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Per- and polyfluoroalkyl substances (PFAS) – unravelling exposure sources and demographical exposure patterns in a Swedish adolescent population

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Per- and polyfluoroalkyl substances (PFAS) – unravelling exposure sources and demographical exposure patterns in a Swedish adolescent population

Abstract

Per- and polyfluoroalkyl acids (PFAS) is a group of anthropogenic substances, with some suspected to cause adverse health effects in humans. Little is known about adolescent exposure to PFAS, thus this thesis focuses on exposure sources and demographical exposure patterns of PFAS in a nationally representative Swedish adolescent (age 10-21 years) population (*Riksmaten Adolescents 2016-17*). Legacy PFAS, perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS) were detected in serum from 70-100% of the participants (n=1098). Concentrations of PFOA, PFHxS and PFOS in serum increased with increased concentrations in drinking water (DW). Even though median PFAS concentrations in DW were as low as <1 ng/L, DW appeared as an important exposure source. Participants adhering to a diverse and healthy diet had higher PFNA, PFDA, PFUnDA and PFOS concentrations in serum than those that did not. This association was likely driven by a higher seafood consumption as a part of the healthy diet, supporting the notion that diet, apart from DW, is an important exposure source. Serum PFAS levels were on average highest among participants born in countries with high per capita income as compared to those born in low income countries. Conclusively, PFAS are chemicals of the industrialised world, with DW and seafood emerging as important exposure sources for adolescents. The findings of this thesis are important to consider in future risk assessments, as well as in studies of PFAS exposure and adolescent health.

Keywords: Biomonitoring, PFAA, tap water, healthy diet, exposure, adolescents

Per- och polyfluorerade alkylsubstanser (PFAS) – bedömning av exponeringskällor och demografiska exponeringsmönster hos svenska ungdomar

Abstract

Per- and polyfluoroalkyl syror (PFAS) är en stor grupp antropogena ämnen, där några misstänks vara kopplade till negativa hälsoeffekter hos människa. Idag finns det knapphändigt med information gällande både källor och demografiska mönster för PFAS-exponering bland ungdomar. Detta undersöktes därför i en nationellt representativ ungdomspopulation (*Riksmaten ungdom 2016-17*, ålder 10-21). Perfluoroktansyra (PFOA), perfluornonansyra (PFNA), perfluordekansyra (PFDA), perfluorundekansyra (PFUnDA), perfluorhexansulfonsyra (PFHxS) och perfluoroktansulfonsyra (PFOS) detekterades i serum från 70-100% av ungdomarna (n=1098). Halten av PFOA, PFHxS och PFOS i serum ökade med ökad halt av ämnena i deltagarnas dricksvatten. Medianhalterna i dricksvattnet var så pass låga som <1 ng/L vilket tyder på att lågkontaminerat dricksvatten var en viktig exponeringskälla för ungdomar. De deltagare som konsumerade en mångsidig och hälsosam kost hade högre serumhalter av PFNA, PFDA, PFUnDA och PFOS i jämförelse med de som inte åt denna typ av kost, en association som sannolikt drevs av en högre konsumtion av fisk/skaldjur bland de som följde en hälsosam kost. Dessa resultat styrker att kosten, förutom dricksvatten, är en viktig exponeringskälla för ungdomar i Sverige. Deltagare födda i länder med hög per capita-inkomst hade klart högre medelhalter av PFAS i serum än de som var födda i låg-inkomstländer. Sammanfattningsvis har ungdomarnas födelseland stor påverkan på serumhalterna av PFAS, och dricksvatten och fisk/skaldjur tycks vara viktiga exponeringskällor. Dessa fynd bör beaktas i framtida riskbedömningar av PFAS och i studier av samband mellan ungdomars PFAS-exponering och hälsa.

Nyckelord: Biomonitorering, PFAA, kranvatten, hälsosam kost, exponering, ungdomar

Dedication

To a PFAS free world

"What is a 'weekend'?" – Violet Crawley, Downton Abbey, Season 1

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I. NYSTRÖM, J.*, BENSKIN, J.P., PLASSMANN, M., SANDBLOM, O., GLYNN, A., LAMPA, E., GYLLENHAMMAR, I., MORAEUS, L., LIGNELL, S. (2022). Demographic, life-style and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents. *Environmental Research*, 208, 112674.

II. NYSTRÖM, J.*, BENSKIN, J.P., PLASSMANN, M., SANDBLOM, O., GLYNN, A., LAMPA, E., GYLLENHAMMAR, I., LIGNELL, S., MORAEUS, L. (2022). Healthy eating index and diet diversity score as determinants of serum perfluoroalkyl acid (PFAA) concentrations in a national survey of Swedish adolescents. *Environmental Research*, 212, 113170.

III. NYSTRÖM-KANDOLA, J.*, AHRENS, L., GLYNN, A., JOHANSON, G., BENSKIN, J.P., GYLLENHAMMAR, I., LIGNELL, S., VOGS, C. (2023). Low concentrations of perfluoroalkyl acids (PFAAs) in municipal drinking water associated with serum PFAA concentrations in Swedish adolescents. *Environment International*, 180, 108166.

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The contribution of Jennifer Nyström-Kandola (**JNK**) to the papers included in this thesis was as follows:

I. **JNK** was involved in the planning and conceptualization of the paper. **JNK** carried out the data curation, methodology and formal analysis. **JNK** wrote the first and final draft, with input from fellow authors.

II. **JNK** was involved in the planning and conceptualization of the paper. **JNK** carried out the data curation, methodology and formal analysis. **JNK** wrote the first and final draft, with input from fellow authors

III. **JNK** was involved in the planning and conceptualization of the paper. **JNK** carried out some of the data collection and data curation, methodology and formal analysis. **JNK** wrote the first and final draft, with input from fellow authors.

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1. Introduction

1.1 Food safety

Food and drinking water (DW) are two of the most fundamental prerequisites for human health and well-being. Food and DW safety and security have therefore been recognized as a major public health priority, and play an integral role within the United Nations 2030 Agenda for Sustainable Development. Access to safe food/water and nutritious food is integrated in many of the 17 Sustainable Development Goals (WHO 2022a). Yet, communities all around the world struggle to secure safe food and DW due to the presence of microorganisms and chemicals, leading to both acute and chronic illnesses (WHO 2022a). Pathogens have historically been, and are still today, one of the leading causes of illness and death from food/DW-borne diseases (Newell et al. 2010; Kirk et al. 2015). Pathogens are however not the only source of concern. History is lined with catastrophes where toxic chemicals have entered the farm-to-fork chain. For example, in the 1950's, methylmercury contamination of seafood caused mass poisoning of inhabitants of Minamata Bay, Japan (Harada 1995). Concurrently, over 4000 Turkish citizens were accidentally poisoned through bread made from hexacholorobenzene pre-treated wheat (Gocmen et al. 1989). In the 1960-70s, among many other chemical- related catastrophes, contamination of rice oils with polychlorinated biphenyls (PCB) and polychlorinated dibenzofurans (PCDF) lead to mass poisoning in both Japan and Taiwan (Masuda 1985). These food crises were one of the reasons for the formation of the Codex Alimentarius, the Joint FAO/WHO Food Standards Programme, in order to protect consumer health and promote fair practices (FAO 2024). Decades later, as a consequence of the rising number of

chemical-related crises in the food chain of Europe, the European Food Safety Authority (EFSA) was established in 2002 with the aim to create a food safety system to combat the challenges that arise in the 21st Century (European Commission 2000; Silano & Silano 2008). Around the same time as EFSA was being established, research started emerging showing the presence of per- and polyfluorinated compounds in both human and environmental matrices (Giesy & Kannan 2001; Brennan et al. 2021).

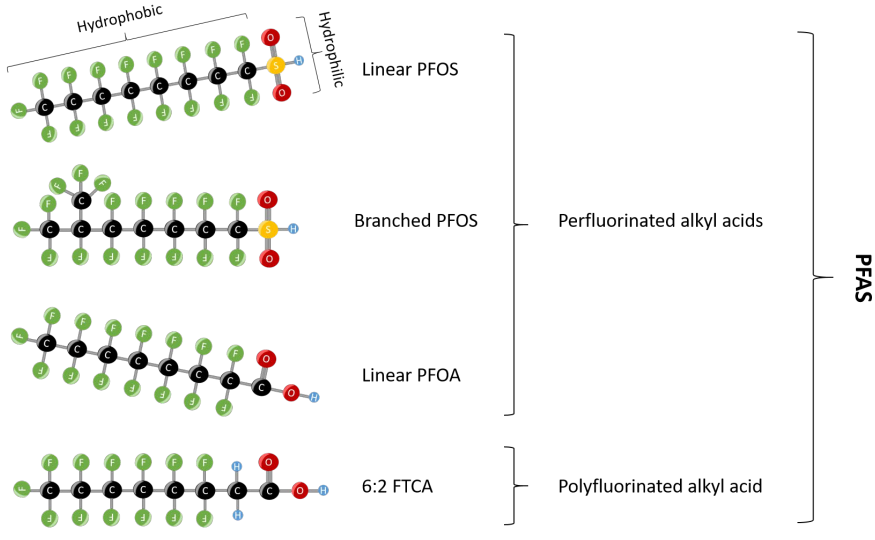

Figure 1. Comparison of the chemical structure between per- and polyfluorinated alkyl acids, and linear and branched isomers of perfluoroocatnesulfonic acids (PFOS). The hydrophobic fluorinated tail, as well as the hydrophilic functional group has been marked out for linear PFOS. Note, 6:2 FTCA is an abbreviation for 6:2 fluorotelomer carboxylic acid.

1.2 Per- and polyfluoroalkyl acids – PFAS

Although there does not exist an international agreement on the definition of PFAS, the Organisation for Economic Co-operation and Development (OECD) defines PFAS as a group of "*fluorinated substances that contain at least one fully fluorinated methyl or methylene carbon atom (without any H/Cl/Br/I atom attached to it)*" (OECD 2021). This definition covers a plethora of substances, 4700 of which have been identified by CAS numbers by the OECD (OECD 2021); the U.S. Environmental Protection Agency's

(US EPA) CompTox Chemicals Dashboard had identified >10,000 PFAS by 2023 (US EPA 2023a).

One of the key traits of PFAS is the hydrophobic fully (per-) or partially (poly-) fluorinated carbon chain which is bound to a hydrophilic functional group (Figure 1). Specifically, the perfluorinated alkyl acids (PFAA) are exceptionally resistant to degradation due to the perfluoroalkyl moiety. Additionally, many polyflourinated alkyl substances have the potential to degrade to form PFAA (Cousins et al. 2020; Zhang et al. 2021). To the legacy PFAA belongs the infamous perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS) (Table 1). Given the functional group, PFAA can be divided into subgroups, such as perfluoroalkyl sulfonic acids (PFSA) and perfluoroalkyl carboxylic acids (PFCA) which contains a $–SO_3H$ group and a $–CO_2H$ group, respectively (Table 1). PFHxS and PFOS belong to the PFSA while PFOA, PFNA, PFDA and PFUnDA belong to the PFCA (Table 1) (Brennan et al. 2021).

Table 1. Legacy perfluoroalkyl acids (PFAA), with full name, acronym, molecular formula and CAS-number provided.

	PFAA	**Acronym**	**Molecular formula**	**CAS-number**
PFCA	perfluorooctanoic acid	PFOA	$C_7F_{15}CO_2H$	335-67-1
	perfluorononanoic acid	PFNA	$C_8F_{17}CO_2H$	375-95-1
	perfluorodecanoic acid	PFDA	$C_9F_{19}CO_2H$	335-76-2
	perfluoroundecanoic acid	PFUnDA	$C_{10}F_{21}CO_2H$	2058-94-8
PFSA	perfluorohexanesulfonic acid	PFHxS	$C_6F_{13}SO_3H$	355-46-4
	perfluorooctanesulfonic acid	PFOS	$C_8F_{17}SO_3H$	1763-23-1

Because of the high stability of the perfluoroalkyl moieties, PFAS are both thermally and chemically stable (Cousins et al. 2020) and the joint hydrophilicity and hydrophobicity gives these compounds excellent surfactant properties (Chen et al. 2024). These desirable properties have resulted in an extensive use of PFAS by industries, with applications in building and construction, manufacturing of electronics, metal plating, mining, oil and gas production, and plastics and semiconductor industries to name a few. PFAS have also been used in a large range of consumer products, including, but not limited to, personal care products, medical

products, textiles, cookware, food packaging, paper, cardboard and fire-fighting foams (Gaines 2023). It is also these desirable properties that makes PFAA persistent with the potential to remain in our environment for centuries (Cousins et al. 2020). The production of PFAS commenced in 1930 with the production of polytetrafluoroethylene (PTFE), commercially known as *Teflon*. Initial production of PFOA and PFOS and related substances began in 1940s, however manufacturing of water resistant products and protective coatings products started in 1950-60s (Renfrew & Pearson 2021). Given the extensive and decades long use, PFAS, specifically PFAA, have been detected in a wide variety of environmental as well as human matrices worldwide (Giesy & Kannan 2001; Banzhaf et al. 2017).

1.3 Toxicity of PFAS

The vast majority of the toxicity data generated for PFAS are limited to only a handful of congeners, predominantly legacy PFAA such as PFOA and PFOS, but recently also PFNA and PFHxS. This is mainly due to the ubiquitous detection of these PFAA in humans. Numerous studies have been undertaken evaluating the health impacts of PFAS on humans, as well as on animals (Fenton et al. 2021). Experimental animal studies of the legacy PFAA report many different toxic effects including liver toxicity, and toxicity on the immune-, developmental-, reproductive- and nervous system. More specifically, toxic effects include delayed eye opening and decreased pup body weight, impaired development of mammary glands, alterations in serum lipid and thyroid hormone levels, hepatocellular necrosis, neurodevelopmental effects and reduced antibody response after antigen challenge, to name a few (ATSDR 2021). Based on human epidemiological studies, PFOS and PFOA have been related to immunosuppressant effects in toddlers and children. More specifically, inverse relationships have been reported between early life exposure to PFOS and PFOA, as reflected in child plasma/serum concentrations, and vaccination antibody levels post-vaccination in standard childhood vaccination programmes (Fenton et al. 2021). Dyslipidemia has been reported amongst both children and adults where observations of a sigmoidal dose-response increase of low- and total density lipoprotein cholesterol levels have been reported (Fenton et al. 2021). For both the immunotoxic and dyslypidemic effects seen in human

populations, comparable effects have been noted *in vivo* in animals (Fenton et al. 2021).

1.4 Risk assessment of PFAS

Given the toxicity of legacy PFAA and the ubiquitous presence in both environment, biota and humans, EFSA revised the health-based guidance value in 2020. In this risk assessment, a tolerable weekly intake was derived for the sum of PFOA, PFNA, PFHxS and PFOS ($\sum_4$PFAA) at 4.4 ng/kg body weight per week. The risk assessors identified the immunomodulating effects observed in epidemiological studies as the most sensitive adverse effect, with toddlers being the most sensitive group. More specifically, the TWI is based on the long-term intake of $\sum_4$PFAA in 35-year-old primiparous women that results in a 10% decrease in diphtheria antibody levels after vaccination in their 1-year-old toddlers who have been breastfed for 12 months. The TWI represents the safe $\sum_4$PFAA intake over the course of a lifetime and protects against other potential toxic effects in humans including increased serum cholesterol, reduced birth weight and high serum levels of liver enzymes (EFSA 2020).

1.5 Human exposure to PFAS

PFAS exposure is multifaceted and can vary greatly both within and between populations. For the general European population, food and DW are considered the major exposure routes of legacy PFOA, PFNA, PFHxS and PFOS (EFSA 2020). Inhalation of indoor dust and air as well as usage of PFAS containing products may contribute to the exposure to a lesser extent. However, for certain populations, exposure patterns might be different to that of the general population. More specifically, for those residing within areas where DW has been highly contaminated with PFAS or those who through their occupation encounter PFAS, the majority of the exposure is directly linked to the highly contaminated exposure sources (DW and working environment) (De Silva et al. 2021; Johanson et al. 2023; Lucas et al. 2023).

Human exposure of toxicants from food and drinking water can be assessed either by calculating the intake from different exposure medias by using data on toxicant concentrations in the medias and data on human consumption of the same medias. Another way to assess exposure is to

measure toxicants in human biomonitoring studies using matrices such as serum/plasma. Human biomonitoring studies provide a good estimate of the cumulative exposure of PFAS before blood sampling (body burden). There exists a great variability in PFAS body burdens within a general population (Toms et al. 2019; Göckener et al. 2020; Fábelová et al. 2023; Hull et al. 2023) which is largely explained by variation in PFAS concentrations within/across exposure mediums that are available for the studied population. Differences in preferences for choice of food, beverages and consumer products further increase the inter-individual variation within the population. These choices are to some extent dependent on the demographics and lifestyle of the study participants, including socio-economic status as well as degree of education, which are known to mediate the human exposure to PFAA (Buekers et al. 2018; Colles et al. 2020). Age is another demographic factor, were the PFAA body burdens are higher in infants and young children compared to adults due to the high exposure that occurs *in utero* and via nursing (Winkens et al. 2017). Other important factors that could affect the inter-individual variation in PFAA body burdens are physiological parameters that may influence the toxicokinetics of PFAS. For example, age, gender and glomerular filtration rate have been suggested to explain some of the variation in PFAA elimination from the body (Li et al. 2022). Although PFAA cannot be metabolised, exposure to and the biotransformation of PFAS precursors to PFAA could potentially also explain certain parts of the variation in PFAA body burdens (Gebbink et al. 2015).

Given the long half-lives of legacy PFAA, i.e. between 2-5 years depending on the specific PFAA (Rosato et al. 2024), measured body burdens reflect the cumulative exposure across the lifetime before sampling. Due to the growing international concern regarding the toxicity, persistence and presence of legacy PFAA in human matrices, a process of phase-out of PFOA, PFOS and by effect also PFHxS, from production and use was initiated in the early 2000s. The phase-out was conducted through a series of policies, regulations as well as "voluntary" phase-out measures taken by industry, across both Europe and North America (Brennan et al. 2021; Renfrew & Pearson 2021). PFNA, PFDA and PFUnDA phase-out was initiated around 2010 (US EPA 2023b). The human exposure of PFOA and PFOS has been estimated to have peaked in the 1990s in Australia and the USA, followed decline at the end of the same decennium (Gomis et al. 2017).

It is reasonable to assume that these temporal exposure patterns were similar in Sweden, as the PFOA, PFHxS and PFOS body burdens among young women started to decrease around the new millennia (Nyberg et al. 2018). Differences in exposure levels over time is therefore another important factor to consider, which could explain a considerable part of the variation in PFAA body burdens within a population of different ages. These differences are also important to consider when comparing results between study populations. For example, a population of 15-year-olds born in 1985, sampled in 2000, would have a considerable different average cumulative exposure of PFOA, PFHxS, PFOS and even PFNA, compared to a population of 15-year-olds born in 2005 and sampled 2020.

1.6 Adolescents – an understudied population

Adolescence is the transitional stage between child and adulthood that is marked by rapid growth and development, in part regulated by different hormonal systems (Cameron 2004; Sawyer et al. 2018). Because of these large physiological changes, adolescents could potentially be more susceptible to exposure of toxicants, which could have long lasting adverse effects on health (Patton & Viner 2007; Schoeters et al. 2008). Human biomonitoring studies investigating demographical/life-style/physiological determinants of PFAA body burdens among adolescents are scarce. Previous studies have mainly focused on adult and child populations (e.g. studies on children include Mogensen et al. 2015; Papadopoulou et al. 2016; Harris et al. 2017; Koponen et al. 2018; Gyllenhammar et al. 2019, and studies on adults include Halldorsson et al. 2008; Ji et al. 2012; Bjermo et al. 2013; Ode et al. 2013; Jain 2014; Kim et al. 2014; Bartolomé et al. 2017). As a consequence, limited information exists on demographic, life-style and physiological determinants that may explain some of the variation of adolescent body burdens of PFAS (Zhou et al. 2016; Averina et al. 2018; Kang et al. 2018) with a growing body of literature as this thesis goes to press (Richterová et al. 2023; Runkel et al. 2023; Sultan et al. 2023; Uhl et al. 2023). Providing information on important determinants explaining at least some of the variation of PFAS body burdens among adolescents is crucial when identifying important exposure sources, groups with high exposure, and potential confounders in future health studies. Moreover, current

measurements of body burdens, in serum/plasma, can be used in future updates for risk assessment of PFAS exposure.

1.7 Objectives

The overarching aim of this thesis was to evaluate demographic/life-style/dietary/drinking water determinants that could explain the variation of PFAA concentrations in serum in the general Swedish adolescent population. The aim was to increase knowledge about drivers of variation in PFAS exposure to produce data that could be used in future studies of PFAS and health of adolescents in Sweden, and in health risk assessments of PFAA. **Paper I** focused on variables related to demographics, lifestyle and physiology to gain more knowledge on the capacity of such variables in mediating PFAS exposure. **Paper II** focused on evaluating specific dietary determinants of PFAA exposure in the adolescent population. As diet consists of a mixture of foods, the relationships with both individual food groups and food indices covering the diet as a whole were evaluated. For **Paper III**, we wanted to evaluate DW as an exposure source of PFAA, specifically of DW that was contaminated by low concentrations of PFAA.

2. Method

This section contains a brief summary of the main material and methods applied for the studies included in this thesis. An in-depth description is provided in each paper.

2.1 Riksmaten adolescents

All papers included in this thesis have utilized data from *Riksmaten adolescents 2016-17* (RMA). RMA is a national dietary survey that was conducted by the Swedish Food Agency (SFA) between autumn of 2016 and spring of 2017, with the help from the regional divisions of Occupational and Environmental Medicine (OEA). In brief, the aim of the survey was to assess the overall dietary habits and nutritional status among Swedish adolescents and using biomonitoring to determine exposures to potentially toxic substances. A representative sample of school-attending adolescents in Sweden were recruited via their schools; selection of schools was based on geographical spread, municipality classification and whether the school was public or charter. To cover the entire adolescent period, school classes from grades 5 (mean age 11.5), 8 (mean age 14.5) and 2nd year of high school (mean age 17.7) were invited to participate (Moraeus et al. 2018). Adolescents that accepted to participate used a validated web-based method (RiksmatenFlex) which included a diet registration section (RiksmatenFlexDiet, RFD) and a questionnaire section (RiksmateFlexQ, RFQ) (Lindroos et al. 2019). In RFD participants retrospectively registered what they ate and drank during two non-consecutive days. In RFQ, participants answered questions concerning demographic, life-style, family and health as well as questions about consumption frequencies of specific foods of interest. Participants were instructed to ask their parents/legal

guardians to fill in parental oriented questionnaires that contained questions the participant might find difficult to answer, i.e. questions such as duration of nursing of participant during infancy and parental educational attainment. Anthropometric measurements (height and weight) were taken on-site by trained staff from SFA and OEA (Moraeus et al. 2018). The Regional Ethical Review Board in Uppsala gave ethical approval (No. 2015/190) and participants, or legal guardians of participants <16 years of age, gave written informed consent to participate in the survey.

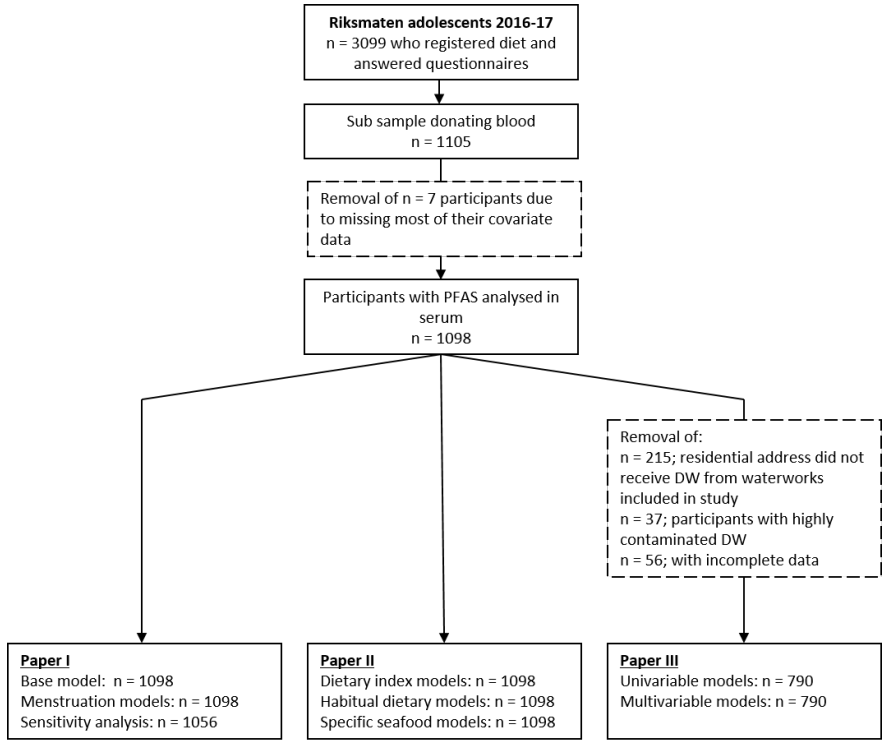

Figure 2. Flowchart of Riksmaten Adolescent 2016-17 study populations included in **Paper I**, **II** and **III**. The flowchart further provides number of participants included in the different regression models included in each paper

Only participants who donated a blood sample were eligible for inclusion in **Paper I**, **II** and **III**. Of the final 3099 participants who completed RFD and RFQ, 1105 participants donated blood samples (SFA 2018) (Figure 2). Out of the participants donating blood, 1098 participants were included in

Paper I and **II**, as these had their serum analysed for PFAS (see section 2.5.1; Figure 2). For inclusion of participants for **Paper III**, participants had to i) only receive municipal distributed drinking water at home, ii) not reside in areas with previous high PFAS contamination in drinking water (Uppsala and Ronneby) and iii) not have any missing data for any covariates included in the models (n=790) (detailed description under section 2.5.1; Figure 2).

2.2 PFAS in serum – sampling and analysis

Trained staff from OEA sampled non-fasting venous blood on-site, during school visits. Following centrifugation, plasma and serum were stored at -20°C during transportation and lastly stored at -80°C awaiting analysis (Moraeus et al. 2018). Serum samples were analysed for PFAS by liquid chromatography-tandem mass spectrometry. The analysis was carried out by the Department of Environmental Science, Stockholm University, Sweden, an included 42 different PFAS, including legacy PFAA and precursor PFAS, and both linear (lin-) and branched (br-) isomers of PFHxS and PFOS, to name a few (**Paper I**, Table S1 in Supplementary Information (SI)). Detailed information of the method is provided in **Paper I**.

2.3 PFAS in drinking water – sampling and analysis

Waterworks that provided RMA schools with drinking water (DW) (n=45) had their incoming raw water (RW) and outgoing DW sampled both in spring and autumn 2018. Grab samples were collected and stored at 4 °C in darkness awaiting analysis. The samples were analysed for 24 PFAS, which included both legacy PFAA, such as PFOA, PFNA, PFHxS and PFOS as well as polyfluorinated precursors, e.g. FOSA and 6:2 FTSA (**Paper III**, Table S1 in SI). The analysis was carried out by the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Sweden, using liquid chromatography and triple quadrupole mass spectrometry. Detailed information on the analytical procedures is found in **Paper III.**

Table 2. Overview of which models that were included in each paper including which covariates that were incorporated into each model.

Paper	Model	Covariate	Comments
Paper I	Base model	Participant/maternal birth country (cat), maternal education level (cat), paternal education level (cat), gender (cat), age (years), BMI status (cat), smoking habits (cat), snuffing habits (cat), alcohol consumption (cat) months fully nursed (cat), Swedish healthy eating index 2015 (SHEIA15) (index)	
	Menstruation model I	Base model covariates + started menstruating (yes/no)	Females only
	Menstruation model II	Base model covariates + length of menstruation (years)	Females only
	Menstruation model III	Base model covariates + ferritin (µg/L plasma) + C-reactive protein (mg/L plasma)	Males and females separately
	Sensitivity analysis	Base model covariates where participants from highly contaminated areas were removed from the analysis	Subsample
Paper II	Dietary index model I	Base model covariates in **Paper I**	
	Dietary index model II	Base model covariates in **Paper I**, where SHEIA15 was replaced by RADDS	
	Habitual dietary models	Base model in **Paper I**, where SHEIA15 was replaced by the habitual consumption (g/day) of the following food groups: cereals, dairy, eggs, fish and seafood, fruits, meats, pastry, potatoes, sweets and vegetables	
	Specific seafood models	Habitual dietary models were habitual consumption of fish and seafood was replaced by daily intake (g/day) of the following seafood groups: anchovies and sardines, Baltic herring, canned tuna, canned herring and mackerel, crab, freshwater fish, large marine fish, lean marine fish, processed fish products, salmonid fish and shellfish	
Paper III	Univariable model	PFAA concentrations in drinking water (ng/L)	
	Multivariable model I	PFAA concentrations in drinking water (ng/L) + participant/maternal birth country (cat) + maternal education level (cat), gender (cat), age (years) + habitual seafood consumption (g/day)	
	Multivariable model II	Multivariable model I + interaction term between PFAA concentration in drinking water and gender	
	Multivariable model III	Multivariable model I + habitual drinking water consumption (mL/day)	

Note: Cat, categorical covariate in which each level description is provided in both Table 1, **Paper I** and Table 1, **Paper II**.

2.4 Data processing

2.4.1 Life-style, demographical and physiological determinants

Life-style, demographical and physiological determinants were selected based on *a priori* knowledge about determinants that could explain some of the variation of PFAS concentrations in serum/plasma. This included participant age and gender, maternal and paternal education level, and participant and maternal birth country, as well as alcohol consumption and tobacco use, months of being breastfed during infancy, menstrual blood loss and plasma ferritin (adjusted for C-reactive protein) as a biomarker for long-term blood loss (**Paper I**). An overview over the specific models and covariates included in **Paper I** is provided in Figure 2 and Table 2, respectively.

The data for the **Paper I** determinants mentioned above, excluding plasma ferritin and C-reactive protein, were extracted from RFD and RFQ. To avoid issues with multicollinearity between participant and maternal birth country (Table 3), a new variable including both participant and maternal birth country was created (BC) based on the per capita gross national income level of the birth countries in accordance with the World Bank Country Classification (World Bank Group, 2018).

2.4.2 Food consumption assessment

Three different types of dietary determinants were evaluated, i.e. dietary indices, habitual (long-term) consumption of commonly consumed food groups, and consumption of specific seafood groups based on the frequency of consumption over the last 12 months prior to participation. An overview over the specific papers and models that evaluated dietary patterns are given in Figure 2 and Table 2.

Table 3. Birth countries of RMA participants and their mothers. The categorisation was carried out in accordance with The World Bank national Income Classification of 2018, which is based on gross national income per capita (World Bank Group 2018).

High income countries	**Upper-middle income countries**	**Lower-middle income countries**	**Low income countries**
Chile	Albania	Bangladesh	Afghanistan
Croatia	Argentina	Bolivia	Eritrea
Czech Republic	Armenia	Egypt	Ethiopia
Denmark	Azerbaijan	India	Democratic Republic of Congo
Finland	Bosnia and Herzegovina	Kenya	Somalia
Germany	Brazil	Laos	Syria
Hungary	Bulgaria	Morocco	Tanzania
Iceland	China	Myanmar	Uganda
Ireland	Colombia	Pakistan	
Israel	Costa Rica	Philippines	
Italy	Iran	Senegal	
Japan	Iraq	Sudan	
Latvia	Kosovo	Tunisia	
Lithuania	Lebanon	Ukraine	
Norway	Libya	Uzbekistan	
Poland	Macedonia	Vietnam	
Portugal	Montenegro	Zimbabwe	
Saudi Arabia	Peru		
Switzerland	Romania		
Singapore	Russia		
South Korea	Serbia		
Spain	Sri Lanka		
Sweden	Thailand		
Taiwan	Turkey		
United Kingdom	Venezuela		
United States of America			

The two dietary indices, Swedish Healthy Eating Index 2015 (SHEIA15; included in **Paper I** and **II**) and Riksmaten Diet Diversity Score (RADDS; **Paper II**), were derived by the SFA (Moraeus et al. 2020). SHEIA15 was developed to quantify diet quality and was based on SFAs dietary guidelines "*Find your way*" and the 2012 Nordic Nutrition Recommendations (Nordic Council of Ministers 2014; Moraeus et al. 2020). The key recommendations are provided in Table 4. SHEIA15 was calculated as the ratio between the registered consumption/intake by the participant and the recommended consumption/intake of each food or nutrient. Consumption was based on the two-day registration in RFD, which was converted to a habitual (long-term) intake using the Multiple Source Method (Moraeus et al. 2020). Each

recommendation could yield a maximum score of 1 and minimum of 0, which were added up to a total maximum score of 9 (Moraeus et al. 2020) (Table 4). RADDS was developed with the aim to quantify diet diversity and was derived in relation to the recommendations in "*Find your way*". The index was based on 17 specific food groups (Table 4), in which participant received one point if they consumed >5 grams of a specific food group during the two days of dietary registration. Thus, the maximum point was 17.

The association between serum PFAA concentrations and habitual consumption of individual food groups (g/day) were evaluated in **Paper II**. Selection of food groups in the habitual consumption calculations were based on the most frequently consumed food groups amongst Swedish consumers, in accordance with the Swedish Market Basket survey (SFA 2017a) and included cereals, dairy, eggs, fruits, meats, pastry, potatoes, seafood, sweets and vegetables. Habitual consumption was estimated using the Multiple Source Method (MSM 1.0.1). This method utilizes the two non-consecutive 24-h dietary recall registrations from which it estimates the daily usual amount of a specific food group that is consumed and the probability of consuming said food group given a random day, adjusting for determinants predicative for consumption of that food group. Such determinants include age and gender as well as data from the food frequency questions in order to separate non-consumers from true non-consumers (Harttig et al. 2011; Haubrock et al. 2011). All participants with complete registration in the RFD (n=3099) were used for the calculations.

Consumption of specific seafood groups were evaluated in **Paper II** (Figure 2; Table 2). This consumption was calculated by using the frequency of consumption over the last 12 months, as registered in the questionnaires in RFQ, and multiplying this frequency with the average portion size for the seafood in question (g) observed by grade and gender in RMA. The daily intake (g/day) was derived for the following seafood groups: shellfish (excluding crab), crab, lean marine fish, salmonid fish, processed fish products, large marine fish, freshwater fish, Baltic herring, canned herring and mackerel, anchovies and sardines and canned tuna.

Table 4. Composition of the dietary indices included in **Paper I** and **II**, Swedish Health Eating Index for Adolescents 2015 (SHEIA15) and Riksmaten diet diversity score (RADDS). For SHIEA15, the seven key dietary advice are provided, on which the nine components are based, alongside the calculation of participant score for each component. For RADDS are each of the 17 food groups accounted for, including specific comments relating to inclusion/exclusion of specific foods for each food group. Note that the information provided in this table is a compilation of Tables 1 and 2 in Moraeus et al. (2020).

SHEIA 15		
Key dietary advice	**Recommendation**	**Calculation**
More seafood	45 g fish and shellfish/day	(Intake g/day)/(45 g/day)
More vegetables and fruits	Minimum of 500 g fruits and vegetables per day (potatoes excluded)	(Intake g/day)/(500 g/day)
	Fiber intake of 2.5 g/MJ	(Intake g/MJ)/(2.5 g/MJ)
Switch to healthy fats	Minimum 7.5% of total energy to come from polyunsaturated fatty acids	(Total energy %)/(7.5%)
Switch to low fat dairy products	Minimum 15% of total energy to come from monounsaturated fatty acids	(Total energy %)/(15%)
	Maximum of 10% of total energy to come from saturated fatty acids	1 – ((total energy% – 10%)/10%)
Switch to wholemeal	Minimum of 75 g whole meal/10 MJ	(Intake g/MJ)/(75 g/MJ)
Less red and processed meats	Maximum of 500 g red and processed meat/week	1 – ((intake g/week – 500 g/week)/500 g/week)
Less sugar	Maximum of 10 % of total energy to come from added sugar	1 – ((total energy% - 10%)/10%)
RADDS		
Food group	**Comment**	
Wholegrain varieties of bread, grains, pasta and rice	Excluding composite dishes	
Berries		
Cabbage		
Pulses		
Root vegetables	Including raw and cooked, as main dish, side dish and in composite dishes	
Other vegetables		
Fruit	Including fruits in smoothies, and excluding deserts, dried fruits and juice	
Milk		
Milk replacements		
Fermented dairy products	Excluding composite dishes	
Egg and egg dishes	Excluding eggs from composite dishes	
Poultry		
Red meat	Including red meat from both main component in composite dish and as main dish	
Vegetarian protein	I.e. dishes with pulses and replacement products	
White fish	Including white fish as a main component in composite dishes or as a main dish	
Shellfish		
Oily fish		

2.4.3 Drinking water exposure assessment

Each participant was matched with the corresponding waterworks that delivered DW to their school and home (**Paper III**). This pairing was carried out using the school and residential postal code, which were cross-referenced against postal codes within the distribution area of the waterworks. The postal codes within the distribution area were provided either directly by the waterworks or derived using names of the areas within the distribution area provided by the water works. In the latter event, the online postal service PostNord (PostNord 2022) was used to match the distribution area names with postal codes of the participants. Only participants receiving DW from the participating waterworks to both their home address as well as their school were included in the analysis. Participants with an alternative DW source at home, e.g. private well, were excluded from the analysis. Above-mentioned exclusions were carried out in order to ensure that the main source of DW came from the participating waterworks.

As conventional RW treatment in most cases fails to remove PFAS from DW (Appleman et al. 2014), we planned to use data on concentrations of PFAS in both DW and RW. To determine if there were differences in RW and DW PFAS concentrations, a paired t-test was used to compare concentrations in RW and DW separately for spring and autumn. As no consistent significant differences were detected, an arithmetic mean concentration was calculated using all the four samples (two DW and two RW) for each waterworks, as described in **Paper III**. If more than one waterworks delivered DW to school or home address, a weighted mean concentration was calculated taking the proportions of DW delivered from each waterworks into account, as reported by the waterworks. If water distribution information was not provided by the waterworks, equal contribution of water from each water works to the finished DW was assumed.

2.4.4 Processing of serum PFAS concentrations

Serum PFAS concentrations below the limit of quantification (LOQ) were replaced with measurable values that were equal to or above the limit of detection (LOD) in order to reduce the systematic bias that is introduced when replacing left-censored data with LOQ/2 or LOQ/$\sqrt{2}$ (RSC, 2001; Bergstrand and Karlsson, 2009). A few participants had concentrations

<LOD (Table 2, **Paper I**), and replacement of such data varied between studies. In **Paper I** and **II**, participants had their PFAS data <LOD replaced with 0.0001 ng/g in order to be included in the statistical analysis. In **Paper III** had participants with concentrations <LOD had their PFAS data replaced with LOD/$\sqrt{2}$.

To produce stable regression models, only PFAS with a detection frequency ≥70% were included in the statistical analysis in **Paper I** and **II**, i.e. lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin-/br-PFOS (Table 2, **Paper I** and Table 3, **Paper II**). In **Paper III**, lin-PFOA, PFNA, lin-PFHxS and lin-PFOS (and the sum of these PFAA; $\sum_4$PFAA) were included in the statistical analysis as PFDA and PFUnDA were not detected in the DW, and br-PFHxS and br-PFOS were not analysed in the DW samples (Table 2, **Paper III**).

2.5 Statistical analysis

To investigate the association between PFAA serum concentrations and determinants of interest, data were fitted using a suitable regression analysis. Statistical analysis was carried out in R, Version 3.6.3 in **Paper I** and **II** and Version 4.0.4 in **Paper III** (R Development Core Team) and statistical significance was set to $p \leq 0.05$. Additional statistical analyses in the corresponding paper, e.g. principal component analysis (PCA) is described in **Paper I**.

2.5.1 Multiple imputation

Around 18% of the 1098 participants had at least one missing data entry. To avoid exclusion of participants in **Paper I** and **II**, the missing data were imputed using multiple imputation by chained equations, in which data were assumed to be missing at random (Rubin 1987; van Buuren & Groothuis-Oudshoorn 2011). An in-depth description of the imputation is provided in **Paper I**. Because of the removal of i) participants who did not receive drinking water from waterworks included in the study and ii) participants from highly contaminated areas, only around 6% of the remaining participants had a least one missing data entry. Consequently, imputation was not deemed necessary in **Paper III**.

2.5.2 Regression model selection

A detailed description of covariate selection and model definition is provided in each respective paper. A schematic overview is provided in Table 2.

In brief, in **Paper I**, a base model was created to investigate the joint influence of socio-demographic/physiological/life-style determinants on serum PFAA concentrations. This model included participant/maternal birth country (BC), maternal and paternal education level (MEL/PEL), gender, age, body mass index (BMI) status, smoking habits, snuffing habits, alcohol consumption and months fully nursed during infancy. Moreover, SHEIA15 was included to adjust the observed associations for the influence of dietary patterns. To evaluate the effect of menstrual blood loss on PFAA body burdens, the number of years since menstrual onset and whether they had started menstruating or not, were included separately to the base model for females only (Figure 2; Table 2). Additionally, plasma ferritin levels were evaluated as a potential determinant for blood loss, while adjusting for C-reactive protein levels (Table 2). To ensure that any potential association was related to blood loss through menstruation, this model was analysed for both genders, separately. As a sensitivity analysis (Figure 2), participants were removed based on the information that they lived in areas with a history of PFAS contaminated DW that was remediated a few years before blood sampling. The intention was to investigate whether these participants influenced the associations observed with the studied determinants.

In **Paper II**, associations between PFAA concentrations and dietary patterns were investigated (Table 2). The regression model included the same demographical/life-style/physiological determinants as in the base model in **Paper I**, with SHEIA15, or RADDS, or all habitual food consumption groups added to the model. Furthermore, in a separate analysis of the habitual food consumption, the habitual consumption of seafood was replaced with consumption of specific seafood groups (Table 2).

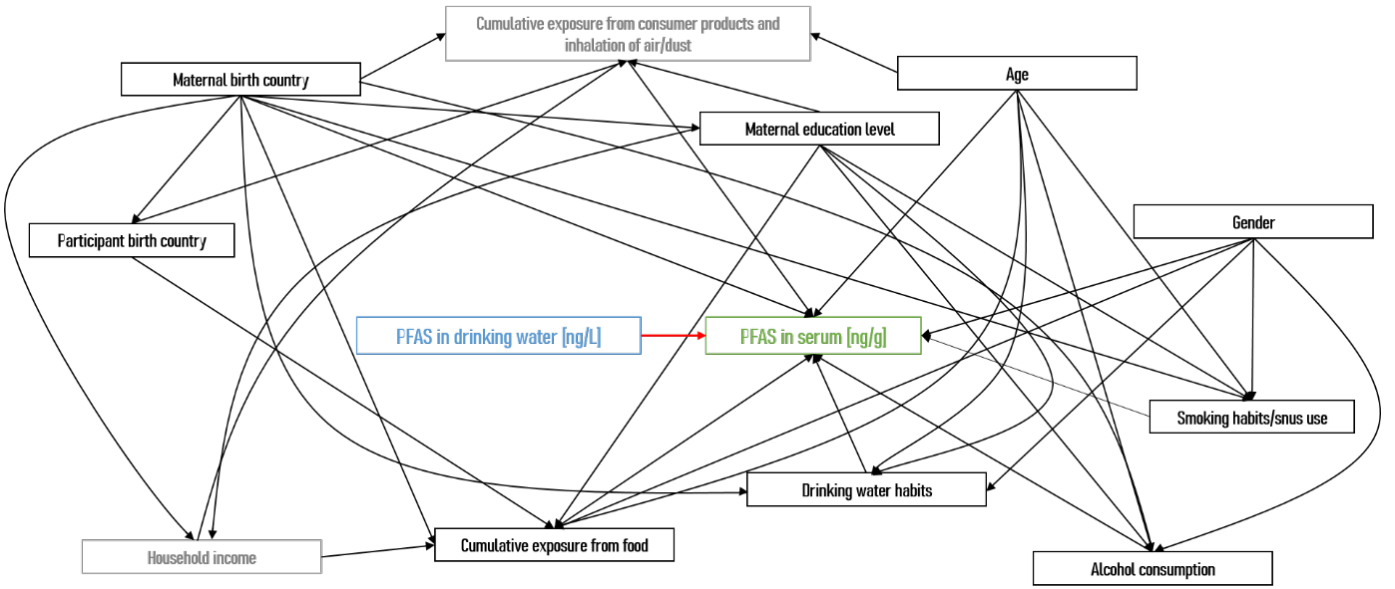

Figure 3. Directed acyclic graph (DAG) illustrating the relationship between PFAS concentrations in drinking water (ng/L) and PFAS concentrations in serum of the RMA participants (ng/g), and how other determinants may or may not influence either the exposure (blue) or the outcome (green). As shown, none of the determinants that influence PFAS concentration in serum, influence the PFAS concentration in DW.

In **Paper III**, using a directed acyclic graph analysis (DAG) (Figure 3), we reasoned that no determinant would likely confound the association between serum PFAA concentrations and PFAA concentrations in DW. Therefore, the main analysis was univariable. However, to determine that this assumption held, the serum and DW PFAA associations were in multivariable models (Figure 2; Table 2) adjusted for age, BC, gender, MEL and habitual seafood consumption, and separately also the habitual DW consumption (Table 2).

Gender specific effects on serum PFAA concentrations were investigated in both **Paper I** and **III** (Table 2). In **Paper I**, determinants relating to the potential impact of female menstruation on serum PFAA concentrations were investigated on females alone (details provided in **Paper I**). Furthermore, in **Paper III**, the interactive effect of PFAA concentrations in DW and gender on PFAA serum concentrations were evaluated (Figure 2; Table 2).

2.5.3 Regression analysis

The associations between serum PFAA concentrations and diet, demographical, life-style and physiological determinants were evaluated using the cumulative probability model (CMP) which fitted data using ordinal logistic regression (OLR). This model was utilised in **Paper I** and **II** as the normality assumption of the residual errors was violated using linear regression models. This method is favourable as the OLR only incorporates the order information of the dependent variable removing the need for monotonic transformations (such as log transformation) to meet regression assumptions while successfully integrating concentrations <LOD without prior need for imputation. The cumulative distribution function was used to transform the model output from log odds ratios to means and confidence intervals (Liu et al. 2017).

In **Paper III**, the associations between serum PFAA concentrations and PFAA concentrations in DW were investigated using weighted linear regression analysis. Much like in **Paper I** and **II**, the normality assumption of the residual errors was not met when fitting the data using ordinary least squares regression. However, by applying weights, the influence of extreme values on the fitted regression lessened. In this way, data could be fitted without the need of transforming the dependant variable. This was desirable,

as we wanted to make predictions based on the direct, non-transformed, relationship between PFAA concentrations in serum and DW (**Paper III**).

2.5.4 Risk assessment

In **Paper II** and **III**, serum $\sum_4$PFAA concentrations of the participants were compared with the serum $\sum_4$PFAA concentration corresponding to a long-term $\sum_4$PFAA intake at the the EFSA TWI level, i.e. of 6.9 ng $\sum_4$PFAA/mL (serum TWI concentration) (EFSA 2020). In both **Paper II** and **III** the percentage of participants exceeding the serum TWI concentration was assessed. In **Paper III**, potential differences in the likelihood of exceeding the serum TWI concentration was investigated among participants receiving DW with $\sum_4$PFAA concentrations above or below the maximum limit in Sweden (4 ng/L; SFA 2022) and Denmark (2 ng/L; DK EPA, 2021) using a χ^2-test of independence.

3. Results and discussion

The following section contains a compilation of the main and most important findings of the papers included in this thesis. The associations provided in the following paragraphs provide insight into possible exposure sources as well as physiological factors that may influence serum concentrations of PFAA, and possible inequalities in exposure in relation to the demographic parameters of the participants. When interpreting the results of the multivariable analyses, it is important to bear in mind that an observed relationship between the dependent and an independent variable has been adjusted for the effect of the other independent variables in the model. Given that the exposure of PFAA may vary geographically in the world (Richterová et al. 2023), the results presented within this thesis are primarily discussed within a Swedish perspective. Nevertheless, results in certain cases can most likely be generalized to other parts of the world.

3.1 Demographical and physiological determinants

3.1.1 Birth country and education

The most important demographical determinant in **Paper I** was participant/maternal birth country (BC), which was significant for all PFAA included in the analysis, i.e. lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS, lin-/br-PFOS (Figure 4, **Paper I**). Participants, who alongside their mothers, originated from high income countries had the highest estimated adjusted mean (EAM) serum PFAA concentrations, whereas participants who were born in low and lower-middle income countries, by mothers from the same countries, had generally the lowest EAM concentrations in serum (Figure 3, **Paper I**). We hypothesised that the difference in serum levels is likely

explained by differences in human exposure levels between high and low/lower-middle income countries. Given that the UN income classification system reflects the level of industrialisation to a degree, it is reasonable to assume that low/lower-middle income countries have lacked PFAS producing industry and have less use of PFAS in industrial production and of PFAS-containing products than high income countries (Shoeib et al. 2016). This would have resulted in a lower degree of PFAS contamination of the human environment, both presently and historically. This notion is supported in research were relatively low body burdens/exposure of PFAS have been found in low/lower-middle income countries such as Afghanistan, Papua New Guinea, Tanzania and Ghana (Hemat et al. 2010; Müller et al. 2019; Dartey et al. 2021; Nguyen et al. 2023). Given the long half-lives of PFAA, it is therefore likely that this determinant catches variation in data that relates to PFAA exposure early in life.

One limitation of our study is the lack of information on duration in Sweden for immigrating participants and mothers. A participant who came to Sweden as a toddler will likely experienced similar exposure levels after coming to Sweden compared to toddlers born in Sweden or other high-income countries. Nevertheless, some differences in PFAA exposure levels even after moving to Sweden cannot be excluded. For example, dietary habits differ considerably between ethnic populations across Europe (Gilbert & Khokhar 2008). Even though such populations incorporate parts of the Western diet into their food regime, namely the unhealthy aspects, staple foods often remain the same as in their native countries (Gilbert and Khokhar, 2008). This is supported by a Swedish study where the parental migration status was found to be a stronger predictor for consumption of specific foods than parental education level, in 6-year-old children (Säfsten et al. 2016). Although the association between PFAA serum concentrations and birth country were adjusted for the healthy eating index SHEIA15, we cannot rule out that this determinant does not catch residual confounding relating to differences in dietary habits between those from high vs low/lower-middle income countries living in Sweden.

Furthermore, birth country might not only reflect the exposure of the adolescent before migrating to Sweden, but also difference in the maternal exposure *in utero* and through nursing from the mother. This can be illustrated to some extent in Figure 3, **Paper I**, were participants from high/upper-middle income countries whose mothers originated from

low/lower-middle income countries had generally lower serum PFAA concentrations than those where the mother-and-child-pair originated from high income countries. Gyllenhammar et al. (2019) found that maternal PFOA and br-PFOS concentrations close to delivery were positively associated with serum concentrations of the PFAA in question in their 12-year-old children, supporting the importance of maternal body burdens as a major exposure source impacting body burdens into adolescence. However, as just discussed, cultural-related differences in both diet and use of products could potentially explain the difference in PFAA body burdens between participants from different birth countries, to some extent.

Given the joint inclusion of covariates in multivariable regression models throughout this thesis (Table 2), it is important to bear in mind that the associations presented within the papers might be altered should one or several determinants be removed from the analysis. Reversely, inclusion of several new variables in the regression models could potentially weaken a previously existing association. Maternal and paternal education levels were only associated to concentrations of lin-PFHxS and PFUnDA in adolescent serum (Table S6-S12 in SI, **Paper I**). Higher education level has previously shown to be associated with higher PFAA body burdens in both adults and adolescents, in the latter case, the highest education level of the adolescents household (Bjermo et al. 2013; Brantsæter et al. 2013; Richterová et al. 2023). Although the level of highly educated immigrants are proportionate to that of native Swedes, a large part of the foreign-born population have low educational attainment (European Commission 2020). Consequently, a certain level of covariation between parental education level and BC cannot be ruled out. To test this notion, the same base models in **Paper I** were rerun in this thesis, however this time removing BC while keeping both maternal and paternal education level in the models. In these runs, the maternal education level was now additionally significantly associated with lin-PFOA, PFNA and lin-/br-PFOS, where participants with highly educated mothers had higher PFAA concentrations than those with a lower educational attainment, except for PFDA (lin-PFOA, $p=0.0429$; PFNA, $p=0.0231$; PFDA, $p=0.2593$; PFUnDA, $p=0.0083$; lin-PFHxS, $p=0.0001$; lin-PFOS, $p=0.0074$; br-PFOS, $p=0.0328$). The associations for serum PFAA concentrations and paternal education level remained unchanged, i.e. still only significantly associated with PFUnDA. These results appear to support the notion of BC catching a significant part of the variation in adolescent

serum PFAA concentrations that could otherwise be explained by the maternal education level. In a recently published article investigating determinants of PFAA body burdens in adolescents across nine European countries, where 300 participants of the total study population originated from RMA, household education level was positively associated with adolescent PFAA body burdens (Richterová et al. 2023). Richterová and colleagues (2023) did not adjust the relation with educational level for birth country of the participant, nor the birth country of the participant's mother.

Education level and the level of birth country development may affect health in different ways. In rich, developed countries, there commonly exists a strong inverse relationship between e.g. BMI and education level. In low income countries, there appears to be a mix of both over- and undernutrition, where more highly educated people are more likely overweight and obese, while those with low education are more likely to be underweight (Cutler & Lleras-Muney 2012). Furthermore, migrants across Europe have higher morbidity and mortality rates due to dietary related diseases, not only compared to the host population but also the population within their native country (Gilbert & Khokhar 2008). In Sweden, immigrants have a higher risk of cardiovascular and coronary heart disease compared to native-born adults (Gadd et al. 2003). It is therefore important to factor in not only the education level, but also the country of origin, in future studies when evaluating the relations between PFAA and adolescent health. Specifically as there likely exists differences in susceptibility and genetic predisposition between ethnicities, as suggested by Gilbert and Kohkhar (2008).

3.1.2 Gender

Gender was in general the second most important determinant in explaining the variation in serum PFAA concentrations in the **Paper I** multivariable regression models (Figure 4, **Paper I**), where males had significantly higher lin-PFOA, PFNA, PFUnDA, lin-PFHxS and lin-/br-PFOS concentrations compared to females (Table S6-12 in SI, **Paper I**). Two potential mechanisms can be hypothesized to explain this difference, i.e. either gender differences in exposure of PFAA and/or differences in toxicokinetics, i.e. absorption, distribution and excretion of PFAS. It could be speculated that the exposure of males is different to that of females given the difference in dietary habits (SLV 2018), amount of food consumption per kg body weight (Shomaker et al. 2010), or in behaviour, which can for example be reflected

in the use of personal care products (Manová et al. 2013). However, males use reportedly less personal care products than females (Manova et al. 2013) and several dietary covariates still explained parts of the variation in PFAA serum concentrations whilst being adjusted for the effect of gender (see section 3.2). This suggest that exposure alone cannot entirely explain the difference in serum PFAA concentrations between genders, and that gender dissimilarities in toxicokinetics of PFAA may be involved. This hypothesis was further supported in **Paper III** were PFAA appeared to bioaccumulate from DW to a higher degree among males then females (Figure 2, **Paper III**), even though males had a slightly lower self-reported habitual DW consumption compared to females (**Paper III**). In **Paper I** and **III**, we therefore hypothesized that the gender difference in PFAA body burden is likely explained by sex-specific differences in toxicokinetics. This hypothesis was supported by **Paper I** were female participants who reported to have started menstruating had significantly lower estimated mean br-PFOS concentrations compare to those females that had not started menstruating (Figure 5, **Paper I**). Menstrual bleeding has been implicated as an elimination route for PFAA among females (Rickard et al. 2022). The same trend was seen for lin-PFOA, lin-PFHxS and lin-PFOS albeit significance was not reached (Figure 5, **Paper I**). Intriguingly, in RMA, no association was observed between the number of menstruating years after menarche and serum PFAA concentrations (Table S13 in SI, **Paper I**). There could be several reasons for the lack of association, including irregular menstrual cycles within the first years of menarche (Carlson & Shaw 2019) and difficulty in providing a correct age for when menstruation commenced. However, using toxicokinetic-modelling approaches, menstruation has been found to only account for roughly 20-30% reduction in PFAS concentration in adult female populations (Wong et al. 2014; Lorber et al. 2015). PFAA clearance through birth and nursing has also been found to significantly decrease PFAA body burdens among females (McAdam & Bell 2023); an unlikely reason for the gender difference in PFAA concentrations among the RMA adolescents. If it is indeed differences in toxicokinetics that drive the difference between sexes, these differences could potentially arise as a result of the physiological/hormonal changes that occur already at an early stage of puberty, i.e. even before females enters menarche. This could explain why the association between serum PFAA concentrations and onset of menstruation among the females, and the years of menstruation was fairly

weak (**Paper I**), as females enters puberty around two years prior to menarche (Martí-Henneberg & Vizmanos 1997). This could also explain why studies on children seldom reports differences in PFAA body burden between genders (Ye et al. 2018; Forthun et al. 2023), nor differences in PFAA half-life (Li et al. 2022). Research has suggested an estrogenic-dependent increase in renal clearance of PFOA, PFHxS and PFOS from serum in both female adolescents and adults (Li et al. 2022). More research is however needed in order to elucidate the mechanism behind the differences in accumulation between genders.

3.2 Dietary variables

PFNA, PFDA, PFUnDA and lin-PFOS concentrations in serum were positively associated with both the healthy eating index SHEIA15 and the diet diversity score RADDS (Figure 1 and 2, **Paper II**). This association is most likely driven by the relatively high levels of PFAA in seafood compared to PFAA levels in other food groups in Sweden (SFA 2017a) and the positive correlation between seafood and both dietary indices (Table 4, **Paper II**). Serum concentrations of PFNA, PFDA, PFUnDA and lin-PFOS also increased with increased habitual seafood consumption among the adolescents (Figure 3, **Paper II**). These findings are supported by the notion that long-chained PFCA, such as PFNA, PFDA and PFUnDA, as well as PFOS, are known to accumulate in seafood (Savoca & Pace 2021). Although the detection frequency of PFDoDA and PFTrDA was around ~10% in RMA and therefore not modelled using regression analysis, it could be speculated that seafood also was a major contributor to the body burden of these PFAA. This is supported by the principal component analysis (PCA) were PFDoDA and PFTrDA clustered strongly with the other long-chained PFCA (Figure 1, **Paper I**). Furthermore, PFDoDA and PFTrDA have been detected in seafood products in Sweden, but not in other foods, albeit less frequently than the detection of PFNA, PFDA and PFUnDA (SFA 2017a).

In Sweden, the recommendation of healthy seafood consumption is 2-3 portions per week due to its content of nutrients beneficial for health and suggestive protective effects against chronic disease (SFA 2017b). Today, no information exists on whether the presence of PFAS in seafood to some extent mitigate these beneficial health effects. A study that followed Swedish adults and seniors between the years of 1998 to 2014 found a U-shaped dose-

response relationship between all-cause mortality and seafood consumption; those who reported a high versus a low consumption had a higher risk of overall mortality compared to the median consumption (Bellavia et al. 2017). The authors speculated that co-exposure of environmental pollutants, including polychlorinated biphenyls (PCB), mercury and dioxins, might explain the increased risk of all-cause mortality with a higher intake of fish above a certain level (Bellavia et al. 2017). This hypothesis was supported by Donat-Vargas et al. (2020) who reported an increased cardiovascular disease mortality with increasing dietary PCB exposure in Swedish adults, after adjustment for intake of fish fatty acids. The reverse was observed for the fatty acids after adjustment for PCB exposure. With this in mind, there is a need to thoroughly assess the overall effect of seafood consumption on human health, taking into account mixture exposure from PFAS as well as other environmental contaminants. Such information is essential when deriving future dietary recommendations.

It is however important to consider temporal trends of exposure when interpreting the results of the observed associations in RMA. The studies conducted by Bellavia et al. (2017) and Donta-Vargas et al. (2020) were conducted on elderly populations who had experienced a considerable higher cumulative exposure to PCB than those that children/adolescents of today will experience during their future life time. This is due to the substantial decrease in human exposure of PCB in Sweden since the early 1970s (Nyberg et al. 2015). Similarly, human exposure to legacy PFAA has declined considerably since early 2000, suggesting that the RMA participants, as well as future adult populations, will have considerably lower body burdens of PFAA than what could be expected for the adult participants in Bellavia et al. (2017) and Donta-Vargas et al. (2020). Nevertheless, a health risk-benefit analysis of seafood consumption is needed in order to determine the net effect of seafood consumption on future health development, and the influence of environmental pollutants such as PFAS on this effect. Presently, EFSA is underway deriving a risk benefit assessment combining exposure to nutrients and contaminants through food which, as this thesis went to press had not been made public (EFSA 2022).

Another interesting finding in RMA was the difference in associations between serum concentrations of linear and branched isomers of PFOS and dietary indices/seafood consumption; neither the dietary indices, nor habitual seafood consumption, were significantly associated with serum of br-PFOS

concentrations among the RMA participants (Figure 1-3, **Paper II**). Habitual meat consumption explained the largest part of the variation of br-PFOS in serum, yet failed to reach statistical significance (Figure 4, **Paper II**). Intriguingly, br-PFOS was detected in low concentrations in fish and eggs in the 2015 Swedish market basket survey (SFA 2017a), yet in RMA, seafood explained very little of the variation of br-PFOS concentrations in serum (Figure 4, **Paper II**). In contrast, lin-PFOS was significantly associated with both habitual seafood consumption and SHEIA15/RADDS (Figure 1-3, **Paper II**). These findings are supported by observations that lin-PFOS in general bioaccumulate to a higher degree in animals, including fish, compared to br-PFOS, although there might be a variation in this aspect between different branched isomers (Sharpe et al. 2010; Schulz et al. 2020). In this context, another interesting observation in RMA was that br-PFOS was clustered with both lin- and br-PFHxS in the cluster analyses of serum PFAA concentrations, as illustrated in Figure 1 (**Paper I**). It has been modelled that DW is a major source of human PFHxS exposure (Vestergren et al. 2012). Due to the higher polarity compared to its linear counterpart, br-PFOS has been found to be more effectively transported in water through the environment and is also more likely to remain solubilized in water (Schulz et al. 2020). It could therefore be speculated that br-PFOS, in contrast to lin-PFOS, to a larger extent had DW as a source of exposure in RMA.

It is important to bear in mind that the lack of a significant association between serum PFAA concentrations and dietary determinants does not mean that some of these dietary determinants are not contributing to the exposure of PFAA (Alderson 2004). A dietary determinant could fail to explain variation in PFAA concentrations in serum if the contribution of this determinant to total PFAA exposure is too low to be detected in the statistical analysis. This is likely the case for lin-PFOA, which has previously been reported in low concentrations in a manifold of food groups, including cereals, eggs, seafood, meat, pastries, fats/oils and sugar sweets in Sweden (SFA 2017a). Among the RMA participants, serum concentrations of PFOA were not positively associated with any of these dietary variables. Instead, an inverse relationship was found for dairy, cereals and pastry (Figure 3, **Paper II**). It is unlikely that higher consumption of certain food groups alters the toxicokinetics of PFAA, and consequently cause lower concentrations in serum. It is however more likely that the inverse relationships represent residual confounding, where high consumption of a certain food with very

low contribution to PFAA exposure covary with a decreased consumption of other food groups/exposure sources which more significantly contribute to the exposure. This was speculated to be the case in **Paper II** were lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin-PFOS was inversely associated with habitual dairy consumption (Figure 3, **Paper II**). More specifically, it may be hypothesized that the inverse relationship was caused by covariation with DW consumption, a significant source of PFAA exposure for the RMA participants (**Paper III**). In this case DW consumption could have been replaced with milk as a preferred beverage, thus explaining the inverse relation with serum PFAA concentrations (**Paper II**). In an additional statistical analysis in this thesis, using Spearman correlation analysis, a significant negative correlation was observed between dairy and habitual DW consumption ($r = -0.22$, $p = <0.0001$). Although the correlation analysis to some extent may support the dairy-DW hypothesis, this may not explain the inverse relationship with serum concentrations of PFDA and PFUnDA, i.e. substances that are rarely detected in DW (Table S1 in SI, **Paper III**). Residual confounding could also be the explanation for the positive association between serum PFNA and PFUnDA concentrations and vegetable consumption since neither of these compounds have been detected in vegetables in Sweden (SFA 2017a).

It is essential to keep in mind that the positive associations between serum PFAA concentrations and a healthy and diverse diet are mainly applicable within a Swedish context, as dietary composition and habits most likely vary across the world. This is also applicable for consumption of individual foods and food groups. Despite the wide international trade of foodstuffs, the level of PFAS contamination of food may vary between regions of the world. Furthermore, there could also be considerable variation in dietary habits due to differences in food access and cultural and food consumption preferences. For example, in an adolescent U.S. population, inverse associations were observed between serum PFAS concentrations and the Healthy Eating Index (HEI) that was designed in conformity with the Dietary Guidelines for Americans. Additionally, no association was seen for the group of seafood and plant protein and PFAS, except for PFNA. The authors reasoned that the lack of association with seafood was likely explained by the low amounts of fish that was consumed (Sultan et al. 2023). It is therefore important to consider the specific dietary composition of the intended population when designing future studies, as well as in future risk assessments.

3.3 Drinking water exposure

The mean lin-PFOA, PFNA, lin-PFHxS and lin-PFOS concentration in DW was <1 ng/L among the waterworks that delivered DW to the RMA participants (Table 4, **Paper III**). Despite the concentrations being this low, the variation of concentrations in DW explained a significant part of the variation of concentrations in serum (Table 5, **Paper III**); the higher the PFAA concentrations in DW the higher the concentrations in serum (Figure 1, **Paper III**). These findings are unique, as the association between PFAA body burdens and PFAA in DW have previously mainly been studied in populations consuming DW with much higher DW PFAA concentrations than in the low-grade PFAA-contaminated DW in RMA (Emmett et al. 2006; Hoffman et al. 2011; Zhang et al. 2019; Xu et al. 2020; Zhu & Bartell 2020; Johanson et al. 2023). These significant associations in RMA remained even when adjusting for determinants such as BC, gender, habitual seafood consumption, maternal education level and age, which in **Paper I** explained parts of the variation of PFAA concentrations in serum (Table 5, **Paper III**).

For this thesis, additional results were extracted from the multivariable regression analyses in **Paper III** to find out the contribution of DW exposure to the variation in PFAA concentrations in serum in relation to the other covariates included in the regression models (Table 5, **Paper III**). For lin-PFOA, lin-PFHxS and lin-PFOS, the concentrations in DW explained the largest part of the variation of PFAA concentrations in serum (Figure 4). This further supports the importance of low-grade PFAA contaminated DW as an exposure source of PFOA, PFHxS and PFOS for the RMA participants. For PFNA, habitual seafood consumption (g/day) and BC explained a larger part of the variation in serum concentrations than DW PFNA (Figure 4). This is likely due to the fact that PFNA concentrations in DW are lower, with a narrower concentration range in DW, compared to PFOA, PFHxS and PFOS (Table 4, **Paper III**). Although the PFAS composition in water varies depending on contamination source, PFNA tends to account for a smaller fraction of the PFAS profile in water compared to PFOA, PFHxS and PFOS (Gobelius et al. 2018). As shown in Figure 4, seafood is a relatively more important determinant of serum PFNA concentrations compared to DW. In cases when PFAA concentrations in foods are much higher than what is currently observed in Sweden (SFA 2017a), it is likely that the association between PFAA concentrations in serum and low-grade PFAA-contaminated DW would be weaker.

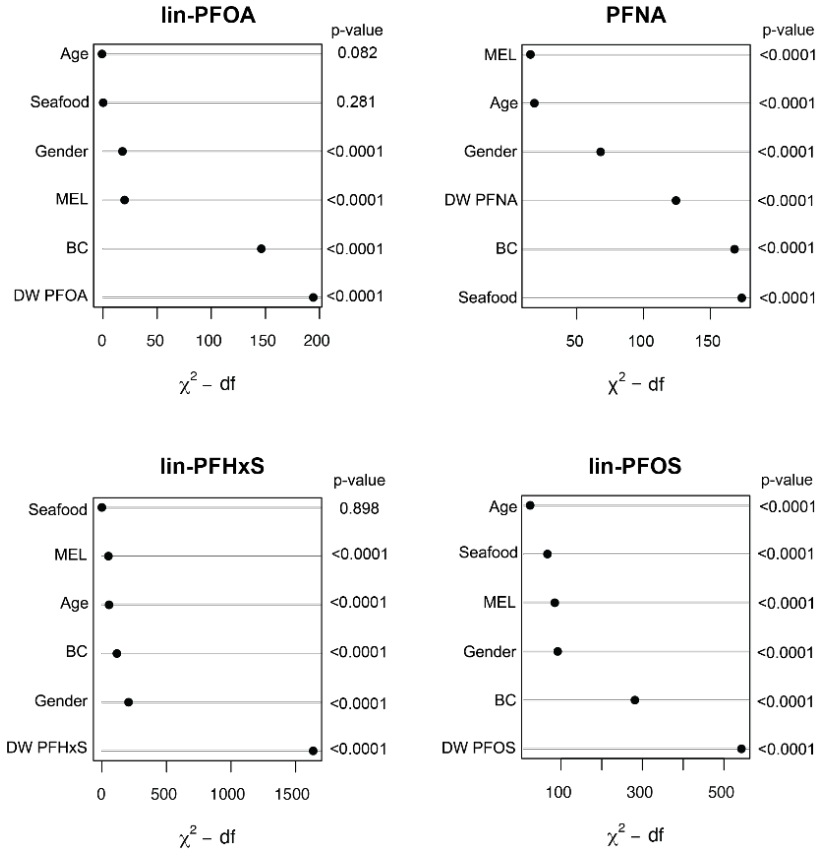

Figure 4. The relative importance of determinants included in Multivariable model I (Table 2) in **Paper III**, expressed asχ^2- df (x-axis). The model was fitted using weighted linear regression analysis, and the determinants in the model are found on the left y-axis and p-value for each determinant is found on the right y-axis. Note that the relative importance of a determinant can only be compared within, and not between different PFAA models.

It may be hypothesized that the relative importance of DW as a PFAA exposure source compared to other exposure sources in Sweden will increase over time in the future. When production and use of PFOA, PFHxS and PFOS and related substances were phased-out in the early 2000s, the decline of human exposure to these PFAA occurred shortly thereafter, as shown in breastmilk samples from women in Sweden (Nyberg et al. 2018). This suggests that a large fraction of the exposure that was removed by the phase-out was likely linked to direct exposure from the use of PFAS containing

products, for instance certain types of food packaging (Gomis et al. 2017). A similar steep decline in PFAA exposure from background-contaminated DW in Sweden is however unlikely to expect, as contamination of important DW sources from the many PFAS hotspots that exist in Sweden most likely will be ongoing for many years to come. This can be illustrated by the situation in Lake Mälaren in Sweden from which surface water is used to produce DW for around 2 million Swedes. A recent report, investigating the mass balance of PFAS in Lake Mälaren, found that a considerable amount of PFAS originated from two sources, i.e. River Fyris and Märsta River (Ekman & Ejhed 2023). Within the catchment areas of both these rivers exists PFAS hotspots which are significantly contributing to the contamination of Lake Mälaren (Ekman & Ejhed 2023), more specifically hotspots in which PFAS containing fire-fighting foam has extensively been used historically (Woldegiorgis et al. 2010; Gyllenhammar et al. 2015). Highly contaminated areas, as well as landfill leachate, sewage treatment plant effluents and run-off from industry (Gobelius et al. 2018) will most likely continue to leach PFAS into both ground- and surface water for a long period of time. If the PFAA exposure from food continues to decline while the exposure from DW remains more-or-less constant, as just reasoned, the exposure from DW ought to account for a larger part of the exposure in the future. This change in contribution of DW PFAA exposure can be illustrated by comparing the average PFAA exposure from diet and DW in 1999 with the exposure in 2015 in Sweden. The per capita intake of PFOA, PFNA and PFOS ($\sum_3$PFAA) from diet was around 133 ng/day in 1999 (103 ng PFOS/day + 26 ng PFOA/day + 4 ng PFNA/day, Vestergren et al. 2012). In 2015, the per capita intake for $\sum_3$PFAA was around 29 ng/day (1.6 ng PFNA/day + 12 ng PFOA/day + 14.8 ng PFOS/day, SFA 2017a). If we assume a daily water consumption of 2 L (WHO 2022b) and a concentration in DW of 2.1 ng/L in both 1999 and 2015 (mean $\sum_4$PFAA, Table 4, **Paper III**), the contribution from DW in relation to the total exposure is 3% and 13% for 1999 and 2015 respectively. PFHxS was not included in the calculation as it is seldom detected in food (Vestergren et al. 2012; SFA 2017a). Even though these rough calculations are uncertain and do not reflect the true complexity of PFAS exposure, they still illustrate the possible shift in relative importance of DW as an exposure source over time.

Unlike PFAS exposure from food in general, DW is a human exposure source that can be directly remediated, thus reducing PFAS exposure within

a short time span. Although traditional water treatment techniques do not remove PFAS from water, remediation using granulated active carbon (GAC) or ion-exchange techniques has shown to successfully remove both long- and short-chained PFAS (Appleman et al. 2014). In areas where highly contaminated DW has been remediated, PFAA levels in serum of the exposed population have decreased shortly after remediation (Gyllenhammar et al. 2015; Li et al. 2018). In 2022, the Swedish Food Agency introduced two maximum limits for PFAS in DW, for 21 PFAS congeners of 100 ng/L and for $\sum_4$PFAA of 4 ng/L (SFA 2022), as a result of EFSAs new, more stringent health-based guidance value. As suggested in RMA, where participants who received DW exceeding the Swedish maximum limit (ML) of $\sum_4$PFAA were more likely to exceed the TWI (section 3.4, **Paper III**), remediation below the ML ought to have a positive impact on the total exposure of PFAS.

Lastly, it is important to also shine a light on the presence of other PFAS in DW than $\sum_4$PFAA, not evaluated in detail in the **Paper III**. It could be speculated that DW was an important exposure source for br-PFHxS and br-PFOS, even though these isomers were not analysed in the RW/DW samples in RMA. This notion is based on the PCA performed in **Paper I** were br-PFHxS/PFOS clustered strongly with lin-PFHxS (Figure 1, **Paper I**), i.e. a PFAA congener for which the major exposure source has been modelled to be DW (Vestergren et al. 2012).

3.4 Risk assessment

A risk assessment of $\sum_4$PFAA exposure was carried out in both **Paper II** and **III**, where serum concentrations in the RMA population were compared to the serum level corresponding to EFSAs TWI, i.e. 6.9 ng $\sum_4$PFAA/mL serum (EFSA 2020). The exceedance of the EFSA serum $\sum_4$PFAA TWI varied between 16-29%, depending on how many of the participants that were included in the studies (section 3, **Paper II** and section 3.4, **Paper III**). Additional analysis in **Paper III** further showed that the proportion of participants exceeding the serum $\sum_4$PFAA TWI was larger for participants that supposedly consumed PFAA-contaminated DW exceeding both the Swedish and Danish MLs of 4 ng/L and 2 ng/L, respectively (SLV 2022; DK EPA 2021), compared to participants with DW $\sum_4$PFAA concentrations below the MLs.

While an exceedance of the serum $\sum_4$PFAA TWI level by a large proportion of the adolescent population in Sweden is a health concern, the exceedances cannot be equated to increases in risks of disease. Although there are both epidemiological studies as well as animal studies that provide evidence of PFAS adversely influencing risk factors for disease, little or insufficient evidence exist on the effect of PFAS on the risk of disease, as seen in EFSAs latest risk assessment (EFSA 2020). However, as of November 2023, the International Agency for Research on Cancer (IARC) classified PFOA as "*carcinogenic to humans*" (Group 1) and PFOS as "*possibly carcinogenic to humans*" (Group 2B), given the strong mechanistic evidence on immunosuppression and induction on epigenetic alternations, with limited evidence for testicular cancer and renal cell carcinoma in humans (Zahm et al. 2024). It is important to bear in mind that the IARC classification only identifies cancer hazards and does not indicate the level of risk that is associated with different exposure levels or scenarios (IARC 2023). A safe threshold, indicating the level of PFOA exposure that may increase risks of cancer in humans, has not been determined. As such, it is impossible to speculate regarding the risk of cancer in any human population. Nevertheless, the IARC classification of PFOA as a human carcinogen will likely have implications for future risk assessments.

Given the latest risk assessment of human exposure to $\sum_4$PFAA by EFSA, the serum $\sum_4$PFAA TWI level represents the highest estimated serum concentration of $\sum_4$PFAA that is not connected to any health concern on a population level (EFSA 2020). Consequently, it is impossible to make predications on the risk development of adverse effects on an individual level. As EFSA choose the most sensitive adverse effect, the TWI is considered protective against other adverse endpoints, including reduced birth weight, increased serum cholesterol and slight effects on the liver, as indicated by increased serum levels of liver enzyme alanine transferase (EFSA 2020). Given the new evidence and classification of the carcinogenicity of PFOA and PFOS on humans, more knowledge is needed to understand the effect PFAA have on human health.

4. General conclusions and future perspectives

The studies comprised in this thesis aimed to investigate potential exposure pathways and demographical and physiological determinants of PFAA concentrations in serum in a general adolescent Swedish population. Such information is imperative when carrying out an assessment of health risks of PFAS, and possibilities to mitigate future exposure. Furthermore, identifying determinants that could influence exposure and excretion of PFAS, and that could simultaneously also influence health and wellbeing, are crucial for the design of future health studies.

Through these studies on adolescents in Sweden, we have identified several determinants that most likely reflect exposure and excretion of legacy PFAA. The most important determinant of PFOA, PFHxS and PFOS body burdens among RMA adolescents where drinking water PFAA concentrations, as shown in **Paper III**. This finding is important as the PFAA levels in DW were within the range normally found in DW in the largest urban areas of Sweden and are considered low within both a Swedish as well as an international context. As discussed in this thesis, assuming that the concentrations of PFAA in DW will decrease more slowly than other exposure sources of PFAA, the relative importance of DW as an exposure source of PFAA in Sweden might increase in the future. Consequently, this hypothesis needs to be followed-up, especially since some of the large Swedish waterworks most probably need to mitigate PFAS concentrations in their DW in order to comply with the Swedish maximum limits of PFOA, PFNA, PFHxS and PFOS in DW. Such follow-up is vital to make accurate future assessments of the importance of DW as a source of PFAS exposure.

Another important exposure source of PFAA for Swedish adolescents is diet, as strongly suggested in **Paper II**. In this study, adolescents adhering to

a diverse and healthy diet had higher PFNA, PFDA, PFUnDA and lin-PFOS concentrations in serum compared to those that did not, were seafood consumption was reasoned to be a major driver of the associations. A healthy diet is a major cornerstone in adolescent health development and prosperity. Considering that both seafood and DW are important foundations of a wholesome and healthy diet, it is imperative to include diet in future studies of PFAA and adolescent health. Furthermore, one of the most important demographical determinants was participant and maternal birth country (**Paper I**). This determinant most likely at least partially catches the exposure that occurs early in life linked to the level of PFAS contamination in the overall environment of the birth country. It further highlights the need to not only address recent exposure, but also the cumulative historical exposure of each individual. Moreover, as there may be discrepancies between ethnicities/nationalities in behaviour and physiology that could affect health, birth country as a determinant of serum PFAS concentrations needs to be considered in future studies investigating health outcomes of PFAS in humans.

In **Paper III**, we showed that males appeared to accumulate PFAA from DW to a higher degree than females. These findings were corroborated in **Paper I**, were excretion of PFAA were suggested to be slightly higher amongst females due to menstrual bleeding. It is however unlikely that menstruation alone explains the differences in PFAA body burdens and PFAA bioaccumulation from DW, and investigations of toxicokinetic differences between male and female due to the onset of puberty are needed. Our studies have additionally provided more information on potential PFOS isomeric differences in relation to exposure and possibly also to toxicokinetics. This was noticeable in **Paper II**, where br-PFOS, unlike lin-PFOS, was not significantly associated with any of the dietary determinants.

To conclude, PFAS is a contaminant of the past, the present, and will likely remain a contaminant for a foreseeable future. This thesis has contributed with important information on determinants that could explain both exposure and excretion of six legacy PFAA. However, as stated in the introduction, thousand other PFAS congeners have been identified for which there is no, or very limited, knowledge on the toxicity and exposure levels of humans. Although there have been important legislative steps taken in order to increase the food safety for certain PFAS, e.g. through the introduction of maximum limits of $\sum_4$PFAA in animal-based foods (European Commission

2022), more exhaustive risk management efforts are needed in order to limit the human exposure to the entire group of PFAS (Cousins et al. 2020). The European Chemicals Agency (ECHA) has recently published such a proposal to restrict and phase-out most uses of PFAS in the European Union (Wollin et al. 2023). The end of the consultation for the restriction proposal was closed on the 25th of September, 2023, and ECHA is presently under way evaluating the roughly 5600 individual comments received during the consultation period (ECHA 2023).

Yet, PFAS is not the only chemical group of concern for human health. Humans are exposed to a plethora of chemical compounds on a regular basis, including industrial chemicals and chemicals found in building materials, household products, cosmetics and hygiene products, to name a few (Panico et al. 2019; Rousseau et al. 2022; Marroquin et al. 2023). There consequently exists a need to understand, not only the potential effects of PFAS exposure on human health but also of possible combined effects that chemical mixtures may have on human health.

My thesis has demonstrated that even though production and use of most of the legacy PFAA have been phased out before the sampling of RMA adolescents, they are still detected in almost all participants. Due to the persistence of PFAA, they will be present in the environment for decades to come. To prevent the future release of toxic and persistent chemicals, like PFAS, into the environment, there exists a dire need for more stringent chemical regulations to prevent the industry from polluting our planet, not only with PFAS but with other similar chemical compounds as well.

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Popular science summary

Humans are exposed to a wide array of chemicals on a daily basis. The exposure can occur via the food we eat, the water/beverages we drink, the products we use, or the air we breathe. This exposure can originate from these multiple sources at once for certain chemicals. Although many of the chemicals are not harmful to human health, some are in fact implicated as possible health risks. One of these groups are per- and polyfluoroalkyl substances (PFAS). This group consists of more than 4700 individual chemicals, with a few of the compounds causing effects on biological processes in the body, with suspected adverse health consequences. Production of PFAS started already back in the early 1940s, and because of their stable, non-stick, surfactant properties, these compounds have been used frequently in both consumer products and by the industry. Due to the decade-long use and the exceptional stability of PFAS, some are detected in blood serum/plasma in almost all humans all over the world.

Today, we have a good understanding of the human exposure to a few of the PFAS, the so-called legacy PFAS. We know that food and drinking water (DW) are important exposure sources for the general adult population and exposure in the womb and through nursing are important initial exposure pathways for children. We do however lack information on the PFAS exposure of adolescent populations, as most of the previous research has been carried out on adult and infant/child populations. Considering that adolescents might be sensitive to PFAS exposure, as they are in a period of rapid physical development, this thesis aimed to investigate which factors that can determine the exposure of PFAS in a Swedish adolescent population.

The study population in this thesis was a subsample of the national dietary survey *Riksmaten Adolescents 2016-17*, and consisted of around one thousand participants at 10-21 years of age. The participants answered

questions about personal characteristics and registered what they ate and drank during two days. They also donated blood samples and had their drinking water sampled, both of which were analysed for PFAS. Using a wide arsenal of statistical methods and models, we could evaluate what factors that explained the variation of PFAS concentrations in the blood of the participants and consequently make interpretations related to the PFAS exposure of the adolescents.

Five of the legacy PFAS were found in 70-100 percent of the participants. For a few of these PFAS, the PFAS concentration in DW, although considered to be relatively low, was the most important driver of exposure. Another important exposure source appeared to be diet, as adolescents who were eating a healthy and diverse diet, rich in seafood, had higher PFAS concentrations in their blood than those who did not. Beside from direct exposure sources, such as water and food, we found other factors that may indirectly influence the concentrations of PFAS in adolescent blood. Birth country of both the participant and the mother of the participant was such an important factor. More specifically, participants and their mothers who originated from high-income countries had higher PFAS concentrations in blood than participants who, alongside their mother, originated from low-/lower-middle income countries. Additionally, males had higher PFAS concentrations in their blood than females.

To conclude, this thesis has provided information on factors that could explain adolescent exposure to PFAS in Sweden. Such information is important for future design of studies evaluating the effect of PFAS on adolescent health, as well as for future risk assessments of PFAS.

Populärvetenskaplig sammanfattning

Vi människor exponeras för många olika kemikalier varje dag. Denna exponering kan ske via den mat vi äter, den dryck eller det vatten vi dricker, de produkter vi använder, eller via den luft vi andas. Ibland kan exponeringen för vissa kemikalier komma från flera källor samtidigt. Även om många av dessa kemikalier inte påverkar vår hälsa så finns det en del som sannolikt gör det. Per- och polyfluorerade alkylsubstanser (PFAS) är en sådan grupp där några av de över 4700 enskilda substanserna har visat sig påverka biologiska processer hos människan. PFAS är en grupp med ytaktiva och stabila kemikalier som har en väldigt god förmåga att stöta bort både smuts, fett och vatten. PFAS har tillverkats sedan början på 1940-talet och har sedan dess använts flitigt inom industrin och i konsumentprodukter. Eftersom PFAS är motståndskraftiga mot nedbrytning och har använts i stora volymer under lång tid så finns PFAS i kroppen hos nästan alla människor på planeten.

Idag vet vi en hel del om hur vissa PFAS, så kallade legat-PFAS, sprider sig till människan. Vi vet att mat och dricksvatten är viktiga exponeringskällor hos vuxna. Vi vet också foster exponeras för PFAS i moderlivet och spädbarn via bröstmjölken. Hittills har dock merparten av exponeringsstudierna fokuserat på vuxna och barn, och väldigt få studier har undersökt exponeringen av ungdomar. Med tanke på att ungdomstiden är en period av snabb fysiologisk utveckling kan ungdomar vara extra känsliga för PFAS-exponering. Syftet med denna avhandling var därför att undersöka vilka faktorer som kan påverka svenska ungdomars PFAS-exponering.

Som studiepopulation användes deltagare från den nationellt täckande kostundersökningen *Riksmaten ungdom 2016-17*. Studiepopulationen bestod av runt tusen deltagare i åldrarna 10-21 år. Deltagarna hade svarat på frågor som rörde dem själva och deras liv samt registrerat vad de åt och drack under två dagar. Både deltagarnas blod och deras dricksvatten samlades in och

analyserades på PFAS. Genom att använda oss av flera olika statistiska metoder och modeller kunde vi undersöka vilka faktorer som kunde förklara variationen av PFAS-halten i blodet hos deltagarna och utifrån dessa göra tolkningar om deltagarnas exponering.

Sex av de mest vanligt förkommande legat-PFAS, detekterades i runt 70-100 procent av deltagarna. För några av dessa PFAS var halten i dricksvattnet den viktigaste förklarande faktorn. Detta tyder på att dricksvattnet varit en viktig exponeringskälla, trots att PFAS-halterna i dricksvattnet får anses vara låga inte bara i Sverige utan även från en utländsk kontext. En annan viktig exponeringskälla tycks ha varit kosten, då deltagare som åt en hälsosam och varierad kost rik på fisk och skaldjur hade högre PFAS-halter i blodet i jämförelse med deltagare som inte åt denna typ av kost. Det finns också faktorer som indirekt påverkade exponeringen. Bland dessa fann vi att det födelseland som deltagaren och deltagarens mamma var födda i påverkade PFAS-halten i blod. Mer specifikt såg vi att de deltagare som, tillsammans med sin mamma, kom från ett höginkomstland hade högre PFAS-halter i blodet än de deltagare, som tillsammans med sin mamma, kom från ett låg-/lägre-medelinkomstland. Därtill hade pojkar en högre PFAS-halter i blodet i jämförelse med flickor.

Sammanfattningsvis har denna avhandling bidragit med information om vilka faktorer som skulle kunna förklara variationen av PFAS-exponeringen hos ungdomar i Sverige. Denna typ av information är viktig att ha i åtanke när man undersöker effekten av PFAS på ungdomars hälsa. Dessutom är denna information viktig att beakta i framtida riskbedömningar.

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Appendix I

Paper I

Demographic, life-style and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents

Appendix II

Paper II

Healthy eating index and diet diversity score as determinants of serum perfluoroalkyl acid (PFAA) concentrations in a national survey of Swedish adolescents

Appendix III

Paper III
Low concentrations of perfluoroalkyl acids (PFAAs) in municipal drinking water associated with serum PFAA concentrations in Swedish adolescents

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Demographic, life-style and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents

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ABSTRACT

Per: and polyfluoroalkyl substances (PFAS) may affect adolescent health, yet factors related to PFAS concentrations in serum are poorly understood. We studied demographic, life-style and physiological determinants of serum PFAS concentrations in Swedish adolescents from a nation-wide survey, Riksmaten Adolescents 2016–17 (RMA, age 10–21 years, n = 1098). Serum samples were analyzed for 42 PFAS, using liquid chromatography-tandem mass spectrometry. The cumulative probability model was used to estimate associations between serum PFAS and determinants, using ordinal logistic regression. Legacy linear (lin-) perfluorooctanoic acid (PFOA), perfluorononaoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), lin-perfluorohexanesulfonic acid (PFHxS) and lin-/branched (br-) perfluorooctanesulfonic acid (PFOS) were quantifiable in ≥70% of the samples. The emerging PFAS 9-chlorohexanedecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS) was quantified in 5.4% of the samples, suggesting initiation of long-range transport far from production sites. Median concentrations of all legacy PFAS were <2 ng/g serum, with a few participants having very high (>100 ng/g serum) lin-PFHxS and lin-/br-PFOS concentrations due to previous high exposure from PFAS-contaminated drinking water. Legacy PFAS exposure was strongly associated with birth country of the participants and their mothers. 2-fold higher estimated adjusted mean (EAM) concentrations were seen among high income country participants with mothers from high income countries than among low/lower-middle income country participants with mothers from the same category. Menstruating females had lower br-PFOS EAM concentrations than those who were not. Iron status (plasma ferritin) among females may be a marker of intensity of menstrual bleeding, but it was not significantly associated with legacy PFAS concentrations among females. Further studies are needed to determine how physiological changes occurring around menstruation affect the toxicokinetics of PFAS in females. In conclusion, PFAS are pollutants of the industrialized world and some of the identified determinants may be overlooked confounders/effect modifiers that should be included in future PFAS/health studies among adolescents.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are ubiquitously detected in biota and humans (Kelly et al., 2009; Ahrens and Bundschuh, 2014; DeWitt, 2015). A wide range of PFAS are linked with adverse health effects in animals, including endocrine disruption, offspring development, immunosuppression and liver toxicity (DeWitt, 2015; Lilienthal et al., 2017). Comparable results have been reported in epidemiological studies on children and adolescents, where PFAS exposure is associated with reduced antibody response post-vaccination (Grandjean et al., 2012; Granum et al., 2013; Abraham et al., 2020), dyslipidemia (Frisbee et al., 2010; Geiger et al., 2014), and reduced birth

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weights in infants (Fei et al., 2007; Darrow et al., 2013). For some of these end-points the literature is however not consistent (EFSA, 2020), and further studies are needed to elucidate the PFAS health risks for children/adolescents.

Of the approximately 5000 registered PFAS (OECD, 2018), the most studied are the persistent legacy chemicals perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). However, due to a nearly complete global phase out of PFOS and PFOA, replacements such as hexafluoropropylene oxide dimer acid (HFPO-DA; GenX) and odecafluoro-3H-4,8-dioxanononanoate (ADONA) are already present in the environment in areas with high contamination due to industrial production (Gebbink et al., 2017; Wang et al., 2019a; Awad et al., 2020). Human biomonitoring of such emerging PFAS have been infrequently conducted due to the relative short production period as opposed to the half a century long production of legacy PFAS (Miaz et al., 2020; Brase et al., 2021). As a result, screening has predominantly been carried out for adults with occupational exposure and those residing within close proximity to fluorotelomer industries or in other highly contaminated areas (Brase et al., 2021; Miaz, 2020). Moreover, biomonitoring of emerging, but also legacy PFAS, has been primarily focused on adult and infant/child populations (Brase et al., 2021). There consequently exists a need to evaluate the prevalence of both emerging and legacy PFAS in a general adolescent population.

Furthermore, human exposure to PFAS is complex and varies both within and between populations. In the general adult population, drinking water from areas with high PFAS contamination of surface/groundwater and, in general, food have shown to contribute substantially to the total exposure (Domingo and Nadal, 2017; Sunderland et al., 2019). Inhalation and/or ingestion of indoor dust, as well as use of products containing PFAS, may also contribute significantly to exposure in some instances (DeLuca et al., 2021). Serum/plasma concentrations of PFAS are generally used as markers of PFAS body burdens in humans, as it reflects the long-term cumulative exposure to bioaccumulating PFAS. Understanding which life-style/demographic/physiological determinants that affects exposure and toxicokinetics of PFAS, such as excretion, is vital for identifying sensitive groups within a population. Identified determinants may also present valuable information on potential confounders in future health studies.

Recognized demographic/physiological determinants explaining some of the variation of serum/plasma PFAS concentrations in adult populations include age, gender (Calafat et al., 2007a; Kärrman et al., 2007), excretion of PFAS via menstruation among women (Wong et al., 2014; Park et al., 2019), ethnicity/race (Calafat et al., 2007b; Jain, 2014), and education level (Calafat et al., 2007b; Bjerregaard-Olesen et al., 2016). Intriguingly, the same determinants have not always been identified in children and adolescents, e.g. contradictory associations have been reported for both age (Ye et al., 2018; Kang et al., 2018; Daly et al., 2018) and gender (Toms et al., 2009; Schecter et al., 2012; Mondal et al., 2012; Ye et al., 2018). Additionally, biomonitoring studies in children and adolescents are often limited up to the age of 13 (Toms et al., 2009; Schecter et al., 2012; Winkens et al., 2017; Harris et al., 2017; Ye et al., 2018), and only a few studies include the entire WHO-defined adolescent period between 10 and 19 years (Zhou et al., 2016; Kang et al., 2018; Averina et al., 2018; Papadopoulou et al., 2019). Due to the large physiological and behavioral changes that occur with the onset of puberty, adolescents are potentially vulnerable to chemical insult (Wild and Kleinjans, 2003; Benedetti and Baltes, 2003). Consequently, more research is warranted on this age group, since determinants of PFAS body burdens in infant/child and adult populations may not be applicable for adolescent populations.

The objectives of the current nation-wide study of Swedish adolescents were two-fold: 1) screen and evaluate serum concentrations of legacy and emerging PFAS; and 2) assess life-style/demographic/physiological determinants that could explain the variation of PFAS concentrations in serum, i.e. variation in cumulative exposure and toxicokinetics. Among female participants, menstruation, an excretion route in adult women, yet rarely studied in adolescent females, was investigated. We additionally assessed the possibility of using plasma ferritin (biomarker of iron-status) as a proxy for menstrual blood loss (Wang et al., 2013).

2. Methods

2.1. Study population

The study population was a subsample of Riksmaten Adolescents 2016–17 (RMA), a nationally representative cross-sectional school-based dietary survey, conducted by the Swedish Food Agency and with approximately 3000 participants. A comprehensive description of the population and study design is given in Moraeus et al. (2018). In brief, students in school grades 5 (ages 11–12 years), 8 (ages 14–15 years) and 11 (ages 17–18 years) were invited to participate in the study between September 2016 and May 2017. Statistics Sweden selected schools based on geographical location, public or charter school, and municipality classification. Participant height and weight were measured by trained staff. A subsample of 2377 students were invited to donate biological samples, and 1176 (49%) completed this part of the study (Moraeus et al., 2018). 1098 participants completed the dietary assessment and donated a blood sample that was available for PFAS analysis. Ethical approval was granted by the Regional Ethical Review Board in Uppsala (No. 2015/190). All participants, or legal guardians of participants <16 years, gave written informed consent to participate in the study.

2.2. Assessment of diet and personal characteristics

A web-based system called RiksmatenFlex (RF), which consisted of the RiksmatenFlexDiet (RFD) and RiksmatenFlexQuestionnaire (RFQ) segments, was used for assessment of personal characteristics and diet. RFD is a validated (Lindroos et al., 2019) 24-h dietary recall method that allows participants to register their consumption of food and beverages on two non-consecutive days, retrospectively (Moraeus et al., 2018). The second day of dietary registration was randomized 2–7 days after the first day of registration. The RFQ covered participants' perceived health status and socio-economic and lifestyle factors. It additionally included food frequency questionnaires focusing on specific foods that are less likely to be consumed regularly and not captured in RFD (Moraeus et al., 2018).

2.3. Blood sampling and chemical analysis

Non-fasting venous blood was sampled in 10 ml tubes with coagulation activator and in 6 ml lithium heparin tubes. After centrifugation, serum and plasma were stored at −20 °C onsite and then transported and stored frozen at −80 °C until analysis (Moraeus et al., 2018).

Serum were analyzed for perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), linear and branched PFOA (lin- and br-), perfluorononoanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluoropentadecanoic acid (PFPeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFOcDA), perfluoropentanesulfonic acid (PFPeS), lin/br-perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), lin/br-PFOS, perfluorononanesulfonic acid (PFNS), lin/br-perfluorodecanesulfonic acid (PFDS), perfluoroundecanesulfonic acid (PFUnDS), lin/br-perfluorooctansulfanomid (FOSA), lin/br-perfluorooctane sulfonamidoacetic acid (FOSAA), lin/br- N-methylperfluoro-1-octanesulfonamideoacetic acid (MeFOSAA), lin/br- N-ethylperfluoro-1-octanesulfonamidoacetic acid (EtFOSAA), 6:2 fluorotelomer phosphate diester (6:2 diPAP), 8:2 fluorotelomer phosphate diester (8:2 diPAP), 6:s/8:2 fluorotelomer phosphate diester (6:2/8:2 diPAP), 9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid (9Cl-PF3ONS), 11-

Table 1
Categorical and continuous base model covariates.

	All *n (%)*	Males *n (%)*	Females *n (%)*
Categorical covariates	1098 (100)	482 *(44)*	616 (56)
	BMI status[a]		
Underweight	72 *(7)*	31 *(6)*	41 *(7)*
Normal weight	793 *(72)*	349 *(72)*	444 *(72)*
Overweight	190 *(17)*	82 *(17)*	108 *(18)*
Obese	43 *(4)*	20 *(4)*	23 *(4)*
	Birth country (participant/mother)		
Both high income countries	898 *(82)*	399 *(83)*	499 *(82)*
High income/upper-middle countries	79 *(7)*	31 *(6)*	48 *(8)*
Both upper-middle income countries	39 *(4)*	15 *(3)*	24 *(4)*
High and upper-middle/low and lower-middle income countries	29 *(3)*	13 *(3)*	16 *(3)*
Both low and lower-middle income countries	42 *(4)*	20 *(4)*	22 *(4)*
Missing	11 *(1)*	4 *(1)*	7 *(1)*
	Education level - mother		
No formal education and primary education	84 *(8)*	34 *(7)*	50 *(8)*
Vocational education or equivalent	162 *(15)*	63 *(13)*	99 *(16)*
3–4 year upper secondary education or equivalent	229 *(21)*	99 *(21)*	130 *(21)*
University education or equivalent	565 *(51)*	256 *(53)*	309 *(50)*
Missing	58 *(5)*	30 *(6)*	28 *(5)*
	Education level – father		
No formal education and primary education	111 *(10)*	43 *(9)*	68 *(11)*
Vocational education or equivalent	215 *(20)*	94 *(20)*	121 *(20)*
3–4 year upper secondary education or equivalent	280 *(26)*	132 *(27)*	148 *(24)*
University education or equivalent	411 *(37)*	179 *(37)*	232 *(38)*
Missing	81 *(7)*	34 *(7)*	47 *(8)*
	Smoking habits		
Non-smoker (incl. 5th graders)[b]	955 *(87)*	430 *(89)*	525 *(85)*
Smoker	123 *(11)*	44 *(9)*	79 *(13)*
Missing	20 *(2)*	8 *(2)*	12 *(2)*
	Snus use		
Does not use snus (incl. 5th graders)[b]	1016 *(93)*	441 *(86)*	575 *(93)*
Snus user	70 *(6)*	33 *(7)*	37 *(6)*
Missing	12 *(1)*	8 *(2)*	4 *(1)*
	Alcohol consumption during last 6 months		
Has never consumed alcohol (incl. 5th graders)[b]	759 *(69)*	352 *(73)*	407 *(66)*
Once	107 *(10)*	48 *(10)*	59 *(10)*
Several times	209 *(19)*	75 *(16)*	134 *(22)*
Missing	23 *(2)*	7 *(2)*	16 *(3)*
	Exclusively breastfed		
Never breastfed and breastfed for <1 month	111 *(10)*	47 *(10)*	64 *(10)*
1–6 months	591 *(54)*	260 *(54)*	331 *(54)*
7 to >12 months	359 *(33)*	156 *(33)*	203 *(33)*
Missing	37 *(3)*	19 *(4)*	18 *(3)*
Continuous covariates	**Median** (range) *(% missing)*	**Median** (range) *(% missing)*	**Median** (range) *(% missing)*
Age (years)	14.6 (10.6, 21.1) *(0)*	14.4 (10.6, 21.0) *(0)*	14.7 (10.8, 21.1) *(0)*
Swedish Healthy Eating Index for Adolescents 2015 (SHEIA15)	5.82 (3.37, 8.86) *(0)*	5.63 (3.76, 8.24) *(0)*	5.93 (3.37, 8.68) *(0)*

[a] For participants <18 years of age, BMI status was classified based on age- and sex dependent cut-offs (Cole and Lobstein, 2012), while for participants ≥18, the WHO cut-offs were used (WHO, 1999).
[b] 5th grade participants were not asked about alcohol and tobacco use due to their young age.

chloroeicosafluoro-3-oxanone-1-sulfonic acid (11Cl-PF3OUdS), ammonium 4,8-dioxa-3H-perfluorononnanoate (ADONA), 3:3 fluorotelomer carboxylic acid (3:3 FTCA), 5:3 fluorotelomer carboxylic acid (5:3 FTCA), 7:3 fluorotelomer carboxylic acid (7:3 FTCA), 4:2 fluorotelomer sulfonate (4:2 FTSA) and 8:2 fluorotelomer sulfonate (8:2 FTSA) by the Department of Environmental Science, Stockholm University. A detailed description of the serum PFAS analysis, including quality control, is provided in the Supplement in connection to Table S1. Briefly, sample extraction was carried out using a method adapted from Powley et al. (2005). The samples were analyzed using a Waters ultra-performance liquid chromatograph coupled to a Waters Xevo TQS triple quadrupole mass spectrometer operated in negative electrospray ionization, multiple reaction monitoring (MRM) mode. Quantification was based on isotope dilution. Targets and their corresponding isotopically labelled internal standards are provided in Table S1. Data for the PFAS were classified as either 'quantitative', 'semi-quantitative', or 'qualitative', based on either availability of authentic standards and/or performance of QC samples (Table S1). 'Quantitative' indicated that an exactly-matched authentic standard was available and that QC samples displayed reasonable accuracy and precision (25 targets). 'Semi--quantitative' targets were those quantified with exactly-matched authentic standards but displaying sub-optimal accuracy and/or precision for QC samples, including 6:2 and 8:2 diPAP, EtFOSAA, 7:3 FTCA, and 8:2 FTSA. 'Qualitative' targets included PFPeS, PFHpS, PFNS, PFUnDS, PFPeDA, PFHxDA, PFOcDA, 6:2/8:2 diPAP, FOSAA, 3:3 and 5:3 FTCA, due to poor accuracy and/or precision in QC samples or lack of authentic standards.

Ferritin and C-reactive protein (CRP) in plasma were analyzed on

Abbott Architect ci8200 analyzers (Abbott Laboratories, Abbott Park, IL, USA) at the accredited laboratory of Department of Clinical Chemistry and Pharmacology, University Hospital, Uppsala, Sweden.

2.4. Regression models

2.4.1. Base model

The associations between PFAS serum concentrations and possible determinants were investigated by ordinal regression analysis (see section 2.5.2.). For more detailed information about the determinants see Table 1. The base model included previously reported determinants of PFAS concentrations, i.e. parental education level, participant/maternal birth country (BC) (Glynn et al., 2020), tobacco use (Eriksen et al., 2011), alcohol consumption (Pitter et al., 2020), body mass index (BMI status) (Hölzer et al., 2008; Eriksen et al., 2011), gender (male or female), age (in years), and months exclusive breastfeeding early in life (Gyllenhammar et al., 2019). Participants (n = 11) exceeding the UN-defined age-span for adolescents (10–19) were included as recruitment was conducted through schools and the observed age range is most likely representative for grades 5, 8 and 11 (Saywer et al., 2018).

In order to account for possible differences in dietary PFAS exposure between different demographic groups, the Swedish Healthy Eating Index for Adolescents 2015 (SHEIA15) was included as a variable in the model. The results for this variable are presented in a separate companion paper investigating the importance of different dietary patterns on PFAS serum concentrations. SHEIA15 reflects healthy eating of RMA participants (Moraeus et al., 2020). In short, SHEIA15 was based on the key dietary advice from the 2012 Nordic Nutrition Recommendations (Nordic Nutrition Recommendations, 2014), calculated as the ratio between the actual consumption (as registered in RFD by participants) and the recommended consumption for each food or nutrient (fruits and vegetables, wholemeal, seafood, dietary fats, low fat dairy products, red and processed meat, added sugar), and summed up to a total continuous score of maximum 9 (Moraeus et al., 2020).

The participant and maternal birth countries were classified according to the World Bank Country Classification (World Bank Group, 2018), by per capita gross national income level with the following categories: high income-, upper-middle- and lower-middle/low income countries. To avoid issues with collinearity between the participant and the maternal birth country, a joint birth country covariate was created (BC) (Table 1).

2.4.2. Sensitivity analysis

A sensitivity analysis was conducted for the base model in order to evaluate potential bias of results due to some participants living in areas with a known history of PFAS-contaminated DW (Gyllenhammar et al., 2015; Li et al., 2018). Consequently, the categorical covariate containing the two categories 'schools in area with previous DW contamination' (i.e. Ronneby and Uppsala, n = 42) and 'schools elsewhere' was added to the base model (DW sensitivity analysis).

2.4.3. Secondary models

We created two separate secondary models with the aim of evaluating the association between menstrual blood loss and serum PFAS concentrations. Females registered in RFQ whether or not they had started menstruating and at what age menstruation began. Menstruation was assumed to have continued from onset to age at RMA participation. Onset of menstruation (yes or no) and years since onset of menstruation (continuous) were separately included in the base model for all females.

In two additional separate models, plasma ferritin (μg/L plasma) was included as a potential marker of long-term menstrual bleeding (Wang et al., 2013). Log-transformed C-reactive protein plasma levels (CRP, mg/L plasma), an infection marker, was included in the ferritin models to adjust for the increase in levels of ferritin during an infection (Khan et al., 2016). Additionally, an interaction term between CRP and ferritin was added to evaluate said relationship, and the significance of ferritin was consequently evaluated by forming contrasts for an inter quartile range increase in ferritin while holding CRP constant at the 10th, 50th and 90th percentiles. CRP $<$ LOD was set to LOD/ $\sqrt{2}$. To ensure that a possible ferritin-PFAS association among the females was solely connected to blood loss, the same ferritin model was also fit for the males.

2.5. Statistical analysis

All data processing and statistical analyses were executed using R (version 3.6.3; R Development Core Team), and statistical significance was set at $p \leq 0.05$. ~18% of participants had single/a few missing data from RFQ or PFAS analyses. Missing data were imputed 5 times using multiple imputation by chained equations assuming data to be missing at random, conditional on the covariates. The imputation was carried out separately by gender as we wanted to ensure that our gender specific analysis (menstruation/ferritin) would not be influenced by any potential gender-dependent variability amongst the covariates. Coefficients and standard errors were estimated using Rubin's rules for pooling multiple imputed data (van Buuren and Groothuis-Oudshoorn, 2011; Rubin, 1987). When PFAS concentrations were <LOQ, reported concentrations $\geq$ LOD were used. Although these data are more uncertain compared to data $\geq$ LOQ, their use tends to reduce bias compared to if concentrations $<$ LOQ are set to a fixed value, for instance zero, ½ LOQ or LOQ/$\sqrt{2}$. The latter replacements will add an undesirable systematic error if used in regression analyses (Bergstrand and Karlsson, 2009; RSC, 2001).

2.5.1. Principal component analysis

Principal component analysis (PCA) was used to visualize exposure patterns between legacy and emerging PFAS. The PCA used a singular value decomposition of the PFAS data matrix including PFAS with detectable concentrations for >2% of the study population. In an additional sensitivity analysis, participants residing in Uppsala and Ronneby were removed. Although analytical quality of PFPeS and PFHpS data was deemed qualitative (due to quantification using an authentic standard of PFHxS), these targets were nevertheless included in the PCA since they were detected in >2% of the participants as well as due to the high degree of confidence in their identification.

2.5.2. Ordinal logistic regression (OLR)

The cumulative probability model (CMP) was used, which fits the continuous response variable using ordinal logistic regression (OLR). Such models are invariant to monotonic transformations (e.g. log) of outcomes as only the order information of the outcome is incorporated. This successfully integrates PFAS concentrations below LOD without the need for prior estimation or imputations of undetectable concentrations. It also removes the need for transformation of the PFAS levels, which showed skewed distributions (Liu et al., 2017). All PFAS data $<$ LOD were substituted with a value of 0.0001 ng/g in order to be included in the analyses. To account for the effect of clustering due to sampling by school, the Huber-White method was applied to the covariance matrices from the model fits. Model fit was evaluated using QQ-plots of the probability scale residuals (Liu et al., 2017). As data were imputed separately for gender, both imputed datasets were joined by stacking before fitting of the ordinal regression models. Being an ordinal logistic model, the OLR provides (log) odds ratios, but as it estimates the entire empirical cumulative distribution function it is possible to obtain conditional means, medians and exceedance probabilities (Liu et al., 2017). The covariate contribution to the variation of serum PFAS concentrations was evaluated by calculating χ^2 - degrees of freedom for each variable for each model separately. This levels the playing field between the variables in a particular model since the expected value of a χ^2 random variable with k degrees of freedom is k. However, this measure can only be compared within, and not across, models.

Table 2
PFAS serum concentrations in the study population, including the extra target analytes included in the PCA.

PFAS (ng/g serum)	*n*	LOD *(%<LOD)*	LOQ *(%<LOQ)*	Detected and quantified Median (range)	Quantified Median (range)
PFCA					
PFHpA	1098	0.020–0.103 *(88.6)*	0.058–0.288 *(92.2)*	<LOD (<LOD, 0.604)	<LOQ (<LOQ, 0.604)
lin-PFOA	1095	0.287 *(0.1)*	0.020–0.288 *(0.2)*	1.203 (<LOD, 9.75)	1.203 (<LOQ, 9.75)
PFNA	1098	0.103–0.176 *(4.9)*	0.058–0.288 *(7.3)*	0.382 (<LOD, 2.80)	0.382 (<LOQ, 2.80)
PFDA	1098	0.028–0.099 *(19.7)*	0.058–0.288 *(37.4)*	0.162 (<LOD, 1.35)	0.140 (<LOQ, 1.35)
PFUnDA	1098	0.020–0.119 *(30.6)*	0.058–0.288 *(52.9)*	0.097 (<LOD, 1.01)	0.097 (<LOQ, 1.01)
PFDoDA	1098	0.014–0.080 *(91.3)*	0.058–0.288 *(98.7)*	<LOD (<LOD, 0.182)	<LOQ (<LOQ, 0.182)
PFTrDA	1098	0.013–0.155 *(91.6)*	0.058–0.288 *(99.4)*	<LOD (<LOD, 0.168)	<LOQ (<LOQ, 0.113)
PFSA					
PFPeS[a]	830	0.044–0.195 *(97.2)*	0.078–3.30 *(99.3)*	<LOD (<LOD, 1.86)	<LOQ (<LOQ, 1.38)
br-PFHxS	1098	0.010–0.061 *(90.6)*	0.022–0.186 *(95.7)*	<LOD (<LOD, 3.74)	<LOQ (<LOQ, 3.74)
lin-PFHxS	1098	0.017–0.216 *(0.7)*	0.022–0.464 *(7.9)*	0.399 (<LOD, 255)	0.397 (<LOQ, 255)
PFHpS[a]	1098	0.021–0.102 *(90.5)*	0.022–3.34 *(97.6)*	<LOD (<LOD, 5.76)	<LOQ (<LOQ, 5.76)
br-PFOS	1098	0.031–0.257 *(0)*	0.056–0.562 *(2.0)*	0.925 (0.031, 110)	0.925 (<LOQ, 110)
lin-PFOS	1098	0.056–0.562 *(0)*	0.056–0.562 *(0)*	1.995 (0.281, 127)	1.995 (0.281, 127)
FASAA					
lin-MeFOSAA	1098	0.013–0.104 *(91.2)*	0.060–3.57 *(98.7)*	<LOD (<LOD, 6.04)	<LOQ (<LOQ, 6.04)
PFESA					
9Cl-PF3ONS	1098	0.005–0.067 *(94.6)*	0.024–3.50 *(99.8)*	<LOD (<LOD, 1.21)	<LOQ (<LOQ, 1.21)

Note: The limit of detection (LOD) and limit of quantification (LOQ) varied between batches; FASAA, perfluoroalkane sulfonamido acetic acid; PFESA, per- and polyfluoroalkyl ether sulfonic acids.
[a] Analytical quality: qualitative.

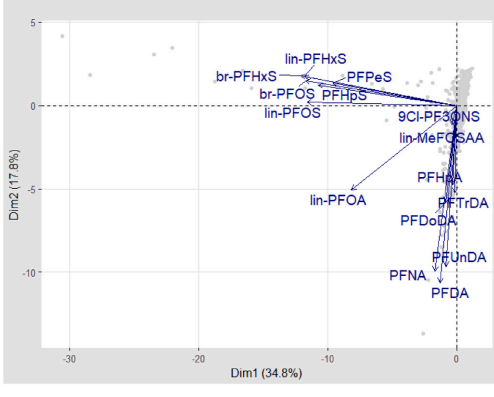

Fig. 1. Principal component analysis (PCA) biplot for PFAS detected in >2% of the adolescent participants, showing loading of each PFAS (arrows) and PC scores of samples (dots) (n = 827).

3. Results

3.1. Study population – PFAS concentrations and demographics

Table 1 presents the base model characteristics and demographics of the study population (n = 1098), while Table S2 presents the secondary and sensitivity analysis model covariates. The legacy PFAS lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin/br-PFOS were detected in ≥70% of the samples and consequently included in the OLR (Table 2). lin-PFOS showed the highest median concentration, being roughly twenty-fold higher than medians of PFDA and PFUnDA. The highest individual concentrations were found among participants from Ronneby for lin-PFHxS, being approximately two-fold higher than the highest concentrations of lin/br-PFOS, and more than 100-fold higher than those of PFNA, PFDA and PFUnDA (Table 2 and S3). Median PFOA, PFHxS and PFOS concentrations were significantly higher in Uppsala and Ronneby participants than in the rest of the study group (Table S3). Lin-MeFOSAA and 9Cl-PF3ONS were detected in about 9% and 5% of the samples, respectively, and were included in the PCA, as were PFHpA, PFDoDA, PFTrDA, br-PFHxS (quantitative data), PFPeS and PFHpS (qualitative data) which were detected in 3–11% of all samples (Table 2). Serum PFAS concentrations not included in either OLRs or PCA, with <2% detected concentrations, are provided in Table S4. Among the PFAS with quantitative analytical quality, the emerging PFAS 11Cl-PF3OUdS and ADONA were detected and quantified in 0.6 and 0.1% of the participants, respectively.

The two first principal components (PCs) of the PCA explained roughly 53% of the total serum PFAS concentration variability (Fig. 1), with a total of eight PC's needed to explain a minimum of 90% of the variance. Based on the directions in the PC space, PFCA with a perfluorinated carbon chain length of ≥8 carbons clustered together and largely explained the variation of the second dimension, while PFSA with ≥5 carbons clustered together to explain a large amount of the variation in the first dimension (Fig. 1). Lin-PFOA did not cluster with any of the large clusters, while PFHpA, lin-MeFOSAA and 9Cl-PF3ONS (all displaying low detection frequency), clustered with the long-chained PFCA, but explained very little of the variation of PC2 (Fig. 1, Table S5). Removing participants from Uppsala and Ronneby reduced the explained total variability in PFAS serum concentrations to roughly 40% (Figure S1). Additionally, PFPeS and PFHpS were excluded from the PCA as they were only detected in a few samples outside of Uppsala/Ronneby. The orientation of the clusters in the PCA changed, with lin-PFOA moving closer to the long-chained PFCA and 9Cl-PF3ONS clustering with the PFSA instead of the PFCA (Figure S1).

3.2. Base model determinants

Birth country (BC) was significantly associated with concentrations of all studied legacy PFAS (Fig. 2, Table S6-S12), and participants whom, together with their mothers, were born in high income countries had the highest estimated adjusted mean (EAM) concentrations (Fig. 3). With the exception of PFDA and PFUnDA, participants whom, together with their mothers, originating from low and lower-middle income countries had the lowest EAM PFAS concentrations (Fig. 3). Moreover, the maternal contribution to the BC association is illustrated among participant born in high income countries, with mothers from upper-middle and lower-middle/low income countries, having lower EAMs than those with mothers from high income countries. BC was the

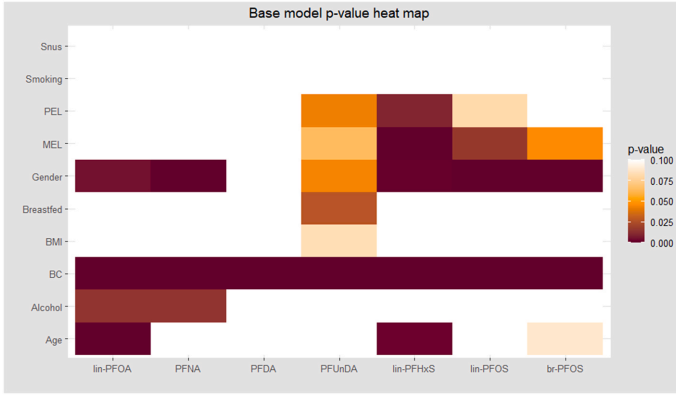

Fig. 2. Heat map of p-values for associations between potential determinants and serum legacy PFAS concentrations in adolescents, estimated in ordinal logistic regression (OLR) analyses (n = 1098). All determinants were included in the regression models and the results were also adjusted for the healthy diet index SHEIA15, Swedish Healthy Eating Index for Adolescents 2015; PEL, paternal education level; MEL, maternal education level; BMI status, body mass index cut-offs; BC, participant/maternal birth country.

strongest determinant of the serum legacy PFAS concentrations (Fig. 4). Females had significantly lower EAM concentrations compared to males, except in the case of PFDA (Fig. 2, Table S6-S12). Gender was the second strongest determinant of PFNA, lin-PFOS and br-PFOS, and third strongest for, lin-PFOS and br-PFOS and lin-PFHxS (Fig. 4). Age was inversely associated with lin-PFOA and lin-PFHxS concentrations

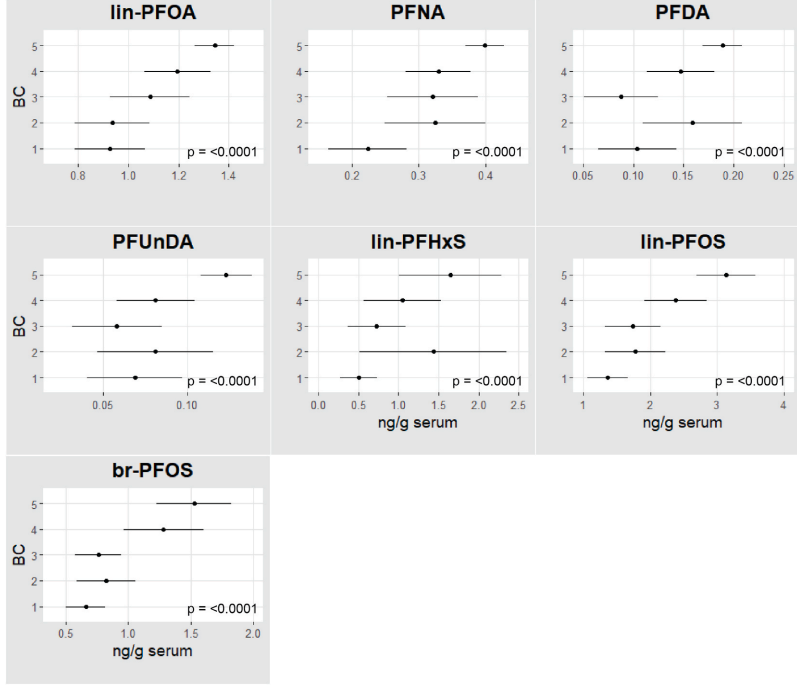

Fig. 3. Estimated adjusted mean serum PFAS concentrations and 95% confidence interval for different categories of birth country (BC) determined in the ordinal regression analysis (OLRs), and adjusted for the covariates age, gender, body mass index cut-offs, maternal and paternal education level (MEL/PEL), months exclusively breastfed, snus and smoking habits, alcohol consumption, and the healthy eating index (SHEIA15). BC; participant/maternal birth country, 5 = both from high income countries, 4 = high/upper-middle income countries, 3 = both upper-middle income countries, 2 = high and upper-middle/low and lower-middle income countries, 1 = both from low and lower-middle income countries. The p-value results from a global test of the variable where the null hypothesis is that all coefficients are equal to zero.

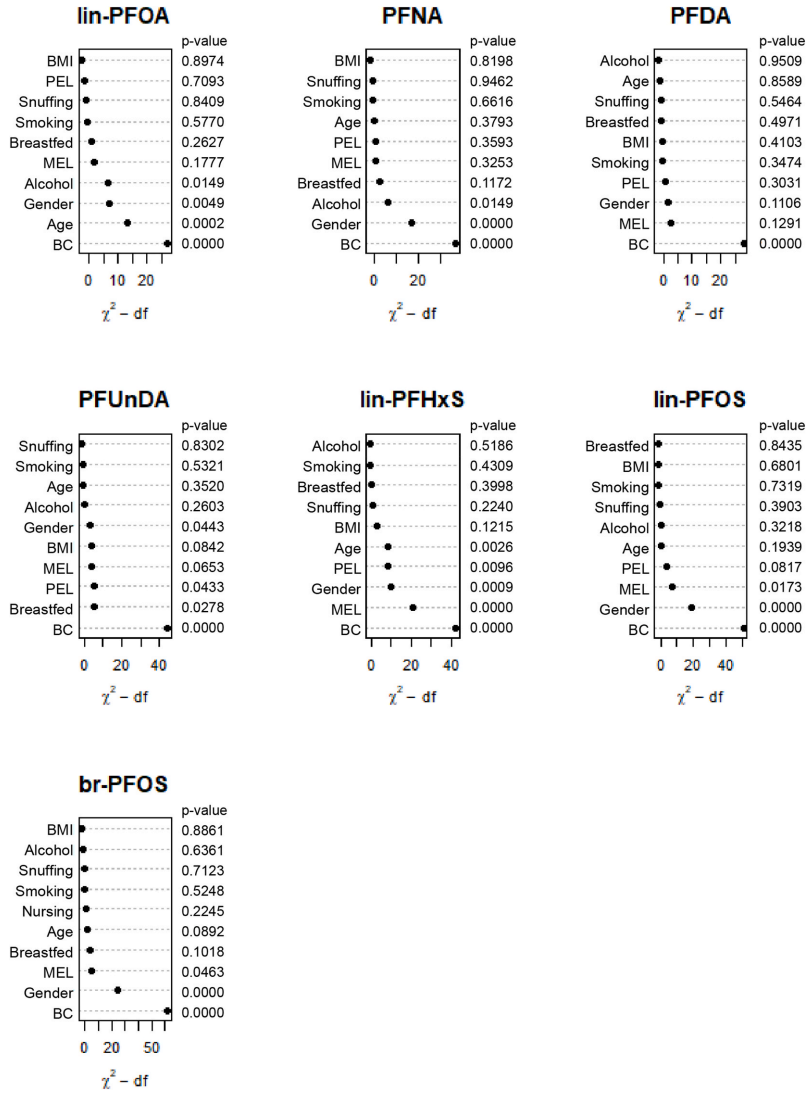

Fig. 4. The relative importance of base model covariates as determinants of PFAS concentrations estimated using ordinal logistic regression (n = 1098), expressed as the χ^2- df (x-axis). Covariates included in the regression models are given on the left y-axis and p-value for each covariate on the right y-axis. Note that the relative importance of a covariate can only be compared within each PFAS model, and not between different PFAS models. SHEIA15, Swedish Healthy Eating Index for Adolescents, was included in the regression model to adjust for differences in dietary patterns among the participants. BMI status, body mass index cut-offs; MEL, maternal education level; PEL, paternal education level; BC, participant/maternal birth country.

(Fig. 2, Table S6-S12), and was the second strongest determinant for lin-PFOA (Fig. 4). Participants who had consumed alcohol more than once in the last six months showed significantly higher EAM concentrations of lin-PFOA and PFNA compared to those not consuming alcohol (Fig. 2, Table S6-S12). EAMs of lin-PFHxS and lin-PFOS were highest among participants with mothers who had attended university/college (Fig. 2, Table S6-S12). Maternal education was the second strongest determinant of lin-PFHxS, and the third strongest for lin-PFOS (Fig. 4). Significant associations with paternal education were observed for PFUnDA and lin-PFHxS (Fig. 2, Table S6-S12), being the third and fourth strongest determinants, respectively (Fig. 4). Months of reported exclusive breastfeeding was significantly associated with PFUnDA (Fig. 2), with the highest EAM concentration found in participants who had the lowest degree of breastfeeding (Table S6-S12). No significant

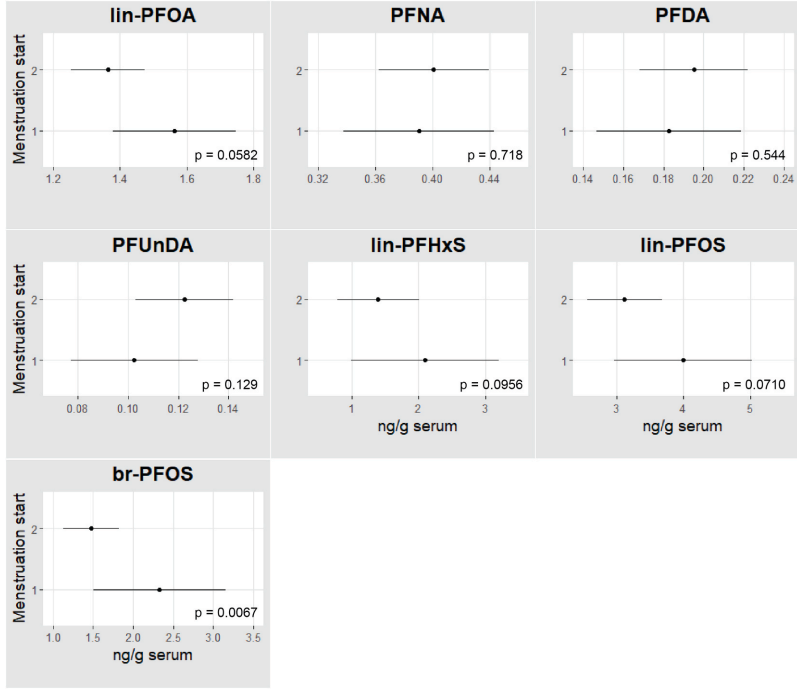

Fig. 5. Estimated adjusted mean serum PFAS concentration and 95% confidence interval for females who had not started menstruating (1) and females who had started menstruating (2) (n = 616). Adjusted for age, participant/maternal birth country (BC), maternal and paternal education level (MEL/PEL), smoking and snus habits, alcohol consumption, months exclusively breastfed, body mass index cut-offs and the healthy eating index SHEIA15. The p-value results from a global test of the variable where the null hypothesis is that all coefficients are equal to zero.

associations were found for BMI status, and smoking and snus (tobacco placed under upper lip) habits (Fig. 2, Table S6-S12).

3.3. Sensitivity analysis

The DW sensitivity analysis did not markedly influence the base model covariate association for lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and br-PFOS (Table S6-S12). However, lin-PFOS and br-PFOS were no longer associated with maternal education.

3.4. Secondary models - menstruation

Females who had started menstruating had significantly lower adjusted br-PFOS serum concentrations compared to females who were yet to start menstruating (Fig. 5, Table S13) (n = 616). A similar tendency, although not statistically significant, was observed for lin-PFOA, lin-PFHxS and lin-PFOS (Fig. 5, Table S13). Number of years since onset of menstruation did not significantly influence PFAS serum concentrations (Table S13). No significant association was found between any of the PFAS and plasma ferritin levels among females at neither the 10th, 50th nor the 90th percentile of CRP (Table S13) (n = 616). However, among males (n = 482), the odds ratio for ferritin and serum PFNA, PFDA and PFUnDA increased significantly with increasing CRP level, a trend which also appeared for lin/br-PFOS (Table S13). Ferritin was also significantly associated with lin-PFOA and lin-PFHxS in males, though in this case the odds ratios appeared to decrease with increasing CRP levels (Table S13).

4. Discussion

The legacy PFAS lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin/br-PFOS were detected in >70% of the serum samples from RMA participants, showing general long-term exposure to these PFAS among Swedish adolescents. The very high PFOS and PFHxS concentrations (>100 ng/ml) observed in some 5th graders from Ronneby were due to serious contamination of one of the two municipal waterworks in the area (Xu et al., 2020). These PFOS and PFHxS concentrations are amongst the highest reported for adolescents globally (Averina et al., 2018; Ramesh et al., 2019; Colles et al., 2020). This highlights the urgency of identifying and mitigating currently unknown hot-spots of PFAS contamination, which may be contributing to very high DW PFAS exposure in populations living in close proximity to the hot-spots. The contamination in Ronneby was discovered and remediated in 2013 (Xu et al., 2020), but the very long half-lives of PFHxS and PFOS (Li et al., 2018) will result in highly elevated body burdens in this exposed population for decades to come. Although production and use of some of the legacy PFAS already has been more or less banned or severely restricted in many countries of the world (Benskin et al., 2010; Stockholm Convention, 2019), the persistence of these PFAS in the environment makes it important to continue assessing human exposure by analyzing legacy PFAS in food and DW, as well as in human matrices.

Inter-study comparisons of PFAS concentrations in humans are

complicated by differences in analytical methods, non-matching age-span between studies, and temporal trends of human exposure (Nyberg et al., 2018). Nevertheless, average lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin/br-PFOS concentrations were within the range of those observed in comparable adolescent populations from industrial countries such as the US, Taiwan, South Korea and Norway sampled in 2009–2011 (Schecter et al., 2012; Ji et al., 2012; Dong et al., 2013; Averina et al., 2018), but on average higher than those reported in a Canadian adolescent population in 2015 (Caron-Beaudoin et al., 2019). Although only quantified in ~5% of all participants, the quantification of 9Cl-PF3ONS in Swedish adolescents is especially disconcerting as this emerging PFAS exhibits a similar bioaccumulation potential as PFOS (Shi et al., 2015; Pan et al., 2021). Used in the Chinese metal electroplating industry as a PFOS replacement, 9Cl-PF3ONS (also known as 6:2 Cl-PFESA) has mostly been observed in human serum and breast milk samples in China (Awad et al., 2020; Brase et al., 2021). However, it was also detected recently in surface water throughout Europe (including Sweden) (Pan et al., 2018) and intermittently in primiparous Swedish women (Miaz et al., 2020). Our results strengthen the suspicion that this compound is becoming a globally dispersed environmental contaminant, with parts of the adolescent population in Sweden already exposed. The number of participants with 9Cl-PF3ONS concentrations in the same range as the legacy PFAS is, however, still low, suggesting that human exposure outside China may be in its initial phase. In addition to this substance, future biomonitoring studies should also include 11Cl-PF3OUdS and ADONA, both of which were detected in a small fraction of the adolescents in the present study. 11Cl-PF3OUdS has previously been observed in fish consumers from China (Shi et al., 2016), while ADONA was detected intermittently in blood donors from Southern Germany (Fromme et al., 2017).

Despite their low detection frequency in RMA, PCA clustering of PFPeS, PFHpS, and br-PFHxS together with lin-PFHxS and lin/br-PFOS suggests that DW was a source of exposure of the adolescents to these PFAS. The almost exclusive detection of PFPeS, PFHpS in Uppsala and Ronneby participants with a history of DW PFAS exposure explains this exposure pattern (Gyllenhammar et al., 2015; Li et al., 2018). While data quality for PFPeS and PFHpS is considered qualitative, we have considerable confidence in these assignments due to a) the presence of multiple product ions for both substances; b) alignment of retention times relative to other PFSA, and c) the observation of other PFSA, which are expected to co-occur with PFPeS and PFHpS. A study of Swedish airport employees exposed to PFAS-contaminated DW at work supports DW as a source of PFPeS and PFHpS exposure (Xu et al., 2020). While we were initially concerned that inclusion of participants from Uppsala and Ronneby might introduce bias in the statistical analyses of PFAS determinants, the DW sensitivity regression analysis showed that the elevated concentrations in Uppsala and Ronneby participants did not markedly affect the overall results for the other determinants studied.

Lower EAM concentrations of legacy PFAS in adolescents was related to a lower degree of industrialization of BC, as indicated by the per capita gross national income UN classification system. Consequently, BC may be an important confounder in population studies of health effects of legacy PFAS among children/adolescents, both influencing health outcomes and PFAS body burdens. The results are in line with studies from less industrialized countries (Hemat et al., 2010; Müller et al., 2019; Macheka et al., 2020; Timmermann et al., 2020) and studies of immigrant mothers from Sweden (Ode et al., 2013). A small study on Swedish 5th graders similarly observed higher average serum concentrations of legacy PFAS in participants born in high income countries than those born elsewhere (Glynn et al., 2020). These BC differences in exposure may be due to a combination of long half-lives of the PFAS (Olsen et al., 2007; Worley et al., 2017; Li et al., 2018) and general differences in PFAS contamination of human environment and food/DW between high income and low income countries. There are most likely differences in the establishment of PFAS-related industry, access and use of modern consumer/industrial products, and in housing and living standards between the different types of countries (Hanssen et al., 2010; Müller et al., 2019).

Placental transfer (Beesoon et al., 2011; Wang et al., 2019b) and breastfeeding (Fromme et al., 2010; Gyllenhammar et al., 2018) are major determinants of PFAS serum/plasma concentrations in infants, toddlers, and children up to 12 years of age in Sweden and elsewhere (Mogensen et al., 2015; Gyllenhammar et al., 2019). In the present study duration of breast-feeding of the participants early in life did not appear to be an important determinants of serum PFAS concentrations. However, the lower PFAS body burdens among mothers from less industrialized countries (Ode et al., 2013), may have resulted in lower early life maternal transfer of these PFAS to the RMA participants. This may have contributed to the BC differences observed among the RMA adolescents, as indicated by the influence of maternal BC on the results.

The observed BC-related differences in PFAS concentrations were not dependent on differences in parental education levels and healthy dietary patterns (SHEIA15) of the participants. BC relations to PFAS concentrations could nevertheless, in addition to differences in maternal PFAS transfer and other exposures of the participants early in life in their BC, reflect more current disparities in PFAS exposures due to BC-related dietary preferences and lifestyles of the adolescents now all living in Sweden (Säfsten et al., 2016). However, daycare and school lunches mitigate gender- and socio-economic differences in diet in Sweden (Eustachio Colombo et al., 2020), thus potentially diminishing dietary-related BC differences in PFAS exposure. The relations between serum concentrations of PFAS and dietary patterns in RMA will be explored in a separate study.

Similarly to RMA, positive associations were observed between PFOA and alcohol consumption in an Italian adolescent/adult population (14–39 years of age) (Pitter et al., 2020), as well as for PFNA in an adult US population (Jian et al., 2017). However, diverging results have been reported in middle-aged Danish men (Eriksen et al., 2011) and U.S. women (Park et al., 2019). Apart from the possibility that the observed associations are coincidental, it is conceivable that alcohol consumption is a direct exposure source if PFAS-contaminated DW is used in the production of alcoholic beverages, such as beer. Moreover, liver effects of alcohol consumption (Maher, 1997) may alter PFAS toxicokinetics, or alcohol consumption may be a marker for dissimilarities in life-style (Fored et al., 2003; Hanson and Chen, 2007), the latter indirectly affecting PFAS exposure.

Previous studies have reported higher serum/plasma PFAS concentrations among highly educated adults than among those with low education level (Bjermo et al., 2013; Brantsæter et al., 2013; Tsai et al., 2018). In RMA, most of the legacy PFAS were not associated with either maternal or paternal education level, suggesting that life-style connected to education level did not markedly affect PFAS exposure after adjustment for the other determinants and healthy eating habits in RMA. Parental education level was associated with a healthy dietary pattern (SHIEA15) of the RMA participants (Moraeus et al., 2020) and, hypothetically, inclusion of SHIEA15 in the regression models may have weakened potential relations between PFAS exposure and parental education level.

Inverse associations between lin-PFOA and lin-PFHxS concentrations and age agree with other studies of children/adolescents (Kato et al., 2009; Kang et al., 2018; Daly et al., 2018; Gyllenhammar et al., 2018), and could partially be due to growth dilution of PFAS accumulated early in life (Wu et al., 2015). However, PFAS and BMI status relations were not observed in RMA, at least partially arguing against a marked growth dilution effect. A higher consumption of food and DW per kilogram body weight at younger ages may also contribute to the inverse PFAS relation with age (Foster et al., 2010).

Gender was one of the strongest determinants of serum PFAS concentrations in RMA, with males generally having higher EAM serum PFAS concentrations than females. Although in line with previous research (Kang et al., 2018; Pitter et al., 2020; Duffek et al., 2020), the reasons behind these findings are not yet fully understood. Besides a

higher overall food consumption per kg body weight among males (Shomaker et al., 2010), menstrual blood loss among females have been suggested to at least partly account for gender differences among adults (Wong et al., 2014). This is supported in our study, as females who had started menstruating had lower EAM serum br-PFOS concentrations than those who had not, with a similar tendency for lin-PFOA, lin-PFHxS and lin-PFOS. Pharmacokinetic modeling has suggested that menstruation accounts for on average 30% of the shorter half-life of PFOS among adult women than among men (Wong et al., 2014), and an on average 22% reduction of PFOA serum concentrations in adult women (Lorber et al., 2015). Our results suggest that the effect of menstrual bleeding on PFAS toxicokinetics in adolescent females was mainly caused by onset of menstrual bleeding rather than years of menstrual bleeding after onset. The frequent irregularity of the menstrual cycle during adolescence may at least partially obscure associations between years of menstruation and PFAS serum concentrations in adolescence (American Academy of Pediatrics, 2006). It is plausible that pharmacokinetic alterations at menarche could potentially explain this association (Wu et al., 2015).

Serum/plasma ferritin may be a marker for degree of blood loss among women, where long-term blood loss due to menstruation could lead to decreasing iron status and consequently lower ferritin levels (Wang et al., 2013). We hypothesized that PFAS concentrations would increase with increased plasma ferritin among RMA females. However, no such associations were observed. Instead, increased lin-PFOA, PFNA, PFDA, PFUnDA and lin-PFHxS concentrations were related to increased ferritin in males. Positive associations between PFOS and serum ferritin have been noted in both male and female Taiwanese uremic patients prior to hemodialysis (Liu et al., 2018), an association which was not found in adults in a representative general U.S. population (Omoike et al., 2020). These diverging results cannot be explained by current knowledge. It has been suggested that ferritin in blood plays a role in PFAS blood transport (Liu et al., 2018), though further studies are needed in order to shed a light on possible gender-related PFAS-ferritin interactions.

A strength of our study is the population-based study design and the large number of participating adolescents. However, there was a slight overrepresentation of participants from densely populated municipalities among those donating blood, compared to those who declined to donate blood (Moraeus et al., 2018). It was nevertheless concluded by Moraeus et al. (2018), that no overall major skewness of distribution in regard to municipality could be seen. The most pronounced difference could be seen for gender, as females were overrepresented amongst those donating blood compared to those declining to donate blood (Moraeus et al., 2018). This should be taken into account in the interpretation of the representativeness of the results. An additional important limitation is that, except for BMI status, data was in most cases self-reported. As many studies predominantly focus on blood concentrations of legacy PFAS, having additional data on many different potentially emerging PFAS greatly adds to the knowledge about human PFAS exposure, especially among adolescents, which can be seen as a considerable strength.

5. Conclusions

Legacy PFAS were detected at high frequency in a nationally representative Swedish adolescent population. The occurrence of emerging PFAS, such as 9Cl-PF3ONS, highlights the importance of screening for PFAS replacements in future biomonitoring studies. Participant and maternal birth country was the most important determinant of legacy PFAS concentrations, which may at least partly be explained by relationships between PFAS environmental contamination and degree of industrialization of birth countries. Gender was an important determinant for adolescents, where females had lower serum PFAS levels as opposed to males. This association could partially be explained by higher excretion due to menstruation, as supported by our present study. However, other toxicokinetics alterations due to onset of puberty may contribute in explaining apparent difference in PFAS body burdens between genders. The determinants identified in this study may be important confounders in future health studies including adolescents.

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Ethical approval

Ethical approval was granted by the Regional Ethical Review Board in Uppsala (No. 2015/190), where participants or legal guardians of participants below 16 years of age, gave written informed consent to participate.

Declaration of competing interest

The authors declare that they have no or actual competing financial interest.

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Appendix A. Supplementary data

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Supplementary Information

Demographic, life-style, dietary and physiological determinants of serum per- and polyfluoroalkyl substance (PFAS) concentrations in a national cross-sectional survey of Swedish adolescents

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Table S1. Target analytes with their quantification and qualifications ions as well as the internal standard used for quantification. lin indicates linear isomers, and br indicates branched isomer. 4

Detailed description of PFAS analyses

Individual samples (~ 0.5 g serum in a polypropylene (PP) centrifuge tubes) were spiked with 0.5 ng each of a suite of isotopically-labelled internal standards (Table S1). The samples were extracted twice with 4 mL of acetonitrile in an ultrasonic bath. Following centrifugation, the supernatant extract was removed and the combined acetonitrile phases were concentrated to 1 mL under a stream of nitrogen. The concentrated extract underwent dispersive clean-up on graphitized carbon and acetic acid. A volume of 0.5 mL of the cleaned-up extract was added to 0.2 mL of 4 mM aqueous ammonium acetate and 0.5 ng of volumetric standards M8PFOA and M8PFOS. The extract was stored at -20˚C prior to analysis. On the day of analysis, the extracts were centrifuged and transferred to an autosampler vial for instrumental analysis. 5 µL aliquots of the final extracts were injected automatically on a Waters Acquity ultra performance liquid chromatograph (UPLC) which was coupled to a Waters Xevo TQS triple quadrupole mass spectrometer. Chromatographic separation of target analytes was achieved on a BEH C18 guard column (1.7 µm, 5 × 2.1 mm; Waters) coupled to a BEH C18 analytical column (1.7 µm particles, 50 × 2.1 mm). The mobile phase consisted of 2 mM ammonium acetate in water (phase A) and 2 mM ammonium acetate in acetonitrile (phase B), the proportions of which were adjusted over the course of the run. The mass spectrometer was operated in negative electrospray ionization, multiple reaction monitoring (MRM) mode. Quantification was based on isotope dilution (see Table S1 for targets and their corresponding isotopically labelled internal standards).

Samples were run in batches consisting of 30 samples plus 2 blanks and 3-4 QC samples. The QC samples consisted of pooled human serum, which were analyzed both with and without a spike of authentic PFAS. Additionally, the NIST Standard Reference Material 1057 was run with every third batch. Limits of quantification (LOQs) were determined using the lowest calibration point. If a signal occurred in the method blanks within a batch, the LOQ was based on the mean blank + 3x standard deviation. LOQs differ between the batches as not all were analyzed on the instrument at the same time. Concentrations <LOQ, with an S/N >3 peak, were used as limit of detections (LODs) and considered to be of semi-quantitative analytical quality.

Blanks were mostly non-detects with only a few very low detects in some batches. Therefore, no blank correction was conducted on the final data. Data for the 45 individual PFAS measured in the present work were classified as either ‘quantitative’, ‘semi-quantitative’, ‘qualitative’, or ‘not-reported’, based on either availability of authentic standards and/or performance of QC samples (Table S1). In all cases, we assumed that data quality for branched and linear isomers were equivalent. ‘Quantitative’ indicated that an exactly-matched authentic standard was available and that QC

samples displayed reasonable accuracy and precision (25 targets). Among these targets, average recoveries ranged from 96 to 133% (RSDs 12-35%), with the best performance observed for C6-C14 PFCAs (105-120%; RSD: 12-25%) and C4, C6, C8, and C10 PFSAs (109-119% RSDs: 20-28%). 'Semi-quantitative' targets were those quantified with exactly-matched authentic standards but displaying sub-optimal accuracy and/or precision for QC samples. For these targets (i.e. 6:2 and 8:2 diPAP, EtFOSAA, 7:3 FTCA, and 8:2 FTSA), average recoveries ranged from 67 – 152% (RSD: 30-63%). 'Qualitative' targets included PFHxDA, PFOcDA, 6:2/8:2 diPAP, FOSAA, 3:3 and 5:3 FTCA, and 6:2 FTS, for which very poor accuracy and precision were observed, as well as PFPeS, PFHpS, PFNS, PFUnDS, and PFPeDA, for which authentic standards were unavailable and therefore method performance could not be assessed. We note that most targets displaying poor accuracy and/or precision did not have exactly matched isotopically labelled internal standards. It is expected that as both authentic and isotopically labelled standards become available, data quality for all of these 'qualitative' targets will improve considerably.

Finally, NIST values were in good agreement with both the certified values and those reported by others previously (Gebbink et al. 2015).

Table S2. Categorical and continuous covariates included in the sensitivity analysis and the secondary model analyses. 6

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Table S6-S12. Odds ratios (OR) and 95% confidence interval (CI) for base and drinkikng water sensitivity model covariates.

The ordinal logistic regression models estimate the adjusted odds ratios (OR) with corresponding 95% confidence intervals (CIs) for both continuous and categorical covariates; any OR>1 for categorical covariates can be interpreted as that category having higher PFAS concentrations compared to the reference category, while the opposite is true for OR<1; OR>1 for continuous covariates denotes an increase in PFAS concentration per unit of increase of the covariate (direct association), while an OR<1 represents a decrease in PFAS concentration per unit of increase of the covariate (inverse association).

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Table S12. Odds ratios (OR) and 95% confidence interval (CI) for br-PFOS base- and drinking water sensitivity model covariates 16

Table S13. Odds ratios (OR) and 95% confidence interval (CI) for predicted PFAS serum levels in the four different ordinal logistic regression models investigating the effect of menstrual blood loss in RMA. 17

The ordinal logistic regression models estimate the adjusted odds ratios (OR) with corresponding 95% confidence intervals (CIs) for both continuous and categorical covariates; any OR>1 for categorical covariates can be interpreted as that category having higher PFAS concentrations compared to the reference category, while the opposite is true for OR<1; OR>1 for continuous covariates denotes an increase in PFAS concentration with an increase of the covariate (direct association), while an OR<1 represents a decrease in PFAS concentration with an increase of the covariate (inverse association).

Table S14. Cheat sheet for interpretation of codes for the categorical model covariates in Table S6-S13. 18

Figure S1. PCA biplot (n=792) when excluding 42 participants from Uppsala and Ronneby with previous high PFAS drinking water exposure. PFASs detected in >2% of participants included. Arrows = loadings; Dots = PC scores of samples. 19

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Table S1. Target analytes with their quantification and qualifications ions as well as the internal standard used for quantification. lin indicates linear isomers, and br indicates branched isomer.

Target Analyte	Full name	Precursor ion	Quant. product ion	Qual. product ion	Standard	IS	IS transition	Data Quality[1]
PFHxA	Perfluorohexanoic acid	313	269	119	lin-PFHxA	$^{13}C_2$-PFHxA	315/270	Quant
PFHpA	Perfluoroheptanoic acid	363	319	169	lin-PFHpA	$^{13}C_4$-PFOA	367/322	Quant
lin-PFOA	Perfluorooctanoic acid	413	369	169	lin-PFOA	$^{13}C_4$-PFOA	417/372	Quant
br-PFOA	Perfluorooctanoic acid	413	369	169	lin-PFOA	$^{13}C_4$-PFOA	417/372	Quant
PFNA	Perfluorononanoic acid	463	419	219	lin-PFNA	$^{13}C_5$-PFNA	468/423	Quant
PFDA	Perfluorodecanoic acid	513	469	269	lin-PFDA	$^{13}C_2$-PFDA	515/470	Quant
PFUnDA	Perfluoroundecanoic acid	563	519	269	lin-PFUnDA	$^{13}C_2$-PFUnDA	565/520	Quant
PFDoDA	Perfluorododecanoic acid	613	569	169	lin-PFDoDA	$^{13}C_2$-PFDoDA	615/570	Quant
PFTrDA	Perfluorotridecanoic acid	662.9	619	169	lin-PFTrDA	$^{13}C_2$-PFDoDA	615/570	Quant
PFTeDA	Perfluorotetradecanoic acid	712.9	669	169	lin-PFTeDA	$^{13}C_2$-PFDoDA	615/570	Quant
PFPeDA	Perfluoropentadecanoic acid	762.9	719	169	lin-PFTeDA	$^{13}C_2$-PFDoDA	615/570	Qual
PFHxDA	Perfluorohexadecanoic acid	813	769	169	lin-PFHxDA	$^{13}C_2$-PFDoDA	615/570	Qual
PFOcDA	Perfluorooctadecanoic acid	913	869	169	lin-PFOcDA	$^{13}C_2$-PFDoDA	615/570	Qual
PFPeS	Perfluoropentaesulfonic acid	348.9	80	99	lin-PFHxS	$^{18}O_2$-PFHxS	403/84	Qual
lin-PFHxS	Perfluorohexanesulfonic acid	398.9	80	99	lin-PFHxS	$^{18}O_2$-PFHxS	403/84	Quant
br-PFHxS	Perfluorohexanesulfonic acid	399	80	99	lin-PFHxS	$^{18}O_2$-PFHxS	403/84	Quant
PFHpS	Perfluoroheptanesulfonic acid	448.9	80	99	lin-PFHxS	$^{18}O_2$-PFHxS	403/84	Qual
lin-PFOS	Perfluorooctanesulfonic acid	498.9	80, 99		lin-PFOS	$^{13}C_4$-PFOS	503/80	Quant
br-PFOS	Perfluorooctanesulfonic acid	498.9	80, 99		lin-PFOS	$^{13}C_4$-PFOS	503/80	Quant
PFNS	Perfluorononanesulfonic acid	548.9	80	99	lin-PFOS	$^{13}C_4$-PFOS	503/80	Qual
lin-PFDS	Perfluorodecanesulfonic acid	598.9	80	99	lin-PFDS	$^{13}C_4$-PFOS	503/80	Quant
br-PFDS	Perfluorodecanesulfonic acid	598.9	80	99	lin-PFDS	$^{13}C_4$-PFOS	503/80	Quant
PFUnDS	Perfluoroundecanesulfonic acid	648.9	80	99	lin-PFDS	$^{13}C_4$-PFOS	503/80	Qual
lin-FOSA	Perfluoroocatanesulfanomid	497.9	78	169	lin-FOSA	$^{13}C_8$-FOSA	506/78	Quant
br-FOSA	Perfluoroocatanesulfanomid	497.9	78	169	lin-FOSA	$^{13}C_8$-FOSA	506/78	Quant
lin-FOSAA	Perfluorooctane sulfonamidoacetic acid	555.9	498	419	lin-FOSAA	D_3-MeFOSAA	573/419	Qual
br-FOSAA	Perfluorooctane sulfonamidoacetic acid	555.9	498	419	lin-FOSAA	D_3-MeFOSAA	573/419	Qual
lin-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	570	419	483	lin-MeFOSAA	D_3-MeFOSAA	573/419	Quant
br-MeFOSAA	N-methylperfluoro-1-octanesulfonamidoacetic acid	570	419	483	lin-MeFOSAA	D_3-MeFOSAA	573/419	Quant
lin-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	584	419	526	lin-EtFOSAA	D_5-EtFOSAA	589/419	Semi-Q
br-EtFOSAA	N-ethylperfluoro-1-octanesulfonamidoacetic acid	584	419	526	lin-EtFOSAA	D_5-EtFOSAA	589/419	Semi-Q
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	531	351	83	lin-9Cl-PF3ONS	$^{13}C_4$-PFOS	503/80	Quant
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxanone-1-sulfonic acid	631	451	83	lin-11Cl-PF3OUdS	$^{13}C_4$-PFOS	503/80	Quant
ADONA	ammonium 4,8-dioxa-3H-perfluorononnoate	377	251	85	ADONA	$^{13}C_4$-PFOS	503/80	Quant
3:3 FTCA	3:3 fluorotelomer carboxylic acid	241	117	177	lin-3:3 FTCA	$^{13}C_4$-PFOA	417/372	Qual
5:3 FTCA	5:3 fluorotelomer carboxylic acid	341	237	217	lin-5:3 FTCA	$^{13}C_4$-PFOA	417/372	Qual
7:3 FTCA	7:3 fluorotelomer carboxylic acid	441	337	148	lin-7:3 FTCA	$^{13}C_4$-PFOA	417/372	Semi-Q
4:2 FTSA	4:2 fluorotelomer sulfonate	327	307	80.6	lin-4:2 FTSA	$^{13}C_2$-6:2 FTSA	429/409	Quant
8:2 FTSA	8:2 fluorotelomer sulfonate	527	507	80.6	lin-8:2 FTSA	$^{13}C_2$-6:2 FTSA	429/409	Semi-Q
6:2 diPAP	6:2 fluorotelomer phosphate diester	789	443	97	lin-6:2 diPAP	$^{13}C_4$-6:2 diPAP	793/445	Semi-Q
8:2 diPAP	8:2 fluorotelomer phosphate diester	989	543	97	lin-8:2 diPAP	$^{13}C_4$-8:2 diPAP	993/545	Semi-Q
6:2/8:2 diPAP	6:2/8:2 fluorotelomer phosphate diester	889	443	543	lin-6:2/8:2 diPAP	$^{13}C_4$-8:2 diPAP	993/545	Qual
$^{13}C_8$-PFOA[2]		421	376					
$^{13}C_8$-PFOS[2]		507	80					

[1]**Quant** = quantitative; indicates that an authentic standard is available and QC samples displayed reasonable accuracy and precision across all batches. Note that the data quality for branched isomers is assumed to be the same as their linear counterpart.

Semi-Q = Semi-quantitative; indicates authentic standard was used but sub-optimal accuracy and/or precision was obtained across all batches. Note that the data quality for branched isomers is assumed to be the same as their linear counterpart.
Qual = qualitative; indicates that an authentic standard was not available in which case quantification was performed with a structurally similar substance, OR that poor accuracy and/or precision was observed for QC samples. Note that the data quality for branched isomers is assumed to be the same as their linear counterpart.
NR = Not Reported. Spikes were not recovered in most QC samples.
[2] $^{13}C_8$-PFOA and $^{13}C_8$-PFOS were used as recovery internal standards

Table S2. Categorical and continuous covariates included in the sensitivity analysis and the secondary model analyses.

Categorical covariates	**All** n	n=1098 n %	**Males** n	n=482 n %	**Females** n	n=616 n %
Sensitivity analysis						
Living in area with previously contaminated drinking water (Ronneby or Uppsala)						
Yes	42	4	22	5	20	3
No	1056	96	460	95	596	97
Secondary analysis						
Started menstruating (females only)						
Yes					440	71
No					168	27
Missing					8	1
Continuous covariates	**Median** (min, max)	n missing (%)	**Median** (min, max)	n missing (%)	**Median** (min, max)	n missing (%)
Ferritin (µg/L plasma)	31.0 (1.70, 797.0)	25 *(2.3)*	40.0 (1.70, 237.0)	14 *(3.0)*	26.0 (1.80, 797.0)	11 *(1.8)*
CRP (mg/L plasma)	0.36 (0.14, 51.0)	25 *(2.3)*	0.38 (0.14, 51.0)	14 *(3.0)*	0.36 (0.14, 37.0)	11 *(1.8)*
No. of years since menstrual onset					2.22 (0.00, 10.1)	11 *(1.8)*

Note: min, minimum; max, maximum; CRP, C - reactive protein.

Table S3. Median (range) serum PFAS concentrations (ng/g serum) for participants residing in areas with a history of high PFAS exposure via drinking water (Ronneby and Uppsala) and for participants residing elsewhere.

PFAS	**Other participants** (n=1056)	**Uppsala** (n=18)	**Ronneby** (n=24)
(ng/g serum)	Median (min, max)	Median (min, max)	Median (min, max)
lin-PFOA	1.18 (<LOD, 6.91)[a]	1.80 (1.01, 3.35)[b]	2.40 (0.680, 9.75)[b]
lin-PFHxS	0.398 (<LOD, 15.8)	9.14 (4.23, 23.7)[b]	27.7 (0.175, 255)[b]
lin-PFOS	1.95 (0.281, 15.4)	2.46 (1.68, 6.56)[b]	19.6 (1.08, 127)[b]
br-PFOS	0.906 (0.031, 10.0)	1.78 (0.683, 4.04)[b]	14.7 (0.652, 110)[b]

Table S4. Serum concentrations of PFAS (ng/g serum) in the RMA study population not included in any statistical analysis.

Analyte	n	LOD (%<LOD)	LOQ (%<LOQ)	Detected and quantified Min, Max	Quantified Min, Max
PFCA					
PFHxA	1095	0.058 – 0.288 (100)	0.058 – 0.288 (100)		
br-PFOA	1095	0.020 – 0.288 (100)	0.020 – 0.288 (100)		
PFTeDA	1098	0.043 – 0.079 (99.7)	0.058 – 0.674 (99.9)	<LOD, 0.136	<LOQ, 0.136
PFPeDA	1098	0.025 (99.6)	0.058 – 0.288 (99.8)	<LOD, 0.359	<LOQ, 0.359
PFHxDA	1098	0.017 – 1.483 (99.5)	0.082 – 5.081 (99.7)	<LOD, 1.482	<LOQ, 0.095
PFOcDA	830	0.311 – 0.860 (99.6)	0.288 – 34.88 (100)	<LOD, 8.520	<LOQ
PFSA					
PFNS	1098	0.022 – 3.338 (100)	0.022 – 3.338 (100)		
lin-PFDS	1098	0.080 – 0.568 (100)	0.080 – 0.568 (100)		
Br-PFDS	1098	0.080 – 0.568 (100)	0.080 – 0.568 (100)		
PFUnDS	1098	0.022 – 3.372 (100)	0.022 – 3.372 (100)		
PAP					
6:2 diPAP	1098	0.118 – 0.257 (99.5)	0.084 – 1.036 (99.9)	<LOD, 0.257	<LOQ, 0.168
8:2 diPAP	1098	1.281 (99.9)	0.616 – 12.60 (100)	<LOD, 1.281	<LOQ
6:2/8:2 diPAP	1098		0.292 – 43.92 (99.9)	<LOD, 0.745	<LOQ, 0.745
FOSA					
lin-FOSA	1098	0.005 – 0.039 (99.1)	0.022 – 0.292 (99.9)	<LOD, 0.100	<LOQ, 0.100
br-FOSA	1098	0.022 – 0.292 (100)	0.022 – 0.292 (100)		
FASAA					
lin-FOSAA	1098	0.025 – 0.091 (99.3)	0.084 – 3.554 (99.9)	<LOD, 0.091	<LOQ, 0.037
br-FOSAA	1098	0.024 – 3.554 (100)	0.024 – 3.554 (100)		
br-MeFOSAA	1098	0.060 – 3.572 (100)	0.060 – 3.572 (100)		
lin-EtFOSAA	1098	0.039 – 0.121 (98.3)	0.024 – 1.014 (99.4)	<LOD, 0.497	<LOQ, 0.497
br-EtFOSAA	1098	0.024 – 1.014 (100)	0.024 – 1.014 (100)		
PFESA					
11Cl-PF3OUdS	1098	0.006 – 0.032 (99.4)	0.060 – 3.492 (100)	<LOD, 0.032	<LOQ
PFECA					
ADONA	1098		0.024 – 3.572 (99.9)	<LOD, 0.066	<LOQ, 0.066
FTCA					
3:3 FTCA	1098	1.002 – 12.50 (100)	1.002 – 12.50 (100)		
5:3 FTCA	949	0.024 – 0.288 (99.7)	0.024 – 0.288 (99.7)	<LOD, 0.151	<LOQ, 0.151
7:3 FTCA	1098	0.069 – 0.170 (99.4)	0.024 – 0.290 (99.6)	<LOD, 0.170	<LOQ, 0.126
FTSA					
4:2 FTSA	1098	0.056 (99.9)	0.060 – 0.288 (99.9)	<LOD, 0.056	<LOQ, 0.056
8:2 FTSA	1098	0.088 (99.6)	0.024 – 3.484 (99.8)	<LOD, 0.420	<LOQ, 0.420

Note: LOD, limit of detection; LOQ, limit of quantification; Min, minimum; Max, maximum; PFCA, perfluoroalkylcarboxylic acids; PFSA, perfluoroalkylsulfonic acids; PAP, fluorotelomer phosphate esters; FOSA, perfluoroctane sulfonamides; FASAA, perfluoroalkane sulfonamido acetic acid; PFESA, per- and polyfluoroalkyl ether sulfonic acids; PFECA, perfluoropolyether carboxylic acids; FTCA, fluorotelomer carboxylic acids; FTSA, fluorotelomer sulfonates. Information on analytical quality for each PFAS can be found in Table S1.

Table S5. Loadings for PC1 and PC2 from the principal component analysis (PCA) (n=827).

PFAS	PC1	PC2
PFHpA	-0.011708287	-0.230339808
lin-PFOA	-0.282096447	-0.244716060
PFNA	-0.058099762	-0.479466606
PFDA	-0.044396218	-0.513364583
PFUnDA	-0.028623074	-0.465397971
PFDoDA	-0.028129379	-0.280939639
PFTrDA	-0.004572359	-0.252302557
lin-PFHxS	-0.408045738	0.083011005
br-PFHxS	-0.418328304	0.085973734
lin-PFOS	-0.400801976	0.009580152
br-PFOS	-0.404977444	0.071589423
PFPeS	-0.331648158	0.064114799
PFHpS	-0.370081833	0.059213882
lin-MeFOSAA	-0.005149012	-0.064194257
9Cl-PF3ONS	-0.013417554	-0.056658986

Table S6. Odds ratios (OR) and 95% confidence interval (CI) for lin-PFOA base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.525 (0.376 , 0.733)	0.0002	0.625 (0.450 , 0.868)	0.0051
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	1.216 (0.755 , 1.960)	0.0149	1.218 (0.755 , 1.964)	0.0286
	3:1	1.910 (1.233 , 2.957)		1.839 (1.175 , 2.877)	
Smoking	1:0	1.139 (0.721 , 1.800)	0.5770	1.147 (0.726 , 1.811)	0.5571
Snus	1:0	0.929 (0.454 , 1.901)	0.8409	0.934 (0.455 , 1.919)	0.8534
Exclusively breastfed	0:1	0.744 (0.522 , 1.061)	0.2627	0.755 (0.530 , 1.074)	0.2947
	2:1	0.977 (0.779 , 1.226)		0.985 (0.795 , 1.220)	
BC	1:5	0.204 (0.095 , 0.438)	<0.0001	0.177 (0.077 , 0.406)	<0.0001
	2:5	0.212 (0.103 , 0.437)		0.219 (0.107 , 0.446)	
	3:5	0.404 (0.217 , 0.750)		0.380 (0.209 , 0.691)	
	4:5	0.608 (0.368 , 1.002)		0.591 (0.367 , 0.951)	
MEL	0:3	0.745 (0.426 , 1.304)	0.1777	0.790 (0.447 , 1.397)	0.2830
	1:3	0.691 (0.461 , 1.036)		0.720 (0.487 , 1.063)	
	2:3	0.748 (0.535 , 1.046)		0.786 (0.564 , 1.095)	
PEL	0:3	0.962 (0.590 , 1.571)	0.7093	0.990 (0.606 , 1.620)	0.6433
	1:3	0.872 (0.524 , 1.451)		0.873 (0.533 , 1.431)	
	2:3	1.123 (0.757 , 1.664)		1.160 (0.758 , 1.774)	
BMI status	0:1	1.072 (0.762 , 1.508)	0.8974	1.079 (0.750 , 1.551)	0.9202
	2:1	1.122 (0.804 , 1.566)		1.104 (0.807 , 1.511)	
	3:1	1.073 (0.688 , 1.673)		1.022 (0.647 , 1.613)	
Gender	0:1	1.385 (1.104 , 1.739)	0.0049	1.366 (1.082 , 1.724)	0.0086
High PFAS contamination	0:1			7.011 (3.004 , 16.36)	<0.0001

Notes: lin-PFOA, linear perfluorooctanoic acid; ref, reference category; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of each category code is found in Table S14.

Table S7. Odds ratios (OR) and 95% confidence interval (CI) for PFNA base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.862 (0.618 , 1.201)	0.3793	0.854 (0.604 , 1.208)	0.3726
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	1.297 (0.848 , 1.983)	0.0149	1.297 (0.848 , 1.984)	0.0148
	3:1	1.805 (1.208 , 2.696)		1.809 (1.210 , 2.705)	
Smoking	1:0	0.891 (0.541 , 1.469)	0.6515	0.892 (0.541 , 1.470)	0.6529
Snus	1:0	1.025 (0.505 , 2.080)	0.9462	1.024 (0.504 , 2.079)	0.9474
Exclusively breastfed	0:1	1.301 (0.975 , 1.737)	0.1172	1.301 (0.975 , 1.736)	0.1172
	2:1	1.181 (0.950 , 1.467)		1.181 (0.951 , 1.467)	
BC	1:5	0.189 (0.102 , 0.349)	<0.0001	0.189 (0.102 , 0.349)	<0.0001
	2:5	0.519 (0.245 , 1.101)		0.518 (0.245 , 1.097)	
	3:5	0.504 (0.244 , 1.039)		0.505 (0.244 , 1.045)	
	4:5	0.545 (0.399 , 0.746)		0.545 (0.398 , 0.746)	
MEL	0:3	0.817 (0.557 , 1.197)	0.3253	0.815 (0.558 , 1.190)	0.3024
	1:3	0.867 (0.611 , 1.230)		0.863 (0.607 , 1.228)	
	2:3	0.741 (0.533 , 1.031)		0.739 (0.533 , 1.025)	
PEL	0:3	0.886 (0.590 , 1.333)	0.3593	0.887 (0.590 , 1.332)	0.3682
	1:3	0.748 (0.520 , 1.077)		0.749 (0.520 , 1.079)	
	2:3	0.984 (0.687 , 1.411)		0.984 (0.685 , 1.412)	
BMI status	0:1	1.155 (0.778 , 1.715)	0.8198	1.155 (0.776 , 1.718)	0.8252
	2:1	1.092 (0.836 , 1.427)		1.091 (0.834 , 1.428)	
	3:1	1.149 (0.615 , 2.146)		1.150 (0.618 , 2.142)	
Gender	0:1	1.786 (1.364 , 2.339)	<0.0001	1.785 (1.364 , 2.336)	<0.0001
High contamination	0:1			0.931 (0.507 , 1.707)	0.8164

Notes: PFNA, perfluorononanoic acid; ref, reference category; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes are found in Table S14.

Table S8. Odds ratios (OR) and 95% confidence interval (CI) for PFDA base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		1.032 (0.728 , 1.462)	0.8589	1.022 (0.715 , 1.459)	0.9069
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	1.014 (0.686 , 1.500)	0.9509	1.015 (0.686 , 1.500)	0.9468
	3:1	1.080 (0.662 , 1.761)		1.083 (0.664 , 1.766)	
Smoking	1:0	1.223 (0.803 , 1.863)	0.3474	1.224 (0.803 , 1.864)	0.3475
Snus	1:0	1.240 (0.616 , 2.497)	0.5464	1.240 (0.616 , 2.496)	0.5474
Exclusively breastfed	0:1	1.197 (0.882 , 1.623)	0.4971	1.197 (0.884 , 1.622)	0.4941
	2:1	1.053 (0.843 , 1.315)		1.053 (0.844 , 1.314)	
BC	1:5	0.304 (0.169 , 0.547)	<0.0001	0.305 (0.170 , 0.548)	<0.0001
	2:5	0.681 (0.405 , 1.145)		0.679 (0.405 , 1.140)	
	3:5	0.231 (0.110 , 0.483)		0.231 (0.111 , 0.484)	
	4:5	0.581 (0.393 , 0.860)		0.582 (0.393 , 0.860)	
MEL	0:3	1.251 (0.774 , 2.020)	0.1291	1.247 (0.770 , 2.018)	0.1247
	1:3	0.884 (0.608 , 1.285)		0.880 (0.603 , 1.284)	
	2:3	0.758 (0.574 , 1.000)		0.755 (0.570 , 0.999)	
PEL	0:3	0.853 (0.547 , 1.330)	0.3031	0.853 (0.547 , 1.329)	0.3126
	1:3	0.739 (0.496 , 1.100)		0.739 (0.496 , 1.101)	
	2:3	1.077 (0.806 , 1.440)		1.076 (0.804 , 1.439)	
BMI status	0:1	0.989 (0.671 , 1.459)	0.4103	0.988 (0.670 , 1.459)	0.4102
	2:1	1.051 (0.806 , 1.371)		1.050 (0.805 , 1.371)	
	3:1	0.586 (0.312 , 1.103)		0.586 (0.311 , 1.104)	
Gender	0:1	1.210 (0.957 , 1.528)	0.1106	1.210 (0.959 , 1.526)	0.1086
High contamination	0:1			0.920 (0.488 , 1.733)	0.7961

Notes: PFDA, perfluorodecanoic acid; ref, reference category BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes are found in Table S14.

Table S9. Odds ratios (OR) and 95% confidence interval (CI) for PFUnDA base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.857 (0.620 , 1.186)	0.3520	0.845 (0.611 , 1.169)	0.3104
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	0.938 (0.597 , 1.473)	0.2603	0.938 (0.597 , 1.473)	0.2532
	3:1	1.337 (0.821 , 2.175)		1.341 (0.825 , 2.181)	
Smoking	1:0	0.867 (0.554 , 1.356)	0.5321	0.867 (0.554 , 1.359)	0.5344
Snus	1:0	0.938 (0.522 , 1.684)	0.8302	0.938 (0.522 , 1.685)	0.8291
Exclusively breastfed	0:1	1.548 (1.024 , 2.340)	0.0278	1.547 (1.024 , 2.339)	0.0276
	2:1	1.327 (1.043 , 1.687)		1.327 (1.045 , 1.686)	
BC	1:5	0.364 (0.229 , 0.579)	<0.0001	0.365 (0.230 , 0.579)	<0.0001
	2:5	0.472 (0.263 , 0.847)		0.470 (0.264 , 0.839)	
	3:5	0.284 (0.147 , 0.547)		0.284 (0.147 , 0.548)	
	4:5	0.471 (0.339 , 0.653)		0.471 (0.339 , 0.654)	
MEL	0:3	0.904 (0.533 , 1.534)	0.0653	0.901 (0.532 , 1.525)	0.0623
	1:3	1.090 (0.842 , 1.413)		1.084 (0.835 , 1.409)	
	2:3	0.735 (0.553 , 0.976)		0.731 (0.551 , 0.970)	
PEL	0:3	0.966 (0.668 , 1.397)	0.0433	0.966 (0.668 , 1.397)	0.0451
	1:3	0.621 (0.435 , 0.888)		0.623 (0.435 , 0.890)	
	2:3	0.846 (0.621 , 1.151)		0.845 (0.621 , 1.149)	
BMI status	0:1	0.910 (0.564 , 1.469)	0.0842	0.910 (0.562 , 1.472)	0.0838
	2:1	0.777 (0.583 , 1.035)		0.777 (0.583 , 1.035)	
	3:1	0.617 (0.373 , 1.021)		0.617 (0.374 , 1.020)	
Gender	0:1	1.255 (1.006 , 1.566)	0.0443	1.255 (1.007 , 1.564)	0.0431
High contamination	0:1			0.897 (0.492 , 1.635)	0.7226

Notes: PFUnDA, perfluoroundecanoic acid; ref, reference category; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes are found in Table S14.

Table S10. Odds ratios (OR) and 95% confidence interval (CI) for lin-PFHxS base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.432 (0.251 , 0.745)	0.0026	0.585 (0.346 , 0.989)	0.0454
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	1.091 (0.759 , 1.568)	0.5186	1.074 (0.741 , 1.557)	0.7385
	3:1	1.274 (0.837 , 1.938)		1.186 (0.763 , 1.843)	
Smoking	1:0	1.202 (0.7601 , 1.902)	0.4309	1.213 (0.757 , 1.944)	0.4223
Snus	1:0	0.721 (0.426 , 1.221)	0.2240	0.709 (0.414 , 1.215)	0.2113
Exclusively breastfed	0:1	0.864 (0.565 , 1.320)	0.3998	0.916 (0.595 , 1.411)	0.3536
	2:1	1.112 (0.865 , 1.429)		1.147 (0.902 , 1.458)	
BC	1:5	0.188 (0.105 , 0.338)	<0.0001	0.147 (0.080 , 0.273)	<0.0001
	2:5	0.836 (0.482 , 1.448)		0.915 (0.533 , 1.569)	
	3:5	0.328 (0.195 , 0.551)		0.297 (0.184 , 0.481)	
	4:5	0.550 (0.324 , 0.935)		0.523 (0.327 , 0.837)	
MEL	0:3	0.954 (0.555 , 1.640)	<0.0001	1.038 (0.610 , 1.766)	0.0002
	1:3	0.572 (0.432 , 0.759)		0.595 (0.452 , 0.784)	
	2:3	0.583 (0.455 , 0.746)		0.634 (0.505 , 0.796)	
PEL	0:3	0.831 (0.518 , 1.332)	0.0096	0.899 (0.545 , 1.483)	0.0089
	1:3	0.562 (0.397 , 0.795)		0.579 (0.409 , 0.821)	
	2:3	0.749 (0.551 , 1.020)		0.776 (0.562 , 1.072)	
BMI status	0:1	1.312 (0.908 , 1.895)	0.1215	1.307 (0.919 , 1.857)	0.1694
	2:1	1.219 (0.879 , 1.691)		1.273 (0.931 , 1.740)	
	3:1	1.695 (0.958 , 2.998)		1.585 (0.864 , 2.906)	
Gender	0:1	1.889 (1.296 , 2.753)	0.0009	1.885 (1.294 , 2.747)	0.0010
High contamination	0:1			345.2 (116.7 , 1021.2)	<0.0001

Notes: lin-PFHxS, linear perfluorohexanesulfonic acid; ref, reference covariate; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes is found in Table S14.

Table S11. Odds ratios (OR) and 95% confidence interval (CI) for lin-PFOS base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.805 (0.580 , 1.117)	0.1939	1.006 (0.729 , 1.387)	0.9722
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	0.935 (0.630 , 1.388)	0.3218	0.917 (0.621 , 1.353)	0.4487
	3:1	1.284 (0.864 , 1.910)		1.219 (0.807 , 1.842)	
Smoking	1:0	1.106 (0.621 , 1.973)	0.7319	1.080 (0.602 , 1.937)	0.7966
Snus	1:0	0.796 (0.472 , 1.340)	0.3903	0.794 (0.468 , 1.348)	0.3927
Exclusively breastfed	0:1	0.970 (0.616 , 1.528)	0.8435	1.031 (0.641 , 1.657)	0.7918
	2:1	0.937 (0.754 , 1.165)		0.936 (0.757 , 1.158)	
BC	1:5	0.110 (0.040 , 0.301)	<0.0001	0.098 (0.039 , 0.252)	<0.0001
	2:5	0.238 (0.127 , 0.445)		0.247 (0.135 , 0.454)	
	3:5	0.224 (0.111 , 0.455)		0.211 (0.106 , 0.420)	
	4:5	0.518 (0.361 , 0.744)		0.489 (0.336 , 0.712)	
MEL	0:3	0.786 (0.385 , 1.602)	0.0173	0.863 (0.426 , 1.744)	0.0829
	1:3	0.666 (0.485 , 0.915)		0.707 (0.517 , 0.967)	
	2:3	0.680 (0.526 , 0.880)		0.736 (0.562 , 0.963)	
PEL	0:3	0.984 (0.625 , 1.548)	0.0817	1.023 (0.649 , 1.611)	0.1309
	1:3	0.656 (0.439 , 0.979)		0.659 (0.430 , 1.011)	
	2:3	0.723 (0.519 , 1.008)		0.743 (0.521 , 1.060)	
BMI status	0:1	0.873 (0.578 , 1.319)	0.6801	0.841 (0.537 , 1.320)	0.4126
	2:1	1.106 (0.827 , 1.481)		1.167 (0.879 , 1.550)	
	3:1	0.852 (0.545 , 1.332)		0.828 (0.535 , 1.281)	
Gender	0:1	2.182 (1.551 , 3.072)	<0.0001	2.157 (1.500 , 3.110)	<0.0001
High contamination	0:1			18.79 (1.040 , 339.3)	0.0470

Notes: lin-PFOS, linear perfluorooctanesulfonic acid; ref, reference category; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes is found in Table S14.

Table S12. Odds ratios (OR) and 95% confidence interval (CI) for br-PFOS base- and drinking water sensitivity model covariates.

		Base model		***Drinking water sensitivity analysis***	
		OR (CI)	*p-value*	*OR (CI)*	*p-value*
Continuous covariates					
Age		0.697 (0.460 , 1.057)	0.0892	0.935 (0.643 , 1.358)	0.7243
Categorical covariates	Category code[a] (tested:ref)				
Alcohol	2:1	0.882 (0.542 , 1.435)	0.6361	0.852 (0.523 , 1.387)	0.7266
	3:1	1.126 (0.735 , 1.725)		1.045 (0.666 , 1.640)	
Smoking	1:0	1.207 (0.676 , 2.154)	0.5248	1.183 (0.654 , 2.139)	0.5792
Snus	1:0	0.904 (0.530 , 1.543)	0.7123	0.897 (0.512 , 1.571)	0.7044
Exclusively breastfed	0:1	0.719 (0.494 , 1.046)	0.2245	0.774 (0.518 , 1.156)	0.4543
	2:1	0.939 (0.738 , 1.194)		0.944 (0.755 , 1.180)	
BC	1:5	0.131 (0.054 , 0.317)	<0.0001	0.114 (0.051 , 0.256)	<0.0001
	2:5	0.238 (0.127 , 0.447)		0.247 (0.131 , 0.465)	
	3:5	0.194 (0.106 , 0.355)		0.181 (0.100 , 0.328)	
	4:5	0.691 (0.491 , 0.972)		0.652 (0.460 , 0.926)	
MEL	0:3	0.863 (0.487 , 1.530)	0.0463	0.954 (0.544 , 1.674)	0.1988
	1:3	0.688 (0.498 , 0.950)		0.740 (0.540 , 1.013)	
	2:3	0.741 (0.583 , 0.942)		0.816 (0.641 , 1.040)	
PEL	0:3	0.966 (0.613 , 1.524)	0.1018	1.019 (0.636 , 1.633)	0.1296
	1:3	0.685 (0.477 , 0.984)		0.694 (0.471 , 1.024)	
	2:3	0.757 (0.553 , 1.036)		0.791 (0.563 , 1.112)	
BMI status	0:1	1.106 (0.777 , 1.573)	0.8861	1.061 (0.738 , 1.525)	0.6810
	2:1	1.083 (0.852 , 1.376)		1.147 (0.908 , 1.450)	
	3:1	1.014 (0.660 , 1.559)		0.966 (0.639 , 1.461)	
Gender	0:1	2.357 (1.684 , 3.299)	<0.0001	2.379 (1.690 , 3.349)	<0.0001
High contamination	0:1			40.84 (4.929 , 338.3)	0.0006

Notes: br-PFOS, branched perfluorooctanesulfonic acid; ref, reference category; BC, participant/maternal birth country; MEL/PEL, maternal/paternal education level; BMI status, body mass index cut-offs. OR and CI were derived from ordinal logistic regression models and p-values using Wald statistics. For categorical covariates, the p-value results from a global test of the variable where the null hypothesis is that for all coefficients are equal to zero. n = 1098.

[a] Explanation of category codes is found in Table S14.

Table S13. Odds ratios (OR) and 95% confidence interval (CI) for predicted PFAS serum levels in the four different ordinal logistic regression models investigating the effect of menstrual blood loss in RMA.

Covariate		lin-PFOA[a]		PFNA[a]		PFDA[a]		PFUnDA[a]		lin-PFHxS[a]		lin-PFOS[a]		br-PFOS[a]	
	Quantile	OR (CI)	p	OR (CI)	p	OR (CI)	p	OR (CI)	p	OR (CI)	p	OR (CI)	p	OR (CI)	p
Menstruation start (1:2)[b, c]		1.710 (0.982, 2.977)	0.0582	0.916 (0.569, 1.475)	0.7182	0.859 (0.525, 1.405)	0.5444	0.707 (0.452, 1.106)	0.1289	1.698 (0.911, 3.163)	0.0956	1.735 (0.954, 3.145)	0.0710	2.385 (1.273, 4.469)	0.0067
Menstruation length (years)[b]		0.785 (0.367, 1.679)	0.4325	1.425 (0.742, 2.739)	0.2878	1.161 (0.645, 2.089)	0.6180	1.417 (0.747, 2.687)	0.2858	0.998 (0.522, 1.908)	0.9949	0.995 (0.514, 1.925)	0.9873	0.955 (0.487, 1.872)	0.8930
Ferritin in females (µg/ L plasma)[d]	25	1.088 (0.778, 1.520)	0.6231	1.020 (0.587, 1.772)	0.9435	1.137 (0.693, 1.865)	0.6107	0.964 (0.692, 0.134)	0.8295	0.963 (0.807, 1.149)	0.6726	1.063 (0.834, 1.354)	0.6215	1.111 (0.893, 1.381)	0.6215
	50	1.082 (0.866, 1.352)	0.4881	1.027 (0.645, 1.637)	0.9103	1.070 (0.712, 1.67)	0.7460	1.103 (0.887, 1.199)	0.8606	1.031 (0.879, 1.209)	0.6874	1.036 (0.859, 1.250)	0.7107	1.077 (0.922, 1.256)	0.7107
	75	1.071 (0.662, 1.501)	0.6907	1.042 (0.655, 1.656)	0.8636	0.944 (0.647, 1.378)	0.7661	1.130 (0.852, 1.653)	0.9360	1.207 (0.663, 2.197)	0.3110	0.984 (0.748, 1.294)	0.9047	1.010 (0.757, 1.348)	0.9074
Ferritin in males (µg/ L plasma) [e]	25	1.730 (1.302, 2.298)	0.0002	1.342 (0.904, 1.991)	0.1441	1.217 (0.893, 1.659)	0.2128	1.130 (0.722, 1.767)	0.5933	1.503 (1.094, 2.065)	0.0120	1.055 (0.693, 1.605)	0.8035	1.219 (0.826, 1.798)	0.3810
	50	1.622 (1.269, 2.074)	0.0001	1.556 (1.191, 2.032)	0.0013	1.391 (1.147, 1.687)	0.0008	1.323 (1.007, 1.738)	0.0443	1.446 (1.126, 1.857)	0.0039	1.181 (0.894, 1.560)	0.2424	1.232 (0.945, 1.605)	0.1230
	75	1.464 (1.044, 2.052)	0.0273	1.975 (1.287, 3.030)	0.0121	1.725 (1.127, 2.641)	0.0121	1.706 (1.011, 2.879)	0.0453	1.359 (0.936, 1.975)	0.1071	1.416 (0.856, 2.342)	0.1754	1.253 (0.796, 1.973)	0.3307

Notes: lin-PFOA, linear perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecaonic acid; PFUnDA, perfluoroundecanoic acid; lin-PFHxS, linear perfluorohexanesulfonic acid; lin/br-PFOS, linear/branched perfluorooctanoic acid. N = 1098 for all models. OR (CI) were derived from ordinal logistic regression analyses and p-values were derived using Wald statistics.

[a] All models included covariates age, body mass index cut-offs (BMI status), participant/maternal birth country, maternal and paternal education level, snus habits, smoking habits, alcohol consumption, months exclusively breastfed, and the Swedish Healthy Eating Index for Adolescents 2015 (SHEIA15).
[b] Model only tested on females in the study population (n=616).
[c] Explanation of category code is found in Table S14.
[d] Model tested on females (n=616) and it additionally included covariates c-reactive protein (CRP) concentrations (mg/L plasma) and an interaction term between ferritin and CRP.
[e] Model tested on males (n=482) and it additionally included the covariates c-reactive protein (CRP) concentrations (mg/L plasma) and an interaction term between ferritin and CRP.

Table S14. Cheat sheet for interpretation of codes for the categorical model covariates in Table S6-S13.

Categorical Covariates	Levels	Code
Alcohol	Has never consumed alcohol	1 (reference)
	Has consumed alcohol once in the last 6 months	2
	Has consumed alcohol more than once in the last 6 times	3
Smoking habits	Non-smoker	0 (reference)
	Previous/occasional/current smoker	1
Snus habits	Non-snus user	0 (reference)
	Previous/occasional/current snus user	1
Exclusively breastfed	Never and < 1 month	0
	1-6 months	1 (reference)
	6-12 months	2
BC; participant/maternal birth country	Mother/participant; both low and lower middle income countries	1
	Mother/participant; low and lower middle/upper middle and high income countries	2
	Mother/participant; both upper middle income countries	3
	Mother/participant; upper middle/high income countries	4
	Mother/participant; both high income countries	5 (reference)
MEL/PEL; maternal/paternal education level	Lacks formal education and elementary school level	0
	2 year upper secondary education, vocational or equivalent level	1
	Minimum of 3 year upper secondary education level	2
	Higher education or equivalent level	3 (reference)
BMI status; body mass index cut-offs	Underweight	0
	Normal weight	1 (reference)
	Over weight	2
	Obese	3
Gender	Males	0
	Females	1 (reference)
Menstruation models		
Menstruation start	Has not started menstruating	1 (reference)
	Has started menstruating	2
Drinking water sensitivity analysis		
High contamination	Schools in areas with previous high contamination of PFAS in drinking water (Ronneby and Uppsala)	0 (reference)
	Schools elsewhere	1

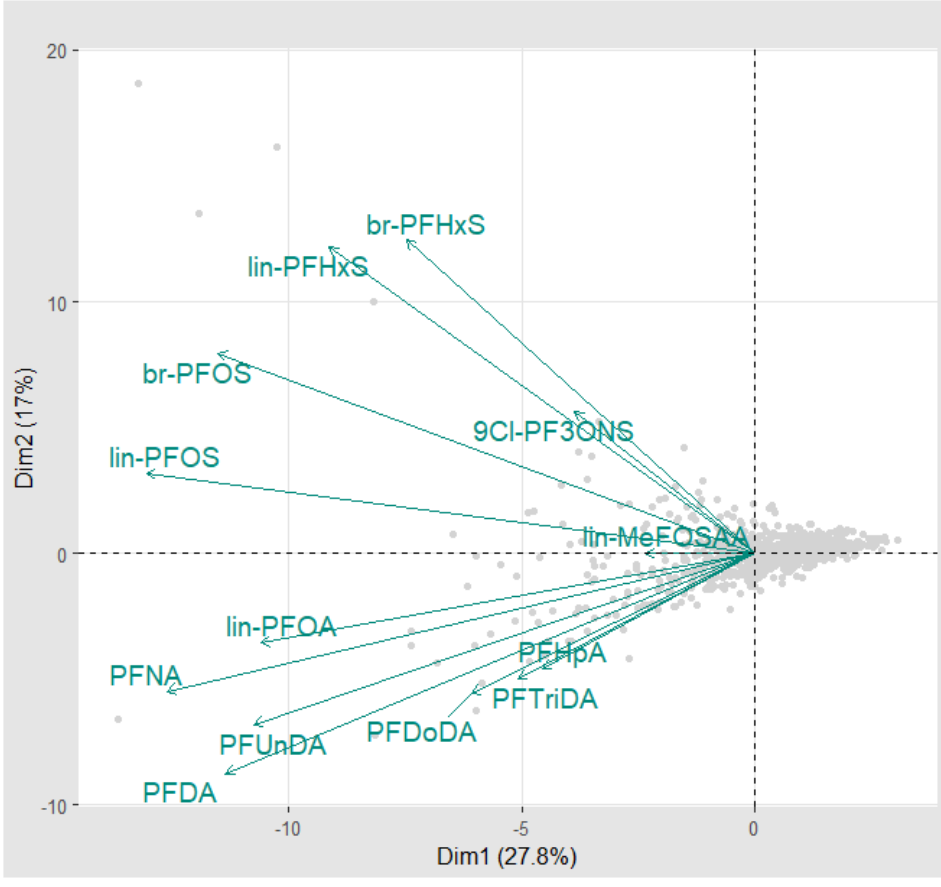

Figure S1. PCA biplot (n=792) when excluding 42 participants from Uppsala and Ronneby with previous high PFAS drinking water exposure. PFASs detected in >2% of participants included. Arrows = loadings; Dots = PC scores of samples.

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Healthy eating index and diet diversity score as determinants of serum perfluoroalkyl acid (PFAA) concentrations in a national survey of Swedish adolescents☆

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ABSTRACT

Food is an important source of perfluoroalkyl acid (PFAA) exposure for the general adult population, but few data exist for adolescents. Healthy food habits established during adolescence may positively influence health later in life. Associations between serum PFAA concentrations and a healthy eating index (SHEIA15), as well as a diet diversity score (RADDS), were determined in a nationally representative adolescent population from Sweden (Riksmaten Adolescents 2016–2017, RMA). Using consumption data from food registrations and frequency questionnaires, we additionally analyzed associations with commonly consumed food groups. Associations were analyzed by fitting a cumulative probability model using ordinal regression. Among the seven PFAAs detected in ≥70% of the 1098 participants (age 10–21 years), median concentrations ranged from <1 ng/g serum of perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perflurorundecanoic acid (PFUnDA), linear (lin-) perfluorohexanesulfonic acid (PFHxS) and branched (br-) perfluorooctanesulfonic acid (PFOS) to 1–2 ng/g serum of lin-perfluorooctanoic acid (PFOA) and lin-PFOS. PFNA, PFDA, PFUnDA and lin-PFOS concentrations were positively associated with both SHEIA15 and RADDS, a finding most likely driven by higher consumption of seafood. PFDA, PFUnDA and lin-PFOS concentrations were positively related to commonly consumed fish/shellfish groups, such as lean marine fish and shellfish. Inverse associations between PFAA concentrations and dairy consumption suggest an underlying factor behind dairy consumption that similarly affects adolescent exposure to the different PFAAs. Isomeric differences in dietary exposure between lin-PFOS and br-PFOS were suggested, as br-PFOS concentrations, in contrast to lin-PFOS, were not associated with SHEIA15, RADDS and consumption of different food groups. We conclude that Swedish adolescents, adhering to a diverse and healthy diet, appears to be more highly exposed to legacy PFAAs than those eating less healthy. Additional research is necessary for a better understanding of the health implications of healthy eating from a PFAA exposure perspective.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a vast group of more than 4700 registered compounds (OECD, 2018). The stability and combined oil-/water repelling properties of perfluorinated carbon chains have led to wide-spread use of PFAS in both consumer and industrial applications since the 1950s (Glüge et al., 2020). This widespread use of PFAS in society has led to their global occurrence in biota and humans (Houde et al., 2011). Perfluoroalkyl acids (PFAAs), such as the well-studied perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been intentionally produced, but they are also formed by degradation of certain of PFAA-precursors

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(Martin et al., 2010; D'eon and Mabury, 2011). PFAAs are very persistent in the environment, leading to contamination of food and drinking water (Dhore and Murthy, 2021; Kurwadkar et al., 2021). In addition to PFOA and PFOS, other long-chained PFAAs, such as perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorohexanesulfonic acid (PFHxS), are considered bioaccumulative in humans (Conder et al., 2008; ITRC, 2020). Epidemiological studies have suggested that certain long-chain PFAAs alter lipid levels in children and adolescents (Geiger et al., 2014; Frisbee et al., 2010), impair the immune system in children (Grandjean et al., 2012; Abraham et al., 2020), and restrict fetal growth (Fei et al., 2007; Marks et al., 2019; Zhuang et al., 2021).

Prenatal PFAA exposure through the placenta and exposure via breast milk are major contributors to serum/plasma PFAA burdens in children, at least up to 12 years of age (Gyllenhammar et al., 2018; Balk et al., 2019). As the child ages through adolescence, exposure sources become more complex (Balk et al., 2019). Among adults, dietary exposure, predominantly from seafood and drinking water, contributes significantly to PFAA body burdens (Domingo and Nadal, 2017; Sunderland et al., 2019). However, associations between these body burdens and food consumption in the transition period between childhood and adulthood, in adolescence, have rarely been studied (Averina et al., 2018; Pitter et al., 2020; Duffek et al., 2020). Due to the large physiological/life-style changes that occur during adolescence, dietary habits of adolescents seem to differ from those of children and adults (von Post-Skagegård et al., 2002; Harris et al., 2015). Moreover, adolescence may be a sensitive PFAA exposure window due to the large physiological changes occurring. Considering the European Food Safety Authority's (EFSA) recent conclusion that the dietary intake of the sum of PFOA, PFNA, PFHxS and PFOS is a health concern in Europe (EFSA Contam Panel et al., 2020), a better understanding of dietary PFAA exposure pathways for adolescent populations is urgently needed.

A healthy diet, rich in vegetables, fruits, wholegrains, legumes, seafood and vegetable oils, provides an essential foundation for the prevention of cardiovascular disease, obesity and cancer (WHO, 2003). Recent research has reported elevated perfluoroundecanoic acid (PFUnDA) and PFOS concentrations in blood from 6 to 11 year-old children that consumed more than two servings of fish/seafood per week (Papadopoulou et al., 2019). Simultaneously, less healthy foods, such as snack foods (Susmann et al., 2019; Park et al., 2019) and fast food (Averina et al., 2018), have been proposed as exposure sources for certain PFAAs, at least partially due to the use of PFAS in paper-based food contact materials (Trier et al., 2011; Poothong et al., 2020; Seshasayee et al., 2021). Moreover, EFSA identified healthy food groups, including fish/shellfish, fruit and fruit products, and egg and egg products, as significant contributors to the overall exposure to PFOA, PFNA, PFHxS and PFOS in the general population (EFSA Contam Panel et al., 2020). There is therefore a need to capture the holistic influence of diet on PFAS exposure (Halldorsson et al., 2008; Liu et al., 2017a), since healthy dietary patterns are important determinants of positive health development. Improved knowledge on the relationship between a healthy diet and PFAS exposure is important when assessing the possible health effects of PFAS, and also for future health risk-benefit analyses of dietary patterns.

In the present study, we aimed to evaluate the association between PFAA serum concentrations and the overall adolescent diet, using a healthy food index and a healthy diet diversity score, in a nation-wide Swedish adolescent population. Individual associations between PFAA body burdens and commonly consumed food and seafood groups were also evaluated, in an effort to determine which food groups that may give a large contribution to adolescent PFAA exposure.

2. Methods

2.1. Study population

This study utilized a subsample of participants from the nationally representative school-based dietary survey Riksmaten Adolescents 2016–17 (RMA), conducted by the Swedish Food Agency (SFA). A detailed account of the population and study design is given in Moraeus et al. (2018), Lindroos et al. (2019) and Nyström et al. (2022).

In short, 3099 adolescents from grades 5 (on average ages 11–12 year), 8 (ages 14–15 year) and 11 (ages 17–18 years) were recruited via their schools between September 2016 and May 2017. Selection of schools was carried out by Statistics Sweden and was based on school type (public or charter), municipality classification and geographical spread (Moraeus et al., 2018). Most of the participants resided in southern Sweden or other densely populated areas, such as around the Swedish capital Stockholm, with fewer participants residing in the far north. Of the 2377 individuals invited to donate biological samples, 1098 had provided blood samples available for PFAS analysis (Nyström et al., 2022). The present study population consisted of a total of 482 male and 616 female participants, with an average age of 14.6 years for the population as a whole (Table 1). Participants exceeding the UN-defined adolescent upper age limit of 19 years of age (n = 11) were included in the study population, as it has previously been reported that their dietary and life-style most likely resembles that of their fellow classmates (Sawyer et al., 2018).

Written, informed consent was obtained from all participants or legal guardians for those below 16 years of age. The Regional Ethical Review Board in Uppsala granted ethical approval (No. 2015/190).

2.2. Personal characteristics and dietary assessment

Information on the personal characteristics and dietary habits of the participants were collected using the SFA-developed and validated web-based system RiksmatenFlex, which consisted of RiksmatenFlexDiet (RFD) and RiksmatenFlexQuestionnaire (RFQ) (Lindroos et al., 2019; Moraeus et al., 2018). Dietary registration in RFD was based on the repeated 24-h dietary recall method which allowed for retrospective registration of consumption of both food and beverages. The registration was carried out over two non-consecutive days; the first registration occurred during a weekday while the second registration was randomized to occur 2–7 days after the first registration. The second registration could consequently occur during the weekend as well, taking possible differences in dietary habits between weekdays and weekends days into account. Additional information on less frequently consumed foods was collected using a non-quantitative food propensity questionnaire (FPQ) in RFQ. The FPQ was designed to collect dietary data with a consumption frequency over the last 12-months. RFQ also contained additional questions concerning the participants' lifestyle, socio-economic factors, and other personal characteristics and demographics (Moraeus et al., 2018).

2.3. Sampling and chemical analysis of blood

Comprehensive accounts of the blood sampling and the method for chemical analysis are given in Moraeus et al. (2018) and Nyström et al. (2022), respectively.

In short, non-fasting venous blood was drawn in 10 ml tubes coated with coagulation activators. Once centrifuged on site, serum was stored and transported at -20°C until final storage at -80°C awaiting PFAS analysis (Moraeus et al., 2018).

Serum samples were extracted with acetonitrile followed by a dispersive clean-up using graphitized carbon (Powley et al., 2005) and analysis by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). A detailed description of the serum PFAS analysis, including quality control, is provided in a companion article

Table 1
Demographical, lifestyle and physiological determinants of the RMA study population, included in the base model and secondary models, reproduced from Nyström et al., (2022).

	All *n (%)*
Categorical covariates	1098 *(100)*
Alcohol consumption during last 6 months	
Has never consumed alcohol (incl. 5th graders)[a]	759 *(69)*
Once	107 *(10)*
Several times	209 *(19)*
Missing	23 *(2)*
Birth country (participant/mother)	
Both high income countries	898 *(82)*
High income/upper-middle countries	79 *(7)*
Both upper-middle income countries	39 *(4)*
High and upper-middle/low and lower-middle income countries	29 *(3)*
Both low and lower-middle income countries	42 *(4)*
Missing	11 *(1)*
BMI status[b]	
Underweight	72 *(7)*
Normal weight	793 *(72)*
Overweight	190 *(17)*
Obese	43 *(4)*
Education level – father	
No formal education and primary education	111 *(10)*
Vocational education or equivalent	215 *(20)*
3–4 year upper secondary education or equivalent	280 *(26)*
University education or equivalent	411 *(37)*
Missing	81 *(7)*
Education level - mother	
No formal education and primary education	84 *(8)*
Vocational education or equivalent	162 *(15)*
3–4 year upper secondary education or equivalent	229 *(21)*
University education or equivalent	565 *(51)*
Missing	58 *(5)*
Exclusively breastfed	
Never breastfed and breastfed for <1 month	111 *(10)*
1 to 6 months	591 *(54)*
7 to >12 months	359 *(33)*
Missing	37 *(3)*
Gender	
Males	482 *(44)*
Females	616 *(56)*
Smoking habits	
Non-smoker (incl. 5th graders)[a]	955 *(87)*
Smoker	123 *(11)*
Missing	20 *(2)*
Snus use	
Does not use snus (incl. 5th graders)[a]	1016 *(93)*
Snus user	70 *(6)*
Missing	12 *(1)*
Continuous covariates	**Median** (min, max) *(% missing)*
Age (years)	14.6 (10.6, 21.1) *(0)*

Note: min, minimum; max, maximum; BMI, body mass index.
[a] 5th grade participants were not asked about alcohol and tobacco use due to their young age but they were assumed to be non-consumers of alcohol and non-smokers.
[b] For participants <18 years of age, BMI was classified based on age- and sex dependent cut-offs (Cole and Lobstein, 2012), while for participants ≥18, the WHO cut-offs were used (WHO, 1999).

(Nyström et al., 2022). A total of 42 PFAS were included in the analysis but only linear (lin) PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin/-branched (br)-PFOS are included in the present study as they were the only PFAS detected at high enough frequency (≥70% of the RMA participants) to produce stable regression models. Furthermore, these PFAS are those that have been "priority" listed by EFSA (EFSA Contam Panel et al., 2020). The measured serum concentrations for all 42 PFAS are accounted for in Nyström et al. (2022).

2.4. Model selection and processing of determinants

Associations between serum PFAA concentrations and dietary determinants were evaluated using ordinal logistic regression (see section 2.5.). We built upon our previous work (Nyström et al., 2022), and created a base model encompassing possible determinants which could arguably explain some of the variation in serum PFAA concentrations. This included participant/maternal birth country (BC), maternal and paternal education level (MEL/PEL), body mass index cut-offs (BMI status), gender (male/female), age (years), months exclusively breastfed early in life, alcohol consumption, smoking habits, and snus use (Table 1) (Nyström et al., 2022). To investigate the association between serum PFAA and healthy and diverse food habits as a whole, a healthy food index (the Swedish Healthy Eating Index for Adolescents 2015, SHEIA15), and a diet diversity score index (Riksmaten Adolescent Diet Diversity Score, RADDS) (Moraeus et al., 2020), were separately explored. Moreover, in order to investigate associations between serum PFAA concentrations and consumption of important food groups, long-term (habitual) consumption of commonly consumed food, and seafood groups were added to the base model, in this case excluding SHEIA15 or RADDS (see section 2.4.1–2.4.4).

As roughly 40 of the RMA participants were residing in areas with PFAS-contaminated municipal drinking water remediated a few years before the blood sampling in RMA (Nyström et al., 2022), we performed a sensitivity analysis on the SHEIA15 and RADDS model adjusting categorically for those who were residing in previously highly contaminated areas (n = 42) and those who were not (n = 1056).

2.4.1. Healthy food index – SHEIA15

SHEIA15 was developed by the SFA and is an index that quantifies diet quality. Further details of SHEIA15 are described in Moraeus et al. (2020). Briefly, SHEIA15 is based on key dietary advice from the 2015 SFA dietary guidelines "Find your way" , i.e. eat more fruits and vegetables, switch to whole grain, eat more seafood, switch to healthy fats, switch to low fat dairy products, eat less sugar and less red and processed meats. The index is a ratio between the actual consumption of food or nutrient intake registered by the participants in RFD and the recommended consumption of each food and nutrient intake included in the 2012 Nordic Nutrition Recommendations (Nordic Council of Ministers, 2014; Moraeus et al., 2020). The scores were summed up (maximum score: 9), where a high score shows a stronger compliance to the recommendations and, as a result, a healthier diet (Moraeus et al., 2020). SHEIA15 was included as a continuous variable in the regression models (Table 2).

2.4.2. Healthy diet diversity score – RADDS

RADDS was also developed by the SFA and quantifies diet diversity. Additional details on RADDS is provided in Moraeus et al. (2020). A diverse and varied diet has been recognized to ensure nutritional adequacy preventing nutritional imbalances. RADDS was included in the base model with the aim to evaluate whether the adolescent serum PFAA concentrations were associated with a healthy diverse diet. RADDS was derived in relation the encouragement of a varied diet in the 2015 SFA dietary guidelines "Find your way", and is based on a variation between 17 food groups, such as cabbage, fruit, wholegrain products, vegetables, fish and milk, to name a few. The participants were given one point if they consumed more than 5 g per day of each subgroup based on the dietary registration in RFD. The points were then summed up, allowing for a maximum score of 17 (Moraeus et al., 2020). RADDS was included as a continuous covariate in the regression models (Table 2).

2.4.3. Habitual consumption of commonly consumed food groups

We also evaluated associations between serum PFAA concentrations and habitual consumption of individual food groups (g/day), i.e. eggs, dairy, meat, cereals, pastry, vegetables, potatoes, sweets, fruits and seafood (Table 2). These groups represent the major food groups

Table 2
Dietary determinants included in the base and secondary regression analysis.

Dietary determinants	All	n = 1098
	Median (min, max)	*% missing*
Dietary indices		
Swedish Healthy Eating Index for Adolescents 2015 (SHEIA15)	5.8 (3.4, 8.7)	*0*
Riksmaten Adolescent Diet Diversity Score (RADDS)	6 (1, 12)	*0*
Habitual consumption determinants[a]		
Habitual consumption of eggs (g/day)	19 (2.0, 95)	*0*
Habitual consumption of dairy (g/day)	410 (24, 1800)	*0*
Habitual consumption of meats (g/day)	120 (0, 380)	*0*
Habitual consumption of fruits (g/day)	69 (1.8, 350)	*0*
Habitual consumption of vegetables (g/day)	150 (19, 650)	*0*
Habitual consumption of potatoes (g/day)	250 (62, 930)	*0*
Habitual consumption of cereals (g/day)	80 (16, 300)	*0*
Habitual consumption of sweets (g/day)	31 (3.9, 220)	*0*
Habitual consumption of pastry (g/day)	13 (1.6, 110)	*0*
Habitual consumption of fish and seafood (g/day)	21 (0, 100)	*0*
Daily fish consumption determinants[b]		
Daily consumption of lean marine fish (g/day)	4.4 (0, 110)	*0.2*
Daily consumption of processed fish (g/day)	2.3 (0, 49)	*0.4*
Daily consumption of canned herring/mackerel (g/day)	0 (0, 49)	*0.5*
Daily consumption of salmonid fish (g/day)	4.4 (0, 49)	*0.5*
Daily consumption of canned tuna (g/day)	0 (0, 110)	*0.5*
Daily consumption of anchovies/sardines (g/day)	0 (0, 39)	*0.7*
Daily consumption of Baltic herring (g/day)	0 (0, 33)	*0.7*
Daily consumption of freshwater fish (g/day)	0 (0, 39)	*0.8*
Daily consumption of large marine fish (g/day)	0 (0, 26)	*0.7*
Daily consumption of crab (g/day)	0 (0, 12)	*1.0*
Daily consumption of shellfish (excluding crab) (g/day)	0.3 (0, 14)	*0.5*

Note: min, minimum; max, maximum.
[a] Based on dietary recall data in RFD.
[b] Based on data from the food propensity questionnaire in RFQ.

consumed by Swedish consumers according to the Swedish Market Basket Survey (SFA, 2017). The habitual food consumption variables were derived from the two independent days of dietary registration in RFD and were transformed to long-term consumption using the Multiple Source Method (MSM, Version 1.0.1). By using the two 24-h recall dietary registrations, the MSM computes long-term intake distributions by combining the probability and amount consumed (Harttig et al., 2011; Haubrock et al., 2011). In the MSM model, all participants were assumed consumers of all foods except fish and meat, where frequency of consumption was collected from the FPQ. Only 50 percent had consumed fish during the two registration days but 90% stated that they generally eat fish. The FPQ was thus used to identify true non-consumers. All 3099 participants with complete RFD registration were used in the calculations, and each school grade was considered to be an individual population.

2.4.4. Daily consumption of specific seafood groups

In order to evaluate if consumption of specific seafood groups contributed to the overall variation in the adolescent serum PFAA concentrations, habitual seafood consumption in the regression models was replaced by the daily intake (g/day) of 11 defined groups of seafood, i.e. lean marine fish, processed fish products, canned herring and mackerel, salmonid fish, anchovies and sardines, Baltic herring, freshwater fish, large marine fish, canned tuna, shellfish (excluding crab) and crab (Table 2). The daily consumption was estimated from the FPQ for consumption of the specific seafood categories consumed over the last 12-months and calculated to daily consumption (g/day) using the average seafood portions (g) by gender and grade derived from the food registration in RFD (Table S1).

2.5. Statistical analysis

Data processing and statistical analysis were carried out in R (version 3.6.3; R Development Core Team), with a statistical significance set at $p \leq 0.05$. A detailed description of data processing is found in Nyström et al. (2022). In brief, missing data of both the dependent and independent variables were imputed 5 times for each gender separately using multiple imputation by chained equations (van Buuren and Groothuis-Oudshoorn, 2011; Rubin, 1987), and joined by stacking prior to the regression analysis. For PFAA concentrations < LOQ, concentrations ≥ LOD were used when possible in the regression analysis, instead of being set to a fixed value (e.g. zero or LOQ/$\sqrt{2}$) (RSC, 2001; Bergstrand and Karlsson, 2009; Nyström et al., 2022).

Ordinal logistic regression (OLR) fitting the cumulative probability model (CMP) was used to evaluate the association between PFAA serum concentrations and the dietary determinants. The OLR can model

Table 3
Legacy serum PFAA concentrations in RMA participants, reproduced from Nyström et al., (2022).

PFAA	***n***	**LOD-range** *(%<LOD)*	**Concentration**(ng/g serum)[a] Median (min, max)
PFCA			
lin-PFOA	1095	0.287 *(0.1)*	1.20 (<LOD, 9.75)
PFNA	1098	0.103–0.176 *(4.9)*	0.382 (<LOD, 2.80)
PFDA	1098	0.028–0.099 *(19.7)*	0.162 (<LOD, 1.35)
PFUnDA	1098	0.020–0.119 *(30.6)*	0.097 (<LOD, 1.01)
PFSA			
lin-PFHxS	1098	0.017–0.216 *(0.7)*	0.399 (<LOD, 255)
br-PFOS	1098	0.031–0.257 *(0)*	0.925 (0.031, 110)
lin-PFOS	1098	0.056–0.562 *(0)*	1.995 (0.281, 127)
Σ_4PFAS[b]	1095		5.137 (0.477, 494)

Note: The limit of detection (LOD) varied between batches; Min, minimum; Max, maximum; PFCA, perfluoroalkylcarboxylic acids; PFSA, perfluoroalkylsulfonic acids.
[a] Median (min, max) concentrations contain serum PFAA samples which were both quantified (>LOQ) and detected (>LOD).
[b] Σ_4PFAS is the summation of serum lin-PFOA, PFNA, lin-PFHxS, br-PFOS and lin-PFOS concentration, i.e. the sum PFAS that EFSA has used to determine the tolerable intake (TWI) in their risk assessment (EFSA Contam Panel et al., 2020).

continuous data by only including the order information of the dependent variable making it a more robust alternative to ordinary least squares. An in depth description of the model and procedure is found in Liu et al. (2017b) and Nyström et al. (2022), respectively. PFAA data < LOD were replaced by 0.0001 ng/g serum (an arbitrary value lower than the lowest detectable concentration) in order to be included in the regression analysis. Using the ORL, the entire empirical cumulative distribution function conditional on the covariates is estimated from which conditional means, medians and exceedance probabilities along with confidence intervals for these quantities can be acquired. The effect of clustering due to sampling by school was accounted for using the Huber-White method (Nyström et al., 2022). Furthermore, we computed the relative contribution of the habitual food groups to the variation of serum PFAA concentrations by calculating χ^2 - degrees of freedom for each of the model determinants separately.

We additionally carried out a Spearman Rank Correlation analysis, examining the correlations between all the habitual dietary determinants and SHEIA15 and RADDS, separately, as well as the correlation between SHEIA15 and RADDS.

3. Results

Serum concentrations of lin-PFOA, PFNA, PFDA, PFUnDA, lin-PFHxS and lin/br-PFOS are found in Table 3. Lin-PFOS had the highest median concentration followed by lin-PFOA and br-PFOS, with br-PFOS median slightly less than 1 ng/g serum. The other PFAAs showed medians of <1 ng/g serum. Although the median lin-PFHxS concentration was similar to the median of PFNA, and only 2.7-fold higher than that of PFDA, the highest lin-PFHxS concentration (>200 ng/g serum) was approximately 2-3 orders of magnitude higher than the highest concentrations of PFNA, PFDA and PFUnDA (Table 3). A similarly large variation as for lin-PFHxS was observed for lin- and br-PFOS with maximum concentrations >100 ng/g serum. Furthermore, median serum concentration of the sum of lin-PFOA, PFNA, lin-PFHxS, br-PFOS and lin-PFOS (Σ_4PFAS) was approximately 5 ng/g serum (n = 1095) (Table 3). About 29% of the RMA adolescents (n = 315) had serum Σ_4PFAS concentrations exceeding the benchmark concentration level 6.9 ng/mL serum (Table 3), corresponding to the EFSA TWI of 4.4 ng/kg bw/week (EFSA Contam Panel et al., 2020).

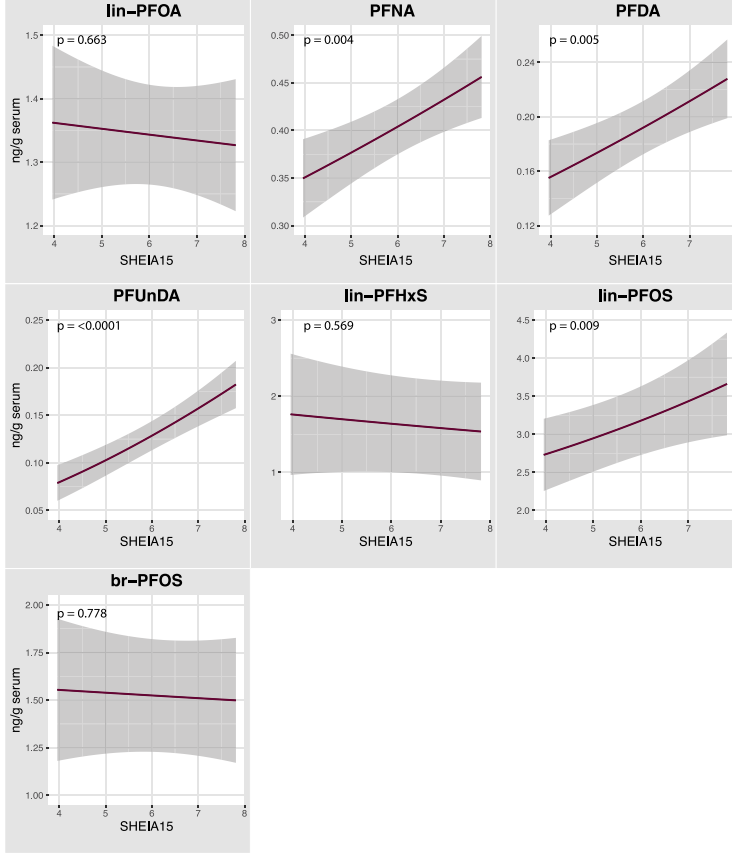

Fig. 1. Associations between adolescent serum PFAA concentrations and the Swedish Healthy Eating Index for Adolescents 2015 (SHEIA15) determined using ordinal logistic regression (OLR) and presented as estimated adjusted mean regression line with 95% confidence interval. The results were adjusted for age, BMI status, gender, maternal and paternal education level, participant/maternal birth country, months exclusively breastfed, snus and smoking habits and alcohol consumption.

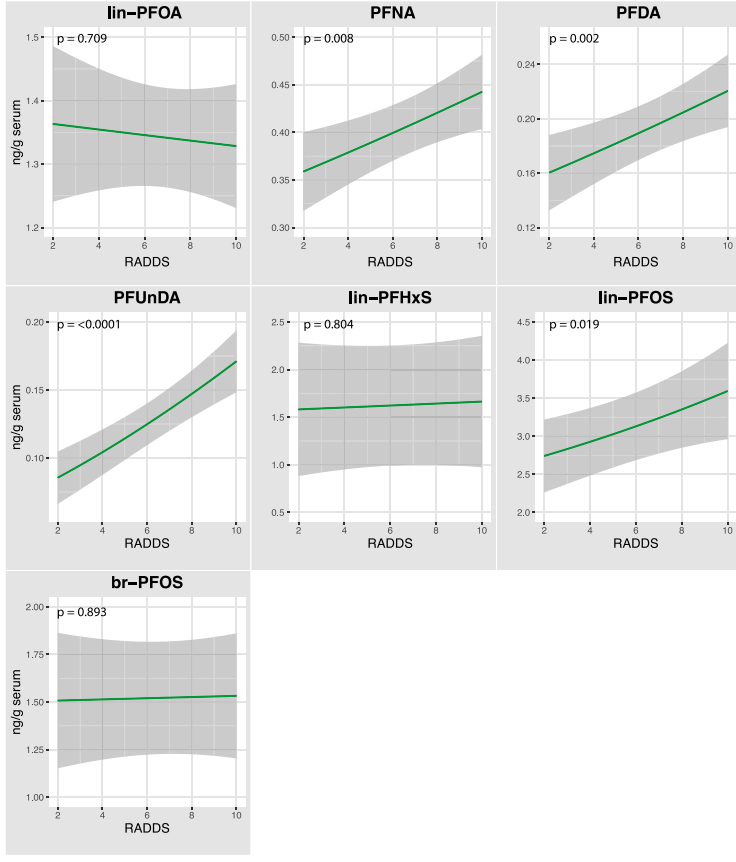

Fig. 2. Associations between adolescent serum PFAA concentrations and the Riksmaten Adolescent Diet Diversity Score (RADDS) determined using ordinal logistic regression (OLR) and presented as estimated adjusted mean regression line with 95% confidence interval. The results were adjusted for age, gender, BMI-status, participant/maternal birth country, maternal and paternal education level, months exclusively breastfed, snus and smoking habits and alcohol consumption.

Table 4
Spearman correlation coefficients for correlations between SHEIA15/RADDS as well as both indices and the habitual food consumption groups.

	SHEIA15		RADDS	
	ρ	p-value	ρ	p-value
Egg	0.02	0.493	0.13	<0.0001
Seafood	0.37	<0.0001	0.26	<0.0001
Meat	−0.40	<0.0001	−0.08	0.013
Dairy	−0.11	0.00035	0.24	<0.0001
Fruits	0.30	<0.0001	0.47	<0.0001
Vegetables	0.34	<0.0001	0.35	<0.0001
Potatoes	−0.03	0.347	−0.02	0.454
Cereals	0.08	0.0078	0.09	0.0454
Sweets	−0.16	<0.0001	0.07	0.020
Pastry	−0.03	0.391	0.07	0.016
SHEIA15			0.48	<0.0001

Note: SHEIA15, Swedish Healthy Eating Index for Adolescents 2015; RADDS, Riksmaten Diet Diversity Score. The p-value was determined using a two-tailed *t*-test.

3.1. Healthy eating index - SHEIA15

The scores of the healthy eating index, SHEIA15, ranged from approximately 3.5 to 8.5 (Table 2). PFNA, PFDA, PFUnDA and lin-PFOS serum concentrations were positively and significantly associated with SHEIA15 (Fig. 1; Table S2), with the highest average increase for PFUnDA (ca 2-fold) between the lowest and highest score. Average PFNA, PFDA and lin-PFOS concentrations increased approximately 1.3- to 1.4-fold. No statistically significant associations were observed for lin-PFOA, lin-PFHxS and br-PFOS. None of these associations changed in the sensitivity analysis (Fig. 2; Table S2). SHEIA15 was positively correlated with habitual consumption of seafood, fruits, vegetables and cereals and negatively correlated with consumption of meat, dairy and sweets (Table 4). Furthermore, a moderate positive correlation was found between the two dietary indices (Table 4).

3.2. Diet diversity score - RADDS

Serum PFNA, PFDA, PFUnDA and lin-PFOS concentrations were positively and significantly associated with RADDS scores, the latter of

Fig. 3. p-value heat map for the associations between serum legacy PFAA concentrations and habitual consumption of food groups (g/day) (A: green/yellow), and for the associations between PFAA concentrations and consumption of specific seafood groups (g/day) (B: purple/pink). + = positive association, - = negative association. A. was determined using ordinal logistic regression analysis (OLR), with all food groups included in the regression models and adjusted for snus use and smoking habits, alcohol consumption, gender, age, months exclusively breastfed, BMI, maternal and paternal education level, participant/maternal birth country. B. All seafood groups were included in the regression model A instead of habitual seafood consumption and adjusted for the same personal characteristics as in A, as well as habitual consumption of eggs/dairy/meat/fruits/vegetables/potatoes/cereals/sweets/pastry. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

which ranged from 1 to 12 (Table 2; Fig. 2; Table S2). The highest average increase between the lowest and the highest scores was observed for PFUnDA (ca 2-fold) and the lowest for PFNA (ca 1.2-fold). Lin-PFOA, lin-PFHxS and br-PFOS did not show significant associations with RADDS (Fig. 2; Table S2). None of the associations changed in the sensitivity analysis (Table S2). RADDS was negatively correlated with habitual meat consumption and positively correlated to habitual consumption of eggs, seafood, dairy, fruits, cereals, sweets and pastry (Table 4).

3.3. Habitual food consumption

The habitual consumption of the different food groups are given in Table 1. Significant inverse associations were observed between concentrations of all the legacy PFAA and dairy consumption, except for br-PFOS (Fig. 3; Table S3). Similarly, lin-PFOA and PFNA were inversely associated with cereal consumption, lin-PFOA with pastry, and PFNA and PFUnDA with sweets consumption (Fig. 3; Table S3). Significant positive associations were observed between PFNA and PFUnDA concentrations and vegetable consumption, and PFNA lin-PFHxS and meat consumption (Fig. 3; Table S3). Lin-PFHxS and lin-PFOS concentrations were positively associated with egg consumption, while PFNA, PFDA, PFUnDA and lin-PFOS were positively associated with seafood consumption (Fig. 3; Table S3). None of the legacy PFAAs were associated with habitual consumption of potatoes and fruits (Table S3).

Of the habitual food groups, seafood was the determinant explaining the largest part of the variation in serum concentrations of PFNA, PFDA and PFUnDA, while being the second most important determinant of lin-PFOS (Fig. 4). Habitual dairy consumption was the most important determinant of lin-PFOS and lin-PFOA, and the second most important in explaining the variation of PFNA, PFDA, lin-PFHxS and br-PFOS serum concentrations (Fig. 4). Meat consumption contributed most to the variation in br-PFOS, whilst egg consumption explained most of the variation for lin-PFHxS (Fig. 4). Meat consumption was the most important determinant of br-PFOS, but none of the relationships were statistically significant.

3.4. Consumption of specific seafood groups

Data on daily fish/seafood consumption based on the FPQ are given in Table 2. PFDA and lin-PFOS concentrations were positively associated with consumption of lean marine fish, while PFUnDA and lin-PFOS were inversely associated with consumption of processed fish (Fig. 3; Table S4). PFNA and lin-PFHxS were positively associated with consumption of canned tuna, as were PFUnDA, lin-PFHxS and lin-PFOS with shellfish consumption (Fig. 3; Table S4). PFUnDA was positively associated with pickled herring and mackerel consumption (Fig. 3; Table S4). Neither lin-PFOA nor br-PFOS were significantly associated with consumption of any of the individual seafood groups.

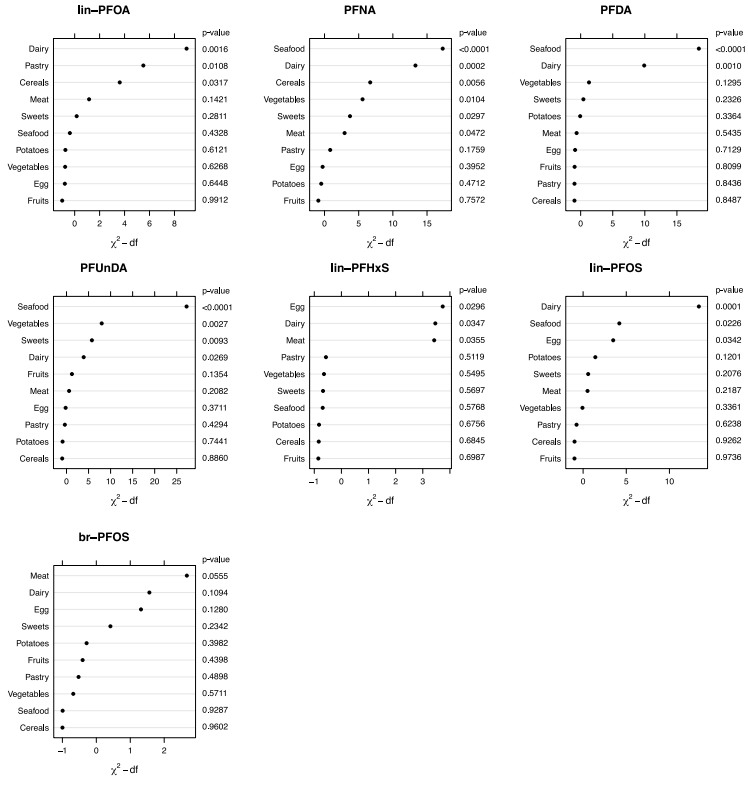

Fig. 4. The relative importance of the contribution for each habitual food group in explaining the variation in the RMA serum PFAA concentrations when included in the same regression model, adjusted for participant/maternal birth country, BMI-status, gender, age, maternal and paternal education level, snus use and smoking habits, alcohol habits and months exclusively breastfed. The relative importance is expressed as χ^2- df (x-axis), where a higher number indicates a higher relative importance. The relative importance of a determinant can only be compared within each PFAA model and not between models. Note that other base model determinants, not shown in this figure, could contribute more or less in explaining the variation in PFAA serum.

4. Discussion

Adolescence is a life stage of special interest, since health status at older ages is at least partially determined by habits established during adolescence (Kim et al., 2020; van Sluijs et al., 2021). Since dietary habits of adolescents may differ from that of adults due to the large physiological/life-style changes that occur during this time of life, it is important to gain knowledge about the relationships between PFAS exposure and dietary habits in adolescents. RMA participants who consumed an overall healthier diet, as suggested by a higher SHEIA15 score, had on average higher PFNA, PFDA, PFUnDA and lin-PFOS serum concentrations. Similar positive associations were found for the same PFAAs and consumption of a diverse diet, as determined by RADDS. Approximately one third of the RMA adolescents had Σ_4PFAS concentrations in serum that exceeded the 6.9 ng/ml serum benchmark concentration determined as no health concern by EFSA Contam Panel et al. (2020). Consequently, the relations between PFAA exposure and healthy diets may be regarded as problematic from a health development perspective. Although it is not possible from the present study to elucidate how much of the present serum Σ_4PFAS body burdens that are attributable to only dietary exposure sources, it is plausible that the RMA adolescent PFAA body burdens at least partially originate from dietary exposure. These findings highlight the problematic relationship between PFAA exposure and a healthy diet that is essential for a healthy human population.

To the best of our knowledge, associations between serum/plasma concentrations of PFAS and healthy diets have not been studied in adolescent populations, and scarcely among adults. Comparisons of results between dietary studies are made difficult due to differences in composition of diet scores and study populations (Lin et al., 2020). Nevertheless, in a U.S population of pre-diabetic adults, Lin et al. (2020) observed no statistically significant relations between plasma PFAS concentrations and Mediterranean-Like Diet Scores (MDS), and a positive association between plasma PFHxS and the Low-Carbohydrate and High-Protein (LCHP) diet. On the contrary, in an elderly Swedish cohort (PIVUS study), positive associations were observed between serum PFOA, PFNA, PFDA, PFUnDA and PFHxS, and MDS, and between serum PFNA, PFDA, PFUnDA and PFOS, and LCHP (Sjogren et al., 2016). In PIVUS, adherence to both MSD and LCHP was characterized by a higher seafood consumption (Sjogren et al., 2016), and much like PIVUS, seafood consumption in RMA correlated positively with both SHEIA15 and RADDS. Furthermore, habitual seafood consumption was one of the most important determinants in explaining the variation in PFAA concentrations in RMA, except for lin-PFOA,lin-PFHxS and br-PFOS. This corroborates with seafood consumption as a substantial contributor to the background PFNA, PFDA, PFUnDA and lin-PFOS exposure in adults in Sweden and other Nordic countries Haug et al. (2010a); Vestergren et al. (2012); (Bjermo et al., 2013; Papadopoulou et al., 2019). Several studies of health risk-benefits of fish consumption, including the toxic contaminants methyl-mercury and polychlorinated biphenyls and the health beneficial component long chain n-3 polyunsaturated fatty acids, have suggested that environmental pollution of fish may counteract the

positive health effects of fish consumption (Wennberg et al., 2012; Donat-Vargas et al. 2017, 2019; Noger-Huet et al., 2022). Hypothetically, PFAS in seafood may also to some degree counteract the beneficial health effects of seafood consumption, included in a healthy diet.

In Swedish market basket studies, seafood has been the dominating source of per capita intake of PFNA, PFDA, PFUnDA and PFOS, but not of PFOA and PFHxS (SFA, 2017; Vestergren et al., 2012). This could potentially explain the lack of associations between serum concentrations of these latter PFAAs and habitual seafood consumption, as well as with SHEIA15 and RADDS. Similarly as for PFOA and PFHxS, serum concentrations of br-PFOS, in contrast to lin-PFOS, was neither associated with SHEIA15 and RADDS nor seafood consumption, shows an isomer-specific difference in PFOS associations with seafood consumption. It has been reported that br-PFOS bioaccumulates to a lesser degree in fish than lin-PFOS, most probably resulting in enrichment of lin-PFOS in fish tissues (Sharpe et al., 2010; Greaves and Letcher, 2013; Beesoon and Martin, 2015; Shan et al., 2016).

The positive associations between body burdens of certain PFAAs and daily consumption of specific seafood groups in RMA further strengthens the role of seafood as a contributor to the PFAA body burdens among the adolescents. Similar to RMA, Duffek et al. (2020) reported lower plasma PFOA/PFHxS/PFOS concentrations in German children/adolescents (ages 3–17) who never consumed fish compared to those who do consumed fish. However, in contrast to RMA, a study on Norwegian adolescents aged 15–19 years (the Tromsø study) found that only serum PFUnDA concentrations were positively associated with consumption of lean marine fish (Averina et al., 2018). Moreover, serum concentrations of several PFAAs, including PFNA, PFDA, PFUnDA and PFOS, were positively associated to consumption of fatty fish, predominantly consisting of salmonids, mackerel and herring (Averina et al., 2018). Amongst the RMA participants, no associations were observed between PFAA body burdens and salmonid fish consumption, despite the fact that the average daily consumption of salmonids in RMA were at the same high level as consumption of lean marine fish, i.e. about 4 g/day. Due to the lack of comprehensive data on PFAS concentrations in seafood on the Swedish and Norwegian market it is not possible to determine if between-country variation in seafood PFAA concentrations may at least partly explain observed differences in PFAA associations with seafood consumption. This comparison is also made uncertain by differences in food groups included in the regression models and different composition of the included food groups. For instance, the fatty fish group in the Tromsø study consisted not only of salmonids but also of herring and mackerel (Averina et al., 2018). In RMA, serum concentrations of PFUnDA were positively associated with herring and mackerel consumption. Furthermore, given that the Tromsø study included a local population from an Arctic district of Norway (Averina et al., 2018), as opposed to the nation-wide study design of RMA, overall large differences in life-style and food habits further complicates comparisons of results. Albeit these differences in study populations and study design, both RMA and the Tromsø study contribute to the disconcerting evidence that seafood consumption, regarded as a healthy dietary component, is an important exposure source to some of the toxic legacy PFAA in adolescents.

Considering the lack of data on PFAA concentrations in seafood on the Swedish market, discussions about PFAA concentrations in seafood in relation to adolescent PFAA exposure can only be hypothetical. Even though daily consumption of shellfish was on average relatively low (median: 0.3 g/day), we could still see positive associations between PFUnDA, lin-PFHxS and lin-PFOS body burdens and shellfish consumption. It has previously been reported that crustaceans, such as shellfish, caught in Norwegian and Dutch waters have higher lin-PFOS and PFUnDA concentrations compared to white marine fish species (Carlsson et al., 2016; Zafeiraki et al., 2019). In RMA, inverse relations were observed between PFUnDA and PFOS serum concentrations and consumption of processed fish products, although this seafood type was one of the most frequently consumed (median: 2.3 g/day). This could be due to chance or possible residual confounding by other factors not studied by us. There are very limited information about PFAA concentrations in fish products, which in RMA mainly was composed of fish sticks and the seafood equivalent to meatballs, i.e. fish balls. In a total diet study from the US, only low concentrations of PFOS and PFNA was detected in fish sticks (Genualdi et al., 2021). Comparable observations have been made for fish sticks sampled in Norway (Haug et al., 2010b).

Even though higher PFAA concentrations have been reported in Swedish freshwater fish species, e.g. perch, burbot and freshwater salmon, compared to the same species caught in the Baltic Sea (Berger et al., 2009), no significant associations were found between serum PFAA and freshwater fish consumption in RMA. This is likely explained by the low median consumption of fresh water fish (0 g/day) among the adolescents. This explanation is also probable when pondering the lack of associations for the daily consumption of anchovies and sardines, large marine fish, Baltic herring and crab. However, even though the median daily consumption of canned tuna was 0 g/day, positive associations were still seen for serum PFNA and lin-PFHxS. This could suggestively be explained by a larger range of canned tuna consumption (0–110 g/day), as opposed to e.g. freshwater fish (0–33 g/day).

Significant associations between PFNA, PFDA, PFUnDA and lin-PFOS body burdens and seafood consumption were observed both in the food registration data (RFD) and in the data from the food frequency questionnaire (RFQ). The habitual seafood consumption variable, estimated from the 24-h recall method in RFD, takes the amount of seafood consumed during two independent days of food registration into account. The RFQ only account for the self-reported frequency of seafood consumption over the last year. However, the median daily consumption of all seafood groups together were only slightly lower in the RFQ data than in the habitual seafood consumption data; 17 g/day as opposed to 21 g/day. Nevertheless, a larger recall bias (Smith et al., 1991) when answering the RFQ than the RFD cannot be ruled out.

In RMA, serum concentrations increased with increasing habitual consumption of heńs eggs (lin-PFHxS and lin-PFOS) and meat (PFNA and lin-PFHxS). Egg consumption was not a component of the SHEIA15 index (Moraeus et al., 2020), which is corroborated by the finding of no relation between habitual egg consumption and the healthy food index in the present study. Consequently, egg consumption did most likely not contribute to a large extent to the observed positive associations between lin-PFOS body burdens and SHEIA15 scores. However, egg consumption could have contributed to the positive PFOS and RADDS association since habitual egg consumption increased with increasing RADDS. Positive associations between plasma PFOS concentrations and egg consumption have also been reported in a 3-17 year-old German population (Duffek et al., 2020). In Sweden, heńs egg and meat consumption have historically made a significant contribution to the average intake of PFHxS and PFOS from food, although decreasing in importance during the last decades (Vestergren et al., 2012; Johansson et al., 2014; SFA, 2017). Similar associations between PFOS body burdens and heńs egg consumption, as observed in RMA, have previously been reported for adults from other industrialized countries (Jain, 2018; Eriksen et al., 2011; Colles et al., 2020; Pitter et al., 2020). Positive associations between PFHxS body burdens and egg consumption are less frequently reported, though shown to be a determinant in pregnant Chinese women (Yang et al., 2019) and to contribute to roughly 20% of the PFHxS intake from food in an adult Spanish population (Arrebola et al., 2018). For heńs eggs, PFOS concentrations likely differ between production systems, since evidence, although limited, suggests much higher concentrations in home-produced compared to conventionally produced eggs (D'Hollander et al., 2011; Zafeiraki et al., 2016; Su et al., 2017; Fillol et al., 2021; Gazzotti et al., 2021). As with fish consumption, no association between br-PFOS and egg consumption was observed among the RMA participants, which might be attributable to lin-PFOS enrichment in heńs eggs compared to other PFOS isomers (Wang et al., 2019).

Inverse associations were found between serum concentrations of

almost all PFAAs and dairy consumption, and was also found to give a relatively large contribution to the variation of the serum PFAA concentrations in the RMA population. In the Tromsø study, no data on associations between PFAA concentrations and dairy consumption were reported (Averina et al., 2018). Similarly, Duffek et al. (2020) reported no significant associations with milk consumption in the child/-adolescent German population. However, similarly to RMA, inverse associations were observed between milk/dairy consumption and plasma PFOS concentrations in Danish middle-aged men (Eriksen et al., 2011), serum PFOA and PFOS concentrations in U.S. adolescents/adults (Jain, 2014) and serum PFOA, PFHxS and PFOS in an Italian adolescents/adult population (Pitter et al., 2020). Contrarily, studies from Spain and the Netherlands have suggested that dairy products may contribute to overall dietary PFAA exposure (Ericson et al., 2008; Noorlander et al., 2011). Apart from differences in study design and study populations, the diverging associations between studies could potentially be explained by regional differences in contamination of agricultural products, for instance due to variation in the use of biosolids and/or irrigation (Ghisi et al., 2019). From our results it is not possible to draw conclusions about the reasons behind the inverse association with dairy products. It could however be speculated that replacement of drinking water consumption, a suggested PFAS exposure source (Sims et al., 2021), with consumption of less PFAA-contaminated milk (Tao et al., 2008; Clarke et al., 2010) offered in school lunches (Patterson and Elinder, 2015) and at home in Sweden, may contribute to lower serum PFAA concentrations. However, this hypothesis fails to explain the negative association for PFDA and PFUnDA in RMA, which are infrequently detected in drinking water (Zafeiraki et al., 2015; Gobelius et al., 2018). In contrast to lin-PFOS, br-PFOS was not associated with dairy product consumption, further supporting the hypothesis of differences in exposure sources between linear and branched PFOS isomers.

It should be noted that some of the aforementioned relationships between serum PFAA concentrations and food habits may be coincidental, or at least partially confounded by life-style/dietary factors not studied in RMA. Apart from fish stick/balls and dairy consumption, this is likely also the reason behind inverse associations between legacy PFAA concentrations and consumption of cereals (lin-PFOA, PFNA), sweets (PFNA, PFUnDA), and pastry (lin-PFOA), and positive associations with vegetable consumption (PFNA, PFUnDA). For instance, vegetables are not an important source of dietary exposure to PFNA and PFUnDA in Sweden (Vestergren et al., 2012; SFA, 2017). Similar to RMA, no significant relationships were found between serum PFAA concentrations and consumption of fruits/vegetables amongst the Tromsø adolescents (Averina et al., 2018). In contrast to our results, a stronger adherence to a diet rich in vegetables was previously reported to be related to a decrease in PFAS exposure among adults, most probably caused by replacement of products of animal origin with higher PFAS contamination (Skuladottir et al., 2015; Tian et al., 2018; Yang et al., 2019; Lin et al., 2020; Menzel et al., 2021). Moreover, non-dietary exposure sources could at least partially be involved in the observed associations, due to the connection between a vegetable-rich diet and high socio-economic status (Lallukka et al., 2007; Hanson and Chen, 2007).

Considering that EFSA Contam Panel et al. (2020) highlighted fruit and fruit products as an important exposure source of PFOA, PFNA, PFHxS and PFOS, it is noteworthy that no significant associations were found between adolescent serum PFAA concentrations and habitual consumption of fruits in RMA. These results are further substantiated by habitual consumption of fruits being one of the dietary determinants that contributed the least in explaining the variation in serum PFAA concentrations. Amongst the many PFAS measured in a Swedish market-basket survey from 2015, PFHxA was the only PFAA with low but quantifiable concentrations in fruits (SFA, 2017) and in 1999, 2005 and 2010 only very low PFOA concentrations were reported (Vestergren et al., 2012). While PFAAs in fruits might be a significant exposure source in some European countries, this does not appear to be the case in Sweden.

The large number of participating adolescents is a strength of our study, including the population-based study design allowing us to generalize the findings for the school-attending adolescent population as a whole in Sweden (Lindroos et al., 2019). However, considering the school-based recruitment of participants, it should be highlighted that an additional dietary survey has been conducted including adolescents not attending upper-secondary school (SFA, 2018). Large discrepancies in socio-demographical factors and dietary habits were found between participants attending school and those not attending school (SFA, 2018). This suggests that the generalization of our results may not include adolescents not attending school. A strength of our study is that detailed food consumption data were collected by a validated recall method (Lindroos et al., 2019) and in agreement with the current European Food Safety Authority standard (Moraeus et al., 2018). However, it is always difficult for study participants to measure and report food consumption, which may add uncertainty to our results. It is also possible that the results were influenced by non-dietary sources of PFAA exposure not studied by us, or other unknown factors that may be associated with serum PFAA concentrations. A limitation of our study was that the regression models were not adjusted for caloric intake. However, several demographic/life-style factors that may explain some of the variation in consumption of different food groups, such as BMI, age, gender, participant and maternal birth country and parental education level (Nyström et al., 2022), were included in the regression models. To the best of our knowledge, associations between dietary determinants and serum concentrations of branched and linear isomers of PFOS have not previously been studied separately in adolescents, and our results showed isomer-related differences in associations with diet. It should however be highlighted that any isomer-related differences in associations between lin- and br-PFOS and food consumption could be confounded by discrepancies in human pharmacokinetic properties between branched and linear PFOS isomers (Benskin et al., 2010).

5. Conclusions

Swedish adolescents adhering to healthy and diverse diets had higher serum concentrations of the legacy PFAAs that have been reported as health concerns in Europe. The associations were most likely driven by a positive relation between seafood consumption and the healthy/diverse diet scores, including lean marine fish species and shellfish. Differences in relationships between br-PFOS and lin-PFOS serum concentrations and healthy and diverse diet scores was observed, showing the importance of analyzing different PFAS isomers separately. As a healthy diet during adolescence lays the foundation of a healthy adult life, more research is warranted on the health benefits of a healthy diet in relation to negative effects of PFAA exposure.

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Ethical approval

Participants ≥16 years, or legal guardians of participants <16 years of age, gave written informed consent for participation. Ethical approval was obtained from the Regional Ethical Review Board in Uppsala (No. 2015/190).

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.113170.

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Supplementary Information

Healthy eating index and diet diversity score as determinants of serum perfluoroalkyl acid (PFAA) concentrations in a national survey of Swedish adolescents

Authors

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Table of content

Table S1. Daily fish and shellfish consumption portions (g/day) calculated separately for grade and gender from the RFQ frequency questionnaire on consumption of specific fish and shellfish types 2

Daily consumption of different types of seafood was derived from the RiksmatenFlexQuestionnaire (RFQ) frequency registration of consumption of seafood groups (i.e. lean marine fish (e.g. cod, Alaska Pollock, saithe, hoki, plaice, tilapia and pangasius), processed fish products (e.g. fish sticks and Swedish fish balls), canned herring and mackerel, anchovies and sardines. (e.g. anchovies, sardines and sardelles), Baltic herring (fresh/canned), freshwater fish (e.g. burbot, pike, perch and pike-perch), canned tuna, salmonid fish (e.g. rainbow trout and salmon), large saltwater fish (e.g. shark, ray, fresh tuna, large halibut and swordfish), crab, and shellfish (e.g. mussels/clams, shrimps, crayfish and lobster/langoustine, excluding crab). The consumption frequency of seafood per year/month/week/day was converted to daily consumption (g/day) using average portion sizes derived from the RFD (n=3099) for each school grade (5th, 8th and 11th) and gender. For females, the average portion size of fish was 84.0, 83.5 and 67.5 g, respectively, for 5th, 8th and 11th grades, and 26.5, 26.0 and 25.5 g for shellfish, respectively. For males, the average portion size of fish for the same school grades was 90.5, 114.5 and 107.5 g, respectively, and 18.5, 28.0 and 47.5 g for shellfish

Table S2. Odds ratios and corresponding 95% confidence interval for the association between legacy serum PFAA concentrations and the healthy food index SHEIA15, and the diet diversity score RADDS, in RMA adolescents, including the sensitivity analysis. 3

Table S3. Odds ratios (OR) and 95% confidence interval (CI) for associations between PFAA serum concentrations and habitual food consumption variables[a]. 4

Table S4. Odds ratios (OR) and 95% confidence interval (CI) for associations between PFAA serum concentrations and consumption of different types of seafood[a] 5

References 6

Table S1. Daily fish and shellfish consumption portions (g/day) calculated separately for grade and gender from the RFQ frequency questionnaire on consumption of specific fish and shellfish types

Frequency interval	**No. times/year**	**5th grade**				**8th grade**				**11th grade**			
		Fish (g/day)		*Shellfish (g/day)*		*Fish (g/day)*		*Shellfish (g/day)*		*Fish (g/day)*		*Shellfish (g/day)*	
		Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
Never	0	0	0	0	0	0	0	0	0	0	0	0	0
1-3 times/year	2	0.5	0.1	0.5	0.1	0.5	0.1	0.6	0.2	0.4	0.1	0.6	0.3
4-8 times/year	6	1.4	0.4	1.5	0.3	1.4	0.4	1.9	0.5	1.1	0.4	1.8	0.8
9-11 times/year	10	2.3	0.7	2.5	0.5	2.3	0.7	3.1	0.8	1.8	0.7	2.9	1.3
1-3 times/month	24	5.5	1.7	6.0	1.2	5.5	1.7	7.5	1.8	4.4	1.7	7.1	3.1
1 time/week	52	12.0	3.8	12.9	2.6	11.9	3.7	16.3	4.0	9.6	3.6	15.3	6.8
2 times/week	104	23.9	7.6	25.8	5.3	23.8	7.4	32.6	8.0	19.2	7.3	30.6	13.5
3 times/week	156	35.9	11.3	38.7	7.9	35.7	11.1	48.9	12	28.8	10.9	45.9	20.3
>1 time/day	365	84	26.5	90.5	18.5	83.5	26	114.5	28	67.5	25.5	107.5	47.5

Table S2. Odds ratios (OR)[a] and corresponding 95% confidence interval (CI) for the association between legacy serum PFAA concentrations and the healthy food index SHEIA15, and the diet diversity score RADDS, in RMA adolescents, including the sensitivity analysis.

	SHEIA15						***RADDS***					
	Base model analysis[b]			*Sensitivity analysis[c]*			*Base model analysis[b]*			*Sensitivity analysis[c]*		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
lin-PFOA	0.967	0.839 – 1.118	0.6634	0.970	0.841 – 1.118	0.6714	0.973	0.844 – 1.122	0.7090	0.982	0.850 – 1.134	0.8019
PFNA	1.291	1.085 – 1.536	0.0040	1.290	1.085 – 1.534	0.0040	1.190	1.046 – 1.353	0.0079	1.189	1.047 – 1.352	0.0078
PFDA	1.297	1.083 – 1.553	0.0047	1.296	1.083 – 1.551	0.0047	1.204	1.068 – 1.357	0.0023	1.204	1.069 – 1.357	0.0023
PFUnDA	1.615	1.373 – 1.900	<0.0001	1.614	1.372 – 1.899	<0.0001	1.409	1.228 – 1.618	<0.0001	1.408	1.226 – 1.618	<0.0001
lin-PFHxS	0.950	0.797 – 1.133	0.5689	0.963	0.804 – 1.154	0.6824	1.065	0.893 – 1.157	0.8043	1.035	0.914 – 1.171	0.5923
lin-PFOS	1.215	1.050 – 1.405	0.0089	1.211	1.033 – 1.421	0.0185	1.168	1.026 – 1.331	0.0192	1.183	1.040 – 1.347	0.0109
br-PFOS	0.979	0.843 – 1.137	0.7782	0.965	0.825 – 1.130	0.6596	1.008	0.893 – 1.139	0.8927	1.017	0.903 – 1.145	0.7857

Notes: n=1098. The associations in both analysis were determined using ordinal logistic regression (OLR), whilst p-value was derived using Wald statistics.

[a] An OR <1 suggest a negative association while an OR>1 suggests a positive association.

[b] Adjusted for age, gender, BMI, participant/maternal birth country, maternal and paternal education level, alcohol consumption, snus use, smoking habits and months fully breastfed early in life.

[c] Adjusted for residence in area with previous high level contamination of PFAS in drinking water (residing vs not residing), as well as adjusted for the above-mentioned base model covariates.

Table S3. Odds ratios (OR)[a] and 95% confidence interval (CI) for associations between PFAA serum concentrations and habitual food consumption variables[b].

Covariate (g/day)	**lin-PFOA** OR (CI)	p	**PFNA** OR (CI)	p	**PFDA** OR (CI)	p	**PFUnDA** OR (CI)	p	**lin-PFHxS** OR (CI)	p	**lin-PFOS** OR (CI)	p	**br-PFOS** OR (CI)	p
Eggs	0.969 (0.847, 1.109)	0.6448	1.062 (0.924, 1.221)	0.3952	1.026 (0.894, 1.178)	0.7129	1.058 (0.935, 1.197)	0.3711	1.150 (1.014, 1.305)	0.0296	1.143 (1.010, 1.293)	0.0342	1.097 (0.974, 1.235)	0.1280
Dairy	0.804 (0.703, 0.921)	0.0016	0.738 (0.630, 0.864)	0.0002	0.802 (0.704, 0.914)	0.0010	0.888 (0.800, 0.987)	0.0269	0.872 (0.768, 0.990)	0.0347	0.801 (0.714, 0.898)	0.0001	0.906 (0.802, 1.023)	0.1094
Meats	0.908 (0.799, 1.033)	0.1421	1.147 (1.002, 1.313)	0.0472	0.956 (0.827, 1.105)	0.5435	0.900 (0.763, 1.061)	0.2082	1.178 (1.011, 1.373)	0.0355	1.100 (0.945, 1.282)	0.2187	1.145 (0.997, 1.315)	0.0555
Fruits	1.001 (0.859, 1.166)	0.9912	1.025 (0.876, 1.199)	0.7572	1.017 (0.888, 1.164)	0.8099	1.099 (0.971, 1.245)	0.1354	0.972 (0.839, 1.125)	0.6987	1.002 (0.879, 1.142)	0.9736	0.954 (0.846, 1.075)	0.4398
Vegetables	1.034 (0.904, 1.183)	0.6268	1.233 (1.050, 1.446)	0.0104	1.120 (0.968, 1.296)	0.1295	1.251 (1.081, 1.448)	0.0027	0.955 (0.823, 1.109)	0.5495	1.078 (0.925, 1.256)	0.3361	0.960 (0.833, 1.106)	0.5711
Potatoes	0.960 (0.819, 1.125)	0.6121	0.943 (0.803, 1.107)	0.4712	0.928 (0.796, 1.081)	0.3364	0.972 (0.818, 1.154)	0.7441	0.972 (0.850, 1.111)	0.6756	0.896 (0.781, 1.029)	0.1201	0.939 (0.812, 1.087)	0.3982
Cereals	0.845 (0.724, 0.985)	0.0317	0.820 (0.713, 0.944)	0.0056	1.013 (0.886, 1.159)	0.8487	0.990 (0.863, 1.135)	0.8860	0.971 (0.841, 1.121)	0.6845	0.994 (0.874, 1.130)	0.9262	0.997 (0.881, 1.128)	0.9602
Sweets	1.077 (0.940, 1.238)	0.2811	0.863 (0.756, 0.986)	0.0297	0.905 (0.768, 1.066)	0.2326	0.850 (0.752, 0.961)	0.0093	0.954 (0.810, 1.123)	0.5697	0.913 (0.793, 1.052)	0.2076	0.915 (0.790, 1.059)	0.2342
Pastry	0.852 (0.753, 0.964)	0.0108	0.902 (0.778, 1.047)	0.1759	0.988 (0.873, 1.118)	0.8436	1.055 (0.924, 1.204)	0.4294	0.937 (0.772, 1.138)	0.5119	0.957 (0.803, 1.140)	0.6238	0.942 (0.796, 1.116)	0.4898
Seafood	0.943 (0.816, 1.091)	0.4328	1.435 (1.216, 1.693)	<0.0001	1.479 (1.243, 1.760)	<0.0001	1.537 (1.312, 1.802)	<0.0001	1.038 (0.911, 1.183)	0.5768	1.208 (1.027, 1.422)	0.0226	0.993 (0.851, 1.159)	0.9287

Notes: n = 1098 for all models. OR (CI) were derived using ordinal logistic regression and p-values were derived using Wald statistics.

[a] An OR <1 suggest a negative association while a OR >1 suggest a positive association.

[b] All models included age, body mass index (BMI), gender, participant/maternal birth country (BC), maternal and paternal education level (MEL/PEL), snus habits, smoking habits, alcohol consumption and months exclusively breastfed, as well as all of the habitual food variables (i.e. all food variables were added simultaneously to the base model).

Table S4. Odds ratios[a] (OR) and 95% confidence interval (CI) for associations between PFAA serum concentrations and consumption of different types of seafood[b].

Covariate (g/day)	lin-PFOA OR (CI)	p	PFNA OR (CI)	p	PFDA OR (CI)	p	PFUnDA OR (CI)	p	lin-PFHxS OR (CI)	p	lin-PFOS OR (CI)	p	br-PFOS OR (CI)	p
Lean marine fish	0.998 (0.842, 1.183)	0.9838	1.161 (0.995, 1.355)	0.0587	1.257 (1.084, 1.459)	0.0025	1.111 (0.975, 1.265)	0.1157	0.998 (0.891, 1.117)	0.9689	1.159 (1.035, 1.298)	0.0105	1.038 (0.928, 1.160)	0.5165
Processed fish	0.946 (0.844, 1.060)	0.3353	0.933 (0.834, 1.045)	0.2308	0.920 (0.813, 1.042)	0.1886	0.849 (0.751, 0.960)	0.0087	0.947 (0.848, 1.057)	0.3302	0.853 (0.749, 0.972)	0.0169	0.917 (0.822, 1.023)	0.1198
Herring and mackerel	1.010 (0.978, 1.043)	0.5378	1.032 (0.992, 1.074)	0.1163	1.019 (0.979, 1.060)	0.3665	1.058 (1.010, 1.108)	0.0169	1.003 (0.964, 1.043)	0.8863	1.015 (0.972, 1.060)	0.5101	1.002 (0.961, 1.044)	0.9277
Salmonid fish	1.023 (0.925, 1.147)	0.5932	1.026 (0.922, 1.142)	0.6333	1.035 (0.928, 1.156)	0.5344	1.089 (0.970, 1.223)	0.1472	0.983 (0.868, 1.113)	0.7864	0.967 (0.856, 1.093)	0.5918	1.018 (0.884, 1.173)	0.8033
Canned tuna	0.999 (0.992, 1.005)	0.6872	1.013 (1.007, 1.020)	0.0001	1.001 (0.974, 1.028)	0.9694	1.005 (0.987, 1.023)	0.5770	1.009 (1.003, 1.015)	0.0046	1.005 (0.998, 1.012)	0.1431	1.002 (0.995, 1.009)	0.5681
Anchovies etc.	0.555 (0.098, 3.156)	0.5071	0.314 (0.030, 3.301)	0.3348	0.787 (0.037, 16.94)	0.8786	1.109 (0.025, 48.70)	0.9574	3.082 (0.077, 122.9)	0.5495	1.477 (0.167, 13.08)	0.7258	1.164 (0.182, 7.456)	0.8729
Baltic herring etc.	15.21 (0.401, 576.9)	0.1423	7.621 (0.346, 168.1)	0.1982	1.191 (0.062, 23.01)	0.9078	1.860 (0.045, 76.93)	0.7437	0.744 (0.105, 5.273)	0.7675	1.362 (0.253, 7.342)	0.7195	2.003 (0.480, 8.356)	0.3403
Freshwater fish	0.997 (0.972, 1.022)	0.8046	1.012 (0.984, 1.040)	0.4140	1.018 (0.981, 1.057)	0.3399	1.008 (0.977, 1.040)	0.6243	0.994 (0.967, 1.021)	0.6591	1.031 (0.994, 1.069)	0.0988	1.019 (0.992, 1.047)	0.1737
Large saltwater fish	3.512 (0.039, 313.1)	0.5835	2.152 (0.350, 13.22)	0.4080	0.587 (0.050, 6.924)	0.6719	2.151 (0.698, 6.625)	0.1822	1.331 (0.181, 9.779)	0.7785	1.270 (0.139, 11.71)	0.8307	1.620 (0.116, 22.72)	0.7203
Crab	0.996 (0.968, 1.025)	0.7934	1.013 (0.984, 1.043)	0.3988	0.992 (0.954, 1.032)	0.6926	0.981 (0.957, 1.005)	0.1271	0.988 (0.943, 1.036)	0.6277	1.005 (0.977, 1.035)	0.7129	1.001 (0.977, 1.023)	0.9464
Shellfish	0.949 (0.875, 1.029)	0.2042	1.028 (0.938, 1.127)	0.5543	1.131 (0.993, 1.288)	0.0635	1.216 (1.072, 1.379)	0.0024	1.099 (1.020, 1.185)	0.0138	1.116 (1.001, 1.244)	0.0488	1.025 (0.913, 1.150)	0.6794

Notes: n = 1098 for all models. OR (CI) were derived using ordinal logistic regression and p-values were derived using Wald statistics.

[a] An OR <1 suggest a negative association while a OR >1 suggest a positive association.

[b] All models included age, body mass index (BMI), gender, participant/maternal birth country (BC), maternal and paternal education level (MEL/PEL), snus habits, smoking habits, alcohol consumption, months exclusively breastfed, the habitual food consumption variables eggs/dairy/meats/fruits/vegetables/potatoes/cereals/sweets/pastry as well as all of the individual fish groups.

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Low concentrations of perfluoroalkyl acids (PFAAs) in municipal drinking water associated with serum PFAA concentrations in Swedish adolescents

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ABSTRACT

While highly contaminated drinking water (DW) is a major source of exposure to perfluoroalkyl acids (PFAAs), the contribution of low-level contaminated DW (i.e. < 10 ng/L of individual PFAAs) to PFAA body burdens has rarely been studied. To address this knowledge gap, we evaluated the association between concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS), and their sum ($\sum_4$PFAAs) in DW and serum in Swedish adolescents using weighted least squares regression. We paired serum PFAA concentrations in adolescents (age 10–21 years, n = 790) from the dietary survey Riksmaten Adolescents 2016–17 (RMA) with mean PFAA concentrations in water samples collected in 2018 from waterworks (n = 45) supplying DW to the participant residential and school addresses. The median concentrations of individual PFAAs in DW were < 1 ng/L. Median concentrations of PFNA and PFHxS in serum were < 1 ng/g, while those of PFOA and PFOS were 1–2 ng/g. Significant positive associations between PFAA concentrations in DW and serum were found for all four PFAAs and $\sum_4$PFAAs, with estimated serum/DW concentration ratios ranging from 210 (PFOA) to 670 (PFHxS), taking exposure from sources other than DW (background) into consideration. The mean concentrations of PFHxS and $\sum_4$PFAA in DW that would likely cause substantially elevated serum concentrations above background variation were estimated to 0.9 ng/L and 2.4 ng/L, respectively. The European Food Safety Authority has determined a health concern concentration of 6.9 ng $\sum_4$PFAAs/mL serum. This level was to a large degree exceeded by RMA participants with DW $\sum_4$PFAA concentrations above the maximum limits implemented in Denmark (2 ng $\sum_4$PFAAs/L) and Sweden (4 ng $\sum_4$PFAAs/L) than by RMA participants with DW concentrations below the maximum limits. In conclusion, PFAA exposure from low-level contaminated DW must be considered in risk assessment for adolescents.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a substantial group of > 4,700 anthropogenic substances (OECD, 2018; US EPA, 2022), some with persistent organic pollutant (POP)-like properties (EU, 2019; EU, 2020). PFAS have excellent surfactant properties, are heat-stable and resistant to chemical and UV-light degradation. They have widely been used in products such as aqueous film forming fire-fighting foams, water- and grease repellent coatings, adhesives, and cosmetics, and in various industrial applications, since the 1940s (ITRC, 2020). This has led to a global pollution where PFAS have been ubiquitously detected in air, sediments, soils, ground- and surface waters, indoor dust and air, and in wild-life and human matrices (Kelly et al., 2009; Ahrens and Bundschuh, 2014; DeLuca et al., 2021). Perfluoroalkyl acids (PFAAs), a subgroup of PFAS, are highly persistent and some have very long half-lives in humans (Xu et al., 2020). These long half-lives, in relation to the suggestive immuntoxic and endocrine disruptive effects (among others) in humans (Fenton et al., 2021), have contributed to growing

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international concern surrounding the entire class of chemicals.

Given their widespread occurrence, human exposure to PFAS is frequently multifaceted, thereby complicating exposure assessments. For the general adult population, diet - in particular fish and shellfish - is considered a significant source of exposure to many of the perfluorocarboxylic acids (PFCAs) and perfluorosulfonic acids (PFSAs) with fluorinated chain-length of ≥ 7 carbons and ≥ 6 carbons, respectively (i.e. long-chain PFAAs) (Sunderland et al., 2019). Drinking water (DW) has also been implicated as an important PFAA exposure source, specifically for populations whose DW is impacted by pollution from point sources (Sunderland et al., 2019; Johanson et al., 2023). Extensive use of PFAS-containing fire-fighting foams, industrial emissions, and use of PFAS-contaminated soil conditioners on agricultural lands have resulted in PFAA concentrations in DW exceeding 100 ng/L of individual PFAA (Emmett et al., 2006; Hölzer et al., 2008; Post et al., 2012; Pitter et al., 2020; Li et al., 2020; Johanson et al., 2023). Thus, serum/plasma concentrations measured in populations living close to PFAA hotspots are highly elevated compared to those living far away from major point sources. A large fraction of the European population has an intake that exceeds the European Food Safety Authority's (EFSA) recently established health-based tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week for the sum of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS) ($\sum_4$PFAA) (EFSA, 2020). Meanwhile, few studies have specifically assessed the extent to which low-level contaminated DW (<10 ng/L of single PFAA) contributes to the exceedance of the TWI.

The widespread use of PFAS over the last seven decades has contributed to dispersal beyond major point sources, leading to widespread low-level PFAS contamination in DW (in general, <10 ng/L of individual PFAS) (Zafeiraki et al., 2015; Li et al., 2019; Arinaitwe et al., 2021). Limited research suggests that low-level PFAA contamination of DW contributes to measurable increases in the long-term cumulative exposure of at least some PFAAs (e.g. PFOA, PFNA, PFHxS and PFOS) with long half-lives in the human body. For example, higher PFAA concentrations in serum/plasma were observed in adults and children drinking water with low-level PFAA contamination compared to those drinking water with non-detectable PFAA concentrations (Post et al. 2012; Hu et al., 2019; Gyllenhammar et al., 2019; Glynn et al., 2020). Nevertheless, the contribution of low levels of PFAA in DW to the body burden is difficult to distinguish from contributions from both historical and contemporary exposures. The voluntary industrial and regulatory phase-out of PFOA, PFHxS, PFOS and related substances was initiated in the early 2000s (Buck et al. 2011) and later PFNA as a higher PFOA homologue in 2010–2015 (US EPA, 2023), and it is reasonable to assume that the long-term cumulative exposure to these substances has historically been higher among adults compared to children/adolescents born around the time of phase-out (Nyberg et al., 2018; Miaz et al., 2020; Lin et al., 2021). Low-level exposure from DW may subsequently not contribute equally to long-term cumulative exposure across age groups, with a possible higher relative contribution among children/adolescents than among adults. Johanson et al. (2023) observed significant associations between PFAA concentrations in DW and serum (matched samples) among adults exposed to a wide range of concentrations up to above 1000 ng ∑PFAAs/L, but to the best of our knowledge no study has investigated associations in young human populations exposed to low-level PFAS contaminated DW. Such knowledge is of importance in the risk assessment of human exposure to PFAS.

The first aim of our study was to evaluate the associations between low concentrations of PFOA, PFNA, PFHxS, PFOS and $\sum_4$PFAA in DW and serum concentrations of these PFAA in Swedish adolescents, who participated in the nation-wide dietary survey Riksmaten Adolescents 2016–17 (RMA). The associations were also evaluated from a gender-specific perspective. The results of this analysis were used to determine the bioaccumulation potential of the studied PFAAs from DW to serum by estimating the ratios between PFAA concentrations in DW and serum, taking exposure from other sources that are prevalent in the general population (from here on referred to as background exposure) into account. From these results, we also attempted to determine the lowest mean PFAA concentrations in DW that would cause measurable elevated serum PFAA levels above background variation when sampling other adolescents in Sweden. The second aim was to risk assess adolescent PFAA exposure from DW by estimating the proportion of RMA participants exceeding the EFSA safe serum concentration of 6.9 ng $\sum_4$PFAAs/mL. We also compared the percentages of participants exceeding the safe serum concentration at DW concentrations above or below the Danish and Swedish maximum limits of 2 ng$\sum_4$PFAAs/L and 4 ng $\sum_4$PFAAs/L om DW, respectively (SLV, 2022; DK EPA, 2021).

2. Material and methods

2.1. Study population

We utilized a subsample of the nationally representative school-based dietary survey Riksmaten Adolescents 2016–17 (RMA) conducted by the Swedish Food Agency. In-depth descriptions of the study design and population are provided in Moraeus et al. (2018), Lindroos et al. (2019) and Nyström et al. (2022a). Briefly, schools with grades 5, 8 and 11 were invited to participate in RMA between September 2016 and May 2017. Selection of schools was carried out by Statistics Sweden and was based on municipality classification, geographical spread and whether the school was public or charter. Each participant was asked to retrospectively register their food, DW and beverage consumption over two non-consecutive days in the validated web-based system RiksmatenFlexDiet (RFD). Participants also answered questions regarding infrequently consumed food items and socio-economic and lifestyle factors in the web-based RiksmatenFlexQuestionnaire (Moraeus et al., 2018).

In the 57 participating schools, 2377 pupils were invited to donate blood samples (Moraeus et al., 2018). For 1098 participants, dietary record and questionnaire answers were available together with serum samples, which were analysed for PFAS (Nyström et al., 2022a). Only participants receiving DW from sampled waterworks both at home and at school (see section 2.2) were included in this study. Consequently, 215 participants were excluded as their residences did not receive DW from a participating waterworks. Moreover, 37 participants were excluded since they were going to school and/or living in areas with a known history of high PFAS contamination of municipal DW, and where remediation of contamination had occurred before blood and DW sampling (Nyström et al., 2022a). Finally, 56 participants were excluded from the analyses due to incomplete dietary or questionnaire data. The final number of participants included in the statistical analyses were 790 (Table 1).

2.2. Sampling and chemical analysis of raw and drinking water

Raw water (RW) and DW were sampled (one sample each) in the spring and autumn of 2018 from waterworks (n = 45) providing DW to the participating schools and homes of the adolescents in RMA (see 2.4 Exposure assessment, 2.4.1 Identification of waterworks). Samples were collected in thoroughly rinsed 1 L high density polypropylene bottles by waterworks staff (grab samples), typically at a tap of the pipe representing ingoing RW and outgoing DW. Samples were stored in darkness at 4 °C until analysed by the Department of Aquatic Sciences and Assessment at the Swedish University of Agricultural Sciences.

A total of 24 different PFAS were analysed in DW and RW samples. The following PFAS were analysed: C_4–C_{14}, C_{16} and C_{18} PFCAs, perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTriDA), perfluorotetradecanoic acid (PFTeDA), perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid

Table 1
Personal characteristics of the participants in Riksmaten Adolescents 2016–17, Sweden, who were included in the present study. The table shows categorical and continuous covariates included in the statistical analysis.

Categorical covariates	**n = 790 *(%)***	
Gender		
Male	350 *(44)*	
Female	440 *(56)*	
School grade		
5	253 *(32)*	
8	324 *(41)*	
11 (second year upper secondary school)	213 *(27)*	
Birth country (participant/mother)[a]		
Both low and lower-middle income countries	30 *(4)*	
High and upper-middle and low and lower-middle income countries	23 *(3)*	
Upper-middle and upper-middle income countries	29 *(4)*	
High and upper-middle countries	65 *(8)*	
High and high income countries	643 *(81)*	
Maternal education level		
No formal education or primary education	70 *(9)*	
Vocational education or equivalent	116 *(15)*	
3–4 year upper secondary education or equivalent	174 *(22)*	
University education or equivalent	430 *(54)*	
Continuous covariates	**Median (min - max)**	**Mean (SEM)**
Age (years)	14.5 (10.6 – 21.1)	14.5 (0.9)
Habitual seafood consumption (g/day)	21.5 (0 – 101)	23.8 (0.53)
Habitual long-term drinking water consumption (mL/day)	502 (40.6 – 2780)	583 (13.7)

Note: SEM, standard error of the mean.
[a] Categorization is based on the per capita gross national income level in accordance with the World Bank Country Classification of 2018 (World Bank Group 2018; Nyström et al. 2022a).

(PFOcDA)); C_4, C_6, C_8 and C_{10} PFSAs (PFBS, PFHxS, PFOS, PFDS); 6:2, 8:2, 10:2 fluorotelomer sulfonates (FTSA); perfluorooctanesulfoneamide (FOSA); perfluorooctane sulfonamidoacetic acid (FOSAA); methyl and ethylperfluorooctane sulfonamidoacetic acid (MeFOSAA and EtFOSAA, respectively) (Table 2 and Table S1 in Supporting Information (SI)).

The water samples were analysed using a validated method in accordance with Gobelius et al. (2018), with few modifications. Briefly, 500 mL water sample was filtered (1.2 μm glass fiber filter GFF, GE Healthcare Life Sciences, Whatman UK) and then spiked with 100 μL of mass-labelled internal standard (IS) mixture (20 ng mL^{-1} of each IS, n = 16). The samples were extracted by solid phase extraction (SPE) using Oasis WAX cartridges (6 cc, 500 mg, 60 μm, Waters Corporation, USA) which were preconditioned with 4 mL of 0.1 % ammonium hydroxide in methanol solution (1:1 methanol: Millipore water). After loading, the cartridges were buffered with 25 mM ammonium acetate buffer in Millipore water and then dried in a centrifuge (2000 rpm, 3 min). Thereafter, the cartridges were eluted with 6 mL methanol, followed by 6 mL 0.1% ammonium hydroxide solution in methanol. The extracts were then concentrated to 1 mL and analysed using a DIONEX UltiMate 3000 ultraperformance liquid chromatograph (UPLC) system coupled to a triple quadrupole mass spectrometer (MS/MS) (TSQ Quantiva, Thermo Fischer Scientific, Waltham, MA, USA). As a standard procedure, quality control samples included matrix spiked samples (n = 7), duplicate samples (n = 8), and laboratory blanks (n = 12).

For RW/DW samples, the limits of quantification (LOQ) were calculated for individual PFAS based on the average concentration in the blanks (n = 12) plus three times standard deviation of the blanks. If no PFAS were detected in the blank, the lowest quantifiable concentration in the calibration curves were set as the LOQ. Ranges of LOQs and the number of samples ≥ LOQ for each analysed PFAS are given in Tables 2 and S2 in SI. The LOQs for PFOS were slightly higher in some analytical batches. To improve the statistical power in the analyses of associations between PFOS concentrations in serum and DW, PFOS concentrations in DW < LOQ but > LOD were used when reported by the laboratory. From here on, PFAS concentrations in DW > LOQ, and/or > LOD (PFOS) when reported, will be referred to as detected.

Table 2
PFAA concentrations (ng/L) in drinking water (DW) and raw water (RW) samples from waterworks (n = 45)[a] sampled in spring and autumn 2018, used for calculations of mean PFAA concentrations in DW supplied to the schools and residences of the participants.

		Spring		**Autumn**	
PFAS		**Range of LOQ *(n ≥ LOQ)***	**Median[b] (min, max)**	**Range of LOQ *(n ≥ LOQ)***	**Median[b] (min, max)**
PFOA	DW	0.086 – 0.42 *(29)*	0.56 (0.13, 1.9)	0.070 – 0.67 *(10)*	0.74 (0.47, 2.5)
	RW	0.062 – 0.36 *(30)*	0.88 (0.22, 5.1)	0.062 – 0.69 *(17)*	0.77 (0.26, 1.4)
PFNA	DW	0.031 – 0.22 *(20)*	0.23 (0.11, 0.37)	0.066 – 0.19 *(6)*	0.26 (0.21, 0.32)
	RW	0.047–0.14 *(20)*	0.27 (0.16, 0.42)	0.063 – 0.25 *(16)*	0.38 (0.17, 0.48)
PFHxS	DW	0.041 – 0.20 *(17)*	0.46 (0.19, 7.9)	0.057 – 7.5 *(11)*	0.36 (0.14, 5.6)
	RW	0.099 – 0.52 *(22)*	0.53 (0.14, 6.1)	0.053 – 6.3 *(17)*	0.52 (0.075, 1.5)
PFOS	DW	0.31 – 1.9 *(6)*	2.55 (1.1, 4.2)	0.67 – 2.8 *(22)*[c]	0.60 (0.22, 3.0)[c]
	RW	0.04 – 4.2 *(4)*	2.95 (1.6, 5.6)	0.59 – 3.0 *(6)*	1.1 (0.71, 2.1)
Σ_4PFAAs	DW		0.81 (0.13, 14)		0.81 (0.22, 10)
	RW		1.3 (0.14, 11)		1.1 (0.075, 4.0)

Note: LOQ, limit of quantification; min, minimum; max, maximum. Σ_4PFAA include PFOA, PFNA, PFHxS and PFOS.
[a] Due to analytical issues or missed sampling, n = 42 and 39 in DW samples in spring and autumn respectively, and n = 41 and 42 in spring and autumn RW samples respectively.
[b] The median and min/max was only calculated for samples > LOQ.
[c] Values < LOQ and > limit of detection (LOD) are included.

Table 3
Serum PFAA concentrations (ng/g) for the RMA participants (n = 790).

PFAA	LOD *(%>LOD)*	Median (min, max)	Mean (SEM)[a]
PFOA	0.29 *(99.9)*	1.2 (<LOD, 5.3)	1.30 (0.02)
PFNA	0.10 – 0.18 *(95.0)*	0.39 (<LOD, 2.8)	0.43 (0.01)
PFHxS	0.017 – 0.22 *(99.7)*	0.40 (<LOD, 16)	0.62 (0.04)
PFOS	0.031 – 0.26 *(100)*	1.9 (0.28, 15)	2.5 (0.07)
Σ_4PFAAs	–	4.2 (0.71, 33)	4.9 (0.10)

Note: LOD, limit of detection; min, minimum; max, maximum; SEM, standard error of the mean; Σ_4PFAA, sum of (lin-) PFOA, PFNA, (lin-) PFHxS and (lin-) PFOS.

[a] Values < LOD were assigned LOD/$\sqrt{2}$ for calculations of the mean and SEM.

2.3. Sampling and chemical analysis of blood

A detailed description of the blood sampling and chemical analysis is given in both Moraeus et al. (2018) and Nyström et al. (2022a). In brief, non-fasting blood samples were taken during study visits to the participating schools in 2016–2017. Venous blood samples were drawn by trained staff and stored in 10 mL tubes coated with coagulation activators. Once centrifuged, serum was stored at −20 °C onsite and during transport until final storage at −80 °C awaiting analysis (Moraeus et al., 2018).

We previously reported concentrations of 42 different PFAS in serum among the RMA participants (Nyström et al., 2022a). In the present study, only linear (lin-) PFOA, PFNA, lin-PFHxS and lin-PFOS were included in the statistical analyses as these PFAA (Table 3) i) were detected at enough frequency in both serum (≥95%) (Nyström et al., 2022a) and DW (≥10%) (Table 2) in order to be included in the statistical analysis and ii) were included in the EFSA risk assessment (EFSA, 2020). From here on, lin-PFOA, lin-PFHxS and lin-PFOS will be referred to as PFOA, PFHxS and PFOS, unless otherwise stated.

The analytical method, including quality control, and measured serum PFAS concentrations have previously been accounted for in Nyström et al. (2022a). In brief, serum samples were fortified with 0.5 ng of individual isotopically-labelled internal standards and thereafter extracted twice with 4 mL of acetonitrile in an ultrasonic bath. The extracts were combined and concentrated to 1 mL under a stream of nitrogen, and then purified with graphitized carbon and acetic acid. A portion of the final extract was suplanted with 4 mM aqueous ammonium acetate and 0.5 ng of individual volumetric standards, prior storage at −20 °C. Instrumental analysis was performed using a Waters Acquity ultra performance liquid chromatograph (UPLC) equipped with a C18 column and coupled to a Waters Xevo TQS triple quadrupole mass spectrometer. The mass spectrometer was operated in negative electrospray ionization, multiple reaction monitoring (MRM) mode. Quantification was based on isotope dilution. Quality control included analysis of blanks, spiked samples, and NIST Standard Reference Material 1957 (see Nyström et al., 2022a for details). If a signal occurred in the method blanks within a batch, the reporting limit was based on the limit of quantification (LOQ, mean blank + 3x standard deviation), otherwise, the reporting limit was set as the limit of detection (LOD, concentration at a signal-to-noise ratio of 3).

2.4. Exposure assessment

2.4.1. Identification of waterworks

The waterworks producing DW for the participating schools were identified based on the postal codes of the schools. A questionnaire was sent to the identified waterworks to verify that they were indeed supplying DW to the participating schools. Since we only wanted to include participants receiving DW from identified waterworks both at school and at home, each participant́s home address postal code was matched to the waterworks DW distribution areas. Postal codes within the waterworks distribution areas were retrieved from the DW producers. In the event DW producers could only provide local names of the areas receiving their DW, this information was matched with the postal code of the participants using the online postal service PostNord (PostNord, 2022).

2.4.2. Calculations of mean PFAA concentrations in DW

As explained in section 2.3., only PFOA, PFNA, PFHxS and PFOS were included in the analyses of associations between PFAA concentrations in serum and DW. To increase the n-values of DW PFAA concentrations, we investigated if both RW and DW data could be utilized in the calculation of the averaged PFAA concentration in DW (ng/mL), since it has been reported that standard DW treatment techniques such as disinfection, granular/micro-/ultra-filtration and alum coagulation are largely ineffective in removing PFAS (Appleman et al., 2014). Thus, a two-sided paired *t*-test was carried out investigating whether PFOA, PFNA, PFHxS and PFOS concentrations significantly differed between DW and RW samples or not. This analysis was carried out for spring and autumn samples separately. Although statistically significant differences between RW and DW were found in certain cases, the differences were not consistently in the same direction in the spring and autumn (Table S2 in SI). Therefore, both RW and DW PFAA concentrations were used to calculate an arithmetic averaged DW PFAA concentration (Table 4).

Inter-analytical batch variation in PFAA LOQs in the DW and RW samples made it necessary to use multiple ways to compute a mean DW PFAA concentration, exemplified by three scenarios provided in Table S3 in SI. Firstly, in the case of all samples from a waterworks having concentrations ≥ LOQ, the concentration was calculated as a mean of PFAA concentrations in all spring and autumn RW and DW samples (example 1, Table S3 in SI). Secondly, if at least one sample from a waterworks had a PFAA concentration ≥ LOQ, the concentrations < LOQ were set at LOQ/2 (example 2, Table S3 in SI) before calculation the mean concentration. If the LOQ of one or more samples was higher than the lowest actual detected concentration, then these data were excluded from the calculation of mean DW concentrations (example 2, Table S3 in SI). Lastly, if concentrations in all samples from the waterworks were < LOQ, only the lowest LOQ/2 was used to estimate the mean DW concentration (example 3, Table S3 in SI).

In addition, in the event that more than one waterworks provided DW to a school or home, a weighted mean DW concentration of PFAA was calculated using the reported distribution proportion of each waterworks to the DW and the individual DW concentration for the waterworks. If no such information could be retrieved, the waterworks were assumed to contribute equally to the final mean DW PFAA concentration.

2.4.3. PFAA concentrations in serum

In some analytical batches concentrations < LOQ but ≥ LOD were reported, these data were used as reported (Bergstrand and Karlsson, 2009; RSC, 2001; Nyström et al., 2022a). If concentrations were < LOD they were set to LOD/$\sqrt{2}$. In analytical batches with only LOQ reported, concentrations < LOQ were replaced with LOQ/$\sqrt{2}$.

Table 4
Calculated median and arithmetic mean PFAA concentrations in drinking water from the participating waterworks (n = 45) using both raw and drinking water samples from spring and autumn 2018.

PFAA (ng/L)	Median (min - max)	Mean (SEM)
PFOA	0.45 (0.03 – 1.7)	0.54 (0.01)
PFNA	0.15 (0.03 – 0.32)	0.16 (<0.01)
PFHxS	0.30 (0.03 – 2.5)	0.47 (0.02)
PFOS	0.63 (0.24 – 3.3)	0.92 (0.03)
Σ_4PFAAs	1.5 (0.39 – 6.3)	2.1 (0.06)

Note: Min, minimum; max, maximum; SEM, standard error of the mean; Σ_4PFAA, sum of PFOA, PFNA, PFHxS and (lin-) PFOS.

2.4.4. Estimation of long-term drinking water consumption

The daily habitual (long-term) DW consumption was derived from the RMA dietary registration in the RFD by the Multiple Source Method (MSM, Version 1.0.1) (Harttig et al., 2011; Haubrock et al., 2011), and included direct tap water consumption and consumption of beverages made with tap water registered by the participants during two independent days (24-hour recall registration) (Table 1). The MSM is based on shrinkage models, a method that has been recommended when estimating long-term DW intake (Cuvelier and Bartell, 2021). The beverages made from tap water included tea, coffee and fruit/berry syrups/concentrates diluted with tap water (in Swedish: "saft").

2.5. Statistical analysis

The statistical analyses were executed in R (version 4.0.4; R Development Core Team), except for the two-sided paired *t*-test which was performed in Excel; version 2016. All analyses used a statistical significance level of $p \leq 0.05$. We determined DW and serum PFAA concentration relationships using both univariate and multivariate linear regression analyses. In the univariate analysis, the calculated mean PFAA concentrations in DW (independent variable) were matched and modeled against participants' serum PFAA concentrations (dependent variable). This association was investigated for PFOA, PFNA, PFHxS and PFOS individually, as well as for the sum of these four ($\sum_4$PFAAs) since EFAS's TWI is based on these homologues (EFSA, 2020). In our previous studies (Nyström et al., 2022a; Nyström et al., 2022b), age (years), gender (male/female), maternal education level, habitual seafood consumption (g/day) and birth country (joint covariate including both maternal and participant birth country) were significant determinants of serum PFAA concentrations among the RMA participants (Table 1). These determinants were included in the multivariate regression models, as additional independent variables. Since the normality assumption of the residual errors when fitting the data using an ordinary least squares regression was not met, weighted linear regression was performed as described in Johanson et al. (2023). Weights for serum PFAA concentrations were defined by the inverse absolute residuals obtained in the ordinary least squares regression. Such weights allow extreme values to influence the regression fit to a lesser extent than in the ordinary least square analysis without the need of transforming the dependent data variable. Transformation makes interpretation of the results difficult as back-transformation to its original scale is needed.

The intercept of the regression line was interpreted as the mean background serum concentration (ng/g; we assume a 1:1 ratio between blood volume and weight as 1 mL serum equals 1.06 g serum), originating from other exposure sources than local DW such as food, air/dust and other unidentifiable sources (Johanson et al., 2023). The coefficient of the association between PFAA concentrations in DW and serum (i.e., the slope of the regression line) was defined as the serum:water ratio (SWR) (Bartell, 2017). In order to generalize this association to other demographically comparable adolescents in Sweden, we estimated the minimum PFAA concentration in DW that ought to cause a measurable increase in PFAA concentrations in serum due to DW exposure (DWES). This was achieved by determining the DW PFAA concentration at which the regression line, representing the fitted population-averaged PFAA concentration in serum, reaches the upper 68% and 95% prediction interval at the background serum concentration ($DWES_{68}$ and $DWES_{95}$). By using the prediction intervals, we were able to account for the variation in estimated background PFAA concentrations and consequently make predictions for other/new observations of PFAA concentrations in serum from adolescents in Sweden (Johanson et al., 2023). These estimates was only derived for those PFAA were the fitted regression line reached the 68% and 95% prediction intervals within the range of DW PFAA concentrations studied as extrapolation outside the DW concentration range was considered too uncertain. Furthermore, we derived the fitted population-averaged PFAA serum concentration at each of the upper prediction interval limits, i.e., the mean serum concentration corresponding to $DWES_{68}$ and $DWES_{95}$.

As gender-based differences in serum PFAA concentrations have previously been reported among adolescents in RMA (Nyström et al., 2022a), a multivariate weighted linear regression analysis was performed with an interaction term between PFAA concentrations in serum and gender. Moreover, we evaluated whether long-term DW consumption influenced the associations between concentrations of PFAA in DW and in serum, and if concentrations of PFAA in serum were associated with DW consumption. This was carried out by adding the long-term DW consumption covariate to the existing multivariate regression analysis as an additional independent variable.

2.6. Risk assessment of PFAA exposure from drinking water

The $\sum_4$PFAA concentration among the RMA participants were compared with the serum concentration attained after life-time average intake of $\sum_4$PFAA (35 years) at the TWI level among females (6.9 ng Σ_4PFAAs/mL serum) (EFSA, 2020). We used a χ^2-test of independence to compare the proportions of individuals exceeding 6.9 ng/mL at Σ_4PFAA concentrations in DW below or above established maximum limits in Denmark (2 ng $\sum_4$PFAAs/L) (DK EPA, 2021) and Sweden (4 ng $\sum_4$PFAAs/L) (SLV, 2022).

3. Results and discussion

3.1. PFAS in drinking and raw water

Our analyses of associations between PFAS concentrations in serum and DW only covered PFOA, PFNA, PFHxS and PFOS (Table 3), since the other PFAS measured in DW (Table S1, Supporting Information) were rarely detected in serum (Nyström et al., 2022). Nevertheless, we aimed to present data on a large number of PFAS in municipal DW that have otherwise rarely been published. FOSA and PFOA were the most abundantly detected PFAS among the spring DW samples (detection frequency 67% and 69%, respectively), followed by PFNA and PFHxS (detection frequency > 40%; Table 2 and S1 in SI). Among the autumn DW samples, 6:2 FTSA was the most frequently detected (64%), followed by PFOS (56%), FOSA (46%), PFHxS (28%) and PFOA (26%) (Table 2 and S1 in SI). PFCA with carbon chains longer than PFNA were rarely observed in both RW and DW (Table S1 in SI). A similar pattern was observed for the FTSAs, with almost no samples having 8:2 or 10:2 FTSA concentrations ≥ LOQ (Table S1 in SI). A lower mobility in soil/ground water for the PFAS with longer chain lengths could at least partially contribute to these observations (Baduel et al., 2017; Nguyen et al., 2020; Cai et al., 2022). The relatively frequent detection of 6:2 FTSA and FOSA in Swedish municipal DW (Table S1 in SI) is in line with a survey of surface/groundwater in Sweden (Gobelius et al., 2018), and with DW samples from the Great Lakes/St. Lawrence River in Canada (Kaboré et al., 2018).

The highest median concentration for the sum of all PFAS were found in spring DW samples, with a concentration of 2 ng/L (Table 2 and S1 in SI). The median sum of PFOA, PFNA, PFHxS and PFOS in DW was < 1 ng/L for both spring and autumn, though with a maximum of 14 ng/L in a waterworks sampled in spring (Table 2). The highest individual PFAS concentrations in DW were observed for PFBA (22 ng/L), followed by PFPeA (18 ng/L), 6:2 FTSA (17 ng/L) and PFHxS (~8 ng/L) (Table 2 and S1 in SI). These concentrations are within the range of previous studies (Gellrich et al., 2013; Zafeiraki et al., 2015; Park et al., 2018; Gobelius et al., 2018; Li et al., 2022a). Comparisons of PFAS concentrations and composition in DW between studies are complicated as LOQ may vary between studies and, perhaps more important, different studies have not necessarily measured the same PFAS. Moreover, depending on the distance between the DW and contamination source, along with the nature of the contamination, the occurrence of different PFAS in DW may vary considerably (Gyllenhammar et al., 2015; Gobelius et al., 2018; Zhang et al., 2019; Nickerson et al., 2021). Yet, legacy PFAAs, such as PFOA,

PFNA, PFHxS and PFOS, have frequently been found globally in both ground- and surface water, as well as in finished DW, even in areas with unknown presence of highly contaminated point sources (Kaboré et al., 2018; Ao et al., 2019; Arinaitwe et al., 2021; Sims et al., 2021). Contrastingly, FOSA was not observed in DW samples from Spain, Brazil and France (Schwanz et al., 2016), but has been quantified in about 4% of DW samples in Germany (Gellrich et al., 2013), in around a third of samples from Uganda (Arinaitwe et al., 2021) and detected in 53% in DW samples from Canada (Kaboré et al., 2018).

3.2. PFAAs in serum

As reported in Nyström et al. (2022a), (lin-) PFOA, PFNA, (lin-) PFHxS, and (lin-) PFOS were detected in ≥ 95% serum samples (Table 3). In contrast, other PFAS (e.g. short-chain PFAA and polyfluoroalkyl substances) were detected in only a few serum samples (Nyström et al. 2022a), even though some of the PFAS were detected at relatively high concentrations in single samples from certain waterworks and/or with high frequency in DW/RW (Table 3).

PFOS showed the highest median concentration in serum followed by PFOA, both at > 1 ng/g serum (Table 3). PFNA and PFHxS occurred at median concentrations < 1 ng/g serum (Table 3). The median concentration of $\sum_4$PFAAs was 2.2-fold higher than that of PFOS. The concentration ranges of PFHxS and PFOS in serum spanned over two orders of magnitude, while those of PFNA and PFOA spanned over one order of magnitude (Table 3). The measured concentrations were within the range of those previously reported for adolescent populations in South Korea, Taiwan, Norway and Denmark (Ji et al., 2012; Guang-Hui et al, 2013; Averina et al., 2018; Thomsen et al., 2021).

Even though FOSA, a known PFOS precursor (Benskin et al., 2009), was frequently detected in DW (Table S1 in SI), it was only detected in a few of the RMA serum samples at a maximum concentration of 0.1 ng lin-FOSA/g serum (Nyström et al. 2022a). In pooled serum samples from Swedish first-time mothers, FOSA declined from > 0.05 ng/g serum in 1997 to < 0.01 ng/g serum in 2006–2012 (Gebbink et al., 2015). It has been suggested that FOSA, unlike many of the long-chained PFAA with a high binding affinity for albumin in serum and plasma, favorably binds to red blood cells (Hanssen et al., 2013; Poothong et al., 2017). Among Norwegian adults sampled in 2013–2014, the median FOSA concentration in serum was 0.03 ng/mL (range: <0.002–0.05), and in whole

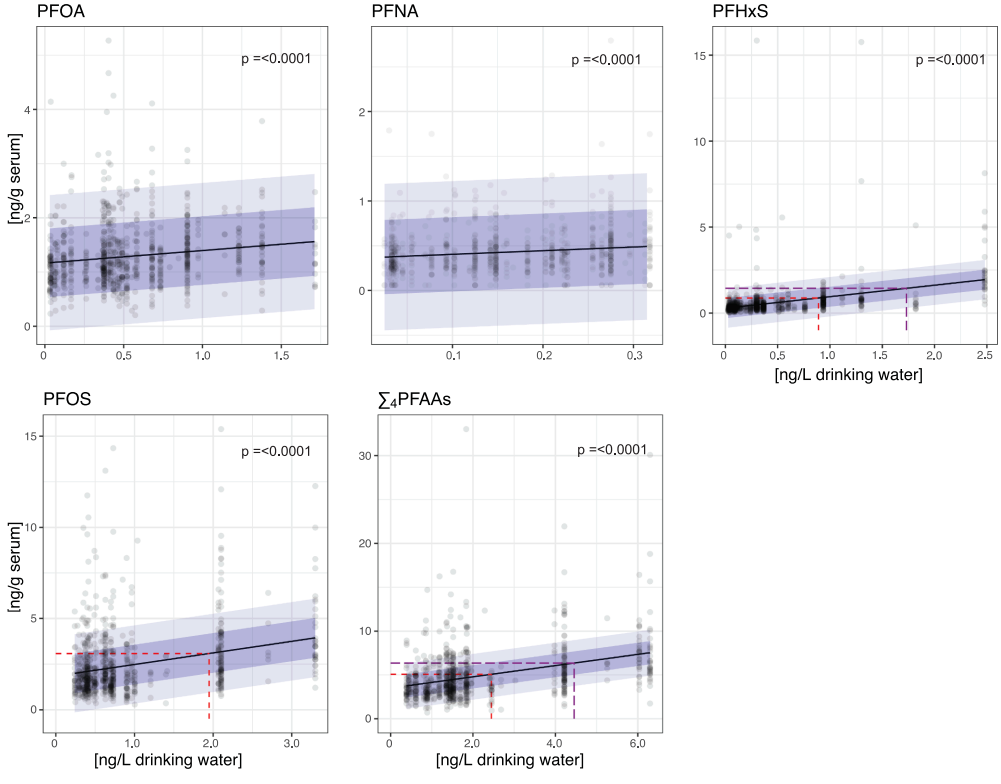

Fig. 1. Univariate weighted linear regression analysis of associations between DW and serum PFAA concentrations (n = 790). The solid black line represents the regression line and the light blue and light grey band represents the 68% and 95% prediction interval (PI), respectively. The horizontal red and purple dashed lines represent the estimated mean serum concentrations at the upper 68% and 95% PI limit of the background PFAA concentration (intercept). The vertical red ($DWES_{68}$) and purple ($DWES_{95}$) line represents the PFAA concentration in DW corresponding to the mean regression line PFAA concentration in serum at the upper PI limit of background PFAA concentration in serum (PI 68% $DWES_{68}$, 95% $DWES_{95}$). Grey circles are individual PFAA concentrations in serum. $DWES_{68}$ and $DWES_{95}$ could not be determined for PFOA, PFNA and PFOS ($DWES_{95}$) since we did not extrapolate DWES outside the ranges of DW concentrations observed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

blood 0.14 ng/mL (0.05–2.35) (Poothong et al., 2017). In RMA, FOSA concentrations in general were not detectable in serum, making it impossible to evaluate the contribution of FOSA in DW to adolescent FOSA body burdens. Since FOSA with current analytical methods seems to be more easily detected in whole blood than in serum, whole blood needs to be considered as an analytical matrix in future studies with the aim to understand whether DW is an important exposure source for this PFOS precursor. Another possibility is to markedly decrease the detection limits of FOSA in serum so that the detection frequency increases in future studies.

3.3. Regression analyses

3.3.1. Associations between PFAA concentrations in drinking water and serum

Concentrations of PFOA, PFNA, PFHxS, PFOS and $\sum_4$PFAAs in DW displayed a significant positive association with concentrations in serum, in both the univariate (Fig. 1, Table 5) and multivariate weighted regression analyses (Table 5). The mean estimated SWR values ranged between 230–670 and 210 – 630 in the univariate and multivariate analyses, respectively, showing that the SWR values of the univariate models were not markedly different from those estimated by multivariate regression analyses (Table 5). The ordinary least squares regression analysis yielded similar results but with high parameter uncertainty (results not shown). The highest SWR was obtained for PFHxS, followed by $\sum_4$PFAA and PFOS (Fig. 1, Table 5), whereas the lowest was estimated for PFOA.

There are however important limitations to consider when interpreting the SWR. First and foremost, PFAA concentrations in DW were measured in the waterworks, instead of at the tap in the schools and homes of the participants. This was due to limited resources but also due to the fact that sampling at participants' residency was impossible as only participants postal codes and not addresses were at our disposal. Therefore, estimated mean PFAA concentrations in the DW represented the area of residence/school more than concentrations of each participanťs home and school. Secondly, water samples were collected 1–2 years after the blood-sampling period, which could influence the exposure assessment and consequently the SWR. For example, if the exposure from DW would be higher at the time of blood sampling than during the time of water sampling, the estimated exposure from DW would have been underestimated, resulting in an underestimation of the SWR, and vice versa. However, as the average PFAA half-lives in humans are > 1 year (Xu et al., 2020; Li et al., 2022b), the concentrations in serum is in fact a result of long-term PFAA exposure for several years, with water being one exposure source. We were however not able to evaluate the long-term exposure of PFAAs from DW, as DW/RW was sampled once in spring and once in autumn in 2018. Lastly, no information was available on how long participants had resided in the area in which the participating waterworks supplied DW. Albeit these uncertainties in DW PFAA exposure assessment, our results show that the measured PFOA, PFNA, PFHxS, PFOS and $\sum_4$PFAA concentrations in DW were representative enough as estimates of long-term PFAA exposure from DW to result in significant positive associations between serum and DW PFAA concentrations.

In addition to these uncertainties, it could be speculated that some waterworks had initiated actions to limit PFAA contamination of DW between the blood and water sampling periods. However, in 2014 the Swedish Food Agency issued an action limit of 90 ng/L of $\sum_{11}$PFAS for DW in Sweden, including PFOA, PFNA, PFHxS and PFOS (SLV, 2021). This action limit was in effect in 2018 when the water sampling in the waterworks occurred. None of the waterworks in the current study had, to the best of our knowledge, PFAS concentrations above the action limit when it was introduced in 2014. Telephone and e-mail contact with the waterworks during the planning of the study did not indicate that actions to limit PFAS concentration in the DW had been taken between the time period of blood sampling 2016–17 and water sampling in 2018. However, 37 participants were excluded from the study since they were going to school and/or living in areas with a known history of high PFAS contamination of municipal DW, and where remediation of contamination had occurred before blood and DW sampling (Nyström et al., 2022a).

Previous studies also suffer from some of the limitations presented above, making estimates of SWRs and comparisons of results between studies uncertain. With this in mind, SWRs have previously been determined for PFOA (Emmett et al., 2006; Hoffman et al., 2011; Zhang et al., 2019), and recently also for PFNA, PFHxS and PFOS (Zhu and Bartell, 2020; Xu et al., 2020; Johanson et al., 2023). Our SWR estimate for PFOA of about 200 (Table 5) is similar to those presented in other studies. Zhang and colleagues (2019) estimated a SWR of 231 by simple regression analysis of matched serum and DW PFOA concentrations for the general Chinese population consuming DW at a median concentration of 9.9 ng PFOA/L. Using adjusted robust regression, Hoffman et al. (2011) estimated the SWR of PFOA to be roughly 142 in the C8 cohort who had been exposed to PFOA in their DW at median concentrations of 200 ng/L. Furthermore, a SWR of 105 was reported for PFOA in residents only consuming DW (median PFOA 3550 ng/L) from the public

Table 5
Output from the univariate (uni) and multivariate (multi) weighted linear regression analyses of associations between PFAA concentrations in drinking water (DW, ng/L) and serum (ng/g) in participants from Riksmaten Adolescents (n = 790).

PFAA		Adj R^2	Serum background concentration[a] (SE)(ng/g)	SWR[b] Mean (SE)	p-value SWR	DWES[c] (ng/L)		Serum concentration at DWES[d]	
						68%	95%	68%	95%
PFOA	Uni	0.22	1.2 (0.011)	230 (15)	<0.0001	NA	NA	–	–
	Multi	0.45	1.0 (0.066)	210 (15)	<0.0001	NA	NA	–	–
PFNA	Uni	0.19	0.36 (0.004)	410 (30)	<0.0001	NA	NA	–	–
	Multi	0.52	0.13 (0.023)	360 (33)	<0.0001	NA	NA	–	–
PFHxS	Uni	0.78	0.28 (0.004)	670 (13)	<0.0001	0.9	1.7	0.9	1.4
	Multi	0.77	0.51 (0.052)	630 (16)	<0.0001	1.2	2.4	1.0	1.8
PFOS	Uni	0.30	1.8 (0.03)	630 (34)	<0.0001	2.0	NA	3.1	–
	Multi	0.61	0.36 (0.19)	620 (26)	<0.0001	1.9	NA	2.3	–
Σ_4PFAA	Uni	0.69	3.5 (0.026)	640 (15)	<0.0001	2.4	4.5	5.1	6.4
	Multi	0.63	1.8 (0.27)	600 (23)	<0.0001	2.3	4.2	4.0	5.1

Note: Adj, adjusted; SE, standard error; SWR, serum:water ratio; NA, not applicable since DWES could not be determined within the range of the measured PFAA concentrations in DW; -, not derived as DWES was not determined.
The multivariate analysis was adjusted for age, gender, participant/maternal birth country, habitual seafood consumption and maternal education level.
[a] The regression intercept is interpreted as the serum background concentration that represents exposure from non-DW sources only.
[b] The SWR represents the slope (coefficient) of the regression line of the association between PFAA concentrations in serum and DW.
[c] DWES is the estimated mean PFAA DW concentration (ng/L) that corresponds to the upper 68% and 95% prediction interval limit of PFAA concentrations in serum at the regression line intercept (background concentrations).
[d] The serum PFAA concentration (ng/g) corresponding to the upper 68% and 95% prediction interval limit of background PFAA concentrations at the DWES.

water systems of Little Hocking, U.S., located near a fluoropolymer manufacturing facility (Emmett et al., 2006). Zhu and Bartell (2020), reported a SWR of 114 for PFOA, but no information was given about the DW PFOA concentration range that this SWR was representative for and how the SWR was modelled. More recently reported SWRs for PFOA, PFNA, PFHxS and PFOS in Swedish adults, exposed to a range of $\sum_4$PFAA concentrations in DW between non-detectable to > 1000 ng/L (Johanson et al., 2023), were approximately ten-fold lower than those reported herein. Moreover, among 26 airport workers in northern Sweden, Xu et al. (2020) estimated median serum/DW concentrations ratios of 30, 107 and 153 for PFOA, PFHxS and lin-PFOS, respectively, which also were considerably lower than in RMA. The reported DW concentrations were 300 ng PFOA/L, 710 ng PFHxS/L and 62 ng lin-PFOS/L (Xu et al., 2020). Although the SWRs in the present study were derived using weighted linear regression analysis as in Johanson et al. (2023), our results are not comprehensive enough to explain the higher SWRs among adolescents than adults in Sweden. It is plausible that the differences in study design, to some degree, can explain the difference in association. For example, the study of Swedish adults had considerably wider PFAA concentration ranges in DW than what was observed in the present study (Johanson et al., 2023; Xu et al., 2020). The RMA adolescents is also a more homogenous group compared to the adults included in Johanson et al. (2023), which consisted of multiple study groups from around Sweden with a large variation in age (18 to ca 80 years of age). Adults, unlike adolescents, have also accumulated PFAA from a multitude of exposure sources for a longer period of time, which also leads to greater variability in PFAA body-burdens.

Intriguingly, the highest SWR outside of Sweden were reported by Zhang et al. (2019) for PFOA, where the population, similarly to the RMA participants, had been exposed to comparatively low PFOA concentrations in DW (9.9 ± 1.8 ng/L, mean and standard deviation). Contrastingly, lower SWRs for PFOA has been reported for the populations exposed from DW with considerably higher PFOA contamination, ranging between 1500 and 7200 ng/L (Emmett et al., 2006), <6 ng/L to 13300 ng/L (Hoffman et al., 2011) and < 0.3 ng/L to 210 ng/L (Johanson et al., 2023). Therefore, it may be speculated that DW PFAAs bioaccumulate to a higher degree in human sera at lower DW PFAA concentrations than at higher concentrations. This hypothesis has previously been proposed by Post et al. (2009), who compared the SWR of PFOA in Little Hocking at 105 (Emmett et al., 2006) with SWR at 185 for a population drinking less PFOA-contaminated DW in Village of Pomeroy, U.S. (average levels in DW of 65 ng/L).

A few studies have suggested that PFAA half-lives are shorter at high PFAA exposures from DW than at low exposures, hypothetically being explained by a multi-compartment mechanism (Seals et al. 2011; Li et al., 2022b). Longer half-lives at low DW PFAA exposures, and consequently a higher relative bioaccumulation of PFAA from DW, may thus hypothetically explain the higher SWR at low PFAA concentrations in DW than at high concentrations. One possible mechanisms is saturable bioaccumulation processes, leading to a lower bioaccumulation from DW with high PFAA concentrations. In support of this hypothesis, toxicokinetic studies of PFAS in monkeys and rats showed that renal reabsorption of PFOA and PFOS is restricted at higher exposure doses leading to a higher excretion (Andersen et al., 2006).

In the univariate regression analyses of PFHxS and PFOS, we derived the mean concentration of PFAA in DW that likely would cause substantially elevated serum PFAA concentrations above background variation when sampling other adolescents in Sweden not participating in RMA (Fig. 1, Table 5). $DWES_{68}$, based on the 68% upper prediction interval for background PFAA concentrations in serum, were estimated to be 0.9 ng PFHxS/L, 2.0 ng PFOS/L and 2.4 ng $\sum_4$PFAA/L. For both PFHxS and $\sum_4$PFAAs, the $DWES_{95}$ was ~ 1.9-fold higher than $DWES_{68}$ (Table 5). $DWES_{68\text{-}95}$ could not be determined for neither PFOA nor PFNA due to a too narrow range of concentrations in DW in relation to the regression fit. If other human biomonitoring studies on adolescent populations, with similar demographical and background PFAA exposure patterns as RMA, detect average PFHxS, PFOS and $\sum_4$PFAA concentrations at or above these elevated levels in sera, the possibility of PFAA contamination of the DW should be investigated. Taken together, the results show that significantly elevated PFHxS, PFOS and $\sum_4$PFAAs concentrations in serum above typical variation in background-exposed adolescents may be expected at DW concentrations far <10 ng/L. The estimated DWES of PFOA, PFHxS and PFOS were considerably higher among the Swedish adults ($DWES_{68}$ > 20 ng/L) (Johanson et al., 2023) than in RMA ($DWES_{68}$ < 5 ng/L). Taking into account uncertainties in comparing results from different studies, the higher $DWES_{68}$ among the adults may be due to higher background, and/or larger variability in, serum PFAA concentrations. In line with this hypothesis, children/adolescents (4–12 years old), living in an area with PFAA-contaminated DW, had significantly elevated concentrations of PFOA and PFOS in serum whereas their mothers had not (Gyllenhammar et al., 2015; Gyllenhammar et al., 2019).

Previously, dietary exposure was suggested as the major exposure route for PFOA and PFOS among adults in Sweden, while the predominant route for PFHxS appeared to be DW (Vestergren et al., 2012). Similar findings have been reported outside of Sweden, where dietary exposure was considered the primary exposure route for PFOS in the general U.S population, with DW only contributing with roughly 20% (Egeghy and Lorber, 2011). However, a theoretical intake calculation of PFAA in a study of Swedish schoolchildren suggested that the intake of PFHxS from DW is two times higher than the estimated average intake from food, assuming a DW concentration of PFHxS at 2 ng/L (Glynn et al., 2020). This is also reflected in the present study where the variation in PFHxS concentration in DW were estimated to explain 78% of the variation in the PFHxS concentration in serum (adjusted R^2) (Table 5); a figure which did not change after including covariates in the regression model (Table 5). The degree of explanation was considerably lower for PFOA and PFOS, with adjusted R^2 values of 22% and 30%, respectively, in the univariate analyses, and 45% and 61%, respectively, in the multivariate analyses (Table 5). These R^2 values indicate that other determinants than concentrations in DW explain a significant part of the variation in PFOA and PFOS concentrations in serum among the RMA participants. Although the relative contribution of DW PFAA exposure to the total PFAA exposure was not investigated in the present study, the R2 values make us speculate that exposure via DW contributed more to the total PFHxS than the total exposure of PFOA, PFNA and PFOS. This speculation needs to be further evaluated in future studies.

The interpretation of the SWR and DWES results for $\sum_4$PFAA (Table 5) is difficult given that RMA participants from many areas of Sweden most likely were exposed to different $\sum_4$PFAA compositions from DW. Nevertheless, the univariate SWR and $DWES_{68\text{-}95}$ of $\sum_4$PFAA, estimated at 640 and ~ 2–4 ng $\sum_4$PFAA/L DW, respectively (Table 5), provide further evidence that low-level PFAA contamination of DW with these EFSA priority-listed PFAA indeed contribute significantly to Swedish adolescent PFAA body burdens. The Swedish Food Agency has recently issued a national maximum limit for $\sum_4$PFAA in DW of 4 ng/L (SLV, 2022), while in Denmark it is set to 2 ng $\sum_4$PFAA/L (DK EPA, 2021). A large-scale screening of PFAA in Swedish DW reported that 14 municipal waterworks had $\sum_{11}$PFAS concentrations higher than 10 ng/L, and almost all of these reported waterworks had at least one DW sample with a $\sum_4$PFAA concentration just below or above 4 ng/L (SLV, 2021). These 14 waterworks distribute DW to over 2 million people in Sweden (SLV, 2021), showing that actions to remediate PFAA in DW, even at low-level contamination, is important to ensure that DW is safe for consumption. Furthermore, lowering of PFAA concentrations in DW to levels below the maximum limits will in the long-run contribute to lower total cumulative PFAA exposures in a large fraction of the population served by the 14 waterworks.

3.3.2. *Influence of reported drinking water consumption in RMA on SWR*

Controlling for long-term DW consumption (Table 1) in the multiple regression analyses did not notably change the SWR for any of the PFAA

analysed (Table 5, Table S4 in SI). Albeit being significant for most of the PFAA, the associations between long-term DW consumption and serum PFAA concentrations were weak (Table S4 in SI). The observed weak negative relationships for PFOA and PFHxS could be due to residual confounding. An important uncertainty with the estimated long-term DW consumption data is that water consumption was self-registered retrospectively in RiksmatenFlexDiet (Lindroos et al., 2019). Furthermore, long-term DW consumption was only comprised of direct consumption of tap water and consumption of beverages made from tap water, e.g. tea, coffee and fruit/berry syrups/concentrate diluted with tap water. Consequently, information about consumption of tap water in cooked food, e.g. sauces, soups and stews, was lacking. An underestimation of long-term DW consumption was indicated by the relatively low average of around 0.6 L/day in RMA (Table 1), a consumption that is considerably lower than the average self-reported direct consumption of cold tap water among Swedish adults of ~ 1 L per day, and ~ 1.9 L per day for cold and heated tap water (Säve-Söderbergh et al., 2018).

To the best of our knowledge, only a few previous studies have investigated the influence of DW consumption on the relationships between PFAA concentrations in DW and serum/plasma, with varied results. PFNA, PFHxS and PFOS concentrations in plasma increased with 13–18% among German children (aged 8–10 years) with a self-reported long-term consumption of > 0.7 L DW per day compared to those consuming < 0.7 L per day (Wilhelm et al., 2015). The concentrations in DW were however slightly higher in the German study compared to RMA, with average levels ranging between 1.3 and 43 ng PFOS/L, <LOQ – 37 ng PFOA/L and ~ 3 ng PFHxS/L (Wilhelm et al., 2015). In the previous study of Swedish adults, concentrations of PFBS and PFHxS in serum were significantly higher among participants reporting consuming on average > 1.4 L/day (7 glasses per day) during the year before blood sampling than among those consuming < 1.4 L/day (Johanson et al., 2023). As in RMA, long-term, self-reported, DW consumption among adults and adolescents in the highly contaminated Veneto Region in Italy contributed only marginally to the variation in PFOA, PFHxS and PFOS concentrations in serum (Pitter et al., 2020).

3.3.3. *Influence of gender on SWR*

The interaction between gender and PFAA concentrations in DW was statistically significant for all PFAAs ($p < 0.002$ for all PFAA and $\sum_4$PFAAs, Table S5 in SI). Serum PFAA concentrations increased more steeply per unit increase in PFAA concentrations in DW for males than females (Fig. 2, Table S5 in SI). As also observed in RMA (Nyström et al., 2022a), males tend to have higher PFAA body-burdens than females (Jain, 2018; Kang et al., 2018; Pitter et al., 2020). Males did on average report a slightly less DW consumption compared to females, i.e. 0.56 L/day (range 0.04 – 2.6 L/day) compared to 0.61 L/day (range 0.05 – 2.8 L/day), respectively. These self-reported DW consumption data are

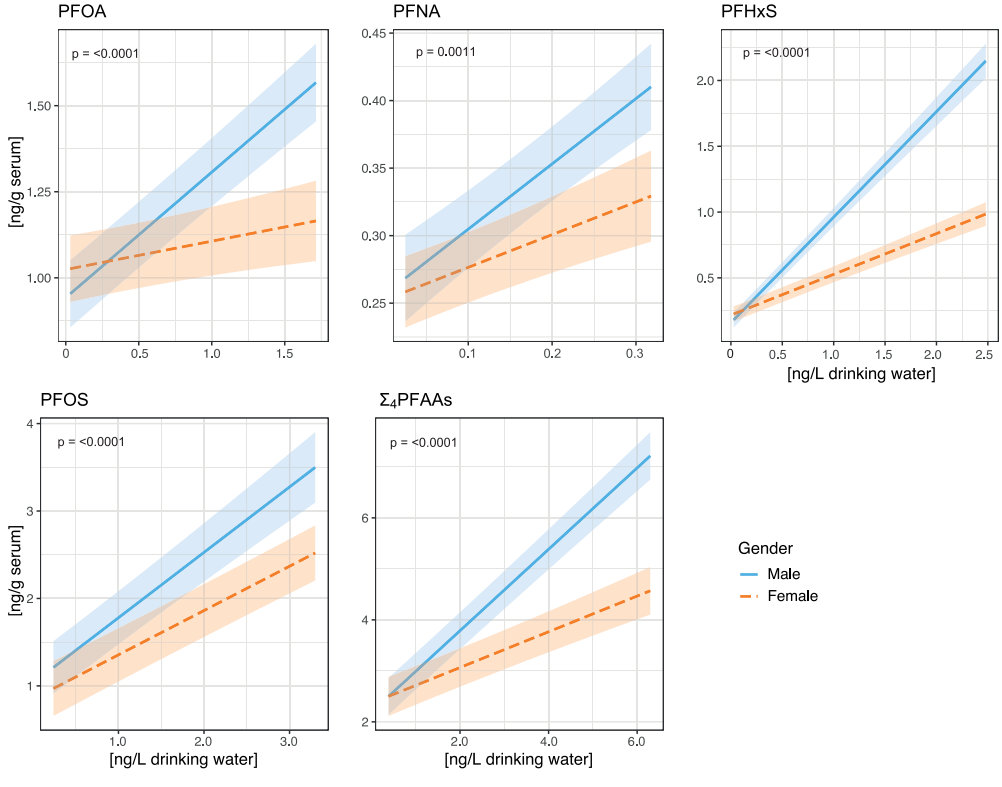

Fig. 2. Gender differences (males n = 350, females n = 440) in the relationship between PFAA in drinking water and serum for the RMA participants (n = 790). Dashed and filled lines show the regression lines for respective gender, shaded areas display the 95% confidence intervals. Associations were adjusted for age, maternal/participant birth country, maternal education level and habitual seafood consumption using weighed linear regression.

uncertain and most likely underestimates, nevertheless they suggest that gender differences in DW consumption were too small to explain the gender differences in the associations between PFAA in DW and serum. This conclusion is further supported by our results showing that the estimated long-term DW consumption of the participants did not appear to substantially influence the PFAA serum concentrations (Table S5 in SI). We hypothesize that the gender difference in the associations of PFAA concentrations in DW and serum may be due to sex differences in the toxicokinetics of PFAAs. Menstruation bleeding has been suggested as a significant excretion route among females (Ding et al., 2020; Park et al., 2019); results that were also to some extent supported in our previous RMA study (Nyström et al., 2022a). However, previous toxicokinetic modelling attempts have suggested that elimination through menstruation cannot entirely explain the gender differences in PFAA concentrations in serum (Wong et al., 2014; Wu et al., 2015; Lorber et al., 2015). Interestingly, a study of PFAA concentrations in urine of adults from China reported that the renal clearance of PFOA, PFNA, PFHxS and PFOS was higher in young females than in men and older females (Zhang et al., 2013). In a study of a Swedish population with high PFAA exposure from drinking water, it was suggested that an estrogenic induction of the renal clearance could contribute to faster elimination (shorter half-lives) of PFOA, PFHxS and PFOS from serum in females than males in the age group 15–50 years (Li et al., 2022b). These sex differences were not observed in younger and older age groups (Li et al., 2022b). More research is needed on possible gender-related differences of PFAA toxicokinetics, including the impact of menstruation and hormonal changes, especially at the onset of puberty.

3.4. Risk assessment of PFAA exposure from drinking water

In the present study, 16% of the participants (n = 790) had $\sum_4$PFAA concentrations in serum > 6.9 ng $\sum_4$PFAA/mL serum, which is the highest estimated serum concentration not connected to health concerns by EFSA (EFSA, 2020). Roughly 19% of the participants were exposed to DW with estimated mean $\sum_4$PFAA concentrations ≥ 4 ng $\sum_4$PFAA/L (Swedish maximum limit), while around 25% were exposed to levels exceeding ≥ 2 ng $\sum_4$PFAA/L (Danish maximum limit). Although only two grab samples (spring and fall) were taken from each participating waterworks, our results suggests that some of the participating waterworks had $\sum_4$PFAA concentrations that were not complying with the Swedish maximum limit that will be enforced in 2026 (Table 2) (SLV, 2022). As a result of the positive relation between serum and DW PFAAs concentrations, 34% of the participants exposed to mean DW concentrations of ≥ 4 ng $\sum_4$PFAA/L had serum levels exceeding 6.9 ng $\sum_4$PFAA/mL serum, while 11% exceeded this level at < 4 ng $\sum_4$PFAA/L in DW. This difference was statistically significant (χ^2-test of independence; $\chi^2 = 48.0$ at one degree of freedom, p-value < 0.0001). Using the slightly more restrictive Danish maximum limit (2 ng/L) (DK EPA, 2021), the corresponding percentages were 30 and 11%, respectively ($\chi^2 = 48.0$ at one degree of freedom, p-value < 0.0001). Taken together, the results show that a higher proportion of the RMA adolescents had $\sum_4$PFAA concentrations in serum above 6.9 ng/mL if the concentrations of $\sum_4$PFAA in DW exceeded the Swedish and Danish maximum limits than when not.

Some RMA participants exposed to very low DW PFAA concentrations (Table 4) still exceeded the 6.9 ng $\sum_4$PFAA/mL serum level (Fig. 1). In these cases, exposure sources other than the local DW most likely contributed to a large fraction of the long-term PFAA exposure. Even at DW PFAA concentrations above the DW PFAA maximum limits exposures from other sources than DW most likely contributed significantly to the exceedances of the 6.9 ng $\sum_4$PFAA/mL serum level. Consequently, efforts are needed to further decrease PFAA exposure, not only from DW but also from other significant sources to ensure that both present and future generations are not exposed to PFAAs at levels of health concern.

4. Conclusion

Drinking water is an indispensable component of the healthy human diet. The present study shows that low-level contaminated DW of the EFSA priority listed PFAAs of health concern, i.e. PFOA, PFNA, PFHxS and PFOS, is a significant exposure source for Swedish adolescents. Furthermore, our results indicate that bioaccumulation of PFAAs from DW in serum appears to be higher at low PFAA contamination levels in DW than at high contamination levels, and that bioaccumulation is higher among males than females. These results need to be further validated and investigated in future studies. Even though other exposure sources contribute to the total adolescent body burden of PFAAs, we show that significantly elevated mean serum PFAA concentrations above background among populations of Swedish adolescents most likely can be attributable to DW exposure at levels ~ 4 ng $\sum_4$PFAAs/L and higher. RMA adolescents having DW with concentrations above the maximum limits implemented in Sweden and Denmark (4 and 2 ng $\sum_4$PFAAs/L) were more likely to have serum PFAA levels exceeding the safe serum concentration estimated by EFSA than participants with DW concentrations below the DW maximum limits. Therefore, it is of considerable importance to reduce $\sum_4$PFAA concentrations in DW to levels below the maximum limits to ensure that DW does not pose a health concern to humans in the future.

CRediT authorship contribution statement

Jennifer Nyström-Kandola: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Lutz Ahrens:** Investigation, Resources, Data curation, Writing – review & editing. **Anders Glynn:** Funding acquisition, Methodology, Conceptualization, Supervision, Writing – review & editing. **Gunnar Johanson:** Conceptualization, Methodology, Writing – review & editing. **Jonathan P. Benskin:** Investigation, Resources, Data curation, Writing – review & editing. **Irina Gyllenhammar:** Conceptualization, Resources, Data curation, Writing – review & editing. **Sanna Lignell:** Conceptualization, Resources, Data curation, Writing – review & editing. **Carolina Vogs:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

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Supplementary Information

Low concentrations of perfluoroalkyl acids (PFAAs) in municipal drinking water associated with serum PFAA concentrations in Swedish adolescents

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Table S1. PFAS concentrations (ng/L) in drinking water and raw water samples from waterworks (n=45)[a] sampled in spring and autumn 2018……..…….**2**

Table S2. Paired two-tailed t-tests comparing PFAA concentrations in drinking water and in raw water samples (year 2018) from the waterworks included in Riksmaten Adolescent 2016-17[a]. Spring and autumn samples were compared separately……...……..……**3**

Table S3. Examples of how the mean PFAA concentrations in drinking water were calculated. Note that all PFAA concentrations presented in this table are hypothetical…….……..…..**4**

Table S4. Multivariate weighted linear regression analysis investigating the relationship between serum PFAAs concentrations (ng/g serum) and habitual long-term drinking water consumption (mL/day) in Riksmaten Adolescent 2016-17 (n=790)[a]. The serum:water ratio (SWR) is also presented for comparison with results from other models not including long-term drinking water consumption covariate…...……..……...……...**5**

Table S5. Multivariate weighted linear regression analysis investigating the interaction between PFAA drinking water concentrations and gender in Riksmaten Adolescents 2016-17 (n_{males} = 350, $n_{females}$ = 440)……....**6**

***Table S1**. PFAS concentrations (ng/L) in drinking water and raw water samples from waterworks (n=45)[a] sampled in spring and autumn 2018*

PFAS		Spring		Autumn	
		Range of LOQ *(n ≥LOQ)*	Median[b] (min, max)	Range of LOQ *(n ≥LOQ)*	Median[b] (min, max)
PFBA	DW	2.5 – 9.7 *(6)*	8.7 (3.5, 22)	0.76 – 3.7 *(1)*	1.6
Perfluorobutanoic acid	RW	1.1 – 9.2 *(1)*	66	1.1 – 14 *(0)*	
PFPeA	DW	3.7 – 55 *(1)*	18	0.59 – 2.9 *(1)*	0.78
Perfluoropentanoic acid	RW	2.9 – 52 *(1)*	34	0.54 -5.3 *(2)*	0.97 (0.96, 0.97)
PFHxA	DW	1.0 – 16 *(0)*		0.074 – 1.8 *(4)*	1.1 (0.4, 1.3)
Perfluorohexanoic acid	RW	2.9 – 40 *(1)*	30	0.068 – 3.1 *(0)*	
PFHpA	DW	0.19 – 2.1 *(3)*	1.2 (0.091, 2.1)	0.068 – 0.63 *(7)*	0.56 (0.071, 1.6)
Perfluoroheptanoic acid	RW	0.13 – 2.9 *(1)*	8.2	0.059 -0.64 *(6)*	0.89 (0.38, 1.1)
PFOA	DW	0.086 – 0.42 *(29)*	0.56 (0.13, 1.9)	0.070 – 0.67 *(10)*	0.74 (0.47, 2.5)
Perfluorooctanoic acid	RW	0.062 – 0.36 *(30)*	0.88 (0.22, 5.1)	0.062 – 0.69 *(17)*	0.77 (0.26, 1.4)
PFNA	DW	0.031 – 0.22 *(20)*	0.23 (0.11, 0.37)	0.066 – 0.19 *(6)*	0.26 (0.21, 0.32)
Perfluorononanoic acid	RW	0.047 -0.14 *(20)*	0.27 (0.16, 0.42)	0.063 – 0.25 *(16)*	0.38 (0.17, 0.48)
PFDA	DW	0.039 – 0.13 *(0)*		0.077 – 0.18 *(0)*	
Perfluorodecanoic acid	RW	0.055 – 0.16 *(1)*	0.27	0.078 – 0.21 *(0)*	
PFUnDA	DW	0.031 – 0.17 *(0)*		0.083 – 0.28 *(0)*	
Perfluoroundecanoic acid	RW	0.069 – 0.34 *(0)*		0.086 – 0.25 *(0)*	
PFDoDA	DW	0.025 – 0.63 *(0)*		0.084 – 8.4 *(0)*	
Perfluorododecanoic acid	RW	0.050 – 0.50 *(0)*		0.080– 5.8 *(0)*	
PFTrDA	DW	0.064 – 0.77 *(0)*		0.096 – 66 *(1)*	0.56
Perfluorotridecanoic acid	RW	0.050 – 0.57 *(0)*		0.092 – 50 *(0)*	
PFTeDA	DW	0.064 – 1.90 *(0)*		0.33 – 13 *(0)*	
Perfluorotetredecanoic acid	RW	0.13 – 6.6 *(0)*		0.091 – 8.3 *(0)*	
PFHxDA	DW	0.10 – 4.5 *(1)*	0.073	0.35 – 27 *(0)*	
Perfluorohexadecanoic acid	RW	0.064 – 3.3 *(0)*		0.23 – 17 *(0)*	
PFOcDA	DW	0.077 – 110 *(1)*	0.06	0.13 – 110 *(1)*	0.18
Perfluorooctadecanoic acid	RW	0.22 – 320 *(0)*		0.15 – 71 *(2)*	0.16 (0.11, 0.20)
PFBS	DW	0.17 – 4.3 *(8)*	1.2 (0.25, 2.9)	0.075 – 2.4 *(11)*	0.48 (0.094, 2.8)
Perfluorobutane sulfonic acid	RW	0.094 – 4.7 *(5)*	0.57 (0.34, 2.7)	0.068 – 4.4 *(11)*	1.5 (0.16, 2.8)
PFHxS	DW	0.041 – 0.20 *(17)*	0.46 (0.19, 7.9)	0.057 – 7.5 *(11)*	0.36 (0.14, 5.6)
Perfluorohexane sulfonic acid	RW	0.099 – 0.52 *(22)*	0.53 (0.14, 6.1)	0.053 – 6.3 *(17)*	0.52 (0.075, 1.5)
PFOS	DW	0.31 – 1.9 *(6)*	2.55 (1.1, 4.2)	0.67 – 2.8 *(22)*[c]	0.60 (0.22, 3.0)[c]
Perfluorooctane sulfonic acid	RW	0.04 – 4.2 *(4)*	2.95 (1.6, 5.6)	0.59 – 3.0 *(6)*	1.1 (0.71, 2.1)
PFDS	DW	0.21 – 1.6 *(0)*		0.40 – 2.0 *(0)*	
Perfluorodecane sulfonic acid	RW	0.17 – 2.4 *(0)*		0.46 – 1.7 *(0)*	
6:2 FTSA	DW	0.026 – 0.11 *(5)*	2.6 (0.097, 17)	0.021 – 0.13 *(25)*	0.13 (0.029, 1.4)
6:2 fluorotelomer sulfonate	RW	0.05 – 0.77 *(11)*	0.48 (0.43, 14)	0.03 – 0.22 *(25)*	0.28 (0.079, 2.5)
8:2 FTSA	DW	0.13 – 1.00 *(0)*		0.27 – 0.80 *(0)*	
8:2 fluorotelomer sulfonate	RW	0.17 – 1.10 *(1)*	0.084	0.24 – 0.98 *(0)*	
10:2 FTSA	DW	0.052 – 0.24 *(0)*		0.17 – 1.0 *(0)*	
10:2 fluorotelomer sulfonate	RW	0.076 – 0.57 *(0)*		0.19 – 1.3 *(0)*	
FOSA	DW	0.18 – 1.2 *(28)*	0.89 (0.2, 4.1)	0.16 – 3.7 *(18)*	0.64 (0.18, 3.7)
Perfluorooctane sulfonamide	RW	0.14 – 5.9 *(10)*	0.35 (0.29, 1.3)	0.16 – 3.5 *(6)*	0.41 (0.23, 0.74)
FOSAA	DW	*(0)*		1.5 – 3.5 *(0)*	
Perfluorooctane sulfonamideoacetic acid	RW	*(0)*		1.4 – 2.6 *(0)*	
Me-FOSAA	DW	0.027 – 0.20 *(0)*		0.10 – 2.9 *(0)*	
N-Methylperfluoro-1-octanesulfonamidoacetic acid	RW	0.056 – 0.21 *(0)*		0.10 – 0.39 *(0)*	
Et-FOSAA	DW	0.02 – 0.19 *(0)*		0.072 – 0.23 *(0)*	
N-Ethylperfluoro-1-octanesulfonamidoacetic acid	RW	0.041 – 0.21 *(0)*		0.068 – 0.34 *(0)*	
Σ_{11}PFAS	DW		1.1 (0.13, 38)		0.91 (0.046, 12)
	RW		1.4 (0.14, 150)		1.5 (0.15, 7.6)
ΣPFAS	DW		2.0 (0.32, 38)		1.7 (0.046, 12)
	RW		1.3 (0.14, 150)		1.5 (0.11, 7.6)

Note: LOQ, limit of quantification; min, minimum; max, maximum. Σ_{11}PFAS include PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS and 6:2 FTSA (SLV 2022a).

ΣPFAS is the sum of all measured PFAS

[a] Due to analytical issues or missed sampling, n=42 and 39 in DW samples in spring and autumn respectively, and n=41 and 42 in spring and autumn RW samples respectively.

[b] The median and min/max was only calculated for samples >LOQ/limit of detection (LOD).

[c] Values <LOQ and >LOD are included.

Table S2. *Paired two-tailed t-tests comparing PFAA concentrations in drinking water and in raw water samples (year 2018) from the waterworks included in Riksmaten Adolescent 2016-17[a]. Spring and autumn samples were compared separately.*

		Spring			Autumn		
		Number of included pairs	Mean (SEM) (ng/mL9	p-value	Number of included pairs	Mean (SEM) (ng/mL)	p-value
PFOA	Drinking water	35	0.59 (0.07)	*0.013*	18	0.54 (0.14)	0.471
	Raw water		0.94 (0.15)			0.67 (0.08)	
PFNA	Drinking water	24	0.20 (0.02)	0.230	18	0.12 (0.03)	*0.001*
	Raw water		0.23 (0.02)			0.31 (0.03)	
PFHxS	Drinking water	25	0.74 (0.23)	0.999	15	0.30 (0.08)	*0.002*
	Raw water		0.75 (0.30)			0.66 (0.09)	
PFOS	Drinking water	5	2.18 (0.64)	0.485	8	1.28 (0.28)	0.481
	Raw water		2.89 (0.72)			1.04 (0.18)	

Note: SEM, standard error of the mean.

[a] The paired t-test was only carried out for those waterworks that had at least one sample with PFAAs above the limit of quantification (LOQ, detectable). If the sample had an LOQ that was lower than the sample with a detectable concentration, the concentration was set to LOQ/2 in order to form a sample pair. If the LOQ in one sample of the pair was larger than the detected concentration in the other sample, the DW/raw water sample pair was excluded from the analysis.

Table S3. *Examples of how the mean PFAA concentrations (ng/L) in drinking water were calculated. Note that all PFAAs concentrations presented in this table are hypothetical.*

	PFAS concentration [ng/L]			
Combination in waterworks	**DW spring**	**DW autumn**	**RW spring**	**RW autumn**
Ex 1.	1.2	0.3	2.4	0.2
If a waterworks had detectable concentrations in all four samples, all four samples were used to calculate PFAA concentration in DW as follows: $$DW_C = \frac{(1.2 + 0.3 + 2.4 + 0.2)}{4}$$				
Ex 2.	2.3	<LOQ (1.0)[a]	1.2	<LOQ (3.1)
If a waterworks had at least one sample with a concentration <LOQ, <LOQ was set to 1/2LOQ. In the calculation of PFAA concentration in DW, samples were excluded if the LOQ of these were higher than the sample with the lowest detectable concentration as follows: $$DW_C = \frac{\left(2.3 + \left(\frac{1.0}{2}\right) + 1.2\right)}{3}$$				
Ex 3.	<LOQ (1.2)	<LOQ (5.1)	<LOQ (0.21)	<LOQ (0.18)
If a waterworks only had undetectable concentrations, the lowest LOQ divided by two was used in the estimation of the PFAA concentration in DW as follows: $$DW_C = \frac{0.18}{2}$$				

[a] The value inside the bracket details LOQ of the sample.

Table S4. *Multivariate weighted linear regression analysis investigating the relationship between serum PFAA concentrations (ng/g serum) and long-term drinking water consumption (mL/day) in Riksmaten Adolescent 2016-17 (n=790)[a]. The serum:water ratio (SWR) is also presented for comparison with results from other models not including the long-term drinking water consumption covariate.*

PFAA	Adj R^2	Serum:water ratio (SE)	Long-term DW coefficient[b] (SE)	p-value
PFOA	0.78	220 (15)	-0.00006 (0.00002)	0.00023
PFNA	0.75	360 (31)	0.00002 (0.000007)	0.042
PFHxS	0.73	620 (16)	-0.00004 (0.00002)	0.016
PFOS	0.72	600 (29)	0.0002 (0.00004)	<0.0001
Σ_4PFAAs	0.65	600 (22)	0.00004 (0.0001)	0.70

Note: Adj, adjusted; SE, standard error.

[a]Adjusted for with the following covariates: PFAA concentration in drinking water, age, gender, participant/maternal birth country, habitual seafood consumption and maternal education level. Note that weights, as defined in the weighted linear regression, was applied.

[b]The long-term DW consumption coefficient estimates how much the DW consumption affects the serum concentrations.

***Table S5**. Multivariate weighted linear regression analysis investigating the interaction between PFAA drinking water concentrations and gender in Riksmaten Adolescents 2016-17 (n_{males} = 350, $n_{females}$ = 440)[a].*

PFAA	Adjusted R^2	Serum:water ratio (SE)		Interaction
		Males	Females	p-value
PFOA	0.44	360 (24)	80 (27)	<0.0001
PFNA	0.55	480 (58)	240 (45)	0.0011
PFHxS	0.67	800 (27)	310 (17)	<0.0001
PFOS	0.77	750 (48)	510 (30)	<0.0001
Σ_4PFAAs	0.63	800 (30)	350 (30)	<0.0001

Note: SE, standard error.

[a]Adjusted for participant/maternal birth country, maternal education level, age and habitual seafood consumption. Note that weights, as defined in the weighted linear regression, was applied.

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Adolescent exposure sources and demographical exposure patterns of per- and polyfluoroalkyl substances (PFAS) are not well studied. In a population-based Swedish adolescent study population, low-grade contaminated drinking water, a healthy and diverse diet rich in seafood, and birth country income level were important determinants of PFAS concentrations in serum. These findings are important to consider in future studies of PFAS effects on adolescent health and in risk assessments.

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