



Levels of per- and polyfluoroalkyl substances (PFAS) in Norwegian children stratified by age and sex - Data from the Bergen Growth Study 2

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ABSTRACT

Background and aim: Due to the persistence, bioaccumulation and potential adverse health effects, there have been restrictions and phase out in the production of certain per- and polyfluoroalkyl substances (PFAS) since the early 2000s. Published serum levels of PFAS during childhood are variable and may reflect the impact of age, sex, sampling year and exposure history. Surveying the concentrations of PFAS in children is vital to provide information regarding exposure during this critical time of development. The aim of the current study was therefore to evaluate serum concentrations of PFAS in Norwegian schoolchildren according to age and sex.

Material and methods: Serum samples from 1094 children (645 girls and 449 boys) aged 6–16 years, attending schools in Bergen, Norway, were analyzed for 19 PFAS. The samples were collected in 2016 as part of the Bergen Growth Study 2. Statistical analyses included Student t-test, one-way ANOVA and Spearman's correlation analysis of log-transformed data.

Results: Of the 19 PFAS examined, 11 were detected in the serum samples. Perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanesulfonic acid (PFHxS) and perfluorononaic acid (PFNA) were present in all samples with geometric means of 2.67, 1.35, 0.47 and 0.68 ng/mL, respectively. In total, 203 children (19%) had PFAS levels above the safety limits set by the German Human Biomonitoring Commission. Significantly higher serum concentrations were found in boys compared to girls for PFOS, PFNA, PFHxS and perfluoroheptanesulfonic acid (PFHpS). Furthermore, serum concentrations of PFOS, PFOA, PFHxS and PFHpS were significantly higher in children under the age of 12 years than in older children.

Conclusions: PFAS exposure was widespread in the sample population of Norwegian children analyzed in this study. Approximately one out of five children had PFAS levels above safety limits, indicating a potential risk of negative health effects. The majority of the analyzed PFAS showed higher levels in boys than in girls and decreased serum concentrations with age, which may be explained by changes related to growth and maturation.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of

synthetic chemicals consisting of several thousands of different substances. Since the 1940s, PFAS have been applied in a variety of industries worldwide due to their useful properties of being chemically

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and thermally stable, as well as being repellent to water, oil, and dirt. For these reasons, PFAS are commonly used in numerous products, including impregnation of paper, textile, and leather, and in firefighting foams, cosmetics, and non-stick kitchenware (Glüge et al., 2020). PFAS have high resistance to degradation, leading to accumulation in the environment. Furthermore, several of the compounds accumulate in the food chain and in humans (Conder et al., 2008; Russell et al., 2013). The fluorinated carbon length is related to the bioaccumulation of PFAS, although other factors may also affect the bioaccumulation potential. In general, perfluoroalkyl carboxylic acids with seven or more fluorinated carbons, e.g. perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), and perfluoroalkane sulfonates with six or more fluorinated carbons, e.g. perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS), are considered bioaccumulative (Conder et al., 2008; Ng and Hungerbühler 2014).

Exposure of PFAS in the general population occurs primarily through ingestion of contaminated food and drinking water, but exposure through inhalation or ingestion of contaminated dust and dermal exposure can also make substantial contributions (EFSA, 2020; Fromme et al., 2009; Haug et al., 2011; Poothong et al., 2020; Trudel et al., 2008). In addition, there is a transplacental transfer of PFAS from the mother to the fetus during pregnancy, and infants are exposed through breastfeeding (Appel et al., 2022; Blomberg et al., 2023; Fromme et al., 2010). PFAS exposure has been confirmed in blood samples from children and adults in numerous countries (EFSA, 2020; Winkens et al., 2017).

Several studies have shown an association between exposure to PFAS and adverse health effects in humans, including reduced vaccine response in children, reduced birth weight, dyslipidemia, and certain types of cancers (Panieri et al., 2022; Rappazzo et al., 2017). In 2020, the European Food Safety Authority (EFSA) set a tolerable weekly intake (TWI) of 4.4 ng/kg body weight per week for the sum of the four PFAS PFOA, PFNA, PFHxS and PFOS (EFSA, 2020). Reduction in vaccination response in children was the basis for setting a cutoff for TWI, as this was the most sensitive health outcome in human cohorts, and the association was considered causal due to similar effects in experimental animal studies. The current dietary exposure to these PFAS exceeds the TWI for large parts of the European population, including Norwegian citizens (EFSA, 2020; VKM Report 2022). Chronic exposure at the TWI corresponds to serum concentrations of 6.9 ng/mL for the sum of the four above-mentioned PFAS in adults (EFSA, 2020). The German Human Biomonitoring Commission has derived health related guidance values for the evaluation of internal exposures to PFOA and PFOS separately. The Human Biomonitoring (HBM)-I values in blood plasma are defined as 2 ng PFOA/mL and 5 ng PFOS/mL in all age groups. These values represent the concentrations below which, according to current knowledge, no adverse health effects are expected to occur (The German Environment Agency, 2020).

The European Commission has recently established maximum levels for certain PFAS in drinking water and food (The European Commission, 2020; 2022). PFOA, PFOS and their precursors are encompassed by the Stockholm Convention on Persistent Organic Pollutants, and further restrictions on PFAS are under development in Europe (European Chemicals Agency, 2023; Stockholm Convention on persistent organic pollutants, 2019). However, despite future restrictions or bans on more PFAS and their precursors, they will persist in the environment and may affect humans for generations to come.

Norwegian data showed a steady increase in the concentration of PFAS in serum samples from the general population of adults between 1977 to the mid-1990s (Haug et al., 2009a). Further, there was a clear decline in the PFOA, perfluoroheptanesulfonic acid (PFHpS) and PFOS concentrations from around the year 2000, which is consistent with the phase-out of these compounds. A clear increase was also observed for PFHxS, PFNA, perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFUnDA) until the early 1990s. Pooled serum samples from children aged 5–14 years, collected in 1976, 1987, 1998 and 2007, were

also included in the study. The PFAS concentrations in these children showed similar trends as for the adults (Haug et al., 2009a). The Norwegian findings for PFOA and PFOS are in line with the reported trends in North America and Europe (Land et al., 2018). In contrast, biomonitoring data from China, where the production has continued, indicate a further increase in serum concentrations of PFOA and PFOS after the year 2000. Concentrations of longer-chained perfluoroalkyl carboxylic acids (PFCAs), including PFNA, PFDA and PFUnDA, are generally increasing in all parts of the world (Land et al., 2018). Published serum levels of PFAS during childhood vary strongly and may reflect the impacts of body weight changes and energy needs, sex, sampling year, and history of exposure due to changes in the production of PFAS (Haug et al., 2018; Nøst et al., 2014; Winkens et al., 2017).

The Bergen Growth Study 2 (BGS2) is a puberty reference study conducted in Norway in 2016. As part of this cross-sectional study, blood samples were collected from 1094 children aged 6–16 years to study hormonal changes in puberty, and exposure to endocrine disruptive chemicals, including PFAS (Júlfússon et al., 2023). Previous data on PFAS exposure in Norwegian schoolchildren were limited to either pooled samples or narrow age groups (Averina et al., 2018; Haug et al., 2009a; Richterová et al., 2023). The aim of the current study was therefore to map PFAS exposure in a critical developmental window by determining serum concentrations in Norwegian children according to a wide age range of 6–16 years, and sex.

2. Materials and methods

2.1. Study design and sampling

Serum samples from 1094 children (645 girls and 449 boys) aged 6–16 years were collected in January–June 2016 as a part of the Bergen Growth Study 2 (BGS2). The BGS2 is a cross-sectional study of growth and pubertal development in Norwegian children. The children were recruited from six randomly selected public schools in Bergen – the second largest city in Norway. All children in the selected schools were invited to participate in the study, and all children who agreed to participate were included. The overall participation rate was 43% and ranged from 29% to 53% across schools. Numbers of participants by age and sex are presented in Supplementary Table 1. The selected schools in BGS2 covered varied socioeconomic backgrounds, but children of parents with an academic degree were overrepresented (80.4%) compared to the general population of Bergen (40.8%, Statistics Norway, 2023). The proportion of children with parents of Norwegian, European, and non-European origin was 80.7%, 7.6% and 11.7%, respectively. Based on the criteria for weight status defined by the International Obesity Task Force (Cole et al., 2000), 7.4% of the participants were children with underweight, 11.2% with overweight and 2.1% with obesity (Júlfússon et al., 2023). An experienced biomedical laboratory scientist collected blood samples for hormonal, genetic and endocrine disruptive chemicals analysis, including PFAS analysis, by venipuncture from an antecubital vein. Further details of the study design and measurement protocol of the BGS2 have recently been described (Júlfússon et al., 2023).

2.2. Analysis of PFAS

Nineteen PFAS in total, including perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, PFNA, PFDA, PFUnDA, perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA), perfluorobutanesulfonic acid (PFBS), PFHxS, PFHpS, PFOS, perfluorodecanesulfonic acid (PFDS), perfluorooctanesulfonamide (PFOSA), N-methylperfluorooctanesulfonamide (MeFOSA) and N-ethylperfluorooctanesulfonamide (EtFOSA), were analyzed at the Norwegian Institute of Public Health using high-performance tandem mass spectrometry, by a previously described

method (Haug et al., 2009b). In brief, the method involves transferring 150 μ L serum to a centrifugation tube, adding internal standards and methanol to make up a total volume of 150 μ L methanol, before mixing using a whirl mixer. The samples are then centrifuged, and the supernatant is transferred to a glass autosampler vial added 500 μ L 0.1 M formic acid and mixed on a whirl mixer. The samples are analyzed by injection of 80 μ L extract on a column switching liquid chromatography (LC) system coupled to a triple quadrupole mass spectrometer (MS). For quantification of PFOS, the total area of the linear and branched isomers is integrated. The limit of quantification (LOQ) was 0.1 ng/mL for PFBA, 0.2 ng/mL for PFTeDA and PFDS, and 0.05 ng/mL for the remaining 16 PFAS.

The laboratory participates regularly in interlaboratory comparisons, was through an extensive process (Nübler et al., 2022), and was approved for measuring a range of PFAS in the HBM4EU project (HBM4EU, 2022). Procedure blanks and in-house controls, as well as two samples from the interlaboratory comparisons in the EU project HBM4EU, were analyzed along with the samples. The procedure blanks ($n = 66$) did not contain PFAS above the limit of quantification, except for PFOA and PFNA in a few samples in concentrations close to LOQ. The relative standard deviations (RSDs) for the in-house controls ($n = 42$) varied between 10 and 15%. For the interlaboratory comparison samples ($n = 11$ per sample), the mean % differences from the assigned values, except PFPeA, were in the range of -14 to $+6\%$, demonstrating high quality of the analyses. The results of PFPeA were deemed unsatisfactory due to a high deviation from the assigned values.

2.3. Statistical analysis

The distribution of all PFAS in serum failed the Shapiro-Wilk test of normality due to positive skewness. The natural logarithm of the values was used in further analysis. Concentrations below the LOQ were set to LOQ divided by the square root of two for analysis (Duffek et al., 2020; Papadopoulou et al., 2016). Statistical analyses were performed using IBM SPSS Statistics version 26. The geometric mean concentration (GM) and 95% confidence interval (CI) were calculated by back transforming the geometric mean and the CI of the log-transformed data to the original scale of measurement. The children were grouped according to age as 6–7 years, 8–9 years, 10–11 years, 12–13 years, and 14–16 years. Statistical significance of the differences in geometric means between age groups was tested with one-way ANOVA, and Student t-test was used to compare PFAS concentrations between sexes. Further, Spearman's correlation analysis was used to explore correlations among different PFAS. For PFHpA and PFHpS, detected vs. non-detected were compared using the Pearson's chi-squared test.

Table 1
Serum concentrations (ng/mL) of PFAS in 1094 children aged 6–16 years.

	LOQ	N > LOQ	% > LOQ	P2.5	P50	P97.5	Max	AM	GM	95%CI GM
PFHpA	0.05	301	27	< LOQ	< LOQ	0.17	0.40	0.05	< LOQ	< LOQ-0.116
PFOA	0.05	1094	100	0.6	1.38	2.60	3.95	1.44	1.35	0.664–2.761
PFNA	0.05	1094	100	0.28	0.66	1.93	4.49	0.77	0.68	0.259–1.783
PFDA	0.05	1082	99	0.06	0.16	0.36	0.74	0.17	0.15	0.062–0.384
PFUnDA	0.05	976	89	< LOQ	0.11	0.32	1.03	0.13	0.11	< LOQ-0.347
PFDoDA	0.05	62	6	< LOQ	< LOQ	0.08	0.27	< LOQ	< LOQ	< LOQ-0.058
PFTrDA	0.05	58	5	< LOQ	< LOQ	0.07	0.21	< LOQ	< LOQ	< LOQ-0.051
PFTeDA	0.20	2	0.2	< LOQ	< LOQ	< LOQ	0.25	< LOQ	< LOQ	
PFHxS	0.05	1094	100	0.20	0.47	1.64	11.04	0.58	0.49	0.171–1.284
PFHpS	0.05	602	55	< LOQ	0.05	0.14	0.29	0.06	0.05	< LOQ-0.126
PFOS	0.05	1094	100	0.08	2.66	6.83	18.5	2.96	2.67	1.098–6.475
Σ 4PFAS				20.62	5.41	11.4	22.4	5.76	5.38	2.64–11.0

LOQ = limit of quantification; AM = arithmetic mean; GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); 95%CI = 95% confidence interval; PFHpA = perfluoroheptanoic acid; PFOA = perfluorooctanoic acid; PFNA = perfluorononaic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFDoDA = perfluorododecanoic acid; PFTrDA = perfluorotridecanoic acid; PFTeDA = perfluorotetradecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid; Σ 4PFAS = sum of PFOA, PFNA, PFHxS and PFOS.

2.4. Ethical considerations

This study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics West (reference number 2015/128). Written informed consent was obtained from a parent or legal guardian of each participating child, and from the participants themselves when they were 12 years of age and older. The children received age-appropriate information ahead of participation. Assent from the participants themselves was an additional requirement for inclusion. A cinema voucher was given as an incentive to participate in the study.

3. Results

3.1. PFAS concentrations in serum

The distributions of PFAS concentrations in serum samples from the 1094 children (645 girls and 449 boys) included in BGS2, are shown in Table 1. PFOS, PFOA, PFHxS and PFNA were detected in all samples. PFOS was the substance showing the highest serum concentrations, with a GM of 2.67 ng/mL, followed by PFOA (1.35 ng/mL), PFNA (0.68 ng/mL) and PFHxS (0.47 ng/mL). The concentration of PFDA, PFUnDA and PFHpS was substantially lower. For PFBA, PFHxA, PFBS, PFDS, PFOSA, MeFOSA and EtFOSA serum concentrations were below the LOQ in all children. PFPeA was detected in a small number of samples, but the measurements were deemed unreliable according to the quality assurance system and are therefore not reported (see section Analysis of PFAS for further information). Only PFAS that were quantifiable in at least 50% of the samples were included in further analyses.

In total, 132 (12%) and 90 (8%) out of the 1094 children had serum concentrations above the HBM I values of 2 ng/mL for PFOA and 5 ng/mL for PFOS respectively, and 203 (19%) children had concentrations above HBM I values for PFOA, PFOS or both. Furthermore, 241 children (22%) had serum concentrations above 6.9 ng/mL (level corresponding to the EFSA TWI) for the sum of PFOA, PFNA, PFHxS and PFOS. The highest proportion of children exceeding the limit of 6.9 ng/mL was found in the youngest age groups, respectively 181 out of 705 children (26%) below 12 years of age, versus 60 out of 389 children (15%) above 12 years of age. Fig. 1 shows a scatter plot of the sum of PFOA, PFNA, PFHxS and PFOS in all 1094 children by age and sex. Supplementary Table 2 gives a more detailed presentation of the proportion of children exceeding the PFAS safety limits by age and sex.

3.2. Correlation among PFAS in serum

There was a significant positive correlation among all the seven PFAS, as shown in Table 2. The strongest correlation was for PFDA with PFUnDA (Spearman correlation coefficient: 0.782), and for PFDA with

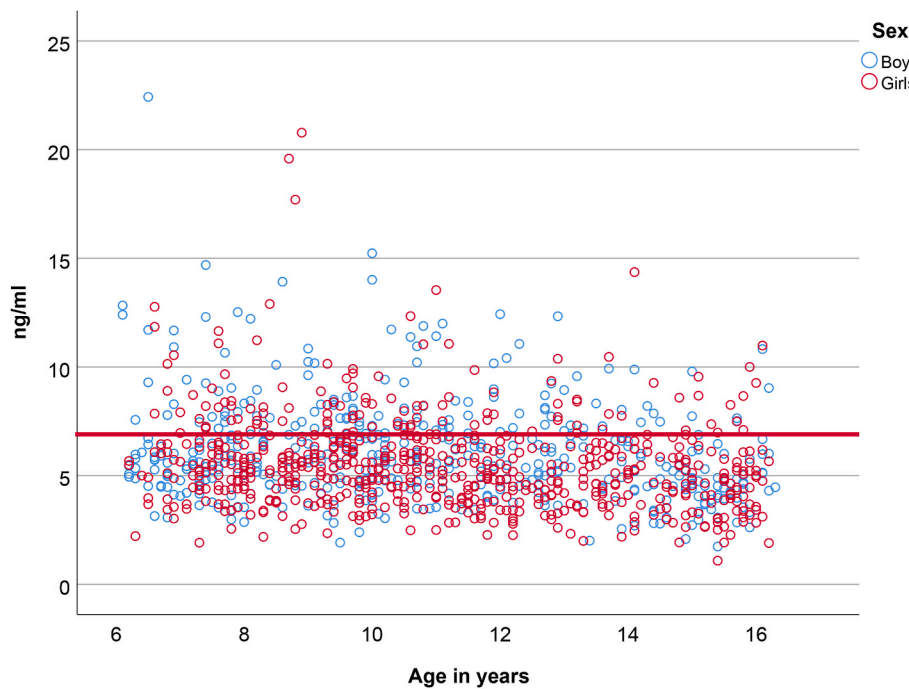


Fig. 1. Sum of PFOA, PFNA, PFHxS and PFOS serum concentrations
 Scatter plot showing the sum of perfluorooctanoic acid (PFOA), perfluorononaic acid (PFNA), perfluorohexanesulfonic acid (PFHxS) and perfluorooctanesulfonic acid (PFOS) in all 1094 children by age and sex. The horizontal line at 6.9 ng/mL is the estimated serum concentration in adults resulting from chronic exposure at the tolerable weekly intake of 4.4 ng/kg body weight per week for the sum of the four PFAS, set by the European Food Safety Authority.

Table 2
 Spearman’s correlations among PFAS in serum (n = 1094).

	PFNA	PFDA	PFUnDA	PFHxS	PFHpS	PFOS
PFOA	0.442	0.535	0.301	0.415	0.372	0.457
PFNA		0.462	0.403	0.235	0.296	0.342
PFDA			0.782	0.308	0.367	0.621
PFUnDA				0.301	0.348	0.608
PFHxS					0.525	0.604
PFHpS						0.595

PFOA = perfluorooctanoic acid; PFNA = perfluorononaic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. All correlations are significant at the 0.01 level (2-tailed).

PFOS (Spearman correlation coefficient: 0.621).

3.3. PFAS concentrations by age and sex

Serum concentrations of PFOS, PFOA, PFDA, PFHxS, PFHpS and PFUnDA were significantly higher in children under the age of 12 years than in children that were above 12 years (Fig. 2). For several of the PFAS the decreasing age trend started about two years earlier in girls compared to boys.

Serum concentrations of PFNA, PFHxS, PFHpS and PFOS were significantly higher in boys compared to girls in all age groups combined (Table 3). After stratification by age group, there were no significant differences between the sexes in the youngest age group, (6–7 years), for any of the PFAS. For PFOA, significantly higher values in boys than girls were observed in the age group 12–13 years specifically. PFDA was the only PFAS that showed significantly lower levels in boys compared to girls, more specifically in the age group 14–16 years (Fig. 2). GM by age and sex are presented in Supplementary Table 3. In addition, detected versus non-detected were examined for PFHpA and PFHpS. A higher proportion of boys had detected levels of PFHpS compared to girls (63% vs. 50%), and a higher proportion of children under 12 years of age had detected levels of PFHpS compared to older children (62% vs. 43%), both significant at the 0.05 level. No significant differences were found for PFHpA.

4. Discussion

Through the Bergen Growth Study 2, we assessed PFAS exposure data from 1094 children aged 6–16 years, living in Bergen, Norway. PFOS, PFOA, PFNA and PFHxS were detected in all samples, which demonstrated widespread exposure in the sample population of Norwegian children analyzed in this study. PFOS had the highest serum concentration, followed by PFOA, PFNA and PFHxS. About one out of five children had serum concentrations above levels considered safe. Furthermore, serum concentrations were generally higher in the youngest age groups compared to the oldest. In addition, there were significant differences between boys and girls, in particular for PFNA, PFHxS, PFHpS and PFOS. The significant positive correlations between the PFAS point to similar exposure sources and pathways, e.g., contaminated food, which based on prior studies has been considered the main source of exposure (EFSA, 2020).

A previous Norwegian study in pregnant women found that variables related to socioeconomic status (i.e., higher maternal education and household income) were related to higher levels of various PFAS (Brantsæter et al., 2013). Higher PFAS concentrations were also found in European teenagers from households with a higher educational level (Richterová et al., 2023). Because children of parents with a higher level educational level were overrepresented in the current study, the PFAS levels might be higher in children participating in BGS2 compared to non-participants and the general population of children in Bergen.

The PFAS levels in the current study are comparable to those of other European studies that have analyzed samples from the same period and age range (Haug et al., 2018; Richterová et al., 2023). Previous publications on PFAS exposure in Norwegian schoolchildren have been limited to either pooled samples or narrow age ranges (Averina et al., 2018; Haug et al., 2009a; Richterová et al., 2023). The Norwegian national data of 12-year-old children included in the European Human Biomonitoring Initiative (HBM4EU) aligned studies, show similar concentrations compared to our data, i.e., PFOA of 1.28 vs 1.35 ng/mL, PFNA of 0.44 vs 0.68 ng/mL, PFHxS of 0.48 vs 0.47 ng/mL and PFOS of 2.79 vs 2.67 ng/mL (Richterová et al., 2023). In our study, the proportion of children exceeding serum levels of 6.9 ng/mL for the sum of PFOA, PFNA, PFHxS and PFOS was 22% in total and 15% for children above 12 years of age. In comparison, 14% of children aged 12–18 years

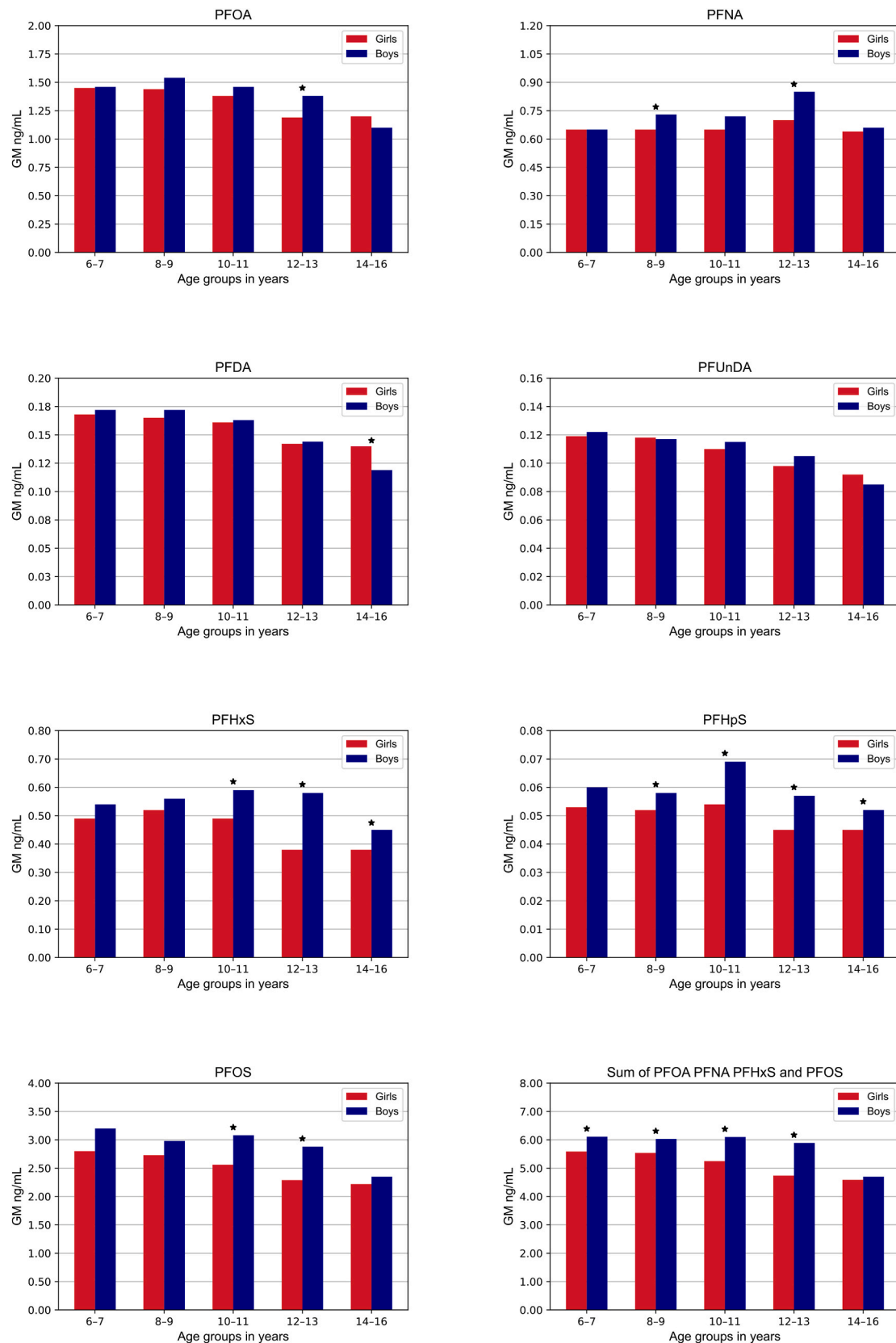


Fig. 2. Geometric mean concentrations for PFAS by age and sex
 GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); PFOA = perfluorooctanoic acid; PFNA = perfluorononaic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. The stars denote p-values < 0.05 for sex differences.

Table 3

Geometric mean concentrations (ng/mL) and 95% confidence intervals (CI) for PFAS by sex.

	Boys GM (95%CI) (n = 449)	Girls GM (95%CI) (n = 645)	P value
PFOA	1.38 (0.70–2.73)	1.33 (0.64–2.78)	0.107
PFNA	0.71 (0.27–1.90)	0.66 (0.25–1.70)	0.007*
PFDA	0.15 (0.06–0.37)	0.16 (0.06–0.39)	0.748
PFUnDA	0.11 (0.03–0.35)	0.11 (0.03–0.34)	0.814
PFHxS	0.54 (0.20–1.44)	0.45 (0.17–1.24)	<0.001*
PFHpS	0.06 (0.02–0.15)	0.05 (0.02–0.11)	<0.001*
PFOS	2.89 (1.20–6.98)	2.52 (1.05–6.05)	<0.001*
Σ4PFAS	5.74 (2.84–11.58)	5.15 (2.54–10.45)	<0.001*

GM = geometric mean (back-transformed arithmetic mean of the natural logarithm of concentrations); PFOA = perfluorooctanoic acid; PFNA = perfluorononaic acid; PFDA = perfluorodecanoic acid; PFUnDA = perfluoroundecanoic acid; PFHxS = perfluorohexanesulfonic acid; PFHpS = perfluoroheptanesulfonic acid; PFOS = perfluorooctanesulfonic acid. Σ4PFAS = sum of PFOA, PFNA, PFHxS and PFOS. Stars denote p-values that are significant at the 0.05 level.

in the HBM4EU studies had serum concentrations above 6.9 ng/mL (Richterová et al., 2023). The proportion of children in the HBM4EU studies exceeding the health-based guidance value of EFSA was calculated per country, and ranged from 1% in Spain, to the maximum of 24% in France (Lobo Vicente et al., 2023).

EFSA showed results from toxicokinetic (TK) modeling of PFOA and PFOS serum concentration under constant exposure conditions that indicated a decline in serum concentration after the breastfeeding period until age 5–6 years, followed by a gradual increase over time until it stabilized after age 30–40 years. The impact of breastfeeding for 12 months on serum concentrations disappeared around the age of 10 years according to the model used by EFSA (EFSA, 2020). Our data showed higher serum concentrations in children under 12 years of age compared to children in the older age groups, which deviates from EFSA's TK model. Both duration of breastfeeding and dietary intake will affect the expected change in serum concentration with age. In Norway, breastfeeding rates are high compared to several other European countries (Theurich et al., 2019). In addition, Norwegian national survey data show that many women continue breastfeeding after the first twelve months (Norwegian Institute of Public Health 2020; Revheim et al., 2023). Longer breastfeeding duration leads to later decrease in serum concentrations. In the current study, we have not accessed data on food intake or breastfeeding. Inverse associations between PFAS concentrations and age in schoolchildren have been reported in previous publications, and can, at least partly, be explained by growth dilution (Koponen et al., 2018; Nyström et al., 2022).

Reported sex differences in biomonitoring data in children are variable (Winkens et al., 2017). In our study, the majority of the PFAS showed higher levels in boys compared to girls. Published data from adults generally show higher PFAS levels in men compared to women, which is primarily attributed to blood loss via menstruation, transfer of PFAS to the fetus during pregnancy, and excretion via breast milk (Winkens et al., 2017). Pregnancy and breastfeeding are not relevant for our study population. However, menstruation can contribute as shown in previous studies for PFOS (Nyström et al., 2022; Wong et al., 2014). In addition, females have lower energy intake per kilogram of body weight compared to males, implying a lower PFAS intake per kilogram of body weight (Shomaker et al., 2010). As girls are entering puberty earlier than boys, their growth spurt and following growth dilution will occur earlier. This, in addition to menstruation, could explain the different age patterns between the sexes. Future research should include further exploration of the relationships of PFAS in relation to growth and maturation.

A limitation of the present study is that we only included children living in Bergen. Our findings may therefore not be representative for the entire Norwegian population of children. However, our data

correspond well with the national data from Norwegian children in the HBM4EU studies (Richterová et al., 2023). As this is a cross-sectional study, the observed differences between age groups could be due to changes related to growth and maturation, behavioral factors, as well as differences in exposure levels and duration. On the other hand, a cross-sectional design with sample collection during a narrow time frame might be more beneficial than a longitudinal design, given that exposure levels have changed during the last decades. Finally, a cross-sectional design might give a more complete picture. Strengths of our study include a relatively large number of participants and a wide range of PFAS assessed using a sensitive analytical method. All blood samples were collected during a narrow time frame which avoids interference of the sample time point when comparing age groups or sexes.

5. Conclusions

In the current study, 11 of the 19 analyzed PFAS were detected in the serum samples of children recruited through the BGS2. The children covered a wide age range, from 6 to 16 years of age, and PFAS exposure was stratified by age and sex. PFOS, PFOA, PFHxS and PFNA were present in all samples demonstrating widespread exposure in the sample population of Norwegian children analyzed in this study. Approximately one out of five children had serum concentrations above safety levels, indicating a potential risk of negative health effects. The majority of the analyzed PFAS showed higher levels in boys than in girls, and higher levels in the youngest age groups compared to the oldest. Future analyses should include examining the relationships between PFAS, growth and maturation.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2023.114199>.

References

- Appel, M., Forsthuber, M., Ramos, R., Widhalm, R., Granitzer, S., Uhl, M., Hengstschläger, M., Stamm, T., Gundacker, C., 2022. The transplacental transfer efficiency of per- and polyfluoroalkyl substances (PFAS): a first meta-analysis. *J. Toxicol. Environ. Health B Crit. Rev.* 25 (1), 23–42. <https://doi.org/10.1080/10937404.2021.2009946>.
- Averina, M., Brox, J., Huber, S., Furberg, A.S., 2018. Perfluoroalkyl substances in adolescents in northern Norway: lifestyle and dietary predictors. The Tromsø study, Fit Futures 1. *Environ. Int.* 114, 123–130. <https://doi.org/10.1016/j.envint.2018.02.031>.
- Blomberg, A.J., Norén, E., Haug, L.S., Lindh, C., Sabaredzovic, A., Pineda, D., Jakobsson, K., Nielsen, C., 2023. Estimated transfer of perfluoroalkyl substances (PFAS) from maternal serum to breast milk in women highly exposed from contaminated drinking water: a study in the ronney mother-child cohort. *Environ. Health Perspect.* 131 (1), 17005 <https://doi.org/10.1289/EHP11292>.
- Brantsæter, A.L., Whitworth, K.W., Ydersbond, T.A., Haug, L.S., Haugen, M., Knutsen, H. K., Thomsen, C., Meltzer, H.M., Becher, G., Sabaredzovic, A., Hoppin, J.A., Eggesbø, M., Longnecker, M.P., 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. *Environ. Int.* 54, 74–84. <https://doi.org/10.1016/j.envint.2012.12.014>.
- Cole, T.J., Bellizzi, M.C., Flegal, K.M., Dietz, W.H., 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br. Med. J.* 320 (7244), 1240–1243. <https://doi.org/10.1136/bmj.320.7244.1240>.
- Conder, J.M., Hoke, R.A., De Wolf, W., Russell, M.H., Buck, R.C., 2008. Are PFCAs bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environ. Sci. Technol.* 42 (4), 995–1003. <https://doi.org/10.1021/es070895g>.

- Duffek, A., Conrad, A., Kolossa-Gehring, M., Lange, R., Rucic, E., Schulte, C., Wellmitz, J., 2020. Per- and polyfluoroalkyl substances in blood plasma - results of the German Environmental Survey for children and adolescents 2014-2017 (GerES V). *Int. J. Hyg Environ. Health* 228, 113549. <https://doi.org/10.1016/j.ijheh.2020.113549>.
- European Chemicals Agency, 2023. Per- and Polyfluoroalkyl Substances (PFASs). <https://echa.europa.eu/hot-topics/perfluoroalkyl-chemicals-pfas>. accessed: 20. February.23.
- European Commission, 2020. Commission staff working document. Poly- and perfluoroalkyl substances (PFAS). https://ec.europa.eu/environment/pdf/chemicals/2020/10/SWD_PFAS.pdf accessed: 20.February.23.
- European Commission, 2022. Commission regulation (EU) 2022/2388 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R2388> (accessed: 20.02.23).
- Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds—exposure assessment for the general population in Western countries. *Int. J. Hyg Environ. Health* 212 (3), 239–270. <https://doi.org/10.1016/j.ijheh.2008.04.007>.
- Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczeny, O., Koletzko, B., Völkel, W., 2010. Prenatal and postnatal exposure to perfluorinated compounds (PFCs). *Environ. Sci. Technol.* 44 (18), 7123–7129. <https://doi.org/10.1021/es101184f>.
- Glüge, J., Scheringer, M., Cousins, I.T., DeWitt, J.C., Goldenman, G., Herzke, D., Lohmann, R., Ng, C.A., Trier, X., Wang, Z., 2020. An overview of the uses of per- and polyfluoroalkyl substances (PFAS). *Environ. Sci. Process. Impacts* 22 (12), 2345–2373. <https://doi.org/10.1039/d0em00291g>.
- Haug, L.S., Thomsen, C., Becher, G., 2009a. Time trends and the influence of age and gender on serum concentrations of perfluorinated compounds in archived human samples. *Environ. Sci. Technol.* 43 (6), 2131–2136. <https://doi.org/10.1021/es802827u>.
- Haug, L.S., Thomsen, C., Becher, G., 2009b. A sensitive method for determination of a broad range of perfluorinated compounds in serum suitable for large-scale human biomonitoring. *J. Chromatogr. A* 1216 (3), 385–393. <https://doi.org/10.1016/j.chroma.2008.10.113>.
- Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds—comparing exposure estimates with biomarkers of exposure. *Environ. Int.* 37 (4), 687–693. <https://doi.org/10.1016/j.envint.2011.01.011>.
- Haug, L.S., Sakhi, A.K., Cequier, E., Casas, M., Maitre, L., Basagana, X., Andrusaityte, S., Chalkiadaki, G., Chatzi, L., Coen, M., de Bont, J., Dedele, A., Ferrand, J., Grazuleviciene, R., Gonzalez, J.R., Gutzkow, K.B., Keun, H., McEachan, R., Meltzer, H.M., Petravicene, I., Robinson, O., Saulnier, P.J., Slama, R., Sunyer, J., Urquiza, J., Vafeiadi, M., Wright, J., Vrijheid, M., Thomsen, C., 2018. In-utero and childhood chemical exposure in six European mother-child cohorts. *Environ. Int.* 121 (Pt 1), 751–763. <https://doi.org/10.1016/j.envint.2018.09.056>.
- HBM4EU, 2022. The European Human Biomonitoring Initiative. Online library. <https://www.hbm4eu.eu/online-library/>.
- Júlíusson, P.B., Bruslerud, I.S., Oehme, N.H.B., Madsen, A., Forthun, I.H., Balthasar, M.R., Rosendahl, K., Viste, K., Jugessur, A., Schell, L.M., Bjerknes, R., Roelants, M., 2023. Deep phenotyping of pubertal development in Norwegian children: the Bergen Growth Study 2. *Ann. Hum. Biol.* <https://doi.org/10.1080/03014460.2023.2174272> (in press).
- Koponen, J., Winkens, K., Airaksinen, R., Berger, U., Vestergren, R., Cousins, I.T., Karvonen, A.M., Pekkanen, J., Kiviranta, H., 2018. Longitudinal trends of per- and polyfluoroalkyl substances in children's serum. *Environ. Int.* 121 (Pt 1), 591–599. <https://doi.org/10.1016/j.envint.2018.09.006>.
- Land, M., de Wit, C.A., Bignert, A., Cousins, I.T., Herzke, D., Johansson, J.H., Martin, J. W., 2018. What is the effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl acids and their precursors in the environment? A systematic review. *Environ. Evid.* 7, 4. <https://doi.org/10.1186/s13750-017-0114-y>.
- Lobo Vicente, J., Ganzleben, C., Gasol, R., Marnane, I., Gilles, L., Buekers, J., Bessems, J., Colles, A., Gerofke, A., David, M., Barouki, R., Uhl, M., Sepai, O., Loots, I., Crabbé, A., Coertjens, D., Kolossa-Gehring, M., Schoeters, G., 2023. HBM4EU results support the chemicals' strategy for sustainability and the zero-pollution action plan. *Int. J. Hyg Environ. Health* 248, 114111. <https://doi.org/10.1016/j.ijheh.2023.114111>.
- Ng, C.A., Hungerbühler, K., 2014. Bioaccumulation of perfluorinated alkyl acids: observations and models. *Environ. Sci. Technol.* 48 (9), 4637–4648. <https://doi.org/10.1021/es404008g>.
- Statistics Norway, 2023. SSB (Statistisk Sentralbyrå). Personer 16 år og over, etter region, kjønn, alder, utdanningsnivå, statistikkvariabel og år. <https://www.ssb.no/statbank/table/08921> accessed: 30.March.23.
- Norwegian Institute of Public Health, 2020. Småbarnskost 3. Landsomfattende undersøkelse av kostholdet blant 2-åringer i Norge. <https://www.fhi.no/publ/2020/smabarnskost-3/> accessed: 20.February.23.
- Nøst, T.H., Vestergren, R., Berg, V., Nieboer, E., Odland, J.Ø., Sandanger, T.M., 2014. Repeated measurements of per- and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: assessing time trends, compound correlations and relations to age/birth cohort. *Environ. Int.* 67, 43–53. <https://doi.org/10.1016/j.envint.2014.02.011>.
- Nübler, S., Esteban López, M., Castaño, A., Mol, H.G.J., Haji-Abbas-Zarrabi, K., Schäfer, M., Müller, J., Hajslova, J., Dvorakova, D., Antignac, J.P., Koch, H.M., Haug, L.S., Vorkamp, K., Göen, T., 2022. Interlaboratory Comparison Investigations (ICIs) and External Quality Assurance Schemes (EQUASs) for human biomonitoring of perfluoroalkyl substances (PFASs) in serum as part of the quality assurance programme under HBM4EU. *Sci. Total Environ.* 847, 157481 <https://doi.org/10.1016/j.scitotenv.2022.157481>.
- Nyström, J., Benskin, J.P., Plassmann, M., Sandblom, O., Glynn, A., Lampa, E., Gyllenhammar, I., Moraes, 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. *Environ. Res.* 208, 112674 <https://doi.org/10.1016/j.envres.2022.112674>.
- Panieri, E., Baralic, K., Djukic-Cosic, D., Buha Djordjevic, A., Saso, L., 2022. PFAS molecules: a major concern for the human health and the environment. *Toxics* 10 (2), 44. <https://doi.org/10.3390/toxics10020044>.
- Papadopoulou, E., Sabaredzovic, A., Namork, E., Nygaard, U.C., Granum, B., Haug, L.S., 2016. Exposure of Norwegian toddlers to perfluoroalkyl substances (PFAS): the association with breastfeeding and maternal PFAS concentrations. *Environ. Int.* 94, 687–694. <https://doi.org/10.1016/j.envint.2016.07.006>.
- Poothong, S., Papadopoulou, E., Padilla-Sánchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): from external exposure to human blood. *Environ. Int.* 134, 105244 <https://doi.org/10.1016/j.envint.2019.105244>.
- Rappazzo, K.M., Coffman, E., Hines, E.P., 2017. Exposure to perfluorinated alkyl substances and health outcomes in children: a systematic review of the epidemiologic literature. *Int. J. Environ. Res. Publ. Health* 14 (7), 691. <https://doi.org/10.3390/ijerph14070691>.
- Revheim, I., Balthasar, M.R., Akerkar, R.R., Stangenes, K.M., Almenning, G., Nygaard, E., Markestad, T., Overland, S., Roelants, M., Juliusson, P.B., 2023. Trends in the prevalence of breastfeeding up to 6 months of age using structured data from routine child healthcare visits. *Acta paediatrica* (Oslo, Norway 112 (1), 100–105. <https://doi.org/10.1111/apa.16367>, 1992.
- Richterová, D., Govarts, E., Fábelová, L., Rausová, K., Rodriguez Martin, L., Gilles, L., Remy, S., Colles, A., Rambaud, L., Riou, M., Gabriel, C., Sarigiannis, D., Pedraza-Diaz, S., Ramos, J.J., Kosjek, T., Snoj Tratnik, J., Lignell, S., Gyllenhammar, I., Thomsen, C., Haug, L.S., Kolossa-Gehring, M., Vogel, N., Franken, C., Vanlarebeke, N., Bruckers, L., Stewart, L., Sepai, O., Schoeters, G., Uhl, M., Castaño, A., Esteban López, M., Göen, T., Palkovičová Murfinová, L., 2023. PFAS levels and determinants of variability in exposure in European teenagers - results from the HBM4EU aligned studies (2014-2021). *Int. J. Hyg Environ. Health* 247, 114057. <https://doi.org/10.1016/j.ijheh.2022.114057>.
- Russell, M.H., Nilsson, H., Buck, R.C., 2013. Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. *Chemosphere* 93 (10), 2419–2425. <https://doi.org/10.1016/j.chemosphere.2013.08.060>.
- Schrenk, D., Bignami, M., Bodin, L., Chipman, J.K., Del Mazo, J., Gras-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Leblanc, J.-C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregård, L., Ceccatelli, S., Cravedi, J.P., Schwerdtle, T., EFSA, 2020. Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), n.d. Risk to human health related to the presence of perfluoroalkyl substances in food. *EFSA J. Eur. Food Saf. Auth.* 18 (9), e06223. doi: 10.2903/j.efsa.2020.6223.
- Shomaker, L.B., Tanofsky-Kraff, M., Savastano, D.M., Kozlosky, M., Columbo, K.M., Wolkoff, L.E., Zocca, J.M., Brady, S.M., Yanovski, S.Z., Crocker, M.K., Ali, A., Yanovski, J.A., 2010. Puberty and observed energy intake: boy, can they eat. *Am. J. Clin. Nutr.* 92 (1), 123–129. <https://doi.org/10.3945/ajcn.2010.29383>.
- Stockholm Convention on persistent organic pollutants, 2019. All POPs listed in the Stockholm Convention. <http://pops.int/TheConvention/ThePOPs/AllPOPs/tabid/2509/Default.aspx>. (Accessed 20 February 2023).
- The German Environment Agency, 2020. Human biomonitoring commission (HBM commission). <https://www.umweltbundesamt.de/en/topics/health/commissions-working-groups/human-biomonitoring-commission-hbm-commission> accessed: 20.February.23.
- Theurich, M.A., Davanzo, R., Busck-Rasmussen, M., Díaz-Gómez, N.M., Brennan, C., Kylberg, E., Bærug, A., McHugh, L., Weikert, C., Abraham, K., Koletzko, B., 2019. Breastfeeding rates and programs in Europe: a survey of 11 national breastfeeding committees and representatives. *J. Pediatr. Gastroenterol. Nutr.* 68 (3), 400–407. <https://doi.org/10.1097/MPG.0000000000002234>.
- Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., Hungerbühler, K., 2008. Estimating consumer exposure to PFOS and PFOA. *Risk Anal.* : an official publication of the Society for Risk Analysis 28 (2), 251–269. <https://doi.org/10.1111/j.1539-6924.2008.01017.x>.
- VKM Report, 2022. Norwegian Scientific Committee for Food and Environment. Benefit and risk assessment of fish in the Norwegian diet. <https://vkm.no/english/riskassessments/allpublications/benefitandriskassessmentoffishinthenorwegiandiet.47b65040716afa27d7ec5d3a.html> accessed 20.February.23.
- Winkens, K., Vestergren, R., Berger, U., Cousins, I.T., 2017. Early life exposure to per- and polyfluoroalkyl substances (PFASs): a critical review. *Emerging Contaminants* 3 (Issue 2), 55–68. <https://doi.org/10.1016/j.emcon.2017.05.001>.
- Wong, F., MacLeod, M., Mueller, J.F., Cousins, I.T., 2014. Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. *Environ. Sci. Technol.* 48 (15), 8807–8814. <https://doi.org/10.1021/es500796y>.