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Per- and polyfluoroalkyl substances (PFAS), thyroid hormones, sexual hormones and pubertal development in adolescents residing in the neighborhood of a 3M factory

Nicolas van Larebeke[1,2*], Bianca Cox[3], Sylvie Remy[3], Stefan Voorspoels[4], Elly Den Hond[5], Ann Colles[3], Martine Leermakers[1], Greet Schoeters[6] and Veerle Verheyen[3]

Abstract

Background Near Antwerp a 3M factory has been active since 1971 emitting PFAS, mainly PFOS, in the local environment. Production of C8 compounds was stopped in 2002, production of other PFAS continued until 2024. This study aimed to examine the association between internal PFAS concentrations and thyroid hormones, sexual hormones, and pubertal development in adolescents living in the neighborhood of the factory.

Methods We measured PFAS in serum of 146 female and 139 male adolescents. For males sex hormones (LH, testosterone, estradiol, progesterone, inhibin B, FSH) and SHBG were measured in serum. For males and females we assessed serum thyroid hormone levels (TSH, T3, T4 and T3/T4) and pubertal development parameters self-assessed through a standardized questionnaire. Associations between PFAS concentrations and effect biomarkers/health effects were assessed through Generalized Estimating Equations (GEE), using linear models for continuous outcomes, logistic models for binary outcomes, and proportional odds models for ordinal outcomes.

Results For males LH, total and bioavailable testosterone showed significant negative associations with PFHxS and PFOA. LH and bioavailable testosterone also showed significant negative associations with other PFAS compounds. SHBG showed significant positive associations with PFDA, PFNA, PFHxS, PFOS and the sum of the linear forms of PFOS, PFOA, PFNA and PFHxS. Males' length and growth spurt showed significant negative associations with PFOS, PFOA and PFAS sum parameters and length and growth spurt separately also with other PFAS compounds. For females growth spurt showed significant negative association with PFOA and a significant positive association with PFOS(branched). For both males and females body hair development showed significant negative associations with PFHxS, and, for males and females separately also with other PFAS compounds. For females, breast development showed significant negative associations with PFOA, pubertal development scale showed significant negative associations with PFOA, PFHxS, PFOS(linear) and the sum of 4 PFAS. For males, TSH showed a significant negative association with PFDA and FT3 showed significant positive associations with PFOA, PFOA and PFNA. For females, FT3 showed a significant negative association with PFOS(branched).

Conclusion We observed significant, consistent and biologically relevant associations of PFAS serum concentrations with sex hormone and SHBG levels in male adolescents. Moreover, a significant delay in physiological processes

*Correspondence:
Nicolas van Larebeke
Nicolas.vanlarebeke@ugent.be
Full list of author information is available at the end of the article

occurring in puberty was observed in females and males. Associations with thyroid hormones differed significantly by sex

Keywords PFOS, PFOA, PFHxS, PFNA, PFDA, Length, Growth spurt, Breast development, FT3, Luteinizing Hormone

Background

Per- and polyfluoroalkyl substances (PFAS) are synthesized since more than 70 years and were a new class of heat-resistant, fireproof, extremely stable surfactants repelling water, oil and stains. This allowed production of innovative consumer products such as textile coatings, non-stick cookware, electronics, and firefighting foams [1]. Emissions during manufacturing, usage, and disposal, resulted in the widespread abundance of PFAS in the environment. By now it is documented that PFAS affect many biological mechanisms such as binding to estrogen and other types of receptors, disruption of gap-junctional intercellular communication and epigenetic effects (see discussion). Consistent with the many biological interactions, PFAS were also shown to be associated with several health effects including endocrine disrupting, immunotoxic and genotoxic effects, disturbance of lipid profiles, and increased risk of cancer, male infertility, diabetes and cardiovascular diseases [2–18], but, at least up to recently, the evidence for an association between PFAS exposure and timing of puberty remained inconclusive as reported in a systematic review by Lee et al. [19].

Due to their multiple carbon–fluorine (C–F) bonds and their chemical and thermal stability, PFAS are commonly referred to as "forever chemicals". PFAS also have the potential to bioaccumulate, which tends to increase with increasing chain length. In Zwijndrecht, near Antwerp (Flanders) an important 3M PFAS manufacturing site has been active since 1971, emitting significant quantities of PFAS in local waters, the local environment and the river Scheldt. From the 1970 s till 2000, mainly PFOS was produced. Production of PFOS and other C8 PFAS was stopped in 2002, while production of C4 PFAS continued until 2024 (https://www.vlaanderen.be/en/pfas-in-flanders). During infrastructure works near 3M (the Oosterweel project), soil contamination with PFAS was discovered. This lead to severe environmental health concerns among local residents and authorities. As part of their response the Flemish government's Planning Agency for Environment commissioned a cross-sectional human biomonitoring study on 300 adolescents living within 5 km from the 3M factory. Adolescence is a developmental period with significant hormonal changes that are essential for growth and development. Therefore, adolescents are particularly sensitive to endocrine disruption by chemicals [20]. The aim was to measure the extent of the adolescents' internal exposure to a comprehensive set of PFAS, to study determinants of PFAS-exposure, and to assess exposure-health effects associations. The selection of health-related parameters was based on the literature [6, 8, 21–27] and on the findings of the Flemish Environment and Health Studies. Health outcomes included data on the incidence of immune system related health problems and of ADHD, parameters of immune response, cardiometabolic health, liver function kidney function, thyroid function, and sex hormones and pubertal development. The study was conducted by an interdisciplinary consortium of Flemish universities and research institutes.

Here we report the observations that were made concerning the association between internal PFAS concentrations and thyroid hormone levels measured in male and female adolescents, sex hormone levels measured in male adolescents and pubertal development parameters assessed in both female and male adolescents.

Methods

Study population

The study area (Fig. 1) comprised a circular area with a radius of 5 km round the center of the 3M site. The inhabited areas are mainly situated to the west, south and east of 3M. These areas have about 90,000 inhabitants. The aim was to include 300 adolescents of 14–15 years. Originally only adolescents born in 2007-2008 received an invitation letter to participate in the study, but due to insufficient response and time limits the invitation was extended to adolescents born in 2006 and 2009. The invitation letter included an informed consent form (ICF) and a flyer. Adolescents were included in the study on condition that they themselves as well as one of the parents signed the ICF and had sufficient knowledge of the Dutch language to fill out extensive questionnaires, and that they inhabited the study area for at least 5 years. Exclusion criteria were pregnancy and treatment with growth hormones. Multiple adolescents from the same household were eligible. The study protocol was approved by the ethical committee of the University of Antwerp (Reference BUN B3002022000041). 303 adolescents participated in the study.

Collection of samples

Sampling took place at central locations in the study area between 28 June and 31 August 2022, was carried

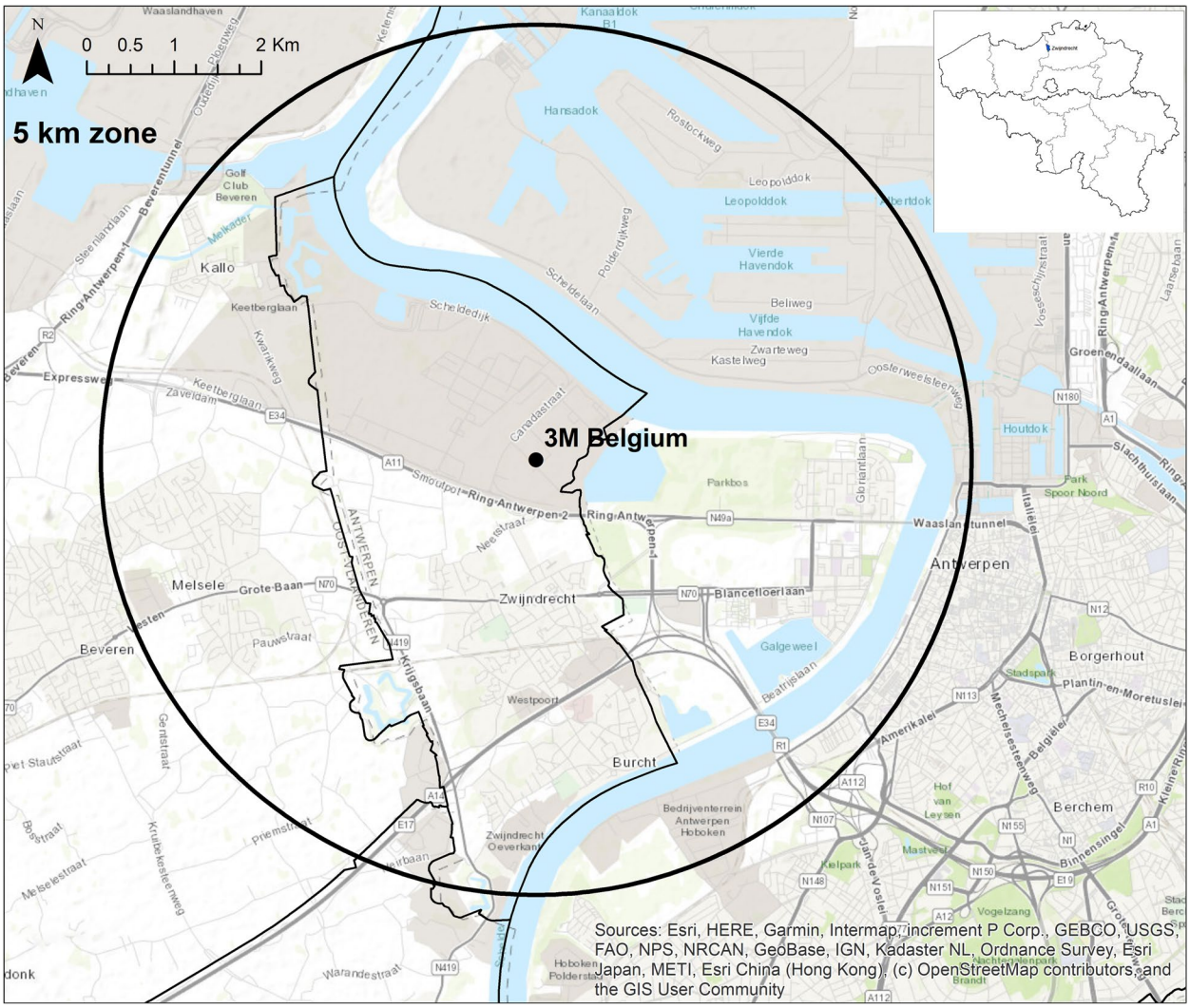

Fig. 1 Map of the study area. In the centre of the circle the position of the 3M factory is indicated. The big city of Antwerp is situated to the right (east) of the 3M factory. The main locality affected by the pollution is the Zwijndrecht municipality

out by an experienced team of nurses and took about 20 minutes per person. A blood sample of 39 ml was collected and the length and weight of participants was measured. All male adolescents were sampled before 11 a.m. to account for the diurnal variation in sex hormones. The blood samples were immediately processed and aliquoted. For PFAS analysis, serum was aliquoted in a cryotube, kept at 4 °C, transported on dry ice and stored at −80 °C within 12 h in the Biobank@vito, until analysis in batch. For hormone analysis, serum was aliquoted in a Sarstedt tube, stored at 4°C and at the end of the field work day transported to the clinical lab Algemeen Medisch Laboratorium for analysis within 24 hours after sampling.

Questionnaires

Two weeks prior to sampling, participants received an email with digital links to three electronic general questionnaires. Adolescents and their parents were asked to complete the questionnaires before the sampling. The questionnaire for the parents contained questions concerning residential history, social-financial situation, level of education, birth and first two life years of the participating adolescent, characteristics of house and garden, use of products and health problems in the biological family. The questionnaire for the adolescent addressed his/her origin, habits, use of products, opinion on environmental pollution and health, including questions on puberty development (see section: Assessment of

pubertal development parameters). The third questionnaire was to be completed by the adolescents with help from their parents and assessed dietary habits and consumption of locally produced food. The dietary questionnaires were designed by dietary experts and the multidisciplinary team of the research consortium and are based on a literature review with the aim to assess potential dietary sources for biomarkers of exposure. They have been used in previous HBM studies in Flanders, and thus are harmonized and validated internally. The questionnaire on dietary habits is included in supplementary material Note S1 and the questionnaire concerning consumption of locally produced food in supplementary material Note S2.

At the sampling day, a short fourth questionnaire had to be filled out by the participant. This questionnaire concerned recent exposures including smoking, alcohol consumption, medication, and food intake during the last 3 days.

Measurements of PFAS in blood

The selection of the PFAS that were measured in serum of the participants was based on: 1) an extensive preliminary study comprising measurements of PFAS in depositions collected around the 3M site; 2) the list of PFAS previously and/or currently produced by 3M, made available by 3M; and 3) the technical feasibility of the measurement methods available in the VITO GOAL laboratory. Measurements were performed by the VITO GOAL laboratory according to the Flemish reference method (WAC/IV/A/025; https://emis.vito.be/nl/erkende-laboratoria/water-gop/compendium-wac). In short, the method uses isotope-labeled PFAS as internal standards and liquid chromatography coupled to a mass spectrometry (UPLC-MS/MS, Waters Acquity Xevo TQ-(X)S in electrospray ionisation (ESI) negative mode). The system was equipped with a Waters Acquity Ultra Performance Liquid Chromatography (UPLC) BEH Shield RP18 (1.7 µm, 2.1 x 100 mm column) and a Waters Vanguard pre-column (Acquity UPLC BEH C18; 2.1 x 5 mm, 1.7 µm). Analysis was performed using water- and MeOH-based gradient elution at a flow rate of 300 µL/min and column temperature of 40 °C. All measurements were done in Multiple Reaction Monitoring (MRM). The method used has a previously validated measurement range of 0.2 - 30 µg/L PFAS in serum. The limit of quantification (LOQ) was between 0.01 and 0.03 µg/L depending on the measured PFAS compound (see supplementary materials Table S1). Each measurement series consisted of 20 samples and was accompanied by the necessary quality control measurements: control standards (measurement standards for calibration and integration standards for evaluation of branched isomers) and a control serum sample. The method quality is ensured through a Belgian accreditation system (BELAC) ISO 17025 accreditation (BELTEST-045 11 PFAS at the time of first accreditation).

Twenty-one PFAS compounds were measured in this study: for 16 PFAS compounds only the linear isomer was measured, for 5 compounds both the linear isomer and the sum of the linear and branched isomers. Only the 11 compounds which were detected in at least 50 (17% of the) participants were considered for further statistical analysis in order to have a sufficient number of participants in the "detected" category for dichotomized exposure variables. These PFAS parameters (reported analytical entities) are the following: linear Perfluorobutanoic acid (PFBA), linear Perfluoroheptanoic acid (PFHpA), linear Perfluorooctanoic acid (PFOA), linear + branched Perfluorooctanoic acid (PFOA total), linear Perfluorononanoic acid (PFNA), linear Perfluorodecanoic Acid (PFDA), linear Perfluorohexanesulfonic acid (PFHxS), linear + branched Perfluorohexanesulfonic acid (PFHxS total), linear Perfluorooctanesulfonic acid (PFOS), linear + branched Perfluorooctanesulfonic acid (PFOS total),), branched Perfluorooctanesulfonic acid (PFOS branched) calculated by subtracting linear PFOS from PFOS (total). Chemical measurements below the LOQ were imputed by single random imputation from a censored lognormal distribution if the detection rate was at least 30% and there were at least 10 distinct values. Compounds with a detection rate <60% were dichotomized (<LOQ versus ≥LOQ) in further exposure-effect analyses. This was only the case for PFHpA (detection rate 21%).

In addition to these 11 measured compounds, 3 sum parameters were calculated: the sum of the linear PFOA, PFNA, PFHxs, and PFOS (referred to as 4PFAS), the sum of total PFOA, linear PFNA, total PFHxS, and total PFOS (referred to as lb4PFAS), and the sum of linear N-Methylperfluorooctanesulfonamide (MePFOSAA), linear PFHxS, total PFOA, linear PFDA, linear Perfluoroundecanoic acid (PFUnDA), total PFOS, and linear PFNA (referred to as 7PFAS). Imputed variables were used for the calculation of sum parameters 4PFAS and lb4PFAS since all individual compounds were well quantifiable (PFNA 99% >= LOQ, all others 100% >= LOQ). For 7PFAS, the values below LOQ in the individual parameters were imputed by the LOQ/sqrt(2), since the quantification rate of MePFOSAA and PFUnDA was below 30%. These sum parameters are used in clinical study of PFAS exposed persons. The 4PFAS comprise the linear forms of the 4 best known PFAS, the lb4PFASAS both the linear and the branched forms of these PFAS, and the 7PFAS is a sum parameter stipulated in the "*Guidance on PFAS Exposure, Testing, and Clinical Follow-Up (2022)*"

published by the USA National Academies of Sciences, Engineering and Medicine (http://nap.nationalacademies.org/26156).

Measurement of thyroid and sex hormones

Hormones were measured at the accredited clinical lab AML (ISO 15189:2022, 030-MED).

Thyroid hormones were measured in males and females: thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4). The FT3/FT4 ratio was calculated. Sex hormones were assessed in males only: testosterone (TT), bioavailable testosterone (bioavailable TT), free testosterone (free TT), sex hormone-binding globulin (SHBG), inhibin B (INHIBB) follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P4), estradiol (E2). Hormones were measured with an electrochemiluminescence immunoassay (ECLIA) using a Roche Cobas Pro e801. Inhibin B was measured by Enzyme Linked Immune Sorbent Assay (ELISA) using a BEP2000. Free testosterone and bioavailable testosterone were calculated on the basis of the serum concentrations of total testosterone, SHBG and albumin using the association constants proposed by Vermeulen et al. [28]. Hormone concentrations were >LOD/LOQ for all samples and markers, except for P4, which was detected in only 48% of the samples, and was further analysed as a binary variable (values >=LOD versus values <LOD).

Assessment of pubertal development parameters

Length of the participants was measured in cm by a study nurse during the field work. Z-score of length standardized by age and sex was calculated using Belgian reference data [29]. Puberty development (described by Marshall and Tanner [30–32] was estimated using a standardized questionnaire (Pubertal Development Scale) in which the participant self-assessed its stage of development for: growth spurt; skin changes, as at puberty the skin of children acquires the characteristics of adult skin [33, 34]; body hair (excluding head); voice changes (for males); facial hair (for males); breast development (for females); age of first period (menarche). The full questionnaire related to estimation of puberty development can be found in supplementary material Note S3. The original questions (except for age menarche) considered 4 categories: "not yet started", "just started", "in the middle", and "past". In order to ensure sufficient numbers of participants in all categories, the two lower categories (not yet and just started) were combined, and the resulting 3-category variables were used in statistical models. Age of menarche was calculated by subtracting birth date from reported date of first period (year + month).

Pubertal development scale (PDS) was assessed in a sex specific manner. For males, PDS was derived from body hair, facial hair, and voice changes, by summing the original answers to these 3 questions, setting the values for "not yet started", "started", "middle", and "past" to 1, 2, 3, and 4, respectively. The summed parameter was subsequently categorized into 5 categories: pre-pubertal (sum=3), early puberty (sum=4–5), mid-puberty(sum=6–8), late puberty (sum=9–11), and post puberty (sum=12). Next, age standardized PDS was calculated, as a binary variable indicating whether the expected stage for age was reached or not. To be categorized as having reached the expected stage for age, males <12.95 years old, [12.95–13.9] years, [13.9–15.25] years, and >15.25 years should have reached early puberty, mid-puberty, late puberty, and post puberty, respectively. PDS was also classified into 3 categories, i.e. pre-puberty, in-puberty (combining early and mid-puberty), and completing puberty (combining late and post puberty) [35], and into 2 categories, i.e. pre- and in-puberty versus completing puberty. For females, PDS was derived from hair growth, breast development, and age menarche. First, the original answers to hair growth and breast development questions were summed, setting the values for "not yet started", "started", "middle", and "past" to 1, 2, 3, and 4, respectively. Then status of menarche was taken into account: pre-menarche females were categorized into "pre-puberty" when the sum of hair growth and breast development was equal to 2, into "early puberty" when the sum was equal to 3, and into "mid-puberty" when the sum was 4 or more. Post-menarche females were classified as "late pubertal" when the sum was smaller than 7, and as "post pubertal" when the sum was equal to 8. Next, age standardized PDS was calculated, as a binary variable indicating whether the expected stage for age was reached or not. To be categorized as having reached the expected stage for age, females <11.85 years, [11.85–12.75] years, [12.75–14.65] years, and >14.65 years should have reached early puberty, mid-puberty, late puberty, and post puberty, respectively.

Statistical analysis

Participants taking growth hormones (n=7), medication for thyroid disease (n=1), diabetes (n=2), or kidney disease (n=1), and with missing information on household income (used as a proxy for socio-economic status, n=7) were excluded, resulting in a final sample size of 285.

Because of their right skewed distribution, PFAS and hormone concentrations (except the binary variables PFHpA (>= versus <LOQ) and P4) were log-transformed for further analysis. Although normality of residuals is not a requirement of GEE models, the log-transformation of skewed variables reduces the impact of outliers

and often improves the overall fit and performance of the model. Moreover associations between endocrine disrupting chemicals and outcomes are often stronger at lower than at higher exposure levels which is captured by the log-transformation. To describe the correlations between the different PFAS compounds, pairwise Pearson correlations (between log-transformed variables) were calculated. The associations between PFAS concentrations and effect biomarkers/health effects were assessed through Generalized Estimating Equations (GEE) using an independence working correlation and the robust (Huber-White) variance estimator to account for the clustering within households (there were 43 households with 2 adolescents participating in the study). Linear models were used for continuous outcomes, logistic models for binary outcomes, and ordinal logistic regression (proportional odds models) for ordinal outcomes. 21 outcomes were considered in the main analysis. 8 of these were measured in males and females: length (continuous), growth spurt (ordinal), hair growth (ordinal), skin changes (ordinal), and the 4 thyroid hormone-related variables (TSH, FT3, FT4, ratio FT3/FT4; continuous). 10 of the main outcomes were measured only in males: male PDS standardized for age (binary), voice changes (ordinal), facial hair (binary), and 7 sex hormones (TT, bioavailable TT, SHBG, INHIBB, FSH, LH, P4; continuous except the binary P4). The remaining 3 outcomes were specific for females: female PDS standardized for age (binary), breast development (ordinal), and age menarche (continuous). Z-score of length (age- and sex-standardized; continuous), male PDS (not standardized for age) analyzed in 3 categories (ordinal) and in 2 categories (binary), and free TT (continuous) were considered as secondary outcomes.

All models were stratified by sex and adjusted for age (except for the age-standardized outcomes) and the binary variable "making ends meet with the income – manage to live comfortably". Age was categorized as 12.5–14.5, 14.6–15.5, and >15.5 years. We tested other confounders including body weight, BMI, alcohol consumption, physical activity, complications during pregnancy, weight at birth and covid infection but the inclusion of these covariates gave similar results. We did however not test confounding by breastfeeding and diet because these variables may be important sources of PFAS *and the inclusion of exposure sources in the model may result in overadjustment (i.e. the effect of the exposure on the outcome gets captured by the source variable instead of the exposure variable*). Estimates of the strength of associations (effect estimates) were calculated for an interquartile fold change in exposure biomarker concentration (effect for P75/P25 change in exposure). For log-transformed outcomes, the estimates represent the fold change in the outcome, for untransformed continuous outcomes the unit change, for binary outcomes the odds ratio (OR), and for ordinal outcomes the proportional OR.

In our description of results the associations are considered marginally significant if $p<0.1$ or significant if $p<0.05$.

Analyses of continuous and binary outcomes were performed in R (version 4.4.1), using 'gee' package for gee models. Ordinal outcomes were modelled using the genmod procedure in SAS version 9.4 (SAS Institute, Cary, NC).

Results

The main data concerning characteristics of the study population and the internal exposure to PFAS of the participants are summarized in Table 1. The study population consisted of 155 females and 148 males, of which 146 females and 139 males were included in the analyses presented here. Around 40% of both females and males was between 12.5 and 14.5 years old, a slightly smaller fraction of females compared to males was aged >15.5 years (20% females versus 29% males). One third of the parents reported having it difficult to make ends meet with the income.

The PFAS occurring in the highest concentration in serum was PFOS (total) followed by PFOS, PFOA (total), PFOA, PFHxS (total), PFHxS, PFNA, PFBA, PFDA with respectively geometric mean concentrations in serum of 8.4, 3.3, 1.1, 1.1,0.6, 0.6, 0.3, 0.1, 0.1 µg/L.

Pearson correlations between PFAS serum concentrations are summarized in Supplementary Materials Fig S1. Correlations were mostly above 0.4, except for the correlations with PFOS (branched), with PFHpA, and with PFBA. As in FigS1 values for PFHpA were binary (>= versus <LOQ), correlations limited to persons (n= 64) who had a PFHpA concentration above the limit of quantification were presented in Supplementary Materials Table S2. PFHpA shows less correlation with other PFAS than most other PFAS. Only PFBA shows even less correlation. Correlations between total concentrations (linear + branched) and linear forms of individual PFAS were always above 0.8 (e.g., r=0.99 between total and linear PFOA, r=0.88 between total and linear PFOS), and correlations between the 3 sum parameters were all above 0.9. Correlations between individual compounds and sum parameters were above 0.8 for PFHxS (r=0.82 with 4PFAS), for linear PFOS (r=0.98 with 4PFAS; r=0.90 with lb4PFAS; r=0.90 with 7PFAS), and for total PFOS (r=0.87 with 4PFAS; r=0.99 with lb4PFAS; r=0.99 with 7PFAS).

The main data concerning health outcomes and biomarkers of effect are summarized in Table 2. Females were on average smaller than males (median length 163

Table 1 Characteristics of the study population and biomarkers of exposure to PFAS

	Whole Group			**Females**		**Males**	
Parameter	N (%)	% >= LOQ	Median (P25 - P75)	N (%)	Median (P25 - P75)	N (%)	Median (P25 - P75)
Sex	285			146		139	
Age (years)	285			146		139	
[12.5, 14.5]	115 (40%)			60 (41%)		55 (40%)	
[14.5, 15.5]	100 (35%)			57 (39%)		43 (31%)	
> 15.5	70 (25%)			29 (20%)		41 (29%)	
Making ends meet with the income – manage to live comforably	285			146		139	
No	96 (33%)			53 (36%)		43 (31%)	
Yes	189 (66%)			93 (64%)		96 (69%)	
PFBA	283	72	0.15 (0.10 - 0.19)	145	0.14 (0.08 - 0.19)	138	0.16 (0.11 - 0.19)
PFHpA	283	21	<LOQ (<LOQ - <LOQ)	145	<LOQ (<LOQ - <LOQ)	138	<LOQ (<LOQ - 0.11)
PFOA	283	100	1.10 (0.87 - 1.40)	145	1.00 (0.83 - 1.40)	138	1.20 (0.99 - 1.60)
PFOA (total)	283	100	1.20 (0.92 - 1.50)	145	1.00 (0.85 - 1.40)	138	1.25 (1.00 - 1.60)
PFNA	283	99	0.26 (0.19 - 0.34)	145	0.24 (0.18 - 0.31)	138	0.27 (0.20 - 0.36)
PFDA	283	73	0.14 (0.10 - 0.20)	145	0.14 (0.09 - 0.19)	138	0.15 (0.10 - 0.21)
PFHxS	283	100	0.51 (0.36 - 0.84)	145	0.47 (0.35 - 0.81)	138	0.54 (0.39 - 0.90)
PFHxS (total)	283	100	0.54 (0.38 - 0.88)	145	0.50 (0.37 - 0.86)	138	0.58 (0.41 - 0.92)
PFOS	283	100	2.40 (1.40 - 5.20)	145	2.60 (1.50 - 5.60)	138	2.10 (1.30 - 5.00)
PFOS (branched)	283		4.20 (2.60 - 6.90)	145	4.10 (2.60 - 6.20)	138	4.40 (2.80 - 7.00)
PFOS (total)	283	100	7.10 (4.70 - 13.00)	145	7.20 (4.70 - 12.00)	138	7.10 (4.80 - 13.00)
4PFAS: sum(PFOA; PFNA; PFHxS; PFOS)	283		4.40 (3.17 - 7.80)	145	4.29 (3.01 - 7.74)	138	4.66 (3.23 - 7.80)
lb4PFAS: sum(PFOA (total); PFNA; PFHxS (total); PFOS (total))	283		9.44 (6.60 - 14.98)	145	9.24 (6.32 - 14.35)	138	9.84 (6.65 - 15.46)
7PFAS: sum(MePFOSAA; PFHxS; PFOA (total); PFDA; PFUnDA; PFOS (total); PFNA)	283		9.65 (6.80 - 15.33)	145	9.48 (6.56 - 14.62)	138	10.06 (6.92 - 15.73)

PFAS biomarker levels were assessed in serum and are expressed in µg/L

Abbreviations: *LOQ* limit of quantification, *N* = number

versus 172 cm) and were on average in a further stage of sexual development than males: 54% of females had reached the final stage of growth spurt versus 24% of males, and corresponding numbers were 38% versus 10% for hair growth (head excluded), 13% versus 5% for skin changes, and 48% versus 25% for pubertal development scale standardized for age.

Results of the main exposure-effect analysis are graphically presented in Figs. 2, 3 and 4, those of the secondary analysis in supplementary Figure S2. All regression estimates and p-values are given in supplementary Table S3.

Brief summary of observed associations

Overall, the most well-studied PFAS, PFOS, FOA, PFNA and PFHxS and also the sum parameters showed a clear trend towards lower sex hormone concentrations (only measured in males) and higher SHBG concentrations. In males they also tended to be associated with a shorter length and a delay in growth spurt and to a lesser extent with a delay in the development of some secondary sexual characteristics, mainly body hair development.

In females the best known PFAS and to a lesser extent also the sum parameters showed a clear trend towards a delay in pubertal development and the most well-studied PFAS showed also a trend towards a delay in breast development and a delay in growth spurt and development of some secondary sexual characteristics. No significant associations with length were observed for females.

PFAS showed, in males but not in females, an association with increased thyroid function with higher FT3 and lower TSH concentrations

For PFHpA, PFOS (branched), PFBA and, to a lesser extent, PFDA, some associations were observed that were opposite to those observed for the best known PFAS and the sum parameters.

Table 2 Health outcomes and biomarkers of effect

	Whole Group		**Females**		**Males**	
Parameter	N (%)	Median (P25 - P75)	N (%)	Median (P25 - P75)	N (%)	Median (P25 - P75)
Length (cm)	284	167 (162 - 173)	145	163 (160 - 167)	139	172 (165 - 178)
Z-score of length (age and sex standardised)	284	0.08 (−0.46 - 0.58)	145	0.05 (−0.53 - 0.44)	139	0.13 (−0.40 - 0.73)
Growth spurt	254		124		130	
Not yet started or just started	46 (18%)		17 (14%)		29 (22%)	
In the middle	110 (43%)		40 (32%)		70 (54%)	
Past	98 (39%)		67 (54%)		31 (24%)	
Hair growth (head excluded)	277		139		138	
Not yet started or just started	51 (18%)		13 (9%)		38 (28%)	
In the middle	159 (57%)		73 (53%)		86 (62%)	
Past	67 (24%)		53 (38%)		14 (10%)	
Skin changes	269		137		132	
Not yet started or just started	77 (29%)		29 (21%)		48 (36%)	
In the middle	167 (62%)		90 (66%)		77 (58%)	
Past	25 (9%)		18 (13%)		7 (5%)	
TSH (mIU/L)	282	1.43 (1.05 - 2.12)	144	1.37 (0.93 - 1.85)	138	1.58 (1.17 - 2.29)
FT3 (ng/dL)	283	0.33 (0.30 - 0.36)	145	0.31 (0.29 - 0.33)	138	0.36 (0.34 - 0.38)
FT4 (ng/dL)	283	0.95 (0.89 - 1.02)	145	0.94 (0.88 - 1.01)	138	0.97 (0.90 - 1.02)
Ratio: FT3/FT4	283	0.35 (0.32 - 0.39)	145	0.33 (0.30 - 0.36)	138	0.37 (0.34 - 0.41)
Male pubertal development scale in 3 categories	N/A		N/A		135	
Pre-puberty					7 (5%)	
In-puberty					75 (56%)	
Completing-puberty					53 (39%)	
Male pubertal development scale in 2 categories	N/A		N/A		135	
Pre-puberty or in-puberty					82 (61%)	
Completing-puberty					53 (39%)	
Male pubertal development scale standardised for age	N/A		N/A		135	
Expected stage not yet reached					101 (75%)	
Expected stage reached					34 (25%)	
Voice changes	N/A		N/A		138	
Not yet started or just started					39 (28%)	
In the middle					59 (43%)	
Past					40 (29%)	
Facial hair growth	N/A		N/A		137	
Not yet started or just started					100 (73%)	
In the middle or past					37 (27%)	
TT (ng/dL)	N/A		N/A		137	457 (360 - 576)
Bio available TT (ng/dL)	N/A		N/A		137	200 (132 - 263)
Free TT (ng/dL)	N/A		N/A		137	8.55 (5.65 - 11.23)
SHBG (nmol/L)	N/A		N/A		137	42.00 (28.00 - 52.00)
INHIBB (pg/mL)	N/A		N/A		137	189 (152 - 233)
FSH (IU/L)	N/A		N/A		137	3.10 (2.00 - 4.30)
LH (IU/L)	N/A		N/A		137	2.50 (1.90 - 3.30)
P4 (µg/L)	N/A		N/A		137	<LOD (<LOD - 0.30)
Below detection limit (< 0.2 µg/L)					71 (52)	
Above detection limit (>= 0.2 µg/L)					66 (48)	

Table 2 (continued)

	Whole Group	Females		Males
Female pubertal development scale standardised for age	N/A	133		N/A
Expected stage not yet reached		69 (52%)		
Expected stage reached		64 (48%)		
Breast development in females	N/A	136		N/A
Not yet started or just started		18 (13%)		
In the middle		90 (66%)		
Past		28 (21%)		
Age Menarche	N/A	90	12.77 (12.33 - 13.76)	N/A

Biomarkers of effect were assessed in serum. For all biomarkers, except for P4, levels in all samples exceeded the limit of detection. The number and % above the detection limit is included in the table for P4 only

Abbreviations: *N/A* not applicable (for parameters that were assessed in either males or females), *LOD* limit of detection; *N* number

More detailed description of observed associations

Sex hormones

Associations of PFAS with sex hormones and SHBG were only measured in males.

LH showed negative associations with most PFAS and all 3 tested sum parameters with, for a P75/P25 increase in internal exposure, fold changes in effect between 0.87 (95% CI: 0.79–0.96) for PFHxS and 0.91 (95% CI: 0.86–0.97) for PFOA.

FSH and Inhibin B did not show associations with the analyzed PFAS.

Total testosterone showed negative associations with PFHxS, PFHxS (total), PFOA, PFOA (total), and all 3 tested sum parameters, with fold changes in effect between 0.82 (95% CI: 0.71–0.94) for PFHxS and 0.88 (95% CI: 0.78–1.006) for 4PFAS.

Bioavailable testosterone showed negative associations with PFHxS, PFHxS (total), PFOS, PFOS (total), PFOA (total), PFOA, and all 3 tested sum parameters with fold changes in effect between 0.75 (95% CI: 0.64–0.89) for PFHxS and 0.84 (95% CI:0.74–0.96) for PFOA.

Free testosterone showed almost identical associations compared to bioavailable testosterone, as shown in supplementary material Figure S2.

The progesterone concentration above LOD showed a positive association with PFBA with, for a P75/P25 increase in internal exposure, an OR=1.76 (95% CI: 1.09–2.82) and negative associations with PFOS (total), PFOS, PFOS (branched) and all 3 tested sum parameters with OR's between 0.56 (95% CI: 0.36–0.89) for PFOS (total) and 0.66 (95% CI: 0.45–0.96) for 4PFAS.

SHBG showed positive associations with PFDA, PFNA, PFHxS, PFHxS (total), PFOS, PFOA (total), PFOA and 4PFAS with fold changes in effect between 1.12 (95% CI: 1.02–1.23) for PFDA and 1.07 (95% CI: 0.996–1.17) for PFOA.

Length and growth

Associations of PFAS with length and growth were studied in males and females.

For males, length measured in cm showed negative associations with PFOS (branched), PFOA (total), PFOA, PFOS (total), PFHxS, PFHxS (total), 7PFAS and lb4PFAS with, for a P75/P25 increase in internal exposure, a decrease in length between 2.4 cm (95% CI: 4.1 cm-0.7 cm) for PFOS (branched) and 1.3 cm (95% CI: decrease 2.8- increase 0.2) for PFHxS (total).

The negative trend concerning length observed for males was not present in females.

For males, the odds for reaching a further stage in the growth spurt showed a negative association with most PFAS and all 3 tested sum parameters with proportional OR's between 0.38 (95% CI: 0.23–0.63) for PFHxS and 0.65 (95% CI: 0.44–0.97) for PFOS (total). These negative associations point to a delay in the growth spurt.

Growth spurt in females showed no general trend, the odds for reaching a further stage in the growth spurt showed a negative association with total PFOA (total) and PFOA with proportional OR's of respectively 0.49 (95% CI: 0.28–0.87) and.0.49 (95% CI: 0.28–0.85), pointing to a delay in the growth spurt and on the contrary a positive association with PFOS (branched) with a proportional OR of 1.79 (95% CI: 1.04–3.06), pointing to an advancement of the growth spurt.

Secondary sexual characteristics

Associations of PFAS with secondary sexual characteristics were studied in males and females.

For males, body hair development showed negative associations with PFHxS, PFHxS (total), PFOS, PFNA, PFOS (total), PFDA, PFOS (branched) and all 3 tested sum parameters with proportional OR's between 0.51 (95% CI: 0.31–0.85) for PFHxS and 0.70 (95% CI:

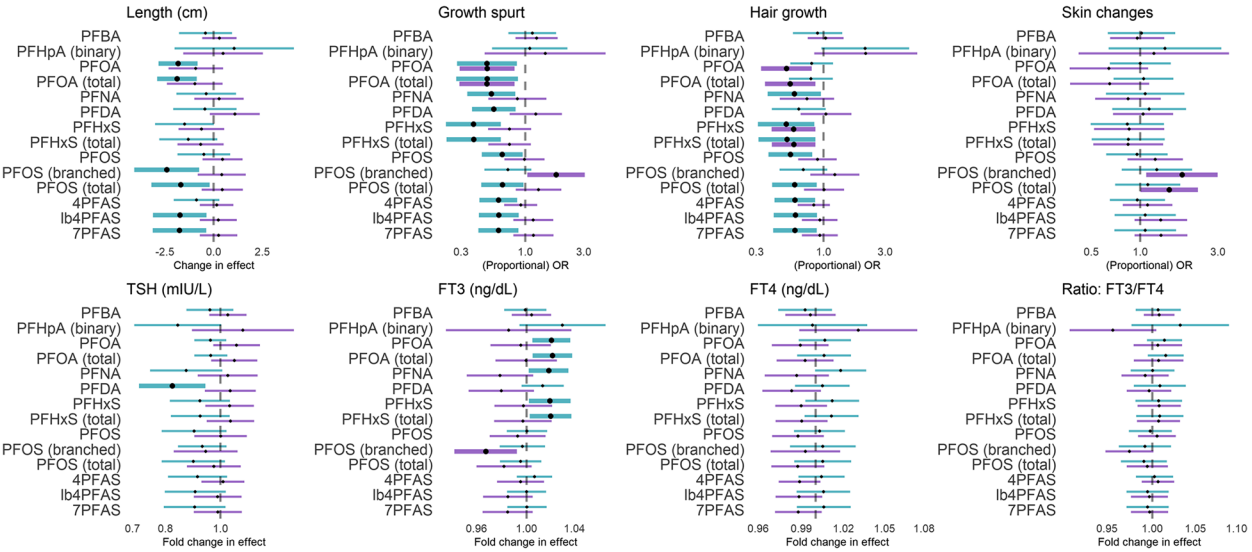

Fig. 2 PFAS and biological and health effects. Observations made for both males and females. Figure 2 shows concerning associations between PFAS serum concentrations and biological and health effects, the effect estimates determined by comparing an exposure at percentile 75 with an exposure at percentile 25. For the log-transformed continuous outcomes TSH, FT3, FT4 and ratio FT3/FT4 the estimates represent the fold change in the effect, for the untransformed continuous outcome length the change in units (cm), for the ordinal outcomes"growth spurt", "hair growth" and "skin changes" the proportional OR. Blue color for boys. Roze color for girls

0.46–1.07) for PFOS (branched). Only one positive association was observed, with PFHpA, with a proportional OR of 2.11 (95% CI: 0.96–4.61).

Body hair development in females showed negative associations with PFOA, PFOA (total), PFHxS and PFHxS (total), with proportional OR's between of 0.52 (95%CI: 0.33–0.81) for PFOA and 0.59 (95%CI: 0.40–0.87) for PFHxS (total).

For males, changes in the skin typical for adolescence showed no associations with PFAS serum concentrations.

For females, changes in the skin typical for adolescence showed positive associations with PFOS (branched) and with total PFOS, with respectively proportional OR's of 1.80 (95%CI: 1.09–2.99) and 1.50 (95%CI: 1.00–2.26).

Development of facial hair in males showed only negative associations with PFOS (total) and PFOS (branched) with respectively proportional OR's of 0.61 (95%CI: 0.35–1.06) and 0.61 (95%CI: 0.36–1.06).

Voice changes in males showed no associations with PFAS serum concentrations.

Breast development in females showed negative associations with PFOA (total), PFOA and PFHxS (total) with proportional OR's between 0.36 (95%CI:0.22–0.58) for PFOA (total) and 0.69 (95%CI: 0.45–1.06) for PFHxS (total).

Pubertal development stage

Associations of PFAS with indicators of pubertal development stage were studied in males and females.

Pubertal development in males (in terms of reaching the minimum pubertal stage expected according to age) showed a positive association with PFHpA with an OR of 2.48 (95%CI: 1.10–5.61) No other associations were observed.

Pubertal development in males, in terms of reaching late puberty or post-puberty or in terms of 3 categories (prepubertal; early pubertal or mid pubertal; late pubertal or post pubertal) showed no associations with PFAS serum concentrations.

Pubertal development in females (in terms of reaching the minimum pubertal stage expected according to age) showed negative associations with PFOA (total), PFOA, PFHxS (total), PFHxS, PFOS, 4PFAS and lb4PAS with OR's between 0.49 (95%CI: 0.29–0.82) for PFOA (total) and 0.73 (95%CI: 0.50–1.06) for lb4PFAS.

Menarche in females showed no associations with PFAS serum concentrations.

Thyroid function

Associations of PFAS with thyroid hormones were studied in males and females.

For males, TSH showed negative associations with PFDA, PFHpA, PFNA and PFOS (total), with fold changes in effect between 0.82 (95%CI: 0.72–0.94) for PFDA and 0.90 (95%CI:0.79–1.02) for PFOS (total).

For females no associations with TSH were observed.

For males, FT3 showed positive associations with PFOA (total), PFOA, PFHxS (total), PFHxS and PFNA

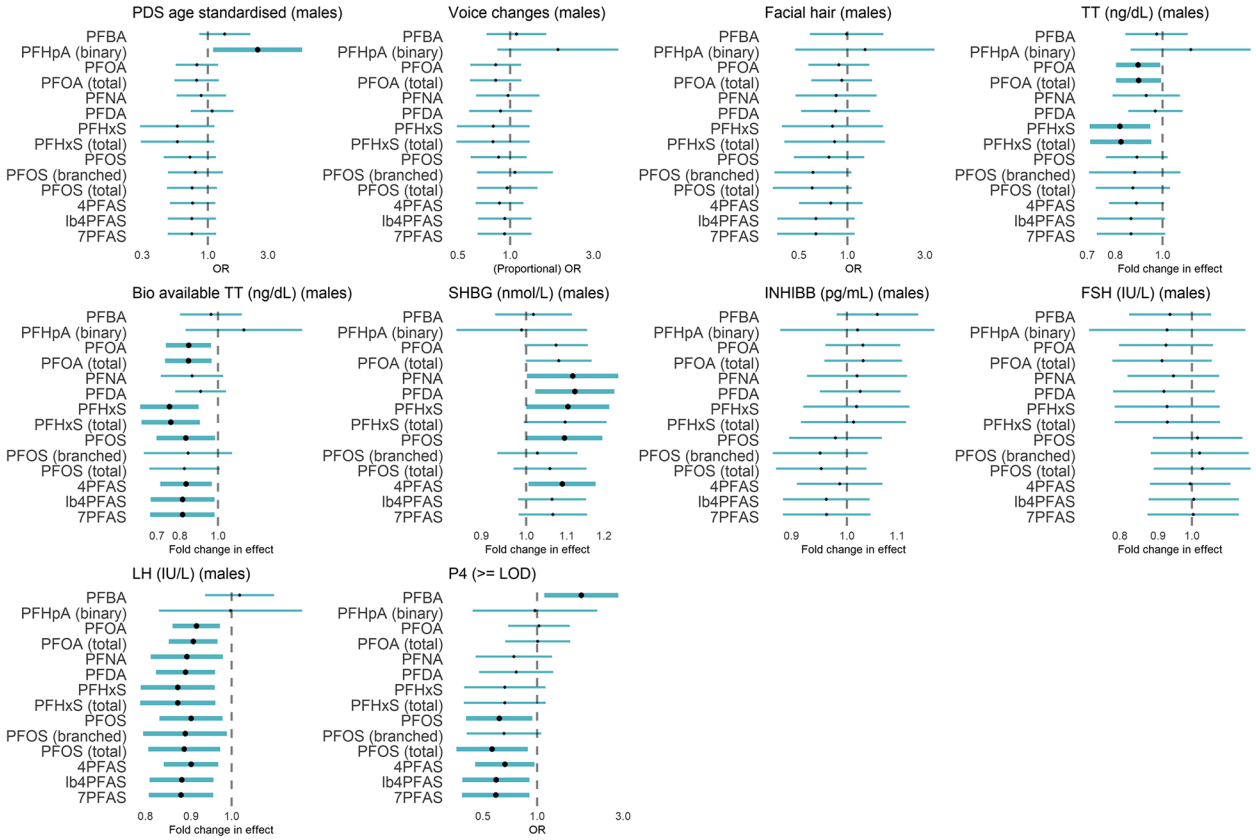

Fig. 3 PFAS and biological and health effects. Observations made only for males. Figure 3 shows, concerning associations between PFAS serum concentrations and biological and health effects, the effect estimates determined by comparing an exposure at percentile 75 with an exposure at percentile 25. For the log-transformed continuous outcomes TT, "Bio available TT", SHBG, INHIBB, FSH and LH the estimates represent the fold change in the effect, for the binary outcomes PDS age standardised, "facial hair" and "P4=LOD" the odds ratio (OR) and for the ordinal outcome "voice changes" the proportional OR

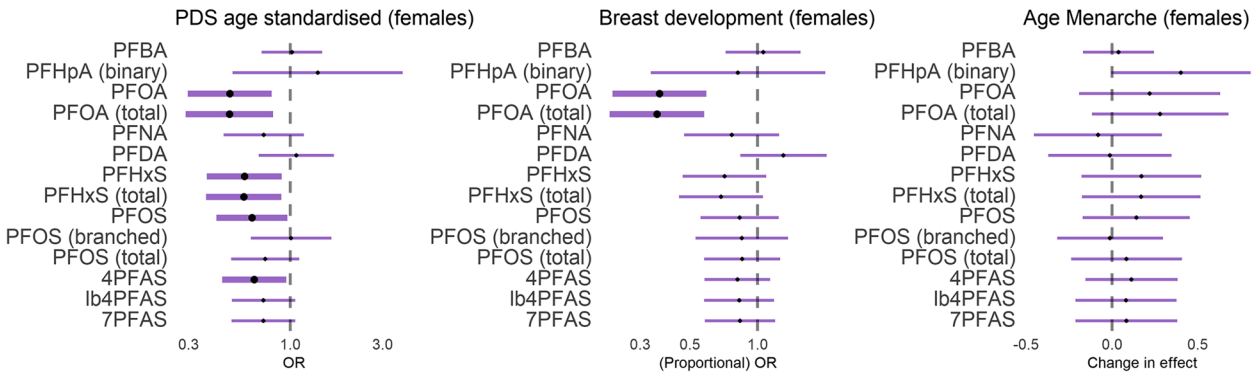

Fig. 4 PFAS and biological and health effects. Observations made only for females. Figure 4 shows, concerning associations between PFAS serum concentrations and biological and health effects, the effect estimates determined by comparing an exposure at percentile 75 with an exposure at percentile 25. For the untransformed continuous outcome "age at menarche" the change in units (years), for the binary outcome 'PDS age standardised" the OR and for the ordinal outcome "breast development" the proportional OR

with fold changes in effect between 1.02 (95%CI:1.01–1.04) for PFOA (total) and 1.02 (95%CI:1.00–1.03) for PFNA. FT4 showed a positive association with PFNA with a fold change in effect of 1.02 (95%CI:1.00–1.04) and the ratio FT3/FT4 showed no associations with the considered PFAS.

For females, FT3 showed a negative association with PFOS (branched) with a fold change in effect of 0.97 (95%CI:0.94–0.99), FT4 showed no associations with PFAS and the ratio FT3/FT4 showed negative associations for PFHpA and PFOS (branched), with respectively fold changes in effect of 0.96 (95%CI: 0.91–1.00) and 0.97 (95%CI: 0.95–1.00).

Discussion

We observed that a higher internal exposure to 11, mainly long chain PFAS, is related to significant changes in thyroid hormones, sex hormones and pubertal development in adolescents residing in the neighborhood of a PFAS manufacturing site. The PFAS serum concentrations in our 3M study population were only slightly higher than those observed in 1957 teenagers (12–18 years) from 9 European countries as part of the HBM4EU aligned studies (2014–2021) [36]. They were for PFOA, PFOS and PFHxS respectively about 13%, 13% and 24% higher, but for PFNA about 13% lower. Compared to Flemish reference values measured in 2017-2018 in a representative sample of Flemish adolescents the serum PFOS concentrations in our 3M study were clearly higher, with a geometric mean and P95 at respectively 3.25 µg/L and 30µg/L against 2.16 µg/L and 7.3 µg/L for the Flemish reference values (see Figure S3).

We observed, for males, statistically significant and robust associations between internal exposure to PFAS and concentrations in blood of LH, TT, P4 bioavailable TT and SHBG. The associations of LH, TT, P4 and bioavailable TT were negative, those with SHBG were positive. Interesting is the finding that internal exposure to some PFAS was associated as well with lower testosterone levels as with lower LH levels, suggesting that lower LH levels might, at least partly, underlie the lower sex hormone levels, in view of the fact that sex hormone levels are known to exert a negative feedback on LH levels [37]. A similar phenomenon was also observed by Pan et al. [38] who found that both levels of testosterone and of LH were negatively associated with phthalates. In addition, higher SHBG concentrations associated with PFAS exposure may partly contribute to the lower level of active sex hormones in the adolescent population included in our study. Consistent with these hormonal findings in males we found significant negative associations between internal exposure to PFAS and boy's body length, growth spurt advancement and body hair development, and a trend towards a negative association with facial hair development. Also, but less directly, consistent with our observations in males, are the negative associations observed in females between internal exposure to linear and total PFOA and growth spurt advancement, linear and total PFOA and linear and total PFHxS and body hair development, linear and total PFOA and (only marginally) total PFHxS and breast development and linear and total PFOA, linear and total PFHxS and linear PFOS and pubertal development.

Analogous findings were reported in the literature. Lopez-Espinosa et al. [39] reported a negative association, in a cross sectional study on males aged 6–9 residing close to a chemical plant in West Virginia, between PFOA and PFOS and serum testosterone concentrations. Joensen et al. [40] reported, in a cross sectional study, a negative association in young healthy men of the general Danish population between PFOS and serum testosterone concentrations. He et al. [41] observed, in a cross-sectional study on 921 children and adolescents aged 6–19 participating in the 2013-2016 National Health and Nutrition Examination Survey (NHANES) in the USA, an inverse association of a low concentration of a PFAS mixture with testosterone and, in boys, a positive association with SHBG. Guo et al. [42] found, in a cross sectional study on 525 adolescents participating in the 2013-2016 NHANES, that mixed exposure to 5 PFASs was negatively associated with testosterone in as well female as male adolescents, but found, in contrast to our study, also a negative association with SHBG. Cui et al. [43] found, in a cross-sectional study on 651 adult men from Nanjing, China, an inverse association between serum concentration of a sum of PFASs and testosterone. However, there is no complete consensus in the literature concerning the association of PFAS and sex hormones. Rodriguez-Carrillo et al. [44] did not find a negative association between serum PFAS levels and serum testosterone concentrations in a cross-sectional study on male European teenagers from the general population. Neither did Liu et al. [45], in a longitudinal study in which PFAS concentrations were assessed from pregnancy to adolescence, observe a pattern for associations between internal PFAS exposure and serum concentrations of reproductive hormones in male children. Di Nisio et al. [46], in a cross-sectional study on 212 exposed males from the Veneto region and 171 nonexposed controls, found a positive association between PFAS serum concentrations and testosterone. Xie et al. [47] found, in a large NHANES 2015-2016 cohort of males and females aged from 12 to 80, that PFAS exposure appears to disrupt sex hormones in a sex-, age-, and compound-specific manner. They reported a positive association between PFDA, PFOS and PFHxS and total serum testosterone among

males of all ages. Of these male participants 17.5% were aged 12–19, and in this age group Xie et al. [47] found a positive association between PFDA and PFOS with estradiol levels whereas the association of PFOA with estradiol was negative.

However it seems likely that in the majority of the epidemiological studies, an increase in internal exposure to PFAS is associated with a decrease in testosterone concentration. This was already suggested by a systematic review by Bach et al. [48] and confirmed by later reviews. A meta-analysis by Sang et al. [49] revealed that PFNA and PFOA exposures were negatively correlated with testosterone. A systematic review and meta-analysis by Li et al. [50] also found a negative association between PFOA and PFOS exposure with male testosterone levels.

The biological mechanisms by which PFAS may impact the endocrine and developmental system are not completely understood. Several biological mechanisms have been put forward. Binding of PFAS to receptors was reported for estrogen receptors [11], vitamin D receptor [2], peroxisome proliferator- activated receptor-alpha [51], constitutive androstane receptor [51] and pregnane X receptor [52]. Also a less specific interaction of PFAS with proteins was reported [53] as well as the infiltration of PFAS in the cellular lipid membranes [54]. PFAS were also observed to disrupt gap-junctional intercellular communication [55]; to have neurotoxic effects [12, 56–59]; and to be associated with an increase in length of telomeres [60–62]. At a somewhat higher level of biological integration epigenetic effects were observed, involving disturbance (often diminution) of DNA methylation [63], increase in acetylation of histones [64] and changes in amounts of micro-RNA [65]. As also neurotoxic effects were observed in animals [66], as PFAS were associated with increased serum concentrations of Kisspeptin in male adolescents [44] and with associations with neuropsychic effects in humans [7, 67–69], it seems plausible that an effect of PFAS on the production of LH in the hypothalamus might be involved in the observed disturbance of sex hormones. In their review Shi et al. [70] state that epidemiological studies confirmed that PFOS and PFOA are not only associated with reduced testosterone levels in humans but also with damage to the integrity of the blood testicular barrier. Although there is still some controverse in epidemiological terms, the evidence from animal studies, indicating that PFAS disturb reproductive hormones and decrease sperm quality, is relatively consistent [70].

That PFAS disturb the functioning of male sex organs is suggested by substantial experimental and epidemiological evidence. A review by Tarapore and Ouyang [18] gives an overview of data stemming from animal experiments on effects of PFAS on Germ cells, Sertoli cells and Leydig cells and of human epidemiological data and suggests that PFAS might contribute to male infertility. Di Nisio et al. [46] found, in a cohort study on 383 young males aged 18–24 residing in 3 area's differing in environmental PFAS pollution, an association between PFAS serum concentrations and a decrease in anogenital distance, length of the penis, testicular volume and sperm quality, and, paradoxically, an increase in serum testosterone concentration. Higher serum concentrations of PFOS and PFOA in young Danish men were associated with lower numbers of normal sperm cells [71]. Also PFAS can, possibly in combination with other pollutants such as polychlorobiphenyls, directly disturb the production of testosterone in the testes [72]. A meta-analysis by Wang et al. [73] found that PFAS were associated with a lower quality of human sperm.

Consistent with the observed disruption of sex hormone physiology (measured in male adolescents only) we also noted, as well in females as in males, a relatively outspoken delay in physiological processes occurring in puberty, such as growth spurt and length (mainly in males), secondary sexual characteristics (both females and males) and pubertal development scale changes (mainly in females). A delay in pubertal development in girls was also reported by Lopez-Espinosa et al. [39], in a cross-sectional study on 2931 girls living near a chemical plant, by Liu et al. [45], in a follow-up study on 200 mother-child pairs from the HOME Study in Cincinnati, Carwile et al. [74] in the Project Viva cohort, a Boston-area prospective cohort, and Pinney et al. [75], in a longitudinal study of young girls in Greater Cincinnati and the San Francisco Bay Area. Lopez-Espinosa et al. [39], in a study on 3076 boys living near a chemical plant and Forthun et al. [76], in a cross-sectional study on 300 Norwegian boys aged 9–16 from the general population in Bergen, reported a delay in puberty for boys and Ernst et al. [77], in a study including prenatal assessment and follow-up until full maturation, also, but only for PFNA and PFDA and not for other PFAS. Liu et al. [45] and Carwile et al. [74] did not find a delay in puberty for boys. There were also studies reporting earlier puberty. Ernst et al. [77] reported earlier puberty in girls, and in boys earlier puberty in association with PFHxS and PFHpS. In a study on 921 adolescents from the general population in Northern Norway [78] PFDA and PFUnDA were positively associated with early menarche, while sum of PFAS and PFOA were positively associated with puberty development score in boys. According to a systematic review by Lee et al. [19] the evidence for an association between PFAS exposure and timing of puberty remains inconclusive. However, the data provided by Liu et al. [45], Carwile et al. [74], Pinney et al. [75], Forthun et al. [76] and those presented in this report were not taken

into account in the Lee et al. [19] systematic review and could possibly be considered as suggesting that PFAS are indeed associated with a delay in pubertal development.

Moreover, we demonstrated that higher PFAS exposure was associated with significant alterations in thyroid hormones. For males, internal exposure to PFAS showed a trend towards higher thyroid hormone concentrations and lower TSH concentrations, whereas for females the observed trend was towards lower thyroid hormone concentrations and slightly higher TSH concentrations.

Our findings concerning a disturbance of thyroid hormones in association with internal expose to PFAS are consistent with at least part of the literature. Freire et al. [79] also found PFAS to be associated in male adolescents with higher free T4 serum concentrations. The interaction of PFAS with thyroid function appears to be very complex, but quite important. Zheng et al. [80] reported a significant association of higher internal exposure to PFAS and an increased risk of thyroid disorders, especially hypothyroidism. Rodriguez-Carrillo et al. [44], in a cross-sectional study on European teenagers from the general population, found in female adolescents an association between serum PFOA and PFAS mixture concentrations with lower FT4 and higher FT3 levels and in male adolescents an association of PFOA with lower FT4 and of a PFAS mixture with higher TSH levels. A meta analysis by Kim et al. [81] revealed complex associations between internal exposure to PFAS and thyroid hormones, that can be different depending on the PFAS concentrations, as also observed by Tillaut et al. [82] in a cross-sectional study on 249 boys and 227 girls of the PELAGIE mother-child cohort in Brittany, France. Lopez-Espinosa et al. [83], in a study on 10,725 children aged 1–17 living near a Teflon manufacturing facility in the Mid-Ohio Valley (USA), observed an increased OR for hypothyroidism in association with higher serum PFOA concentrations in children, but an IQR shift in serum PFOA was not associated with TSH or TT4 levels in all children combined. The complexity of the interaction between PFAS and the thyroid function probably rests in part on the property of PFAS to bind to proteins, and in particular on the capacity of at least some PFAS to bind strongly to albumins [84] and to the thyroid hormone transport protein transthyretin [85]. Chang et al. [86] showed in rats that oral doses of PFOS increased FT4 due to the ability of PFOS to compete with thyroxine for binding proteins resulting in transiently increased tissue availability of the thyroid hormones and turnover of T4 with a resulting reduction in serum T4.

Our observations suggest, as can be seen from Figs. 2, 3, 4 and Figure S2 that the different PFAS congeners show some differences in their biological effects. In particular PFBA, PFHPA and branched PFOS, and to a lesser extent PFDA, show, in our study, some associations with effect parameters that differ from those of the most studied congeners. In Table S4 in supplementary material these associations are listed. Differences in effects are probably due to differences in chemical structure, such as chain length, which influence the toxicodynamic and toxicokinetic properties of PFAS, and their interaction with biological molecules.

Our observations suggest that PFAS have, in relation to pubertal development, essentially similar effects in male and female adolescents. However, also a few differences were observed. PFAS showed a more pronounced negative association with length for males than for females, and for males also a more pronounced association with a delay in growth spurt than for females. However, in relation to other parameters of pubertal development effects were somewhat more pronounced in females. In Table S5 in supplementary material these differences are listed. In relation to thyroid hormones however different associations were observed for males and females. This is in line with previous research in European adolescents, that also strongly suggests that exposure to PFAS may disrupt thyroid hormone homeostasis in a sex-specific manner [44].

We also paid attention to the issue of reverse causality, implying that adolescents with less advanced puberty, weighing less than those with more advanced puberty, would have less dilution of PFAS in their bodies and so have higher serum PFAS levels. Participating male adolescents with a 7PFAS serum concentration above the median value had a mean body weight of 56,52 kg, those with a 7PFAS serum concentration below the median value had a mean body weight of 57.69 kg or 2.1% more. This has to be compared with the range of measured 7PFAS serum concentrations, with p75 and p90 values respectively at 225.1% and 589.4 % of respectively p25 and p10 values. Also, additional correction for body weight had very little impact on the observed associations.

This study has several limitations. The number of adolescents in this study was limited. The cross-sectional nature of the study limits the value of the study with regard to establishing a cause-effect relationship. As we choose not to adjust for dietary factors to avoid capture of the effect of the exposure on the outcome by the source variable instead of the exposure variable we cannot exclude confounding by diet. Information was not available on the iodine status of adolescents, which is essential for thyroid health and may influence the relationship between PFAS exposure and thyroid hormones [44]. The use of single-pollutant models did not account for potential confounding by correlated PFAS nor for potential mixture compositions where the presence of certain compounds may alter the activity of others. So it is likely that the associations observed for some of the

individual PFAS are in fact due to the correlated presence of other PFAS. Confounding bias can be avoided by using specialized mixture methods (e.g. Bayesian Kernel Machine Regression, Bayesian model averaging…). We did however not use such methods because of the high correlations between PFAS substances in combination with the low sample size (stratifying by sex) and relatively weak effects, which makes it very difficult to distinguish the effects of individual substances. Moreover, the estimation of the overall mixture effect was less relevant in this study because of the opposite direction of associations observed for some substances. The assessment of stage of pubertal development rested on self-administered questionnaires and not on examination by specialised physicians.

Strengths of our studies rest on the facts that exposure assessment was done through validated chemical analysis, physiological status related to pubertal development was based on validated clinical biological analysis and that sampling and biological measurements were performed by trained study nurses. Also, support for the credibility of our puberty assessment can be found in the observation by Jones et al. [87] showing that self-assessed pubertal development was positively associated with hormonal biomarkers of puberty.

Conclusions

We observed significant negative associations of PFAS serum concentrations with sex hormone levels and significant positive associations with SHBG levels in male adolescents. Moreover, a significant delay in physiological processes occurring in puberty was observed in females and males. Associations with thyroid hormones differed significantly by sex. Our findings on associations between PFAS internal exposure and biologically relevant differences in blood hormone levels, consistent with our findings on associations of PFAS exposure with different parameters of pubertal development, contribute to the scientific insight in PFAS-related biological and health effects. Our findings in this study population are robust and biologically relevant, they confirm that continued efforts to lower PFAS exposure in this study area are warranted. Further longitudinal research is recommended to establish whether the PFAS exposure in adolescents increases the risk later in life of thyroid disease and lasting developmental health repercussions.

Abbreviations

4PFAS	Sum of the linear PFOA, PFNA, PFHxs, and PFOS
7PFAS	Sum of linear N-Methylperfluorooctanesulfonamide (MePFOSAA), linear PFHxS, total PFOA, linear PFDA, linear Perfluoroundecanoic acid (PFUnDA), total PFOS, and linear PFNA
BELAC	Belgian accreditation system
C8 Compounds	compounds with a chain of 8 carbon atoms
E2	Estradiol
ECLIA	Electrochemiluminescence immuno-assay
ELISA	Enzyme Linked Immune Sorbent Assay
EFSA	The European Food Safety Authority
ESI	Electrospray ionisation
FT3	Free triiodothyronine
FT4	Free thyroxine
GEE	Generalized Estimating Equations
INHIBB	Inhibin B
lb4PFAS	Sum of total PFOA, linear PFNA, total PFHxS, and total PFOS
LH	Luteinising hormone
LOD	Limit of detection
LOQ	Limit of quantification
MeOH	Methanol
MePFOSAA	N-Methylperfluorooctanesulfonamide
MRM	Multiple Reaction Monitoring
NHANES	National Health and Nutrition Examination Survey
OR	Odds ratio
P4	Progesterone
PDS	Pubertal development scale
PFAS	Per- and polyfluoroalkyl substances
PFBA	Perfluorobutanoic acid
PFDA	Perfluorodecanoic Acid
PFHpA	Perfluoroheptanoic acid
PFHxS	Perfluorohexanesulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
SHBG	Sex hormone-binding globulin
TSH	Thyroid stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
TT	Testosterone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12940-025-01188-1.

Supplementary Material 1.

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Authors' contributions

NVL participated in the setting up of the study and was the main contributor in writing the manuscript. BC participated in the setting up of the study, in statistical analyses and in writing the manuscript. SR participated in the setting up of the study, in statistical analyses and in writing the manuscript. SV was responsible for the PFAS measurements. ED participated in the setting up of the study and organised the field work. AC participated in the setting up of the study and was responsible for the general organisation of the study. ML participated in the setting up of the study and in writing the manuscript. GS participated in the setting up of the study and in writing the manuscript. VH participated in the setting up of the study, was involved in the scientific direction of the study and participated writing the manuscript. All authors reviewed the manuscript.

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Data availability
Access to data and materials can be requested from the "Toezichtcommissie van de Vlaamse Humane biomonitoring (Monitoring committee of the Flemish Human Biomonitoring). This committee can be contacted through TZC@provincieantwerpen.be

Declarations

Ethics approval and consent to participate
The study was approved by the Ethical Committee of the University of Antwerp/University Hospital of Antwerp, Belgium (Belgian Registry Number: B3002022000041).
As well the participants as at least one of their parents had to complete a document of informed consent, also stipulating for which aspects of the study they stood as a candidate (human biomonitoring, measurements in house dust, measurements in environmental samples), whether they wanted to receive their personal results, whether these personal results could be transmitted to their family doctor and whether they accepted to be contacted again in the future.

Consent for publication
Not applicable

Competing interests.
The authors declare no competing interests.

Author details
[1]Archeology, Environmental Changes and Geochemistry, Vrije Universiteit Brussel, Pleinlaan 2, Brussels 1050, Belgium. [2]Ghent University Hospital, Study Centre for Carcinogenesis and Primary Prevention of Cancer, De Pintelaan 185, Ghent 9000, Belgium. [3]VITO Health, Flemish Institute for Technological Research (VITO), Mol 2400, Belgium. [4]Materials and Chemistry Unit, Flemish Institute for Technological Research (VITO), Boeretang 200, Mol 2400, Belgium. [5]Provincial Institute of Hygiene, Kronenburgstraat 45, Antwerp 2000, Belgium. [6]Department of Biomedical Sciences & Toxicological Centre, University of Antwerp, Universiteitsplein 1, Wilrijk 2610, Belgium.

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References

1. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, et al. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag. 2011;7:513–41.
2. Di Nisio A, Rocca MS, De Toni L, Sabovic I, Guidolin D, Dall'Acqua S, et al. Endocrine disruption of vitamin D activity by perfluoro-octanoic acid (PFOA). Sci Rep. 2020;10:16789.
3. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A, et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol. 2017;14:188–95.
4. Abraham K, Mielke H, Fromme H, Völkel W, Menzel J, Peiser M, et al. Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch Toxicol. 2020;94:2131–47.
5. Bartell SM, Vieira VM. Critical review on PFOA, kidney cancer, and testicular cancer. J Air Waste Manage Assoc. 2021;71:663–79.
6. Schrenk D, Bignami M, Bodin L, Chipman JK, del Mazo J, Grasl-Kraupp B, et al. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J. 2020;18:e06223.
7. van Larebeke N, Koppen G, Decraemer S, Colles A, Bruckers L, Den Hond E, et al. Per- and polyfluoroalkyl substances (PFAS) and neurobehavioral function and cognition in adolescents (2010–2011) and elderly people (2014): results from the Flanders Environment and Health Studies (FLEHS). Environ Sci Eur. 2022;34:98.
8. Canova C, Barbieri G, Zare Jeddi M, Gion M, Fabricio A, Daprà F, et al. Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environ Int. 2020;145:106117.
9. Barry V, Winquist A, Steenland K. Perfluorooctanoic Acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environ Health Perspect. 2013;121:1313–8.
10. Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, et al. Serum Concentrations of Per- and Polyfluoroalkyl Substances and Risk of Renal Cell Carcinoma. JNCI J Natl Cancer Inst. 2021;113:580–7.
11. Omoike OE, Pack RP, Mamudu HM, Liu Y, Wang L. A cross-sectional study of the association between perfluorinated chemical exposure and cancers related to deregulation of estrogen receptors. Environ Res. 2021;196:110329.
12. Cao Y, Ng C. Absorption, distribution, and toxicity of per- and polyfluoroalkyl substances (PFAS) in the brain: a review. Environ Sci Process Impacts. 2021;23:1623–40.
13. Feng Y, Bai Y, Lu Y, Chen M, Fu M, Guan X, et al. Plasma perfluoroalkyl substance exposure and incidence risk of breast cancer: a case-cohort study in the Dongfeng-Tongji cohort. Environ Pollut. 2022;306:119345.
14. Hoyeck MP, Matteo G, MacFarlane EM, Perera I, Bruin JE. Persistent organic pollutants and β-cell toxicity: a comprehensive review. Am J Physiol Endocrinol Metab. 2022;322:E383-413.
15. Chung SM, Heo D-G, Kim J-H, Yoon JS, Lee HW, Kim J-Y, et al. Perfluorinated compounds in adults and their association with fasting glucose and incident diabetes: a prospective cohort study. Environ Health. 2022;21:101.
16. Meneguzzi A, Fava C, Castelli M, Minuz P. Exposure to perfluoroalkyl chemicals and cardiovascular disease: experimental and epidemiological evidence. Front Endocrinol (Lausanne). 2021;12:706352.
17. Wee SY, Aris AZ. Revisiting the "forever chemicals", PFOA and PFOS exposure in drinking water. NPJ Clean Water. 2023;6:57.
18. Tarapore P, Ouyang B. Perfluoroalkyl chemicals and male reproductive health: do PFOA and PFOS increase risk for male infertility? Int J Environ Res Public Health. 2021;18:3794.
19. Lee YJ, Jung HW, Kim HY, Choi Y-J, Lee YA. Early-life exposure to per- and poly-fluorinated alkyl substances and growth, adiposity, and puberty in children: a systematic review. Front Endocrinol (Lausanne). 2021;12:683297.
20. Lucaccioni L, Trevisani V, Marrozzini L, Bertoncelli N, Predieri B, Lugli L, et al. Endocrine-disrupting chemicals and their effects during female puberty: a review of current evidence. Int J Mol Sci. 2020;21:2078.
21. European Environment Agency. Effects of PFAS on human health [Infographic]. 2020 [updated 09/12/2022. 2022. https://www.eea.europa.eu/signals/signals-2020/infographics/effects-of-pfas-on-human-health/view.
22. Hölzer J, Lilienthal H, Schümann M. Human Biomonitoring (HBM)-I values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) - Description, derivation and discussion. Regul Toxicol Pharmacol. 2021;121:104862.
23. Schümann M, Lilienthal H, Hölzer J. Human biomonitoring (HBM)-II values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) - Description, derivation and discussion. Regul Toxicol Pharmacol. 2021;121:104868.
24. ATSDR. Potential health effects of PFAS chemicals. 2022. [updated 2022/11/01/T02:54:31Z]. https://www.atsdr.cdc.gov/pfas/health-effects/index.html.
25. Gallo E, Barbiellini Amidei C, Barbieri G, Fabricio ASC, Gion M, Pitter G, et al. Perfluoroalkyl substances and thyroid stimulating hormone levels in a highly exposed population in the Veneto Region. Environ Res. 2022;203:111794.
26. Andersson EM, Scott K, Xu Y, Li Y, Olsson DS, Fletcher T, et al. High exposure to perfluorinated compounds in drinking water and thyroid disease. A cohort study from Ronneby, Sweden. Environ Res. 2019;176:108540.
27. Li Y, Barregard L, Xu Y, Scott K, Pineda D, Lindh CH, et al. Associations between perfluoroalkyl substances and serum lipids in a Swedish adult population with contaminated drinking water. Environ Health. 2020;19:33.
28. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab. 1999;84:3666–72.
29. Roelants M, Hauspie R, Hoppenbrouwers K. References for growth and pubertal development from birth to 21 years in Flanders, Belgium. Ann Hum Biol. 2009;36:680–94.

30. Marshall WA, Tanner JM. Growth and physiological development during adolescence. Annu Rev Med. 1968;19:283–300.
31. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44:291–303.
32. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970;45:13–23.
33. Akutsu N, Ooguri M, Onodera T, Kobayashi Y, Katsuyama M, Kunizawa N, et al. functional characteristics of the skin surface of children approaching puberty: age and seasonal influences. Acta Dermato Venereol. 2009;89:21–7.
34. Eady A, Bojar R. Spotting the onset of puberty-the secret's in the skin. Microbiology Today. 2001;28:178–81.
35. Koopman-Verhoeff ME, Gredvig-Ardito C, Barker DH, Saletin JM, Carskadon MA. Classifying pubertal development using child and parent report: comparing the pubertal development scales to tanner staging. J Adolesc Health. 2020;66:597–602.
36. Richterová D, Govarts E, Fábelová L, Rausová K, Martin LR, Gilles L, Remy S, Colles A, Rambaud L, Riou M, Gabriel C. PFAS levels and determinants of variability in exposure in European teenagers–Results from the HBM4EU aligned studies (2014–2021). Int J Hyg Environ Health. 2023;247:114057.
37. Pitteloud N, Dwyer AA, DeCruz S, Lee H, Boepple PA, Crowley WF, et al. Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab. 2008;93:784–91.
38. Pan Y, Jing J, Dong F, Yao Q, Zhang W, Zhang H, et al. Association between phthalate metabolites and biomarkers of reproductive function in 1066 Chinese men of reproductive age. J Hazard Mater. 2015;300:729–36.
39. Lopez-Espinosa M-J, Fletcher T, Armstrong B, Genser B, Dhatariya K, Mondal D, et al. Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environ Sci Technol. 2011;45:8160–6.
40. Joensen UN, Veyrand B, Antignac J-P, Blomberg Jensen M, Petersen JH, Marchand P, et al. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum Reprod. 2013;28:599–608.
41. He Y, Hu C, Zhang Y, Fan X, Gao W, Fang J, et al. Association of perfluoroalkyl and polyfluoroalkyl substances with sex hormones in children and adolescents 6–19 Years of age. Environ Pollut. 2023;329:121707.
42. Guo J, Huang S, Yang L, Zhou J, Xu X, Lin S, et al. Association between polyfluoroalkyl substances exposure and sex steroids in adolescents: the mediating role of serum albumin. Ecotoxicol Environ Saf. 2023;253:114687.
43. Cui Q, Pan Y, Wang J, Liu H, Yao B, Dai J. Exposure to per- and polyfluoroalkyl substances (PFASs) in serum versus semen and their association with male reproductive hormones. Environ Pollut. 2020;266:115330.
44. Rodríguez-Carrillo A, Salamanca-Fernández E, den Hond E, Verheyen VJ, Fábelová L, Murinova LP, et al. Association of exposure to perfluoroalkyl substances (PFAS) and phthalates with thyroid hormones in adolescents from HBM4EU aligned studies. Environ Res. 2023;237:116897.
45. Liu Y, Calafat AM, Chen A, Lanphear BP, Jones NHY, Cecil KM, et al. Associations of prenatal and postnatal exposure to perfluoroalkyl substances with pubertal development and reproductive hormones in females and males: the HOME study. Sci Total Environ. 2023;890:164353.
46. Di Nisio A, Sabovic I, Valente U, Tescari S, Rocca MS, Guidolin D, et al. Endocrine disruption of androgenic activity by perfluoroalkyl substances: clinical and experimental evidence. J Clin Endocrinol Metab. 2019;104:1259–71.
47. Xie X, Weng X, Liu S, Chen J, Guo X, Gao X, et al. Perfluoroalkyl and polyfluoroalkyl substance exposure and association with sex hormone concentrations: results from the NHANES 2015–2016. Environ Sci Eur. 2021;33:69.
48. Bach CC, Vested A, Jørgensen KT, Bonde JPE, Henriksen TB, Toft G. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. Crit Rev Toxicol. 2016;46:735–55.
49. Sang L, Ge Y, Liu F, Wei K, Shen X, Zhang Y, et al. Association between per- and polyfluoroalkyl substances and sex hormone levels in males based on human studies. Ecotoxicol Environ Saf. 2024;271:115998.
50. Li L, Guo Y, Ma S, Wen H, Li Y, Qiao J. Association between exposure to per- and perfluoroalkyl substances (PFAS) and reproductive hormones in human: a systematic review and meta-analysis. Environ Res. 2024;241:117553.
51. Pierozan P, Cattani D, Karlsson O. Tumorigenic activity of alternative per- and polyfluoroalkyl substances (PFAS): mechanistic in vitro studies. Sci Total Environ. 2022;808:151945.
52. Su S, Billy LJ, Chang S, Gonzalez FJ, Patterson AD, Peters JM. The role of mouse and human peroxisome proliferator-activated receptor-α in modulating the hepatic effects of perfluorooctane sulfonate in mice. Toxicology. 2022;465:153056.
53. Fedorenko M, Alesio J, Fedorenko A, Slitt A, Bothun GD. Dominant entropic binding of perfluoroalkyl substances (PFASs) to albumin protein revealed by 19F NMR. Chemosphere. 2021;263:128083.
54. Nouhi S, Ahrens L, Campos Pereira H, Hughes AV, Campana M, Gutfreund P, et al. Interactions of perfluoroalkyl substances with a phospholipid bilayer studied by neutron reflectometry. J Colloid Interface Sci. 2018;511:474–81.
55. López-Arellano P, López-Arellano K, Luna J, Flores D, Jiménez-Salazar J, Gavia G, et al. Perfluorooctanoic acid disrupts gap junction intercellular communication and induces reactive oxygen species formation and apoptosis in mouse ovaries. Environ Toxicol. 2019;34:92–8.
56. Ko MY, Park H, Chon S-H, Kim Y-B, Cha S-W, Lee B-S, et al. Differential regulations of neural activity and survival in primary cortical neurons by PFOA or PFHpA. Chemosphere. 2024;352:141379.
57. Zhou A, Wang L, Pi X, Fan C, Chen W, Wang Z, et al. Effects of perfluorooctane sulfonate (PFOS) on cognitive behavior and autophagy of male mice. J Toxicol Sci. 2023;48:513–26.
58. Basaly V, Hill J, Bihaqi SW, Marques E, Slitt AL, Zawia NH. Developmental Perfluorooctanesulfonic acid (PFOS) exposure as a potential risk factor for late-onset Alzheimer's disease in CD-1 mice and SH-SY5Y cells. Neurotoxicology. 2021;86:26–36.
59. Tian C, Cai H, Ao Z, Gu L, Li X, Niu VC, et al. Engineering human midbrain organoid microphysiological systems to model prenatal PFOS exposure. Sci Total Environ. 2024;947:174478.
60. Eick SM, Goin DE, Cushing L, DeMicco E, Park J-S, Wang Y, et al. Mixture effects of prenatal exposure to per- and polyfluoroalkyl substances and polybrominated diphenyl ethers on maternal and newborn telomere length. Environ Health. 2021;20:76.
61. Huang H, Wang Q, He X, Wu Y, Xu C. Association between polyfluoroalkyl chemical concentrations and leucocyte telomere length in US adults. Sci Total Environ. 2019;653:547–53.
62. Clarity C, Trowbridge J, Gerona R, Ona K, McMaster M, Bessonneau V, et al. Associations between polyfluoroalkyl substance and organophosphate flame retardant exposures and telomere length in a cohort of women firefighters and office workers in San Francisco. Environ Health. 2021;20:97.
63. Leung Y-K, Ouyang B, Niu L, Xie C, Ying J, Medvedovic M, et al. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics. 2018;13:290–300.
64. Alam MN, Han X, Nan B, Liu L, Tian M, Shen H, et al. Chronic low-level perfluorooctane sulfonate (PFOS) exposure promotes testicular steroidogenesis through enhanced histone acetylation. Environ Pollut. 2021;284:117518.
65. Xu Y, Jurkovic-Mlakar S, Lindh CH, Scott K, Fletcher T, Jakobsson K, et al. Associations between serum concentrations of perfluoroalkyl substances and DNA methylation in women exposed through drinking water: a pilot study in Ronneby, Sweden. Environ Int. 2020;145:106148.
66. Sammi SR, Foguth RM, Nieves CS, De Perre C, Wipf P, McMurray CT, et al. Perfluorooctane Sulfonate (PFOS) produces dopaminergic neuropathology in caenorhabditis elegans. Toxicol Sci. 2019;172:417–34.
67. Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, et al. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort. Environ Health Perspect. 2015;123:367–73.
68. Lenters V, Iszatt N, Forns J, Čechová E, Kočan A, Legler J, et al. Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: a multi-pollutant analysis of a Norwegian birth cohort. Environ Int. 2019;125:33–42.
69. Gao Y, Zhang Y, Luo J, Mao D, Lei X, Liu C, et al. Effect modification by maternal vitamin D status in the association between prenatal exposure to per- and polyfluoroalkyl substances and neurodevelopment in 2-year-old children. Environ Int. 2024;185:108563.

70. Shi W, Zhang Z, Li M, Dong H, Li J. Reproductive toxicity of PFOA, PFOS and their substitutes: a review based on epidemiological and toxicological evidence. Environ Res. 2024;250:118485.
71. Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebæk NE, Jørgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect. 2009;117:923–7.
72. Petersen MS, Halling J, Jørgensen N, Nielsen F, Grandjean P, Jensen TK, et al. Reproductive function in a population of young faroese men with elevated exposure to Polychlorinated Biphenyls (PCBs) and Perfluorinated Alkylate Substances (PFAS). Int J Environ Res Public Health. 2018;15:1880.
73. Wang H, Wei K, Wu Z, Liu F, Wang D, Peng X, et al. Association between per- and polyfluoroalkyl substances and semen quality. Environ Sci Pollut Res. 2022;30:27884–94.
74. Carwile JL, Seshasayee SM, Aris IM, Rifas-Shiman SL, Claus Henn B, Calafat AM, et al. Prospective associations of mid-childhood plasma per- and polyfluoroalkyl substances and pubertal timing. Environ Int. 2021;156:106729.
75. Pinney SM, Fassler CS, Windham GC, Herrick RL, Xie C, Kushi LH, et al. Exposure to perfluoroalkyl substances and associations with pubertal onset and serum reproductive hormones in a longitudinal study of young girls in greater cincinnati and the San Francisco Bay Area. Environ Health Perspect. 2023;131:97009.
76. Forthun IH, Roelants M, Knutsen HK, Haug LS, Iszatt N, Schell LM, et al. Exposure to Per- and polyfluoroalkyl substances and timing of puberty in Norwegian boys: data from the Bergen growth study 2. Environ Sci Technol. 2024;58:16336–46.
77. Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, et al. Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish National Birth Cohort. Environ Health Perspect. 2019;127:17004.
78. Averina M, Huber S, Almås B, Brox J, Jacobsen BK, Furberg A-S, et al. Early menarche and other endocrine disrupting effects of per- and polyfluoroalkyl substances (PFAS) in adolescents from Northern Norway. The fit futures study. . Environ Res. 2024;242:117703.
79. Freire C, Vela-Soria F, Castiello F, Salamanca-Fernández E, Quesada-Jiménez R, López-Alados MC, et al. Exposure to perfluoroalkyl substances (PFAS) and association with thyroid hormones in adolescent males. Int J Hyg Environ Health. 2023;252:114219.
80. Zheng L, Wang Z, Yang R, Chen W, Zhang J, Li R, et al. The interference between effects of PFAS exposure on thyroid hormone disorders and cholesterol levels: an NHANES analysis. Environ Sci Pollut Res. 2023;30:90949–59.
81. Kim MJ, Moon S, Oh B-C, Jung D, Ji K, Choi K, et al. Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. PLoS One. 2018;13:e0197244.
82. Tillaut H, Monfort C, Giton F, Warembourg C, Rouget F, Cordier S, et al. Persistent organic pollutant exposure and thyroid function among 12-year-old children. Neuroendocrinology. 2023;113:1232–47.
83. Lopez-Espinosa M-J, Mondal D, Armstrong B, Bloom MS, Fletcher T. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ Health Perspect. 2012;120:1036–41.
84. Jones PD, Hu W, De Coen W, Newsted JL, Giesy JP. Binding of perfluorinated fatty acids to serum proteins. Environ Toxicol Chem. 2003;22:2639–49.
85. Weiss JM, Andersson PL, Lamoree MH, Leonards PEG, van Leeuwen SPJ, Hamers T. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol Sci. 2009;109:206–16.
86. Chang S-C, Thibodeaux JR, Eastvold ML, Ehresman DJ, Bjork JA, Froehlich JW, et al. Thyroid hormone status and pituitary function in adult rats given oral doses of perfluorooctanesulfonate (PFOS). Toxicology. 2008;243:330–9.
87. Yayah Jones N-H, Khoury JC, Xu Y, Newman N, Kalkwarf HJ, Braun JM, et al. Comparing adolescent self staging of pubertal development with hormone biomarkers. J Pediatr Endocrinol Metab. 2021;34:1531–41.

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