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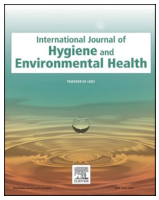

Exposure to perfluoroalkyl substances (PFAS) and association with thyroid hormones in adolescent males

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ABSTRACT

Background: Perfluoroalkyl substances (PFAS) are found in a wide range of consumer products. Exposure to PFAS in children and adolescents may be associated with alterations in thyroid hormones, which have critical roles in brain function.

Objective: This study investigated the association between plasma concentrations of PFAS and serum levels of total triiodothyronine (T3), free thyroxine (T4), and thyroid-stimulating hormone (TSH) in adolescent males.

Methods: In 2017–2019, 151 boys from the Environment and Childhood (INMA)-Granada birth cohort, Spain, participated in a clinical follow up visit at the age of 15–17 years. Plasma concentrations of ten PFAS (PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFOS, and PFHxS) and serum thyroid hormones were measured in 129 of these boys. Linear regression analysis was performed to determine associations of individual PFAS with total T3, free T4, TSH, and free T4/TSH ratio, and quantile g-computation models were performed to assess the mixture effect. Additional models considered iodine status as effect modifier.

Results: PFOS was the most abundant PFAS in plasma (median = 2.22 μg/L), followed by PFOA (median = 1.00 μg/L), PFNA (median = 0.41 μg/L), and PFHxS (median = 0.40 μg/L). When adjusted by confounders (including age, maternal schooling, and fish intake), PFOA and PFUnDA were associated with an increase in free T4 (β [95% CI] = 0.72 [0.06; 1.38] and 0.36 [0.04; 0.68] pmol/L, respectively, per two-fold increase in plasma concentrations), with no change in TSH. PFOS, the sum of PFOA, PFNA, PFOS, and PFHxS, and the sum of long-chain PFAS were marginally associated with increases in free T4. Associations with higher free T4 and/or total T3 were seen for several PFAS in boys with lower iodine intake (<108 μ/day) alone. Moreover, the PFAS mixture was association with an increase in free T4 levels in boys with lower iodine intake (% change [95% CI] = 6.47 [–0.69; 14.11] per each quartile increase in the mixture concentration).

Conclusions: Exposure to PFAS, considered individually or as a mixture, was associated with an increase in free T4 levels in boys with lower iodine intake. However, given the small sample size, the extent of these alterations remains uncertain.

1. Introduction

Perfluoroalkyl substances (PFAS) are man-made chemicals used in a wide range of commercial and industrial products, including fire-fighting foams, semiconductors, water- and oil-repellent textiles, leather, food contact materials, cosmetics, medical devices, biocides, pharmaceuticals, and paints (Glüge et al., 2020). Food, contaminated drinking water, and indoor air and dust are major routes of exposure to

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PFAS in the general population (Cornelis et al., 2012; EFSA, 2020; Haug et al., 2011). Factors associated with internal levels of PFAS such as perfluorooctane sulfonate (PFOS) and perfluorononanoic acid (PFNA) in children include the intake of fast foods and snacks (Halldorsson et al., 2008; Wu et al., 2015) and seafood (Dassuncao et al., 2018), the frequency of wearing waterproof clothing (Wu et al., 2015), and the presence of PFAS in residential dust (Wu et al., 2015). PFAS are highly persistent in the environment and have been detected in adults and children from various countries (Bartolomé et al., 2017; Calafat et al., 2007; Kannan et al., 2004; Lewis al., 2015). The half-life of PFAS in human serum can vary widely, being 3.5 and 4.8 years, respectively, for perfluorooctanoic acid (PFOA) and PFOS, and up to 7.3 years for perfluorohexane sulfonate (PFHxS) (Olsen and Zobel, 2007). In 2009, PFOS was added to the Stockholm Convention, a global treaty to eliminate or restrict the use of several persistent organic pollutants (Stockholm Convention, 2017), PFOA was added to the Convention in 2019 (Regulation (EU) 2019/1021) but had been phased out in the European Union (EU) since 2008 (Commission Regulation (EU) 2017/1000). Thus, the use of PFOA is banned in the EU and the use of PFOS is only allowed for a few applications. Other PFAS such as PFHxS remain under review by the European Chemicals Agency (ECHA, 2019).

Both long (≥8 carbon compounds) and short (<8 carbon compounds) chain PFAS may act as endocrine disrupting chemicals (Diamanti-Kandarakis et al., 2009; Gore, 2016), and some are also suspected of disrupting thyroid hormone homeostasis (Coperchini et al., 2017, 2021). Thyroid homeostasis is controlled by the hypothalamus-pituitary-thyroid axis feedback mechanism, which depends on interactions among thyroid stimulating hormone (TSH), thyroxin (T4), and triiodothyronine (T3). These hormones play critical roles in regulating different physiological functions, including metabolism, circadian rhythm, reproductive function, and fetal and child nervous system development (Zoeller, 2007). Various epidemiological studies have assessed the association between PFAS exposure and thyroid hormone levels in adults, reporting varied results and suggesting that associations are dependent on the compound, dose, age, and sex (Blake et al., 2018; Bloom et al., 2010; Byrne et al., 2018; Ji et al., 2012; Kim et al., 2018; Knox et al., 2011; Lewis et al., 2015; Melzer et al., 2010). However, there has been only limited research in children and adolescents, who are more sensitive than adults to thyroid-disrupting chemicals because their bodies and brains are still developing (Lanphear, 2015). In this way, Lin et al. (2013) found a positive association of serum PFNA concentrations with free T4 levels but not with TSH in adolescents and young Taiwanese adults. Likewise, Lopez-Espinosa et al. (2012) described slight positive associations of serum PFNA, PFOA, and PFOS with total T4 in children living near a petrochemical plant in the USA. In the same line, Caron-Beaudoin et al. (2019) observed that high serum PFNA concentrations were positively associated with free T4 levels but not TSH in children and adolescents from a Native Community in Quebec. Finally, analysis of data from the U.S. National Health and Nutrition Examination Survey (NHANES) revealed positive associations of serum PFNA and PFOS with TSH in male adolescents and a negative association between PFOA and TSH in female adolescents (Lewis et al., 2015). Overall, the role of PFAS as thyroid disrupting chemicals remains controversial, and there has been no consistent evidence of an association between a given PFAS and human thyroid function (Coperchini et al., 2021).

As part of the European Human Biomonitoring Initiative (HBM4EU), serum concentrations of 12 PFAS were measured in adolescents (12–18 years) from nine countries, including Spain, in 2014–2021 (Richterová et al., 2023). Around 14% of the adolescents exceeded the EFSA health-based guidance value of 6.9 μg/L for the total serum concentration of PFOS, PFOA, PFNA, and PFHxS with significantly higher PFAS concentrations in boys *versus* girls and in subjects from Northern and Western Europe (Richterová et al., 2023). The aim of the present study was to assess the relationship of plasma concentrations of ten PFAS, including long and short chain compounds, with serum levels of total T3, free T4, and TSH in adolescent Spanish males aged 15–17 years.

2. Material and methods

2.1. Study population

The Environment and Childhood (INMA) Project is a multicenter population-based birth cohort study designed to investigate the effect of environmental exposures and diet during pregnancy on fetal and child development in different parts of Spain (Guxens et al., 2012). The INMA-Granada birth cohort was established between 2000 and 2002 by recruiting 668 mother-son pairs at delivery in Granada province, Spain (Fernandez et al., 2007). Randomly selected pairs from the baseline cohort were contacted to request their participation in different clinical follow-ups at 4–5 (N = 220, 32.9%) and 9–11 years (N = 300, 44.9%). Those who attended both follow-up sessions (N = 269) were re-contacted and asked to participate in the most recent follow-up at the age of 15–17 years (2017–2019), from which 151 agreed to participate and underwent physical examination (Castiello et al., 2020). The follow-up visit included anthropometric and pubertal measures, questionnaire completion, and the collection of a non-fasting blood sample, which was obtained from 135 (89%) of the boys. Whole venous blood was collected from participants between 17:00 and 20:00 h and processed to obtain plasma and serum samples, which were stored at −80 °C until delivery to the *Instituto de Investigación Biosanitaria de Granada* (ibs.Granada), Granada, Spain, for analyses of PFAS exposure and thyroid hormones. The present study was conducted in 129 of the boys with available data on plasma PFAS and thyroid hormone levels. Further details on study participation were previously described (Castiello et al., 2020; Suárez et al., 2021). The parents of all participants signed informed consent, and the study protocol was approved by the Biomedical Research Ethics Committee of Granada. No significant differences in general characteristics were observed between boys included (N = 129) and not included (N = 26) in the present study (Supplementary material, Table S1).

2.2. Laboratory analysis

A previously described methodology based on salt-assisted liquid-liquid extraction (SALLE) and dispersive liquid-liquid microextraction (DLLME) (Vela-Soria et al., 2020) was used to measure plasma levels of three short chain PFAS (perfluorohexanoic acid [PFHxA], perfluoroheptanoic acid [PFHpA], and PFHxS) and seven long chain PFAS (PFOA, PFOS, PFNA, perfluorodecanoic acid [(PFDA], perfluoroundecanoic acid [PFUnDA], perfluorododecanoic acid [PFDoDA], and perfluorotridecanoic acid [PFTrDA]). After placing 1 mL of plasma sample in a polypropylene centrifuge tube, 1 mL Milli-Q water and 5.0 mL of acetonitrile were added, and the mixture was vortexed for 30 s. SALLE was then performed by adding a salt mixture of 600 mg NaCl, 200 mg disodium hydrogen citrate, and 200 mg trisodium citrate, followed by manual agitation for 60 s and centrifugation at 4000 rpm for 10 min. The supernatant was transferred into a glass 7-mL vial, concentrated to 1 mL under nitrogen stream, and poured into a 15-mL screw cap glass test tube. The sample was then prepared for DLLME by adding 10 mL of 10% NaCl aqueous solution (w/v) at pH 2. Next, 1500 μL of trichloromethane was rapidly injected by syringe, and the mixture was gently shaken for 40 s and then centrifuged for 5 min at 4000 rpm. The entire sedimented phase volume was transferred into a clean glass vial, the organic phase was evaporated under a nitrogen stream, and the residue was dissolved with 100 μL of a mixture of 5 mM ammonium acetate (pH 4.5) and acetonitrile 30:70 (v/v) and then vortexed for 30 s, thereby preparing the sample for injection into the high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) system. HPLC-MS/MS analysis was performed with a NexeraXR LC-20A liquid chromatography system (Shimadztu, Japan) and 4500 QTRAP MS/MS4500 mass spectrometer (ABSciex, USA). A

Gemini C18 column (100 mm × 2 mm i.d., 3-μm particle) from Phenomenex (Torrance, CA, USA) and a gradient mobile phase consisting of 5 mM ammonium acetate aqueous solution with pH of 4.5 (solvent A) and acetonitrile (solvent B) were used for the chromatography. Compounds were determined in negative ion mode. The tandem mass spectrometer was operated in selected reaction monitoring mode. The limits of detection (LOD) and quantification (LOQ) were 0.02 μg/L and 0.05 μg/L, respectively, for all PFAS.

Serum thyroid hormone measurements were performed by electrochemiluminescence immunoassay using a Roche® kit (Elecsys System, Roche Diagnostics). The ratio of FT4 to TSH was calculated by dividing FT4 (pmol/L) by TSH (mU/mL) as a marker of the negative feedback control mechanism of the hypothalamus-pituitary-thyroid axis. Limits of detection were 0.005 μIU/mL for TSH, 0.5 pmol/L for free T4, and 0.300 nmol/L for total T3. Age-specific laboratory reference ranges (11–18 years old) were 10.29–24.45 pmol/L for free T4; 1.23–3.23 nmol/L for total T3; and 0.32–3.0 μIU/mL for TSH (Iwaku et al., 2013).

2.3. Covariate data

The weight, height, and waist circumference of the boys were measured following standardized procedures (Castiello et al., 2020). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2) and converted to age-specific z-scores based on World Health Organization growth reference standards for children aged 5–19 years (de Onis et al., 2007), classifying the boys as underweight (<-1 standard deviation [SD]), normal weight (±1 SD), overweight (>+1 SD, equivalent to BMI≥25 kg/m^2 at 19 years), or obese (>+2 SD; equivalent to BMI≥30 kg/m^2 at 19 years). Weight status was finally categorized into underweight/normal weight or overweight/-obese. The waist-to-height ratio was also calculated by dividing waist circumference (cm) by height (cm), and abdominal obesity was defined as waist-to-height ratio≥0.5 (Browning et al., 2010). Tanner stages of genital development (G) and pubic hair growth (PH) were assessed by a pediatric endocrinologist and categorized as reaching sexual maturity (Tanner G = 5, Tanner PH = 5) or no (Castiello et al., 2023). Information was also obtained from questionnaire responses on the following covariates: maternal schooling (primary [from age of 6–12 years], secondary, or university), and child passive smoking (living with a smoker, yes or no), alcohol intake (never; 1 beverage per month; 2 beverages per month; and ≥3 beverages per month), total fish intake (g/day), iodine intake (μg/day), history of physician-diagnosed thyroid disease (yes or no), and current or recent (<12 months) medication use. Information on fish intake was gathered from a validated semiquantitative food frequency questionnaire (FFQ) completed by the adolescents, who were asked to report the average frequency of consumption for the specified serving or portion size for each food item of the FFQ, based on the previous year (Notario-Barandiaran et al., 2020, 2021). To estimate iodine intake, the published food composition tables of the US Department of Agriculture (USDA) and other published sources for specific Spanish food and portion sizes were used. Average daily iodine intake was estimated by multiplying the frequency of consumption of each food item by iodine composition of the serving size specified in the FFQ and summed the results for all foods (Notario-Barandiaran et al., 2020).

2.4. Statistical analysis

Concentrations of PFAS below the LOD were assigned a value of LD/$\sqrt{2}$ (Croghan and Egeghy, 2003). The total concentration of all PFAS analyzed (∑PFAS), the most abundant PFAS commonly found in human blood samples (∑4 PFAS=PFOA + PFOS + PFNA + PFHxS) (EFSA, 2020), long chain PFAS (∑LC PFAS), and short chain PFAS (∑SC PFAS) were calculated as the sum of molar concentrations of the compounds based on molecular weight and were expressed as PFOA (or PFHxS in the case of ∑SC PFAS).

Data were missing on passive smoking for two participants, on alcohol, fish, and iodine intake for four, and on maternal schooling for eight. Missing data were imputed by using the mode for categorical covariates (passive smoking, alcohol intake, and maternal schooling) and the median for continuous variables (fish and iodine intake). The association between the concentrations of each PFAS and each thyroid parameter (total T3, free T4, TSH, and free T4/TSH) was assessed using linear regression analysis. Concentrations of PFAS, TSH, and free T4/TSH ratio showed a non-normal distribution and were natural log-transformed (ln) before inclusion in models. Total T3 and free T4 were normally distributed and modeled untransformed.

Confounders were selected a priori among the above-reported covariates using a directed acyclic graph (DAG) (Textor et al., 2011) (Figure S1) that included adolescent age (in years, continuous), maternal schooling (primary, secondary, or university), and fish intake (ln-transformed). Models were adjusted for fish intake as a relevant source of both PFAS exposure and iodine (Carlsen et al., 2018; Menzel et al., 2021), which is in turn essential for thyroid hormone biosynthesis. Further regression analyses were conducted by categorizing PFAS exposure in tertiles. All linear regression models were checked for normality and homoscedasticity of residuals, and outliers. Regression coefficients of models of ln(PFAS) on total T3 or free T4 were transformed to represent the average change in total T3 or free T4 associated with a two-fold increase in the concentration of each PFAS compound or group. For TSH and free T4/TSH, regression coefficients were transformed to represent the percentage change (%) in TSH or free T4/TSH associated with a two-fold increase in the concentration of each PFAS compound or group, calculated as the complement of the exponentiated regression coefficient [(expβ-1) x 100]. Results of models based on PFAS tertiles are expressed as the difference in hormone level or the percentage change relative to the first tertile of PFAS exposure.

Quantile g-computation was used to assess the combined effect of PFAS on thyroid hormones. Quantile g-computation estimates the joint effect using a parametric generalized linear model that simultaneously increases all exposures by one tertile (Keil et al., 2020). Advantages of this method include the possibility of assessing individual exposure-effect relationships within the mixture in opposite directions, producing an unbiased estimate of the overall joint effect, and its usefulness for small sample sizes (Eick et al., 2021; Keil et al., 2020). The functioning of quantile g-computation is based on the categorization of urinary biomarkers of exposure to phenols, metals, and pesticides into quartiles. Each biomarker is given a negative or positive weight. If the individual compound shows a different direction of the effect, the weight is interpreted as the proportion of the partial effect in a negative or positive direction.

We examined the potential effect modification by iodine intake through stratification of single-exposure and mixture effect models by using the median of iodine intake (*i.e.*, 108 μg/day). Finally, associations were examined after excluding boys who had ever been diagnosed with thyroid disease (N = 3 hyperthyroidism; N = 2 hypothyroidism) and/or received thyroid medication (N = 1), finding no major differences (results not shown). The significance level was established at 0.05. SPSS version 28 (IBM, Chicago, IL) and R version 4.1.2, package "qgcomp" (https://cran.r-project.org/web/packages/qgcomp/index.html) were used for statistical analyses.

3. Results

Participants had a mean age of 16.2 years; 38% had mothers with primary schooling, 70% resided in an urban area, 42% were passive smokers, 12% consumed at least 2 alcoholic beverages per month; 28% had overweight or obesity, and 23% had a weight-to-height ratio ≥0.50. All boys were in pubertal stage 4 or 5, 45% were in stage G5 and 61% in stage PH5. Average fish and iodine intakes were 79.8 g/day and 104.6 μg/day, respectively (Table 1).

All plasma samples had detectable concentrations of PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFOS, and PFHxS, while PFDoDA was below the

Table 1
Characteristics of 129 adolescents from the INMA-Granada cohort.

Variables	n (%) or mean ± SD
Adolescent age (years)	16.2 ± 0.42
Maternal schooling	
Primary	49 (38.0)
Secondary	48 (37.2)
University	32 (24.8)
Passive smoking	54 (41.9)
Alcohol intake	50 (36.8)
Never	79 (61.2)
1 beverage/month	35 (27.1)
2 beverage/month	7 (5.4)
≥3 beverage/month	8 (6.2)
Weight status[a]	
Underweight/normal weight	93 (72.1)
Overweight/obese	36 (27.9)
Waist-to-height ratio≥0.50	30 (23.3)
Puberty development	
Stage G5	58 (45.0)
Stage PH5	79 (61.2)
Total fish intake (g/day)	79.8 ± 83.7
Iodine intake (μg/day)	140.6 ± 102.9

[a] Based on age-specific z-scores based on World Health Organization growth reference standards.

LOD in 10.7% of samples, and PFHxA and PFTrDA was below in 13.8% of samples (Table 2). PFOS was the PFAS with the highest concentration (median = 2.224 μg/L), which was more than two-fold higher than the concentration of PFOA (median = 0.997 μg/L) and five-fold higher than the concentrations of PFNA and PFHxS (median = 0.408 and 0.398 μg/L, respectively). There was a higher total concentration of LC PFAS than of SC PFAS (median = 3.93 *vs.* 1.04 μg/L). In general, positive correlations were observed between PFAS; however, PFHxA was only weakly correlated with PFOA and PFUnDA and was inversely correlated with PFTrDA (Fig. 1). Median total T3 and free T4 values were 2.15 nmol/L and 16.20 pmol/L, respectively, and median TSH and free T4/TSH ratio values were 1.84 mU/mL and 9.06, respectively. None of the participants had total T3, free T4, or TSH levels outside the age-specific pediatric range (Table 3).

Table 2
Plasma concentrations of PFAS (μg/L) in 129 adolescent males.

PFAS		%> LD	25th percentile	Median	75th percentile
PFHxA	Perfluorohexanoic acid	86.2	0.046	0.110	0.230
PFHpA	Perfluroheptanoic acid	100	0.084	0.139	0.236
PFOA	Perfluorooctanoic acid	100	0.805	0.997	1.338
PFNA	Perfluorononanoic acid	100	0.271	0.408	0.715
PFDA	Perfluorodecanoic acid	100	0.170	0.218	0.267
PFUnDA	Perfluoroundecanoic acid	100	0.134	0.250	0.382
PFDoDA	Perfluorododecanoic acid	89.3	0.043	0.061	0.089
PFTrDA	Perfluorotridecanoic acid	86.3	0.040	0.069	0.119
PFOS	Perfluorohexane sulfonic acid	100	1.626	2.224	3.079
PFHxS	Perfluorooctane sulfonic acid	100	0.244	0.398	0.819
Sum of PFAS[a]					
∑4 PFAS[b]	PFOA + PFOS + PFNA + PFHxS	–	3.238	4.316	5.765
∑SC PFAS[c]	PFHxA + PFHpA + PFHxS	–	0.603	1.040	1.782
∑LC PFAS[d]	PFOA + PFOS + PFNA + PFDA + PFUnA + PFDoA + PFTrDA	–	3.145	3.926	5.224
∑PFAS	Sum of all 10 PFAS	–	4.028	5.263	7.171

LD: Limit of detection.
[a] Sum of the molar concentrations of PFAS based on molecular weight and expressed as PFOA (or PFHxS in the case of ∑SC PFAS).
[b] Most abundant PFAS in human serum (EFSA, 2020).
[c] Long-chain PFAS.
[d] Short-chain PFAS.

In single-exposure models, PFOA and PFUnDA were associated with slight increases in free T4 (β [95% CI] = 0.72 [0.06; 1.38] and 0.36 [0.04; 0.68] pmol/L, respectively, per two-fold increase in plasma concentrations). In addition, PFOS, the sum of PFOA, PFNA, PFOS, and PFHxS (∑4 PFAS), and the sum of LC PFAS were marginally associated with increases in free T4 (β [95% CI] = 0.42 [–0.08; 0.93], 0.54 [–0.03; 1.13], and 0.57 [–0.09; 1.23] pmol/L, respectively) (Table 4). No association was observed for total T3 or TSH (Table 4). Stratification by iodine status revealed that associations were mostly significant in boys with iodine intake <108 μ/day (Table S2). Thus, PFOA and ∑4 PFAS were associated with a slight increase in total T3, and PFNA, PFUnDA, ∑4 PFAS, and ∑LC PFAS with an increase in free T4 in boys with lower iodine intake alone (*e.g.*, free T4 increased by 1.09 and 0.95 pmol/L, respectively, per two-fold increase in ∑4 PFAS and ∑LC PFAS) (Table S2).

Analysis based on PFAS tertiles did not show a clear trend towards higher thyroid hormone levels with increasing PFAS exposure. Nonetheless, second-tertile concentrations of PFOA and ∑LC PFAS were associated with a slight increase in total T3 (β [95% CI] = 0.17 [0.03; 0.32] and 0.16 [0.02; 0.29], respectively); second tertile of ∑4 PFAS with an increase in free T4 (β [95% CI] = 1.07 [0.13; 2.01]); third tertiles of PFOA and PFHxS with an increase in free T4; and third tertile of PFHxS with a decrease in TSH (Table S3). Analysis of residuals and outliers indicated that linear regression assumptions are met (data not shown).

In the quantile g-computation model, the mixture was not associated with thyroid parameters when considering the total sample of boys (Table 5 and Fig. 1). However, after stratification by iodine intake, there was a suggestive positive association between the PFAS mixture and free T4 levels in boys with lower iodine intake (% change [95% CI] = 6.47 [–0.69; 14.11] per each quartile increase in the mixture concentration) (Table 5), with PFTrDA, PFNA, PFOS, and PFHxS contributing most to this effect (Fig. 2).

4. Discussion

This cross-sectional study on the association of plasma PFAS concentrations with thyroid hormone parameters in adolescent males found that higher PFOA, PFUnDA, PFOS, ∑4 PFAS, and ∑LC PFAS concentrations were associated with a slight increase in free T4 that ranged from 0.36 to 0.72 pmol/L per two-fold increase in plasma concentrations. In addition, it was observed that these and other PFAS biomarkers, including PFNA and PFHxS, and the PFAS mixture were positively associated with free T4 and/or total T3 in boys with lower iodine intake alone. These results suggest that exposure to environmentally relevant concentrations of PFAS may be associated with subtle alterations in the thyroid hormone levels of adolescents, notably an increase in free T4, and that this association is modified by their iodine status. Thyroid hormone levels were within normal reference ranges and may not be of clinical significance. Nevertheless, the thyroid-disrupting effects of PFAS in adolescents may have a significant impact on their health, given the major role played by thyroid hormones in growth, metabolism, and brain maturation and in behavior and mood (Anderson, 2001; Bauer et al., 2003).

PFOS was the most abundant PFAS in the present plasma samples, and concentrations were in the range recently reported in sera from European teenagers (median = 2.22 μg/L in plasma *vs.* geometric mean [GM] = 2.13 μg/L in serum) (Richterová et al., 2023). Plasma PFOA, PFNA, and PFHxS concentrations were also similar to those observed in

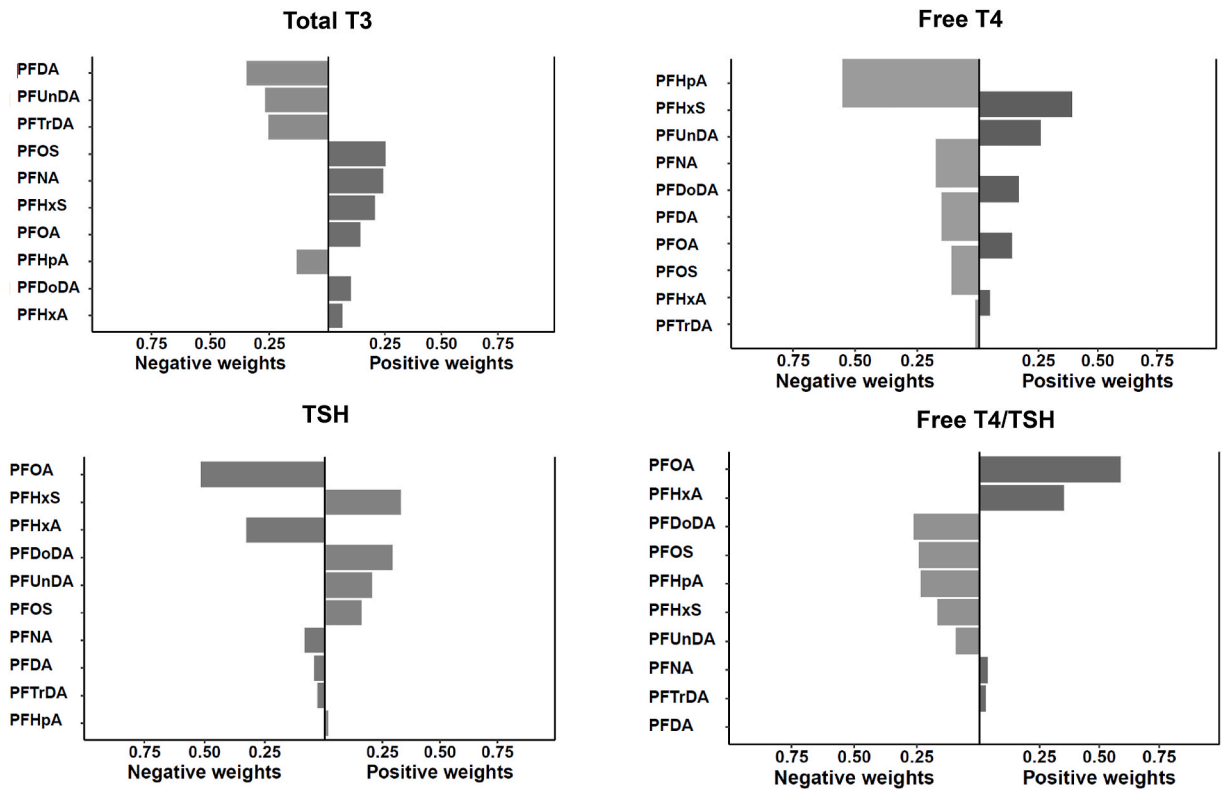


Fig. 1. G-computation models for the mixture effect of PFAS on thyroid parameters among adolescent males (N = 129). Dark-colored bars refer to chemicals with an effect in the same direction to the overall effect. Grey-colored bars refer to chemicals with an effect in the opposite direction to the overall effect.

Table 3
Serum thyroid parameters.

Thyroid hormones	Median	5th-95th percentile	Range
Total T3 (nmol/L)	2.15	1.61–2.73	1.48–3.00
Free T4 (pmol/L)	16.20	13.10–19.62	7.8–22.11
TSH (μIU/mL)	1.84	0.81–3.89	0.63–4.69
Free T4/TSH	9.06	4.23–21.15	3.51–32.6

Reference values (11–18 years): Total T3: 1.23–3.23 nmol/L; free T4: 10.29–24.45 pmol/L; TSH: 0.6–5.8 μIU/mL.

the teenagers (GM = 0.97, 0.30, and 0.41 μg/L in serum, respectively) (Richterová et al., 2023). However, plasma concentrations of PFOA, PFNA, PFDA, PFOS, and PFHxS were around two-fold lower than observed in serum samples collected from Spanish adults in 2009–2010 (Bartolomé et al., 2017), suggesting a decline in PFAS exposure over the past decade due to their regulation in the EU and other countries. Overall, the present data corroborate recent findings from the HBM4EU Project indicating generalized exposure to legacy and newer PFAS among European teenagers.

Epidemiological studies have shown that exposure to individual PFAS has varied effects on thyroid hormones, with differences in their magnitude and direction. However, studies in children and adolescents provide some evidence of a positive association between serum PFNA and higher total or free T4 levels (Caron-Beaudoin et al., 2019; Lin et al., 2013; Lopez-Espinosa et al., 2012), and this association was stronger in males in two of these studies (Caron-Beaudoin et al., 2019; Lin et al., 2013). In the present sample of boys, PFNA was not associated with thyroid hormones in the main analysis, but stratification by iodine intake revealed a significant positive association between PFNA and free T4 and a suggestive positive association with total T3 in subjects with lower iodine intake. Despite the between-study variations in the age of participants, which was 12–30 years in Lin et al. (2013), 1–17 years in Lopez-Espinosa et al. (2012), and 6–19 years in Caron-Beaudoin et al. (2019), our findings are in partial agreement with these results. The associations of PFOS and PFOA with higher free T4 (and higher total T3 for second-tertile PFOA) observed in the main analysis are in line with the positive association of serum PFOA and PFOS with total T4 reported by Lopez-Espinosa et al. (2012) in a large sample of children living near a Teflon manufacturing facility exposed to high PFOA levels. Strikingly, PFOA and PFOS levels in North American children were nearly 30- and 10-fold higher, respectively, than in the present boys. However, no association was found between PFOA or PFOS and T4 or T3 levels in other studies of children or adolescents (Caron-Beaudoin et al., 2019; Lewis et al., 2015; Lin et al., 2013). To our knowledge, this is the first report that links PFUnDA, a 11-carbon compound that has been used as an alternative to the 8-carbon compounds PFOS and PFOA in the fluoropolymer industry, to thyroid function in children or adolescents. Several studies in pregnant women reported inverse association between maternal PFUnDA and thyroid hormones (Boesen et al., 2020), but findings in children are not comparable to those in pregnant women. In the present study, the increase in free T4 or total T3 was not accompanied by a decrease in TSH, and previous studies in children have also failed to find an association between PFAS exposure and TSH levels (Caron-Beaudoin et al., 2019; Lopez-Espinosa et al., 2012; Lewis et al., 2015; Lin et al., 2013). There was a suggestive inverse association of TSH with PFHxA, and suggestive positive associations with PFHxS and ∑4 PFAS in boys with higher iodine intake, but these results may be spurious and should be taken with caution.

Altered thyroid hormone levels following prenatal and postnatal exposure to PFAS have been found in experimental animal studies (Coperchini et al., 2021). For instance, increased T4 and T3 levels were observed in zebrafish larvae after exposure to PFDoDA (Zhang et al., 2018), while serum levels of PFAS, particularly PFOS, were significantly higher in hyperthyroid *versus* non-hyperthyroid cats (D Wang et al., 2018a,b). *In vitro* models reported several effects of legacy and newer PFAS on thyroid function, including cytotoxicity and genotoxicity in thyroid cells and interference with thyroid hormone synthesis, thyroid

Table 4
Single-exposure models[a] for the association between plasma concentrations of PFAS and thyroid parameters.

PFAS	Total T3	Free T4	TSH	Free T4/TSH
	β (95% CI)	β (95% CI)	% change (95% CI)	% change (95% CI)
PFHxA	−0.00 (−0.03; 0.03)	0.06 (−0.15; 0.27)	−2.72 (−7.31; 1.39)	3.51 (−0.69; 7.89)
PFHpA	−0.01 (−0.06; 0.03)	−0.14 (−0.46; 0.17)	0.00 (−6.67; 6.41)	−0.69 (−7.31; 6.41)
PFOA	0.06 (−0.03; 0.15)	**0.72 (0.06; 1.38)****	−5.37 (−17.57; 7.89)	10.90 (−2.72; 27.32)
PFNA	0.02 (−0.03; 0.08)	0.18 (−0.21; 0.57)	2.80 (−4.72; 11.67)	−1.37 (−9.21; 6.41)
PFDA	0.04 (−0.04; 0.12)	−0.08 (−0.66; 0.50)	3.51 (−7.95; 16.39)	−3.39 (−14.67; 8.63)
PFUnDA	0.00 (−0.04; 0.05)	**0.36 (0.04; 0.68)****	2.09 (−4.72; 9.38)	0.00 (−6.02; 7.14)
PFDoDA	0.00 (−0.05; 0.05)	0.01 (−0.28; 0.40)	4.95 (−2.05; 12.45)	−4.06 (−10.45; 2.80)
PFTrDA	−0.01 (−0.05; 0.03)	−0.08 (−0.37; 0.20)	2.09 (−3.39; 8.63)	−2.72 (−8.58; 3.51)
PFOS	0.03 (−0.04; 0.10)	**0.42 (-0.08; 0.93)***	3.51 (−6.67; 14.80)	−0.69 (−11.07; 10.14)
PFHxS	0.03 (−0.01; 0.07)	0.13 (−0.14; 0.41)	4.23 (−1.37; 10.90)	−3.39 (−9.21; 2.09)
∑4 PFAS	0.06 (−0.02; 0.14)	**0.54 (-0.03; 1.13)***	4.23 (−7.31; 18.01)	−0.69 (−12.29; 11.67)
∑SC PFAS	0.02 (−0.03; 0.07)	0.01 (−0.35; 0.36)	0.69 (−6.02; 8.63)	−0.69 (−7.95; 6.41)
∑LC PFAS	0.04 (−0.05; 0.14)	**0.57 (-0.09; 1.23)***	2.09 (−11.07; 17.20)	2.09 (−11.68; 17.20)
∑PFAS	0.05 (−0.04; 0.14)	0.46 (−0.17; 1.08)	2.09 (−10.45; 16.39)	0.69 (−11.68; 15.59)

Models are adjusted by adolescent age (years), maternal schooling (primary, secondary, or university), and (log) total fish intake.
Regression estimates were transformed to represent the average change (total T3, free T4) or percentage change (TSH, free T4/TSH) in thyroid parameter associated with a two-fold increase in PFAS concentration.
**p < 0.05; *p < 0.10.
[a] Each PFAS compound or group was separately modeled with each thyroid parameter.

Table 5
Plasma PFAS mixture effect on thyroid parameters.

Thyroid parameters	% change	95% CI
Total sample (N = 129)		
Total T3	2.02	−3.93; 7.25
Free T4	2.02	−2.96; 7.25
TSH	−0.99	−15.64; 16.18
Free T4/TSH	3.05	−12.19; 22.14
Iodine intake <108 μg/L (N = 64)		
Total T3	4.29	−3.62; 12.91
Free T4	**6.47**	**−0.69; 14.11***
TSH	2.78	−19.21; 30.73
Free T4/TSH	3.50	−18.12; 31.21
Iodine intake ≥108 μg/L (N = 65)		
Total T3	−0.17	−7.38; 7.25
Free T4	−0.99	−8.37; 6.60
TSH	−2.09	−21.53; 22.14
Free T4/TSH	1.10	−19.01; 26.24

Mixture components: PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA. PFTrDA, PFOS, and PFHxS.
All models are adjusted by adolescent age, maternal schooling, and total fish intake.
Estimates are expressed as percentage of change in thyroid hormone parameter. Pear each quartile increase in the mixture concentration.
*p < 0.10.

peroxidase (TPO) function, iodine uptake, and thyroid hormone transport and clearance (Coperchini et al., 2021; Weiss et al., 2009; Yu et al., 2009, 2011). Thus, Kim et al. (2021) suggested that PFUnDA and PFTrDA cause transcriptional changes of thyroid regulating genes that may increase thyroid hormone synthesis (Kim et al., 2021), which could explain the positive association between PFUnDA and free T4.

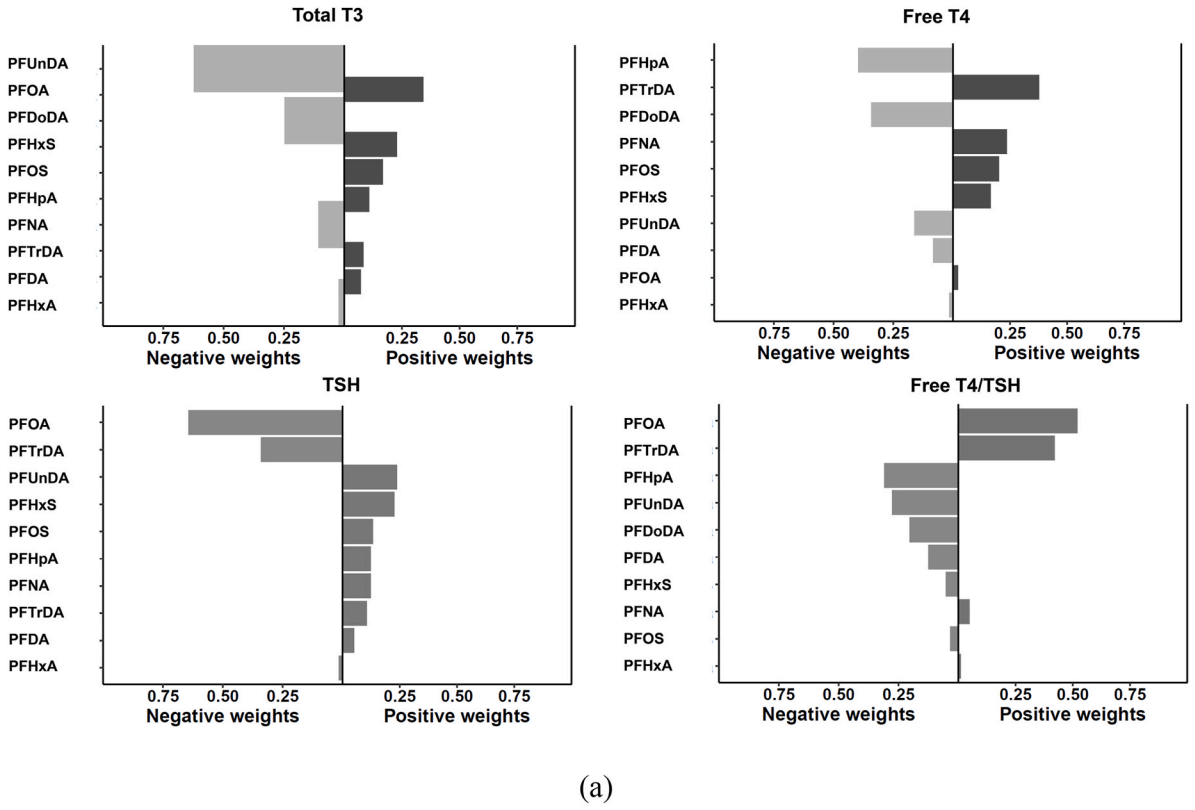
Overall, direct comparisons with previous findings on the effects of PFAS on thyroid hormones in children and adolescents are hampered by differences in the age of participants, their PFAS concentrations, and/or the types of thyroid hormones measured. For instance, regarding age, levels of free T4, free T3, and TSH are generally higher in younger children than in older children, with a gradual decline in concentrations as adult age is approached (Taylor et al., 2023). In addition, complex mechanisms are involved in thyroid homeostasis, and PFAS has been reported to interfere with this endocrine system at several levels (Coperchini et al., 2021).

When stratified by iodine intake, single-exposure and g-computation models suggested that exposure to individual PFAS and their mixture may increase levels of free T4 only in boys with lower iodine intake. In a mother-child cohort study, iodine deficiency did not modify the association between maternal serum PFAS and thyroid hormones (Lebeaux et al., 2020), while serum PFOA, PFOS, PFNA, and PFHxS were positively associated with free T3, total T3, and TSH in adults from the NHANES with high TPO antibody and low urinary iodine levels (Webster et al., 2016). According to Webster et al. (2014), effect modification by iodine status contribute to the "multiple hit hypothesis", a theory that thyroid function may be more susceptible to disruption by chemicals such as PFAS if the system is already impacted by multiple stressors. Although iodine deficiency is still present in some parts of Europe, recent data suggest that the iodine status of the population is optimal in the Southern region of Spain (Andalusia) where the INMA-Granada cohort was established, with higher urinary iodine concentrations in schoolchildren than in adults (Ittermann et al., 2020). Nevertheless, the median iodine intake of the present sample of adolescents was below the recommended level of 120 μg/day, possibly due to low fish intake in the study sample (mean = 80 g/day) while underestimation of iodine intake cannot be ruled out. Further studies using robust mixture models in larger populations are required to corroborate these findings.

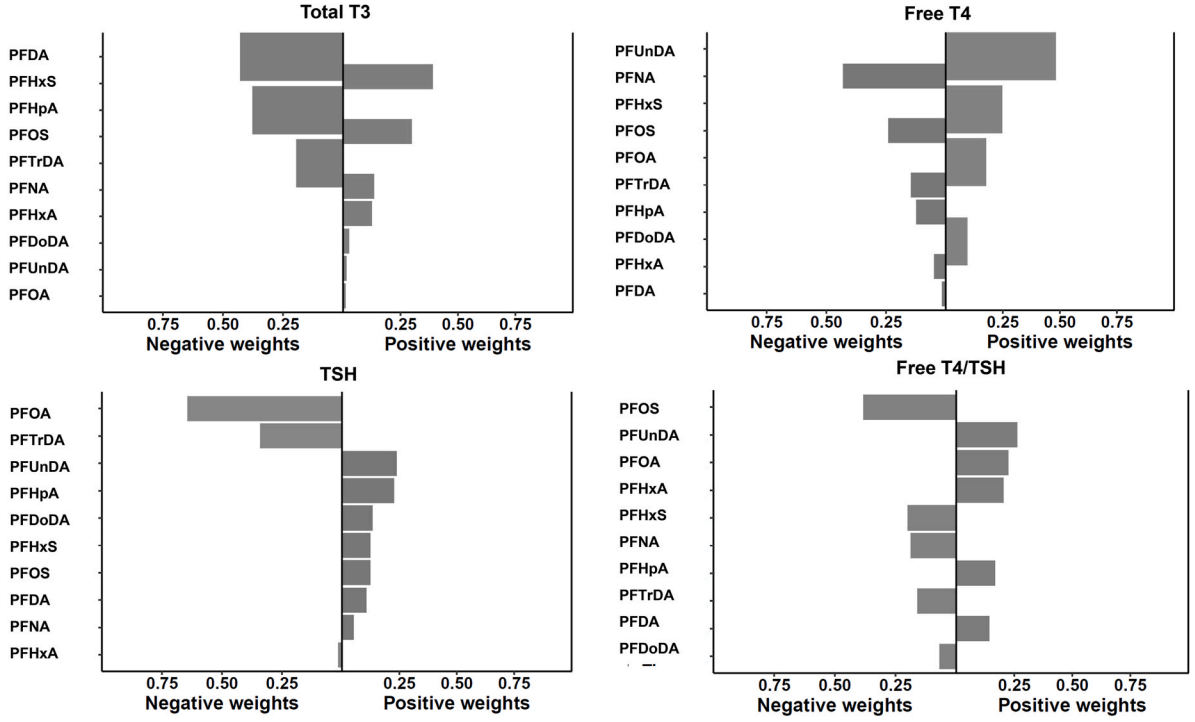
The main strengths of this study include the evaluation of ten different PFAS with high detection frequencies, the performance of mixture effect analysis, examination of the association between PFAS and thyroid hormones in a novel population, and assessment of effect modification by iodine status. The cross-sectional design of the study is a major limitation, preventing evaluation of the potential causality of the association between PFAS exposure and thyroid hormone levels. The small sample size may have resulted in imprecise estimates of the effect size, thus preventing the ascertainment of the extent to which PFAS exposure altered thyroid hormone levels. Additionally, it is unclear whether the associations observed in single-exposure models represent cause-effect relationships or result from the performance of multiple comparisons (*i.e.*, 10 PFAS x 3 thyroid hormones = 30 effective comparisons). However, the mixture analysis corroborated the effect observed in single-exposure models. Furthermore, the sex-specific influence of PFAS on thyroid hormones could not be assessed in the INMA-Granada cohort, which contains only boys.

5. Conclusions

This study found a wide presence of legacy and newer PFAS in plasma samples from Spanish adolescent males and observed that higher concentrations of some PFAS (including PFOA PFOS, and PFUnDA) and the PFAS mixture were associated with a mild increase in thyroid hormone levels, particularly higher free T4 levels in boys with lower iodine intake. Larger longitudinal studies are needed to confirm the associations observed and to improve understanding of the effects of PFAS on thyroid function in young populations.



(a)



(b)

Fig. 2. G-computation models for the mixture effect of PFAS on thyroid parameters by iodine intake: a) lower iodine intake (<108 μg/day, N = 64); b) higher iodine intake (≥108 μg/day, N = 65). Dark-colored bars refer to chemicals with an effect in the same direction to the overall effect. Grey-colored bars refer to chemicals with an effect in the opposite direction to the overall effect.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ijheh.2023.114219.

References

Anderson, G.W., 2001. Thyroid hormones and the brain. Front. Neuroendocrinol. 22 (1), 1–17. https://doi.org/10.1006/frne.2000.0208.

Bartolomé, M., Gallego-Picó, A., Cutanda, F., Huetos, O., Esteban, M., Pérez-Gómez, B., Bioambient, es, Castaño, A., 2017. Perfluorinated alkyl substances in Spanish adults: geographical distribution and determinants of exposure. Sci. Total Environ. 603–604, 352–360. https://doi.org/10.1016/j.scitotenv.2017.06.031.

Bauer, M., London, E.D., Silverman, D.H., Rasgon, N., Kirchheiner, J., Whybrow, P.C., 2003. Thyroid, brain and mood modulation in affective disorder: insights from molecular research and functional brain imaging. Pharmacopsychiatry 36 (Suppl. 3), S215–S221. https://doi.org/10.1055/s-2003-45133.

Blake, B.E., Pinney, S.M., Hines, E.P., Fenton, S.E., Ferguson, K.K., 2018. Associations between longitudinal serum perfluoroalkyl substance (PFAS) levels and measures of thyroid hormone, kidney function, and body mass index in the Fernald Community Cohort. Environ. Pollut. 242 (Pt A), 894–904. https://doi.org/10.1016/j.envpol.2018.07.042.

Bloom, M.S., Kannan, K., Spliethoff, H.M., Tao, L., Aldous, K.M., Vena, J.E., 2010. Exploratory assessment of perfluorinated compounds and human thyroid function. Physiol. Behav. 99 (2), 240–245. https://doi.org/10.1016/j.physbeh.2009.02.005.

Boesen, S.A.H., Long, M., Wielsøe, M., Mustieles, V., Fernandez, M.F., Bonefeld-Jørgensen, E.C., 2020. Exposure to perflouroalkyl acids and foetal and maternal thyroid status: a review. Environ. Health 19 (1), 107. https://doi.org/10.1186/s12940-020-00647-1.

Browning, L.M., Hsieh, S.D., Ashwell, M., 2010. A systematic review of waist-to-height ratio as a screening tool for the prediction of cardiovascular disease and diabetes: 0·5 could be a suitable global boundary value. Nutr. Res. Rev. 23 (2) https://doi.org/10.1017/S0954422410000144, 247–69.

Byrne, S.C., Miller, P., Seguinot-Medina, S., Waghiyi, V., Buck, C.L., von Hippel, F.A., Carpenter, D.O., 2018. Exposure to perfluoroalkyl substances and associations with serum thyroid hormones in a remote population of Alaska Natives. Environ. Res. 166, 537–543. https://doi.org/10.1016/j.envres.2018.06.014.

Calafat, A.M., Wong, L.Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. Population: data from the national health and nutrition examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115 (11), 1596–1602. https://doi.org/10.1289/ehp.10598.

Carlsen, M.H., Andersen, L.F., Dahl, L., Norberg, N., Hjartåker, A., 2018. New iodine food composition database and updated calculations of iodine intake among Norwegians. Nutrients 10, 930. https://doi.org/10.3390/nu10070930.

Caron-Beaudoin, É., Ayotte, P., Laouan Sidi, E.A., 2019. Community of lac simon; community of winneway – long point first nation; CSSS tshukuminu kanani of nutashkuan; community of unamen shipu, gros-louis McHugh N, lemire M. Exposure to perfluoroalkyl substances (PFAS) and associations with thyroid parameters in first nation children and youth from Quebec. Environ. Int. 128, 13–23. https://doi.org/10.1016/j.envint.2019.04.029.

Castiello, F., Olmedo, P., Gil, F., Molina, M., Mundo, A., Romero, R.R., Ruíz, C., Gómez-Vida, J., Vela-Soria, F., Freire, C., 2020. Association of urinary metal concentrations with blood pressure and serum hormones in Spanish male adolescents. Environ. Res. 182, 108958 https://doi.org/10.1016/j.envres.2019.108958.

Castiello, F., Suárez, B., Gómez-Vida, J., Torrent, M., Fernández, M.F., Olea, N., Freire, C., 2023. Exposure to non-persistent pesticides and sexual maturation of Spanish adolescent males. Chemosphere 324, 138350. https://doi.org/10.1016/j.chemosphere.2023.138350.

Commission Regulation (EU). 2017/1000 of 13 june 2017 amending annex XVII to regulation (EC) No 1907/2006 of the European parliament and of the council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards perfluorooctanoic acid (PFOA), its salts and PFOA-related substances. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R1000&from=EN. (Accessed 18 October 2022).

Coperchini, F., Awwad, O., Rotondi, M., Santini, F., Imbriani, M., Chiovato, L., 2017. Thyroid disruption by perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). J. Endocrinol. Invest. 40 (2), 105–121. https://doi.org/10.1007/s40618-016-0572-z.

Coperchini, F., Croce, L., Ricci, G., Magri, F., Rotondi, M., Imbriani, M., Chiovato, L., 2021. Thyroid disrupting effects of old and new generation PFAS. Front. Endocrinol. 11, 612320 https://doi.org/10.3389/fendo.2020.612320.

Cornelis, C., D’Hollander, W., Roosens, L., Covaci, A., Smolders, R., Van Den Heuvel, R., Govarts, E., Van Campenhout, K., Reynders, H., Bervoets, L., 2012. First assessment of population exposure to perfluorinated compounds in Flanders, Belgium. Chemosphere 86 (3), 308–314. https://doi.org/10.1016/j.chemosphere.2011.10.034.

Croghan, C., Egeghy, P.P., 2003. Methods of Dealing with Values below the Limit of Detection Using SAS. Presented at Southern SAS User Group, St. Petersburg, FL. September 22-24.

Dassuncao, C., Hu, X.C., Nielsen, F., Weihe, P., Grandjean, P., Sunderland, E.M., 2018. Shifting Global Exposures to poly- and perfluoroalkyl substances (PFASs) evident in longitudinal birth cohorts from a seafood-consuming population. Environ. Sci. Technol. 52 (6), 3738–3747. https://doi.org/10.1021/acs.est.7b06044.

de Onis, M., Onyango, A.W., Borghi, E., Siyam, A., Nishida, C., Siekmann, J., 2007. Development of a WHO growth reference for school-aged children and adolescents. Bull. World Health Organ. 85 (9), 660–667. https://doi.org/10.2471/blt.07.043497.

Diamanti-Kandarakis, E., Bourguignon, J.P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., et al., 2009. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr. Rev. 30 (4), 293–342. https://doi.org/10.1210/er.2009-0002.

ECHA (European Chemicals Agency), 2019. Candidate list of substances of very high concern for authorisation. Available online: https://echa.europa.eu/candidate-list-table. (Accessed 18 October 2022).

EFSA (European Food Safety Authority), 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. Scientific opinion of the panel on contaminants in the food chain. EFSA J. 18 (9), 6223.

Eick, S.M., Goin, D.E., Cushing, L., DeMicco, E., Park, J.S., Wang, Y., Smith, S., Padula, A. M., Woodruff, T.J., Morello-Frosch, R., 2021. Mixture effects of prenatal exposure to per- and polyfluoroalkyl substances and polybrominated diphenyl ethers on maternal and newborn telomere length. Environ. Health 20 (1), 76. https://doi.org/10.1186/s12940-021-00765-4.

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.

Gore, A.C., 2016. Endocrine-disrupting chemicals. JAMA Intern. Med. 176 (11), 1705–1706. https://doi.org/10.1001/jamainternmed.2016.5766.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., INMA Project, 2012. Cohort profile: the INMA–INfancia y medio ambiente–(environment and childhood) Project. Int. J. Epidemiol. 41 (4), 930–940. https://doi.org/10.1093/ije/dyr054.

Halldorsson, T.I., Fei, C., Olsen, J., Lipworth, L., McLaughlin, J.K., Olsen, S.F., 2008. Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort. Environ. Sci. Technol. 42 (23), 8971–8977. https://doi.org/10.1021/es801907r.

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.

Ittermann, T., Albrecht, D., Arohonka, P., Bilek, R., de Castro, J.J., Dahl, L., Filipsson Nystrom, H., Gaberscek, S., Garcia-Fuentes, E., Gheorghiu, M.L., Hubalewska-Dydejczyk, A., Hunziker, S., Jukic, T., Karanfilski, B., Koskinen, S., Kusic, Z., Majstorov, V., Makris, K.C., Markou, K.B., Meisinger, C., Milevska Kostova, N., Mullen, K.R., Nagy, E.V., Pirags, V., Rojo-Martinez, G., Samardzic, M., Saranac, L., Strele, I., Thamm, M., Top, I., Trofimiuk-Müldner, M., Ünal, B., Koskinen, S., Vila, L., Vitti, P., Winter, B., Woodside, J.V., Zaletel, K., Zamrazil, V., Zimmermann, M., Erlund, I., Völzke, H., 2020. Standardized map of iodine status in Europe. Thyroid 30 (9), 1346–1354. https://doi.org/10.1089/thy.2019.0353. Erratum in: Thyroid. 2022;32(5):603.

Iwaku, K., Noh, J.Y., Minagawa, A., Kosuga, Y., Suzuki, M., Sekiya, K., Matsumoto, M., Ohye, H., Kunii, Y., Yoshihara, A., Watanabe, N., Mukasa, K., Ito, K., Ito, K., 2013. Determination of pediatric reference levels of FT3, FT4 and TSH measured with ECLusys kits. Endocr. J. 60 (6), 799–804. https://doi.org/10.1507/endocrj.ej12-0390.

Ji, K., Kim, S., Kho, Y., Paek, D., Sakong, J., Ha, J., Kim, S., Choi, K., 2012. Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones. Environ. Int. 45, 78–85. https://doi.org/10.1016/j.envint.2012.03.007.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldoust, K.M., 2004. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38 (17), 4489–4495. https://doi.org/10.1021/es0493446.

Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environ. Health Perspect. 128 (4), 47004. https://doi.org/10.1289/EHP5838.

Kim, J., Lee, G., Lee, Y.M., Zoh, K.D., Choi, K., 2021. Thyroid disrupting effects of perfluoroundecanoic acid and perfluorotridecanoic acid in zebrafish (Danio rerio) and rat pituitary (GH3) cell line. Chemosphere 262, 128012. https://doi.org/10.1016/j.chemosphere.2020.128012.

Kim, M.J., Moon, S., Oh, B.C., Jung, D., Ji, K., Choi, K., Park, Y.J., 2018. Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. PLoS One 13 (5), e0197244. https://doi.org/10.1371/journal.pone.0197244.

Knox, S.S., Jackson, T., Frisbee, S.J., Javins, B., Ducatman, A.M., 2011. Perfluorocarbon exposure, gender, and thyroid function in the C8 Health Project. J. Toxicol. Sci. 36 (4), 403–410. https://doi.org/10.2131/jts.36.403.

Lanphear, B.P., 2015. The impact of toxins on the developing brain. Annu. Rev. Publ. Health 36, 211–230. https://doi.org/10.1146/annurev-publhealth-031912-114413.

Lebeaux, R.M., Doherty, B.T., Gallagher, L.G., Zoeller, R.T., Hoofnagle, A.N., Calafat, A. M., Karagas, M.R., Yolton, K., Chen, A., Lanphear, B.P., Braun, J.M., Romano, M.E., 2020. Maternal serum perfluoroalkyl substance mixtures and thyroid hormone concentrations in maternal and cord sera: the HOME Study. Environ. Res. 185, 109395 https://doi.org/10.1016/j.envres.2020.109395.

Lewis, R.C., Johns, L.E., Meeker, J.D., 2015. Serum biomarkers of exposure to perfluoroalkyl substances in relation to serum testosterone and measures of thyroid function among adults and adolescents from NHANES 2011-2012. Int. J. Environ. Res. Publ. Health 12 (6), 6098–6114. https://doi.org/10.3390/ijerph120606098.

Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Hsu, S.H., Chien, K.L., Liao, C.C., Sung, F.C., Chen, P.C., Su, T.C., 2013. The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults. J. Hazard Mater. 244–245, 637–644. https://doi.org/10.1016/j.jhazmat.2012.10.049.

Lopez-Espinosa, M.J., Mondal, D., Armstrong, B., Bloom, M.S., Fletcher, T., 2012. Thyroid function and perfluoroalkyl acids in children living near a chemical plant. Environ. Health Perspect. 120 (7), 1036–1041. https://doi.org/10.1289/ehp.1104370.

Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the U.S. National Health and Nutrition Examination Survey. Environ. Health Perspect. 118 (5), 686–692. https://doi.org/10.1289/ehp.0901584.

Menzel, J., Abraham, K., Dietrich, S., Fromme, H., Völkel, W., Schwerdtle, T., Weikert, C., 2021. Internal exposure to perfluoroalkyl substances (PFAS) in vegans and omnivores. Int. J. Hyg Environ. Health 237, 113808. https://doi.org/10.1016/j.ijheh.2021.113808.

Notario-Barandiaran, L., Freire, C., García-de-la-Hera, M., Compañ-Gabucio, L.M., Torres-Collado, L., González-Palacios, S., Mundo, A., Molina, M., Fernández, M.F., Vioque, J., 2020. Reproducibility and validity of a food frequency questionnaire for dietary assessment in adolescents in a self-reported way. Nutrients 12 (7), 2081. https://doi.org/10.3390/nu12072081.

Notario-Barandiaran, L., Navarrete-Muñoz, E.M., Valera-Gran, D., Hernández-Álvarez, E., Donoso-Navarro, E., González-Palacios, S., García-de-la-Hera, M., Fernández, M.F., Freire, C., Vioque, J., 2021. Biochemical validation of a self-administered food frequency questionnaire to assess diet using carotenoids and vitamins e and d in male adolescents in Spain. Antioxidants 10 (5), 750. https://doi.org/10.3390/antiox10050750.

Olsen, G.W., Zobel, L.R., 2007. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. Int. Arch. Occup. Environ. Health 81 (2), 231–246. https://doi.org/10.1007/s00420-007-0213-0.

Regulation (EU). 2019/1021 of the European Parliament and of the Council of 20 June 2019 on persistent organic pollutants. Available online: http://data.europa.eu/eli/reg/2019/1021/oj. (Accessed 18 October 2022).

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., Palkovičová Murínová, Ľ., 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.

Stockholm Convention, 2017. Available online: http://www.pops.int/Portals/0/download.aspx?d=UNEP-POPS-COP-CONVTEXT-2017.English.pdf (accessed on 18 October 2022).

Suárez, B., Vela-Soria, F., Castiello, F., Olivas-Martinez, A., Acuña-Castroviejo, D., Gómez-Vida, J., Olea, N., Fernández, M.F., Freire, C., 2021. Organophosphate pesticide exposure, hormone levels, and interaction with PON1 polymorphisms in male adolescents. Sci. Total Environ. 769, 144563 https://doi.org/10.1016/j.scitotenv.2020.144563.

Taylor, P.N., Lansdown, A., Witczak, J., Khan, R., Rees, A., Dayan, C.M., Okosieme, O., 2023. Age-related variation in thyroid function - a narrative review highlighting important implications for research and clinical practice. Thyroid Res. 16 (1), 7. https://doi.org/10.1186/s13044-023-00149-5. Erratum in: Thyroid Res. 2023;16 (1):20.

Textor, J., Hardt, J., Knuppel, S., 2011. Dagitty: a graphical tool for analyzing causal diagrams. Epidemiology 22 (5), 745. https://doi.org/10.1097/EDE.0b013e318225c2be.

Vela-Soria, F., Serrano-López, L., García-Villanova, J., de Haro, T., Olea, N., Freire, C., 2020. HPLC-MS/MS method for the determination of perfluoroalkyl substances in breast milk by combining salt-assisted and dispersive liquid-liquid microextraction. Anal. Bioanal. Chem. 412 (28), 7913–7923. https://doi.org/10.1007/s00216-020-02924-x.

Wang, D., Cheng, X., Yu, S., Qiu, L., Lian, X., Guo, X., Hu, Y., Lu, S., Yang, G., Liu, H., 2018a. Data mining: seasonal and temperature fluctuations in thyroid-stimulating hormone. Clin. Biochem. 60, 59–63. https://doi.org/10.1016/j.clinbiochem.2018.08.008.

Wang, M., Guo, W., Gardner, S., Petreas, M., Park, J.S., 2018b. Per- and polyfluoroalkyl substances in Northern California cats: temporal comparison and a possible link to cat hyperthyroidism. Environ. Toxicol. Chem. 37 (10), 2523–2529. https://doi.org/10.1002/etc.4239.

Webster, G.M., Venners, S.A., Mattman, A., Martin, J.W., 2014. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ. Res. 133, 338–347. https://doi.org/10.1016/j.envres.2014.06.012.

Webster, G.M., Rauch, S.A., Marie, N.S., Mattman, A., Lanphear, B.P., Venners, S.A., 2016. Cross-sectional associations of serum perfluoroalkyl acids and thyroid hormones in U.S. adults: variation according to TPOAb and iodine status (NHANES 2007-2008). Environ. Health Perspect. 124 (7), 935–942. https://doi.org/10.1289/ehp.1409589.

Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E., van Leeuwen, S.P., Hamers, T., 2009. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. 109 (2), 206–216. https://doi.org/10.1093/toxsci/kfp055.

Wu, X.M., Bennett, D.H., Calafat, A.M., Kato, K., Strynar, M., Andersen, E., Moran, R.E., Tancredi, D.J., Tulve, N.S., Hertz-Picciotto, I., 2015. Serum concentrations of perfluorinated compounds (PFC) among selected populations of children and adults in California. Environ. Res. 136, 264–273. https://doi.org/10.1016/j.envres.2014.09.026.

Yu, W.G., Liu, W., Jin, Y.H., Liu, X.H., Wang, F.Q., Liu, L., Nakayama, S.F., 2009. Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system. Environ. Sci. Technol. 43 (21), 8416–8422. https://doi.org/10.1021/es901602d.

Yu, W.G., Liu, W., Liu, L., Jin, Y.H., 2011. Perfluorooctane sulfonate increased hepatic expression of OAPT2 and MRP2 in rats. Arch. Toxicol. 85 (6), 613–621. https://doi.org/10.1007/s00204-010-0613-x.

Zhang, S., Guo, X., Lu, S., Sang, N., Li, G., Xie, P., et al., 2018. Exposure to PFDoA causes disruption of the hypothalamus-pituitary-thyroid axis in zebrafish larvae. Environ. Pollut. 235, 974–982. https://doi.org/10.1016/j.envpol.2018.01.015.

Zoeller, R.T., 2007. Environmental chemicals impacting the thyroid: targets and consequences. Thyroid 17 (9), 811–817. https://doi.org/10.1089/thy.2007.0107.