observed ($\log \text{cor} = 0.72$, $p \leq 0.01$). This connection was further validated by regression analysis ($\log r^2 = 0.47$, $p \leq 0.01$).

3.2. Toxicity evaluation

Based on the calculation described in Eq. (2), the environmental quality standard for the weaned pups was 0.09 ng/g ww., and 0.25 ng/g ww. for adults. Both environmental quality standards were exceeded in all the perches and roaches between the years 2012 to 2023 (SI 1 Tables 2 and 3). However, in vendace the environmental quality standard was exceeded for weaned pups and adults in Puruvesi (Pihlajavesi region), but only for weaned pups in Haukivesi. The environmental quality standard for humans, derived from Eq. (1), yielded the result of 9.1 ng/g ww., which was exceeded with only the perches sampled from Southern Saimaa in 2014.

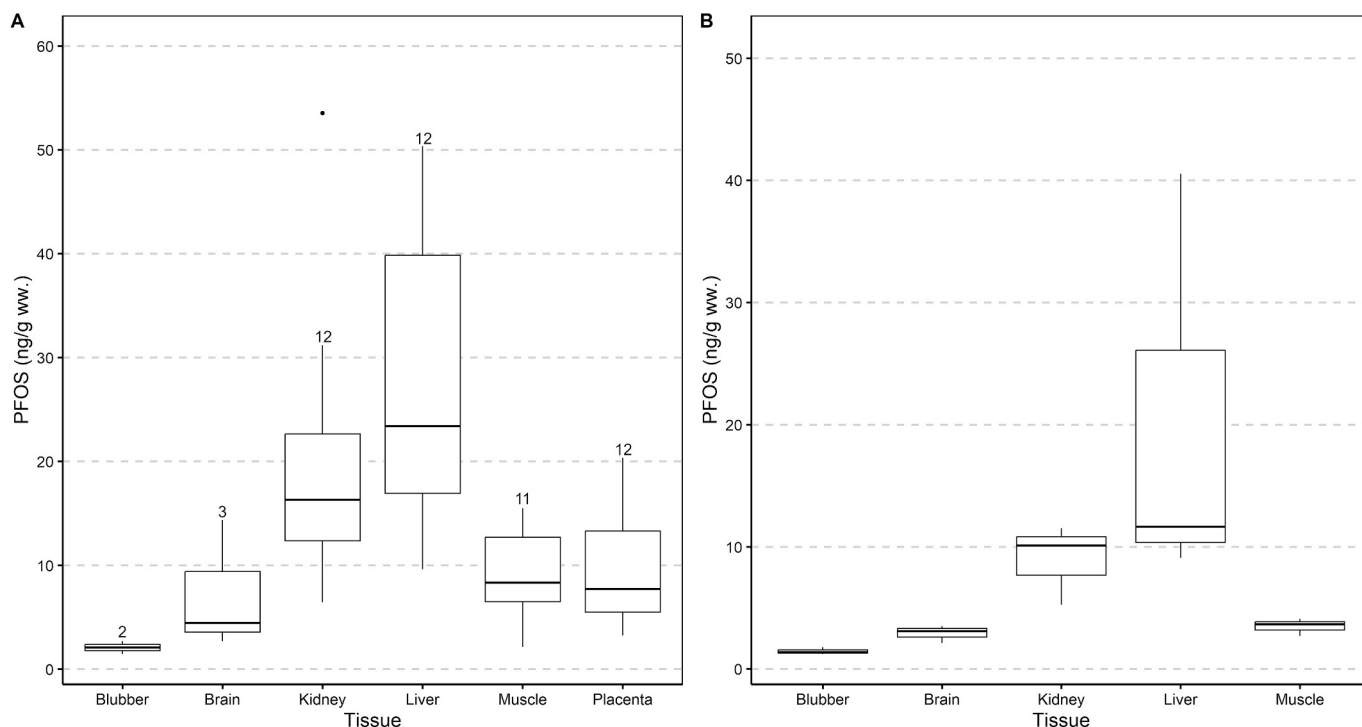


Fig. 2. Perfluorooctanesulfonate (PFOS) concentrations in Saimaa ringed seal lanugo pups ($n = 12$) (A) and adults ($n = 3$) (B). The solid lines within boxes denote medians while the boxes cover the middle 50 % of the data. Whiskers present the minimum and maximum quartiles. Dots show values that the boxplot deemed to be outliers. Numbers above the boxes denote the number of samples per tissue type for lanugo pups. For adults the number of samples for each tissue type was three.

Table 2
Tukey HSD test p-values for PFOS concentrations in different tissues of the Saimaa ringed seals.

	Blubber	Brain	Kidney	Liver	Muscle	Placenta
<i>Lanugo pups</i>						
Blubber (n = 2)		0.39	<0.001 (Ki)	<0.0001 (Li)	0.04 (Mu)	0.03 (Pl)
Brain (n = 3)	0.39		<0.05 (Ki)	<0.01 (Li)	0.93	0.88
Kidney (n = 12)	<0.001 (Ki)	<0.05 (Ki)		0.74	0.03 (Ki)	0.04 (Li)
Liver (n = 12)	<0.0001 (Li)	<0.01 (Li)	0.74		<0.001 (Li)	<0.001 (Li)
Muscle (n = 11)	0.04 (Mu)	0.93	0.03 (Ki)	<0.001 (Li)		<1.0
Placenta (n = 12)	0.03 (Pl)	0.88	0.04 (Li)	<0.001 (Li)	<1.0	
<i>Adults</i>						
Blubber (n = 3)		0.38	<0.01 (Ki)	<0.001 (Li)	0.19	
Brain (n = 3)	0.38		0.07	<0.01 (Li)	0.98	
Kidney (n = 3)	<0.01 (Ki)	0.07		0.42	0.16	
Liver (n = 3)	<0.001 (Li)	<0.01 (Li)	0.42		0.01 (Li)	
Muscle (n = 3)	0.19	0.98	0.16	0.01 (Li)		

Significant results are in bold (p < 0.05). Abbreviations in brackets denote which tissue has higher PFOS concentration. Ki stands for kidney, Li stands for liver, Mu stands for muscle, and Pl stands for placenta. n denotes the number of samples for each tissue type.

Table 3
Tissue PFOS concentrations in Saimaa ringed seals. All concentrations are in ng/g ww.

Tissue	n	Median	Mean	St.dev.	Min	Max
<i>Lanugo pups</i>						
Blubber	2	2.1	2.1	0.6	1.5	2.7
Brain	3	4.4	7.2	5.1	2.7	14.3
Kidney	12	16.3	19.8	12.0	6.4	53.5
Liver	12	23.4	26.8	12.8	9.6	50.3
Muscle	11	8.3	9.1	4.3	2.1	15.5
Placenta	12	7.7	9.8	5.6	3.2	20.3
<i>Weaned pups</i>						
Blubber	2	1.5	1.5	0.5	1.1	2.0
Brain	1				2.4	2.4
Kidney	2	9.3	9.3	7.1	2.1	16.4
Liver	2	26.7	26.7	8.9	17.8	35.6
Muscle	2	4.9	4.9	3.2	1.7	8.1
<i>Adults</i>						
Blubber	3	1.4	1.5	0.2	1.4	1.8
Brain	3	3.1	2.9	0.6	2.1	3.5
Kidney	3	10.1	9.0	2.7	5.2	11.5
Liver	3	11.6	20.4	14.3	9.1	40.6
Muscle	3	3.6	3.5	0.6	4.1	3.6

Tolerable daily intake calculation for adult seals based on EFSA (2008) recommendation, provided a value of 8850 ng/d (Table 1). This threshold was exceeded only with perches in Haukivesi from 2023, Northern Saimaa from 2015, and in Southern Saimaa from 2014. The tolerable weekly intake recommended by EFSA (2018), resulted in a threshold of 767 ng/w, which was exceeded with perches and roaches from all locations between 2012 and 2023. Tolerable weekly intake threshold was exceeded with vendace from Puruvesi from 2023, but not from Haukivesi from 2022. However, the tolerable daily intake recommended by EFSA (2020) resulted in a threshold of 5.9×10^6 ng/d, which was not exceeded with any of the fish samples from Lake Saimaa.

Contrary to the thresholds described above, the PFOS concentrations in all the fish were below the environmental quality standard set for secondary poisoning of predators by EC (2011a). In similar fashion, all the seal PFOS tissue concentrations were below the LOEC and IC50 thresholds provided by Ishibashi et al. (2008b, 2019). Additionally, PFOS concentrations in all three sampling points in Haukivesi from 2014 (SI Table 4) were below the environmental quality standard set for secondary poisoning of predators in freshwaters by EC (2011a) as well as the thresholds proposed by Zhang et al. (2024).

4. Discussion

PFOS was present in all analysed seal tissues, with the highest concentrations in liver and lowest in blubber (Table 3), which is not surprising as these toxins have been shown to accumulate into the tissues with high protein content (Ahrens and Bundschuh, 2014; Pizzurro et al., 2019). The observed high concentrations of PFOS in the kidneys of lanugo pups (Fig. 2), were in contrast with lower levels observed in the older seals, which suggests that the pups may be more vulnerable to PFOS accumulation due to immature detoxification processes or different pathways during their early development. The observed high individual variation in tissue-specific PFOS concentrations, coupled with the limited number of adult samples, suggests that these factors may influence the reliability and generalizability of the study's findings, especially on inter-age comparisons between pups and adults. The samples for adult seals from KERTY (SI 1 Table 5, n = 6) had liver mean PFOS concentrations roughly 14 times higher than the adults from this study, which would indicate a considerable decrease in PFOS concentrations in adult seals during the past few years. This apparent decline in PFOS concentrations may be influenced by confounding factors such as difference in sampling years and regions as well as in analytical methods. Additionally, PFOS was added to the Stockholm Convention on Persistent Organic Pollutants (SC-POPs) annex around the time of sampling of adult seals in KERTY database, which also could be the source for this decline (UNEP, 2024a, 2024b). However, a similar decrease cannot be seen in the perches in Lake Saimaa (SI 1 Tables 2 and 3) (Finnish Environment Institute, 2024; Suomi et al., 2024). PFOS concentrations in the perches from the KERTY database seem to be affected by sampling year and location (Finnish Environment Institute, 2024) but this does not explain the decrease in adult seal tissue concentrations. Whatever the reason, PFOS concentrations in adult Saimaa ringed seals seem to have declined during the last decade even though a similar trend cannot be seen in one of their typical prey fishes, perches.

4.1. Lanugo pups and placentas

Lanugo pups had significantly higher PFOS concentrations than the older seals (Fig. 2 and Table 3), which could be explained by nutrition of the nursed pups. Isotope study by Auttila et al. (2015) discovered that pups are shown to be at a higher trophic level than the adults as pups receive nutrients from their mothers during gestation and via nursing. A similar pattern has been noted in DDE and PCB concentrations in some populations of harbour seals (Neale et al., 2009; Mos et al., 2010). In Alaska and California, the concentrations of these chemicals decreased with age in pups and adult females but on conversely, increased in adult males (Neale et al., 2009). Therefore, it would seem that the exposure to environmental chemicals is high during the nursing period and

decreases as the pups are weaned. Moreover, the study's findings demonstrate that PFOS crosses the placental barrier and accumulates into the pups, indicating that the placenta is ineffective in preventing the transfer of this chemical from mother to foetus, raising concerns about the potential prenatal exposure and developmental risks associated with PFOS. This finding is in accordance with the previous studies from Saimaa ringed seals (Simola et al., 2024) and other marine mammals (Grønnestad et al., 2017; Pizzurro et al., 2019; Taylor et al., 2021; Lee et al., 2023). Current study supports earlier findings by Simola et al. (2024) that placentas could be used as biomonitoring tools for exposure to environmental toxins.

4.2. Toxicity evaluation

In this toxicity evaluation we considered only PFOS as it is in general the most common PFAS in pinnipeds as observed for example in Australian sea lion (*Neophoca cinerea*) and Australian fur seal (*Arctocephalus pusillus doriferus*) pups (Taylor et al., 2021), in grey seal pups from Norway (Defago, 2023) and in the ringed seals from the Northern Baltic Sea (Roos et al., 2019). PFOS contamination is lower in Lake Saimaa than in many other aquatic environments inhabited by pinnipeds (Gebbinck et al., 2016; Roos et al., 2019; Taylor et al., 2021). For example, juvenile Baltic ringed seals (Roos et al., 2019) and Australian sea lion pups (Taylor et al., 2021) had higher liver PFOS concentrations (range 9.4–400 ng/g ww., $n = 69$, and 27.4 ng/g ww., range 10.5–2119 ng/g ww., $n = 20$, respectively) than Saimaa ringed seals. Similarly, ringed seals from the East coast of Greenland (Gebbinck et al., 2016) and the Scoresby Sound (Boisvert et al., 2019) had higher PFOS liver concentrations (93 ng/g ww., $n = 10$, and 108 ng/g ww., $n = 16$, respectively) than seals from Lake Saimaa. However, PFOS concentrations in the liver of adult seals (liver mean 13.0 ng/g ww., range 2.6–38, $n = 44$) from Lake Baikal reported by Ishibashi et al. (2008a) were equivalent to the concentrations observed in the current study for adult seals from Lake Saimaa. Furthermore, the Australian sea lion pups had generally lower liver PFOS concentrations (7.14 ng/g ww., 1.0–16.9 ng/g ww., $n = 28$) than the pups from Lake Saimaa. Altogether, marine pinnipeds seem to have higher PFOS liver concentrations than the seals in Lake Saimaa.

In addition to the evident PFOS contamination in marine and freshwater environments, adverse effects of PFOS exposure have been observed in humans and wildlife (Androulakis et al., 2022; Lee et al., 2023; Lohmann et al., 2024; Shi et al., 2024). These effects have been observed in pinnipeds as changes in hepatic protein function that regulate gene expression (Ishibashi et al., 2008b, 2019) and disturbance in steroid homeostasis (Defago, 2023). These observations support the use of PFOS as the basis of our toxicity evaluation as it is a common PFAS and it has been shown to have adverse effects, especially in other seal species.

The environmental quality standards for PFOS contamination calculated in this study for Saimaa ringed seal prey fish were based on the equation (Eq. (1)) used by the EC (2011a) to calculate a similar standard for humans. Even though the PFOS exposure in fish from Lake Saimaa is currently not considered to be of concern to humans (Suomi et al., 2024), the situation may be different for Saimaa ringed seals as they consume fish in considerably higher quantities than average humans. Therefore, it is not surprising that the environmental quality standard calculations produced clearly lower standards for the seals (0.09 ng/g ww. and 0.25 ng/g ww. for pups and adults, respectively) as compared to those for humans (9.1 ng/g ww.).

The PFOS environmental quality standards for prey fishes were exceeded in perch and roaches throughout Lake Saimaa and in vendace from Puruvesi basin (Fig. 1) for weaned pups and adults alike. This would suggest that the varied diet is not likely to protect the seals from PFOS or PFAS exposure. High PFOS exposure is further suggested as some of the literature thresholds used in this toxicity evaluation (Table 1) were exceeded in prey fish of Saimaa ringed seals.

Furthermore, this study only considered PFOS, but there are a multitude of PFAS present in the fish of Lake Saimaa (Kumar et al., 2022; Suomi et al., 2024), which indicates that the overall PFAS exposure from the prey fish could be significantly higher than what is seen in this study. Moreover, the mixture of PFAS may be more harmful than PFOS alone (Dale et al., 2022; Sadrabadi et al., 2023), which indicates that the overall PFAS exposure may be more detrimental than what the results of the PFOS toxicity evaluation would suggest. Altogether, this evidence would suggest that PFOS and therefore PFAS may have detrimental effects in Saimaa ringed seals. However, as some of the thresholds used for toxicity evaluation were not exceeded and are not calibrated for seals, this result should not be considered unequivocal.

5. Conclusions

PFOS was found in all analysed tissues of the Saimaa seals and the highest PFOS concentrations were found in the liver. Additionally, the lanugo pups seem to also accumulate high PFOS concentrations into the kidneys. The PFOS concentrations in the liver of adult seals observed in this study were lower than those observed at the beginning of 2000's. The source for this decrease may stem from the addition of PFOS to SC-POPs annex list, but confounding factors such as variability in sampling sites and years may hamper the assessment. Furthermore, PFOS was shown to reach the placenta resulting in exposure of the foetuses and a correlation was observed between PFOS concentrations in the placenta and lanugo pup liver. This indicates that a change in the placenta PFOS concentrations should result in a similar change in the lanugo pups, which in turn further validates the possibility to use placentas in the non-invasive biomonitoring of chemicals that can pass the placental barrier as proposed by Simola et al. (2024). The toxicity evaluation revealed that PFOS and therefore PFAS may accumulate into the seals in concentrations high enough to have detrimental effects, but this result cannot be considered unequivocal due to the confounding factors of the toxicity evaluation.

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CRediT authorship contribution statement

Jesse Simola: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mervi Kunnasranta:** Writing – review & editing. **Seppo Auriola:** Writing – review & editing, Visualization, Resources, Methodology. **Jarkko Akkanen:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Research data available as supplementary data to the article

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