Environmental Research 237 (2023) 116897

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Association of exposure to perfluoroalkyl substances (PFAS) and phthalates with thyroid hormones in adolescents from HBM4EU aligned studies

Andrea Rodríguez-Carrillo [a,b], Elena Salamanca-Fernández [c,d,e,f], Elly den Hond [g], Veerle J. Verheyen [a], Lucia Fábelová [h], Lubica Palkovicova Murinova [h], Susana Pedraza-Díaz [i], Argelia Castaño [i], José Vicente García-Lario [j], Sylvie Remy [a], Eva Govarts [a], Greet Schoeters [k], Nicolás Olea [c,d,e,f], Carmen Freire [c,e,f,l,*], Mariana F. Fernández [c,d,e,f]

[a] VITO Health, Flemish Institute for Technological Research (VITO), 2400, Mol, Belgium
[b] Toxicological Centre, University of Antwerp, Universiteitsplein, 1, 2610, Wilrijk, Belgium
[c] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), 18012, Granada, Spain
[d] Department of Radiology and Physical Medicine, University of Granada, 18071, Granada, Spain
[e] Biomedical Research Centre, University of Granada, 18016, Granada, Spain
[f] CIBER de Epidemiología y Salud Pública (CIBERESP), Spain
[g] Provincial Institute for Hygiene, Antwerp, Belgium
[h] Department of Environmental Medicine, Faculty of Public Health, Slovak Medical University, Bratislava, Slovakia
[i] National Centre for Environmental Health, Instituto de Salud Carlos III, Madrid, Spain
[j] Hospital Universitario Clínico San Cecilio, Granada, Spain
[k] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
[l] Department of Legal Medicine, Toxicology and Physical Anthropology, University of Granada, 18071, Granada, Spain

ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Perfluoroalkyl substances
Phthalates
Thyroid hormones
Adolescents
HBM4EU

ABSTRACT

Background: Perfluoroalkyl substances (PFAS) and phthalates are synthetic chemicals widely used in various types of consumer products. There is epidemiological and experimental evidence that PFAS and phthalates may alter thyroid hormone levels; however, studies in children and adolescents are limited.
Aim: To investigate the association of exposure to PFAS and phthalate with serum levels of thyroid hormones in European adolescents.
Methods: A cross-sectional study was conducted in 406 female and 327 male adolescents (14–17 years) from Belgium, Slovakia, and Spain participating in the Aligned Studies of the HBM4EU Project (FLEHS IV, PCB cohort, and BEA, respectively). Concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), free thyroxine (FT4), free triiodothyronine (FT3), and thyroid-stimulating hormone (TSH) were measured in sera from study participants, and urinary metabolites of six phthalates (DEP, DiBP, DnBP, BBzP, DEHP, and DiNP) and the non-phthalate plasticizer DINCH® were quantified in spot urine samples. Associations were assessed with linear regression and g-computational models for mixtures. Effect modification by sex was examined.
Results: In females, serum PFOA and the PFAS mixture concentrations were associated with lower FT4 and higher FT3 levels; MEP and the sums of DEHP, DiNP, and DINCH® metabolites (∑DEHP, ∑DiNP, and ∑DINCH) were associated with higher FT4; ∑DEHP with lower FT3; and the phthalate/DINCH® metabolite mixture with higher FT4 and lower FT3. In males, PFOA was associated with lower FT4 and the PFAS mixture with higher TSH levels and lower FT4/TSH ratio; MEP and ∑DiNP were associated with higher FT4; and MBzP, ∑DEHP, and the phthalate/DINCH® metabolite mixture with lower TSH and higher FT4/TSH. PFOA, mono-(2-ethyl-5-hydroxyhexyl) phthalate (OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (oxo-MEHP), and monocarboxyoctyl phthalate (MCOP) made the greatest contribution to the mixture effect.
Conclusions: Results suggest that exposure to PFAS and phthalates is associated with sex-specific differences in thyroid hormone levels in adolescents.

* Corresponding author. Department of Legal Medicine, Toxicology and Physical Anthropology, University of Granada, Av. de la Investigación 11, 18071, Granada, Spain.
E-mail address: cfreire@ugr.es (C. Freire).

https://doi.org/10.1016/j.envres.2023.116897
Received 30 March 2023; Received in revised form 24 July 2023; Accepted 13 August 2023
Available online 19 August 2023

1. Introduction

Perfluoroalkyl substances (PFAS) are synthetic chemicals used in a wide range of industrial applications and consumer products, including fire-fighting foams, alkaline cleaners, floor polishes, cosmetics, soil- and stain-resistant coatings for fabrics, carpets, and leather, and grease- and oil-resistant coatings for paper products (Bergman et al., 2012; Renner, 2001). PFAS are highly resistant to degradation and compounds with longer chains have a long half-life in humans (*e.g.*, up to 8.5 years for perfluorohexane sulfonate [PFHxS]) (Bartell et al., 2010; Olsen et al., 2007, 2009), and they tend to bioaccumulate and biomagnify in food chains (Bergman et al., 2012). The main exposure source to PFAS in general populations is the diet, particularly food contact materials, drinking water, and specific food items (Fábelová et al., 2023; Domingo et al., 2012; Vestergren et al., 2008), and the indoor environment (Haug et al., 2011). Human biomonitoring studies have shown that virtually all inhabitants of developed and developing countries have measurable blood concentrations of four PFAS: perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), PFHxS, and perfluorononanoic acid (PFNA) (Kannan et al., 2004; Fromme et al., 2009; Kato et al., 2011; Lopez-Espinosa et al., 2012; Manzano-Salgado et al., 2016; Zhao et al., 2012). PFAS regulations are mainly focused on PFOS and PFOA, which are included in the Stockholm Convention on persistent organic pollutants and have been banned in the European Union (EU) since 2008 (*Stockholm Convention - Home page*, n.d.). Other PFAS, such as PFHxS and perfluorohexanoic acid (PFHxA), are also subject to restrictions in the EU (*Perfluoroalkyl chemicals (PFASs) - ECHA*, n.d.).

Phthalates are another class of high-production industrial chemicals widely used as plasticizers in food packaging materials, medical equipment, toys, furniture, and cosmetics (Koch and Calafat, 2009). High-molecular weight phthalates, such as di(2-ethylhexyl) phthalate (DEHP), are used as plasticizers to impart flexibility to polyvinyl chloride (PVC) materials (Coltro et al., 2014), while low-molecular weight phthalates such as di-ethyl phthalate (DEP) are often used as solvents in personal care products, and in adhesives, varnishes, and coatings (Wittassek et al., 2011). In recent years, di-iso-nonyl phthalate (DiNP) and the non-phthalate plasticizer di-iso-nonyl-cyclohexane-1, 2-dicarboxylate (DINCH®) have been replacing DEHP and other phthalates in these applications (*ECHA, 2009. Data on Manufacture, Import, Export, …*- n.d.). Humans are exposed to phthalates through ingestion, dermal absorption, and inhalation (Schettler, 2006); these compounds are rapidly metabolized, and their metabolites have been detected in urine from the general population, including adolescents (Bastiaensen et al., 2021; Johns et al., 2015; Koch et al., 2003).

PFAS and phthalates are well-known groups of endocrine-disrupting chemicals (EDCs), and some disrupt thyroid hormone homeostasis (Breous et al., 2005; Coperchini et al., 2021; Dong et al., 2019; Jensen and Leffers, 2008; Wenzel et al., 2005). Thyroid hormones are crucial for numerous physiological processes in children and adults, including fetal and child growth and development, energy balance, metabolism, and other functions of reproductive, nervous, and cardiovascular systems (Diamanti-Kandarakis et al., 2009; Miller et al., 2009). Thyroid hormones are especially important for neurodevelopmental processes such as myelination, which is not completed until adolescence (Rice and Barone, 2000; Schug et al., 2015). Animal studies demonstrated that exposure to the phthalates DEHP and di-butyl-phthalate (DBP) altered thyroid hormone levels (O'Connor et al., 2002; Sun et al., 2022). Cross-sectional studies in adults (Albert et al., 2018; Choi et al., 2020; Donat-Vargas et al., 2021; Huang et al., 2007; Meeker et al., 2007; Meeker and Ferguson, 2011; Souter et al., 2020; F. Wang et al., 2018; Yue et al., 2023) and longitudinal and cross-sectional analysis in pregnant women (Derakhshan et al., 2021; Huang et al., 2007; Kuo et al., 2015; Nakiwala et al., 2022; Romano et al., 2018) have variously reported positive, negative, or null associations between urinary phthalate metabolites and serum levels of thyroxine (T4), triiodothyronine (T3), or thyroid-stimulating hormone (TSH). Studies in children and adolescents have been more limited but provide evidence of an association between urinary phthalate metabolites, particularly DEHP and DBP metabolites, and concurrent thyroid hormone levels (Boas et al., 2010; Huang et al., 2020a,b; Hyun Kim et al., 2018; Meeker and Ferguson, 2011; Morgenstern et al., 2017; Zhao et al., 2022). However, the direction of the associations is not consistent among studies, and the age of study participants has ranged from 3 years (Morgenstern et al., 2017) to 19 years (Meeker and Ferguson, 2011; Zhao et al., 2022). Likewise, data on the association between prenatal, childhood, or adult exposure to PFAS and thyroid function have been inconclusive (Ballesteros et al., 2017; Blake et al., 2018; Boesen et al., 2020; Byrne et al., 2018; Coperchini et al., 2021; Hyun Kim et al., 2018; Lewis et al., 2015). However, results obtained in adults indicate a mainly positive association of PFAS exposure with TSH, and an inverse association with T3 and T4 (Boesen et al., 2020), while cross-sectional studies in children and adolescents provide some evidence of a positive association of serum PFNA and PFOA with higher total or free T4 levels, observing varied results for other PFAS such as PFOS (Caron-Beaudoin et al., 2019; Freire et al., 2023; Lewis et al., 2015; Lin et al., 2013; Lopez-Espinosa et al., 2012).

In general, there have been few human studies with comparable data on the relationship between exposure to PFAS or phthalates and thyroid hormones, and research in children and adolescents has been limited. The European Human Biomonitoring Initiative HBM4EU (*HBM4EU - science and policy for a healthy future*, n.d.) has listed PFAS and phthalates as priority substances for human biomonitoring and further review of their regulation in the EU. The purpose of the present study was to investigate the association of exposure to PFAS and phthalate metabolites with serum levels of thyroid hormones in European adolescents in the context of HBM4EU. Given that thyroid hormones are modulated by sex steroids (and *vice versa*) (Tahboub and Arafah, 2009), it was also explored whether PFAS and phthalate metabolites are associated with thyroid hormones in a sex-specific manner.

2. Methods

2.1. Study population

This cross-sectional study included participants from HBM4EU aligned studies (Gilles et al., 2022). The aim was to collect and harmonise human biomonitoring (HBM) data to support chemical's risk assessment and risk management in Europe. HBM4EU builds on existing capacity for HBM in the participating countries. The present study included the aligned studies with data available on adolescents (12–19 years old) and their serum PFAS, urinary phthalate/DINCH® metabolites, and serum thyroid hormone levels, *i.e.*, FLEHS IV (Flemish Environment and Health Study IV, Belgium, n: 300, age range: 14–15 years), PCB cohort (Endocrine Disruptors and Health in Children and Adolescents in Slovakia; Slovakia, n: 294, age range: 15–17 years), and BEA (*Biomonitorización en Adolescentes*, Spain, n: 300, age range: 14–16 years). These studies met the following eligibility criteria (Gilles et al., 2021): i) initiated before the start of the HBM4EU project or, if new, fully compliant with HBM4EU protocols; ii) biological sample collection in 2014–2020, to evaluate recent exposure (*i.e.*, FLESH IV: 2017–2018; PCB cohort: 2019–2020; BEA: 2017–2018), and iii) performance of chemical analyses by laboratories in the HBM4EU Quality Assurance/Quality Control (QA/QC) program (Nübler et al., 2022). After excluding participants with no available data on exposure biomarkers or serum thyroid hormones, the final sample comprised 733 male and female adolescents. BEA and FLEHS IV were cross-sectional studies, while PCB study was a longitudinal cohort study. All parents and adolescents signed informed consent. Detailed information on study selection and data harmonization within the HBM4EU was previously reported (Gilles et al., 2021, 2022).

2.2. PFAS, phthalate metabolites, and thyroid hormones

Frozen serum and urine samples were collected from their respective studies and immediately kept at −80 °C until analyses. PFAS and phthalate metabolites were respectively measured in serum and urine samples at laboratories fulfilling HBM4EU QA/QC protocols (Esteban-López et al., 2021). PCB cohort collected fasting serum samples, and FLEHS IV and BEA collected non-fasting serum samples. FLEHS IV and PCB cohort collected random urine samples, while BEA primarily collected first-morning urine samples. In all studies, PFAS were determined with liquid chromatography-tandem mass spectrometry and phthalate metabolites with ultra-performance liquid chromatography-tandem mass spectrometry; the QA/QC protocol and sample measurements are reported in detail elsewhere (Esteban-López et al., 2021; Mol et al., 2022). Twelve PFAS, fifteen phthalate metabolites, and two metabolites of the non-phthalate plasticizer DINCH® were initially measured, but only compounds with detection frequencies above 75% were selected for the present study. Reported detection limits (LODs) and quantification limits (LOQs) varied among studies; therefore, the LOQ served as cut-off value for reporting quantifiable data (Supplementary Material, Table S1 and Table S2). Consequently, the main analysis included three PFAS (PFOA, PFNA, and PFOS); ten urinary monoester metabolites of phthalates: mono-ethyl phthalate (MEP), metabolite of DEP; mono-benzyl phthalate (MBzP), metabolite of BBzP; mono-iso-butyl phthalate (MiBP), metabolite of DiBP; mono-n-butyl phthalate (MnBP), metabolite of DnBP; mono-(2-ethyl-hexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (OH-MEHP), and mono-(2-ethyl-5-oxohexyl) phthalate (oxo-MEHP), primary metabolites of DEHP; mono-(2-ethyl-5-carboxypentyl) phthalate (cx-MEPP), metabolite of MEHP; mono-hydroxyisononyl phthalate (MHNP) and monocarboxyoctyl phthalate (MCOP), secondary metabolites of DiNP; and two DINCH® metabolites: cyclohexane-1, 2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH) and cyclohexane-1,2-dicarboxylate-mono-(7-carboxylate-4-methyl) heptyl ester (cx-MINCH). Additionally, the arithmetic sum of PFOS, PFNA, and PFOA concentrations was calculated ($\sum$PFAS= PFOA + PFNA + PFOS) (μg/L), and the molar-weight (mw) adjusted sum of the three primary urinary metabolites of DEHP was calculated by dividing each metabolite concentration by its molecular weight and then summing. Thus, the sum of DEHP metabolites ($\sum$DEHP) was (MEHP*1/mw) + (OH-MEHP*1/mw) + (oxo-MEHP*1/mw) (μmol/L); and the same procedure was applied for the two metabolites of DiNP, calculating $\sum$DiNP=(MHNP*1/mw) + (MCOP*1/mw) and DINCH®: $\sum$DINCH=(OH-MINCH*1/mw) + (cx-MINCH*1/mw), respectively.

Serum levels of free T4 (FT4), free T3 (FT3), and TSH were measured in all participants, calculating the FT4/TSH ratio as marker of the negative feedback control mechanism of the hypothalamus-pituitary-thyroid (HPT) axis. Thyroid hormones were measured by immunoassay using the UniCel DxI 600 Access Immunoassay System of Beckman Coulter® at the facilities of San Cecilio University Hospital in Granada (Spain). Briefly, FT4 was determined using a ruthenium chelate-labeled anti-T4 antibody (Tris (2,2′-bipyridine) ruthenium (II) chelate (Ru (bpy)), and FT3 with the Elecsys T3 test, which uses a 8-anilino-1-naphthalenesulfonic acid (ANS) that competes with the biotinylated derivative of exogenously added T3 to occupy binding sites on ruthenium complex-labeled antibodies (Tris Complex (2,2′-bipyridine) ruthenium (II) (Ru (bpy)). TSH was measured using the Elecsys TSH test with monoclonal antibodies specifically directed against human TSH. Strict QA/QC protocols were also performed, as previously reported (Fernández et al., 2021). Finally, urinary creatinine levels were determined using the CRJ2U colorimetric kinetic test based on the Jaffé method.

2.3. Statistical analysis

Serum PFAS, urinary phthalate and DINCH® metabolites, and serum thyroid hormones were natural log-transformed to centralize distribution skewness. Concentrations of PFAS and phthalate/DINCH® metabolites below the limit of quantification (LOQ) were imputed by random imputation from the estimated with truncated lognormal distribution (Lubin et al., 2004). Phthalates and DINCH® metabolites were also standardized by urinary creatinine (μg/g for single biomarkers and μmol/g for sum-parameters) to correct for urine dilution (O'Brien et al., 2016). Pairwise Spearman correlation coefficients were used to examine correlations between PFAS and phthalate/DINCH® metabolites (Table S3). The association of each individual PFAS and urinary phthalate/DINCH® metabolite with each thyroid parameter was assessed using linear regression models. All variables were entered in the model as fixed variables. Confounders were selected *a priori* using a directed acyclic graph (DAG) (Textor et al., 2011) (Fig. S1) based on previous evidence of their relevance to thyroid hormones and PFAS/phthalate exposure, and included: age (continuous, in years) and BMI z-score (zBMI) (standardized for age and sex according to the World Health Organization (WHO, 2022) using the 'anthroplus' package in R), which influence several maturational processes (Gillison et al., 2017); sex, to account for sex-dependent differences (Lee et al., 2018); study of origin (FLEHS IV/BEA/PCB), to account for potential differences by country of residence; household educational level, based on the International Standard Classification of Education (ISCED: low, ISCED 0–2/medium, ISCED 3–4/high, ISCED 5–8), as indicator of socioeconomic status (Jackson et al., 2017); and urine creatinine levels, to correct for potential error bias in measurements (O'Brien et al., 2016). To assess potential non-linear relationships, exposure biomarkers were categorized based on quartiles. Regression estimates were transformed to represent the percentage of difference in each thyroid parameter associated with a log-unit increase in each exposure biomarker with corresponding 95% confidence interval (CI) or 4th/3rd/2nd quartile *versus* 1st quartile concentrations.

The mixture effect of PFAS and phthalate/DINCH® metabolites was evaluated by quantile g-computation using the 'qgcomp' package in R (version 3.6.1) with 1000 bootstraps. Quantile g-computation was performed by categorizing urinary biomarkers of exposure to PFAS/phthalates in quartiles. Each exposure biomarker was assigned a weight representing its relative contribution to the estimated effect in a positive or negative direction (Keil et al., 2020). If the individual compound showed a different direction of the effect, the weight was interpreted as the proportion of the partial effect in the negative or positive direction. Because serum PFAS concentrations provide information on long-term PFAS exposure and urinary phthalate metabolites reflect recent exposure to parent compounds, their mixture effects were considered separately.

A sensitivity analysis excluding zBMI from the models was conducted to explore whether results changed substantially from those of the main analyses, possibly reflecting mediation. Finally, we assessed the potential association between PFHxS and thyroid hormones, including the effect of the PFAS mixture, in the sub-sample of participants (FLEHS IV and PCB cohort, N = 463) with available data for PFHxS. P-values were based on two-sided tests, setting the cut-off for statistical significance at 0.05.

3. Results

3.1. Characteristics of study participants

Characteristics of the total sample of participants and those in the different studies (FLEHS IV, PCB cohort, and BEA) are displayed in Table 1. The mean (standard deviation, SD) age and zBMI of adolescents was 14.96 (0.86) years and 0.13 (1.13), respectively; 45% were male and 55% were females. Around 85% of their parents had a medium or high educational level at time of recruitment (Table 1).

None of the adolescents had thyroid hormone levels outside the laboratory reference range (Radicioni et al., 2013; Lewandowski et al.,

Table 1
Sociodemographic characteristics of the study population.

Characteristics	FLEHS IV (Belgium)	PCB cohort (Slovakia)	BEA (Spain)	All participants
	n = 237	n = 226	n = 270	n = 733
	Mean ± SD or n (%)			
Age (years)	14.43 ± 0.58	15.78 ± 0.59	14.74 ± 0.76	14.96 ± 0.86
Sex, male/female	103 (44)/134 (56)	95 (42)/131 (58)	129 (48)/141 (52)	327 (45)/406 (55)
zBMI	−0.02 ± 1.13	0.25 ± 1.17	0.16 ± 1.08	0.13 ± 1.13
Overweight/obese[a]	45 (19.0)	57 (25.2)	62 (23.0)	164 (22.4)
Urinary creatinine (mg/dL)	155.87 ± 71.75	191.64 ± 108.87	128.25 ± 59.63	156.73 ± 85.35
Household education				
Low	17 (7.2)	28 (12.4)	68 (25.2)	113 (15.4)
Medium	90 (38.0)	183 (81.0)	80 (29.6)	353 (48.2)
High	130 (54.9)	15 (6.6)	122 (45.2)	267 (36.4)

[a] Based on BMI z-score standardized for age and sex (WHO, 2022).

2015) (Table 2). The median (interquartile range) of thyroid hormone levels was 1.81 (1.33–2.43) μIU/mL for TSH, 0.84 (0.77–0.94) ng/dL for FT4, 0.35 (0.32–0.38) ng/dL for FT3, and 0.47 (0.34–0.66) for FT4/TSH ratio (Table 2). Central dispersion of serum PFAS and urinary phthalate/DINCH® metabolite concentrations is shown in Table 2. Belgian adolescents had the highest serum PFAS concentrations, whereas Slovakian and Spanish adolescents had the highest urinary concentrations of phthalate and DINCH® metabolites, respectively.

3.2. Association of individual environmental chemicals with thyroid hormones

In single-exposure models, serum PFOA, PFOS, and ∑PFAS were significantly associated with lower FT4 levels [% difference (95% CI): 4.52 (−6.60;-2.40), −9.01 (−11.22;-6.75), and −2.55 (−4.27;-0.79) per log-unit increase in concentrations, respectively]; PFOA and ∑PFAS were also associated with a lower FT4/TSH ratio (Table 3). Urinary MEP was associated with higher FT4 and FT4/TSH levels [% difference (95% CI): 2.69 (1.70; 3.69) and 4.82 (1.55; 8.20), respectively]; MBzP with higher FT4/TSH [% difference (95% CI): 3.93 (0.09; 7.92)]; ∑DEHP with higher FT4 and FT4/TSH [% difference (95% CI): 2.05 (0.38; 3.75) and 10.82 (5.08; 16.88), respectively] and lower TSH and FT3 [% difference (95% CI): 7.92 (−12.32;-3.29) and −2.23 (−3.70;-0.74), respectively]; and ∑DiNP and ∑DINCH® with higher FT4 [% difference (95% CI): 3.05 (1.54; 4.58) and 1.60 (0.16; 3.07), respectively] (Table 3). Analysis based on quartiles showed some non-linear associations of MEP with TSH and of MBzP, MiBP, MEHP, and OH-MINCH with FT4 (Table S4). Sensitivity analysis excluding zBMI from the models showed similar results (Table S5).

In males, PFOA was associated with lower FT4 and FT4/TSH [% difference (95% CI): 6.81 (−10.64;-2.81) and −12.54 (−22.54;-1.25), respectively]; MEP was associated with higher FT4 and FT4/TSH [% difference (95% CI): 2.64 (1.16; 4.14) and 5.32 (0.96; 9.86), respectively], ∑DEHP with lower TSH and higher FT4/TSH [% difference (95% CI): 14.42 (−20.28;-8.13) and 19.35 (10.33; 29.12), respectively], and ∑DiNP with higher FT4 [% difference (95% CI): 3.03 (0.88; 5.22)] (Fig. 1, Table S6). In females, associations were closer to those observed in Table 3, including associations of ∑PFAS, MEP, and ∑DEHP with FT4 [% difference (95% CI): 5.36 (−7.98;-2.67), 2.63 (1.29; 3.99), and 2.18 (0.11; 4.30), respectively]; associations of ∑PFAS and ∑DEHP

Table 2
Concentrations of serum PFAS, urinary phthalate/DINCH® metabolites, and serum thyroid hormones in adolescents from HBM4EU aligned studies.

Chemicals/thyroid hormones	FLEHS IV (Belgium) n = 237			PCB cohort (Slovakia) n = 226			BEA (Spain) n = 270			All participants n = 773		
	Median	P25	P75	Median	P25	P75	Median	P25	P75	Median	P25	P75
Serum PFAS (μg/L)rowhead												
PFOA	1.10	0.88	1.40	0.77	0.55	0.98	0.66	0.52	0.79	0.79	0.59	1.07
PFNA	0.31	0.23	0.44	0.18	0.11	0.27	0.28	0.21	0.39	0.27	0.17	0.38
PFOS	2.20	1.50	3.40	1.36	0.87	2.42	1.38	0.93	1.86	1.60	1.00	2.50
∑PFAS	3.93	2.99	5.45	2.53	1.82	3.97	2.51	1.93	3.17	2.89	2.05	4.18
Urinary phthalate metabolites (μg/g creatinine) and sum of phthalate metabolites (μmol/g creatinine)rowhead												
MEP	15.15	8.77	36.27	34.28	17.81	66.58	68.57	38.00	136.92	36.83	16.33	85.93
MBzP	1.41	0.78	3.08	0.94	0.42	1.62	1.34	0.82	2.27	1.19	0.68	2.18
MiBP	14.09	8.90	24.81	20.75	10.97	39.01	16.46	11.63	25.10	16.07	10.47	29.47
MnBP	10.92	7.42	17.68	57.66	33.60	111.31	12.98	8.19	19.20	16.86	9.19	38.21
DEHP metabolitesrowhead												
MEHP	0.86	0.50	1.36	2.29	1.28	4.17	1.65	1.05	2.59	1.45	0.80	2.64
OH-MEHP	3.88	2.48	6.10	20.16	12.00	33.70	7.09	4.93	10.51	7.38	4.15	15.28
oxo-MEHP	2.39	1.63	3.80	4.27	2.69	7.19	4.99	3.52	7.28	3.85	2.35	6.56
∑DEHP	7.13	4.81	11.18	26.75	16.69	49.43	13.76	9.49	20.37	13.57	7.95	24.15
cx-MEPP	10.07	7.17	14.19	5.42	3.49	9.37	9.30	6.22	13.66	8.46	5.40	12.45
DiNP metabolitesrowhead												
MHNP	2.67	1.70	4.21	9.61	6.01	17.22	3.29	2.13	5.77	4.05	2.31	8.31
MCOP	1.21	0.79	1.79	4.59	2.44	7.42	5.45	3.44	8.13	3.38	1.59	6.38
∑DiNP	3.95	2.49	5.90	14.76	9.20	25.26	8.93	5.81	14.12	7.95	4.44	14.84
DINCH® metabolitesrowhead												
OH-MINCH	0.76	0.48	1.51	0.89	0.53	1.76	1.38	0.97	2.31	1.09	0.63	1.84
cx-MINCH	0.71	0.49	1.06	0.45	0.25	0.87	0.79	0.55	1.46	0.68	0.43	1.09
∑DINCH	1.50	0.98	2.50	1.33	0.82	2.71	2.12	1.53	3.76	1.77	1.07	2.94
Thyroid hormonesrowhead												
TSH (μIU/mL)	1.84	1.42	2.50	2.00	1.43	2.62	1.65	1.24	2.11	1.81	1.33	2.43
FT4 (ng/dL)	0.76	0.70	0.83	0.86	0.80	0.95	0.91	0.84	1.01	0.84	0.77	0.94
FT3 (ng/dL)	0.35	0.33	0.38	0.37	0.34	0.41	0.33	0.31	0.37	0.35	0.32	0.38
FT4/TSH	0.41	0.30	0.55	0.46	0.32	0.60	0.54	0.42	0.77	0.47	0.34	0.66

P25, P75: 25th and 75th percentiles; TSH: thyroid stimulating hormone; FT4: free thyroxine; FT3: free triiodothyronine; FT4/TSH: ratio of FT4 to TSH concentrations.

Table 3
Adjusted associations of serum PFAS and urinary phthalate/DINCH® metabolite concentrations with thyroid parameters (n = 733).

Exposure biomarkers	TSH			FT4			FT3			FT4/TSH		
	%	95% CI		%	95% CI		%	95% CI		%	95% CI	
		LL	UL		LL	UL		LL	UL		LL	UL
PFAS metabolites												
PFOA	2.88	−4.59	10.93	−9.01*	−11.22	−6.75	1.10	−1.21	3.47	−11.56*	−18.48	−4.04
PFNA	0.89	−4.24	6.31	−0.59	−2.33	1.18	0.90	−0.71	2.53	−1.47	−6.91	4.29
PFOS	2.63	−2.67	8.23	**−2.55***	−4.27	−0.79	1.42†	−0.23	3.08	−5.05†	−10.37	0.59
∑PFAS	3.29	−3.29	10.31	−4.52*	−6.60	−2.40	1.65	−0.39	3.72	−7.56*	−13.93	−0.73
Phthalate/DINCH® metabolites												
MEP	−2.03	−4.86	0.88	2.69*	1.70	3.69	−0.72	−1.61	0.18	4.82*	1.55	8.20
MBzP	−3.18	−6.48	0.23	0.63	−0.54	1.81	−0.82	−1.87	0.24	3.93*	0.09	7.92
MiBP	−2.56	−6.47	1.53	0.46	−0.92	1.86	−0.66	−1.90	0.60	3.09	−1.40	7.80
MnBP	−3.56	−7.86	0.94	−0.35	−1.87	1.19	−1.33†	−2.70	0.06	3.33	−1.67	8.59
DEHP												
MEHP	−5.82*	−9.64	−1.84	1.67*	0.26	3.10	−1.69*	−2.94	−0.43	7.96*	3.22	12.92
OH-MEHP	−6.29*	−10.45	−1.93	1.33†	−0.21	2.90	−2.16*	−3.52	−0.79	8.13*	2.92	13.61
oxo-MEHP	−7.91*	−12.17	−3.45	2.88*	1.26	4.53	−1.91*	−3.33	−0.47	11.72*	6.14	17.60
∑DEHP	−7.92*	−12.32	−3.29	2.05*	0.38	3.75	−2.23*	−3.70	−0.74	10.82*	5.08	16.88
cx-MEPP	−2.95	−8.15	2.56	0.05	−1.79	1.93	−0.97	−2.64	0.72	3.09	−2.91	9.46
DiNP												
MHNP	2.62	−1.82	7.26	0.48	−1.01	1.99	0.12	−1.23	1.49	−2.09	−6.69	2.74
MCOP	−3.07†	−6.58	0.58	4.34*	3.09	5.61	−1.18*	−2.29	−0.05	7.64*	3.44	12.02
∑DiNP	−0.07	−4.40	4.45	3.05*	1.54	4.58	−0.54	−1.88	0.83	3.13	−1.72	8.21
DINCH®												
OH-MINCH	−1.39	−5.22	2.58	1.90*	0.56	**3.25**	−0.14	−1.35	1.08	3.34	−1.01	7.87
cx-MINCH	2.01	−1.98	6.16	0.91	−0.44	2.27	0.22	−1.01	1.45	−1.08	−5.28	3.31
∑DINCH	−0.63	−4.79	3.71	1.60*	0.16	3.07	0.12	−1.19	1.44	2.24	−2.40	7.10

All models are adjusted by study of origin (FLEHS IV, PCB, BEA; random effect), age (years), sex (male, female), zBMI (continuous), household education (low, medium, high), and urinary creatinine concentration (only for phthalate metabolites).
Estimates are expressed as the percentage difference in thyroid hormone level for one log-unit increase in the exposure biomarker.
%: percentage difference; CI: Confidence interval; LL: Lower level; UL: Upper level.
*p ≤ 0.05; †p < 0.10.

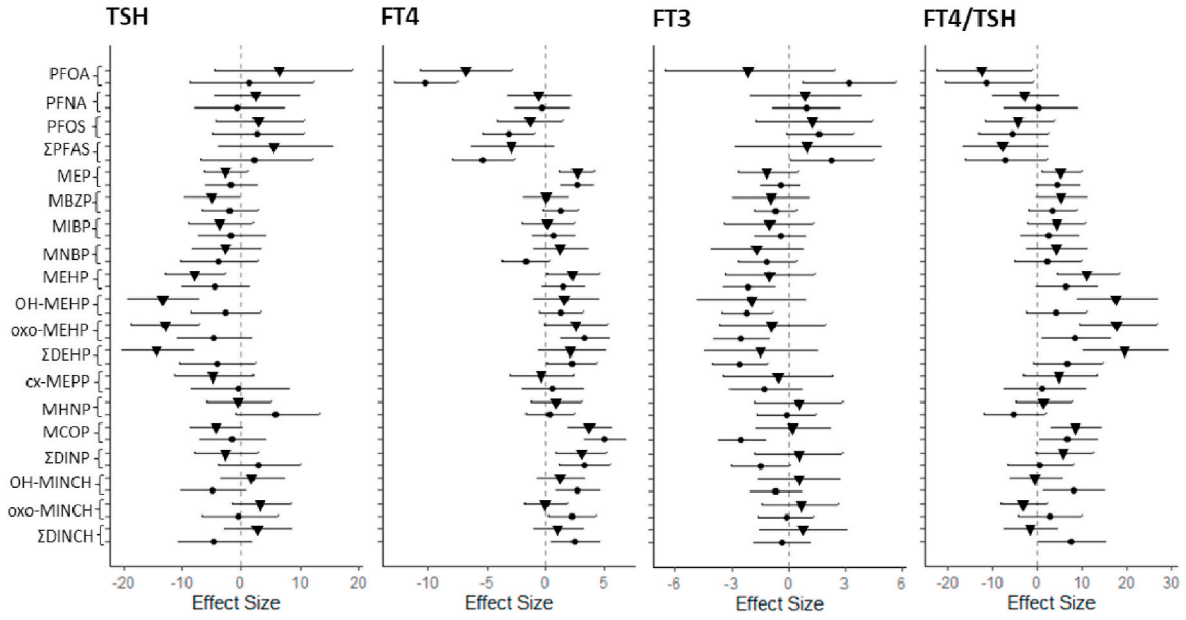

Fig. 1. Association of serum PFAS and urinary phthalate/DINCH® metabolites with thyroid hormones in males (n = 317, squares) and females (n = 406, circles).

with FT3 [% difference (95% CI): 2.30 (0.13; 4.51) and −2.57 (−4.06;-1.06), respectively]; and associations of ∑DiNP and ∑DINCH with higher FT4 [% difference (95% CI): 3.27 (1.15; 5.44) and 2.47 (0.45; 4.52), respectively] (Fig. 1, Table S7).

In the sub-analysis of the effect of PFHxS on thyroid hormones in FLEHS IV and PCB cohorts, PFHxS was associated with higher FT3 and TSH in the total sample and in males and females (Table S8).

Figure shows the % difference (95% CI) per one log-unit increase in exposure according to sex. Models are adjusted by study of origin (FLEHS IV, PCB cohort, BEA; random effect), age (years), sex (male, female), zBMI (continuous), household education (low, medium, high), and urinary creatinine concentration (only for phthalate metabolites). Triangles highlight associations found in male participants and dots associations found in female participants.

3.3. Mixture effect of PFAS/phthalates on thyroid hormones

The mixture of three PFAS was significantly associated with lower

FT4 levels [% difference (95% CI): 2.60 (−3.92;-1.19) per quartile increase in the mixture concentration and marginally associated with a lower FT4/TSH ratio [% difference (95% CI): 4.00 (−7.96; 0.10)] (Table 4), with PFOA being the major contributor to these effects (weights: 0.81 and −0.72, respectively) (Fig. 2). In contrast, the mixture of urinary phthalate/DINCH® metabolites was significantly associated with higher FT4 levels [% difference (95% CI): 2.59 (0.40; 4.81)] and a higher FT4/TSH ratio [% difference (95% CI): 9.13 (0.00; 15.60)] (Table 4), with MCOP and oxo-MEHP contributing most to these effects (weights for MCOP: 0.38 and 0.18; weights for oxo-MEHP: 0.29 and 0.27, respectively; Fig. 2), and marginally associated with lower FT3 [% difference (95% CI): 2.09 (−4.30; 0.20)] (higher weights for OH-MEHP: 0.24 and MCOP: 0.22) (Table 4, Fig. 2). Exclusion of zBMI from the g-computation model showed similar results, although some associations were attenuated (Table S9).

In females, higher concentrations of the PFAS mixture were significantly associated with lower FT4 levels [% difference (95% CI): 3.00 (−4.78;-1.19)]. In males, the mixture was significantly associated with a lower FT4/TSH ratio [% difference (95% CI): 5.67 (−10.95;-0.10)] and marginally associated with higher TSH [% difference (95% CI): 4.81 (−0.50; 10.41)] (Table 4). PFOA was the major contributor to these associations (Supplementary material, Figs. S2 and S3). Regarding phthalate/DINCH® metabolites, higher concentrations of the mixture were significantly associated with lower FT3 [% difference (95% CI): 2.26 (−4.40;-0.10)] and marginally associated with higher FT4 [% difference (95% CI): 2.69 (−0.20; 5.65)] in females (Table 4), with oxo-MEHP and MCOP contributing most to the overall mixture effect (Fig. S3). In males, the phthalate metabolite mixture was significantly associated with a higher FT4/TSH ratio [% difference (95% CI): 10.97 (−0.40; 23.61)] (Table 4), with OH-MEHP and oxo-MEHP being the major contributors (Fig. S2). In the sub-analysis including PFHxS, no significant association was found between the PFAS mixture and any thyroid parameter (Table S8).

4. Discussion

To our best knowledge, this is the first study on the association of PFAS and phthalate exposure with thyroid function in a relatively large sample of European adolescents. In females, higher concentrations of PFOA and the PFAS mixture were associated with lower FT4 and higher FT3 levels; higher MEP, ∑DEHP, ∑DiNP, and ∑DINCH with higher FT4; higher ∑DEHP with lower FT3; and higher concentrations of the phthalate mixture with higher FT4 and lower FT3. In males, higher concentrations of PFOA were associated with lower FT4 and the PFAS mixture with a lower FT4/TSH ratio, whereas MBzP, ∑DEHP, and the phthalate mixture were associated with lower TSH and higher FT4/TSH, and MEP and ∑DiNP with higher FT4. These results support the hypothesis that exposure of adolescents to mixtures of PFAS and mixtures of phthalate metabolites may be associated with mild subclinical differences in thyroid hormone levels and that the effects are sex dependent.

Table 4
Serum PFAS and urinary phthalate/DINCH® metabolite mixture effects on thyroid hormones.

Thyroid parameters	PFAS mixture			Phthalate/DINCH® mixture		
	%	95% CI		%	95% CI	
		LL	UL		LL	UL
All participants (n = 733)						
TSH	1.46	−2.37	5.44	−4.59	−10.86	2.02
FT4	−2.60*	−3.92	−1.19	2.59*	0.40	4.81
FT3	0.73	−0.50	1.92	−2.09†	−4.30	0.20
FT4/TSH	−4.00†	−7.96	0.10	9.13*	0.00	15.60
Males (n = 327)						
TSH	4.81#x2020;	−0.50	10.41	−7.42	−16.05	2.12
FT4	−1.13	−3.40	1.21	2.74	−0.50	6.08
FT3	−0.05	−2.37	2.33	−2.08	−6.01	2.02
FT4/TSH	−5.67*	−10.95	−0.10	10.97†	−0.40	23.61
Females (n = 406)						
TSH	−0.65	−5.73	4.71	−1.97	−12.19	9.42
FT4	−3.00*	−4.78	−1.19	2.69†	−0.20	5.65
FT3	0.96	−0.50	2.43	−2.26*	−4.40	−0.10
FT4/TSH	−2.36	−7.69	3.36	4.75	−6.57	17.47

PFAS mixture components: PFNA, PFOS, PFOA; Phthalate/DINCH metabolite mixture components: MEP, MBzP, MiBP, MnBP, MEHP, OH-MEHP, oxo-MEHP, cx-MEPP, MHNP, MCOP, OH-MINCH, and cx-MINCH. All models are adjusted by study of origin (FLEHS IV, PCB cohort, BEA), age (years), sex (male, female), zBMI (continuous), household education (low, medium, high), and urinary creatinine concentration (only for phthalate metabolites). Estimates are expressed as percentage of difference in thyroid hormone parameter per each quartile increase in the mixture concentration. %: percentage of difference; CI: Confidence interval; LL: Lower level; UL: Upper level. *p ≤ 0.05; †p < 0.10.

4.1. PFAS and thyroid hormones

Data have recently been published on PFAS concentrations in serum or plasma collected in 2014–2021 and related factors in adolescents (12–18 years) from nine European countries participating in HBM4EU aligned studies, including FLEHS IV, PCB cohort, and BEA (Richterová et al., 2023). PFOS was the most abundant PFAS in serum/plasma from adolescents, with concentrations that were two-fold higher than PFOA concentrations, and PFAS exposure levels were higher in Northern and Western European countries (Richterová et al., 2023). Internal PFAS concentrations in European adolescents are similar to those observed in North American adolescents for the same period (Centers for Disease Control and Prevention., n.d.; Health Canada, 2019, 2021).

Among children and adolescents, serum PFNA concentrations several-fold higher than in the present study (mean: 1–3 μg/L serum *vs.* median: 0.27 μg/L plasma) were associated with slight increases in free or total T4 levels in cross-sectional studies conducted in Taiwan (age of participants: 12–30 years) (Lin et al., 2013), a Native Community in Quebec (age: 6–19 years) (Caron-Beaudoin et al., 2019), and the vicinity of a Teflon manufacturing facility in the USA (age: 1–17 years) (Lopez-Espinosa et al., 2012). In the present study, PFOA was the only PFAS associated with thyroid hormones both in males and females, and the association between serum PFOA and lower FT4 (males and females) and higher FT3 (females) is not supported by previous reports on children/adolescents exposed to higher PFOA levels, which showed positive or null cross-sectional associations with FT4 or FT3 (Caron-Beaudoin et al., 2019; Lewis et al., 2015; Lopez-Espinosa et al., 2012). This is of interest, given that associations could be expected to be more evident at higher exposure levels. However, most EDCs are likely to have low-dose effects that are not predicted by effects at higher doses, and nonlinearity is not uncommon after exposure to hormones and endocrine disruptors in experimental and human studies (Vandenberg et al., 2012). However, Freire et al. (2023) recently observed an association of PFOA and the mixture of ten PFAS in plasma with higher rather than lower FT4 levels in Spanish male adolescents (15–17 years) exposed to PFAS concentrations in the range of those in the present study. In addition, the inverse association of PFOS with FT4 observed in this study is not consistent with the positive association found for children and adolescents living near a Teflon factory (Lopez-Espinosa et al., 2012), although differences in the age of participants may hamper direct comparison with previous findings.

Results of g-computation analysis indicated that PFOA made the greatest contribution to the overall mixture effect. In the study of Spanish male adolescents, PFOA was not among the PFAS contributing most to the effect of the PFAS mixture on FT4 levels (Freire et al., 2023). In pregnant women participating in the Project Viva cohort (USA), higher plasma concentrations of a PFAS mixture were associated with lower maternal FT4 index, and PFOA was among the main contributors to this mixture effect (Preston et al., 2020); however, findings in adolescents are not comparable to those in pregnant women. On the other hand, the present observation of a mixture effect of PFAS on higher TSH

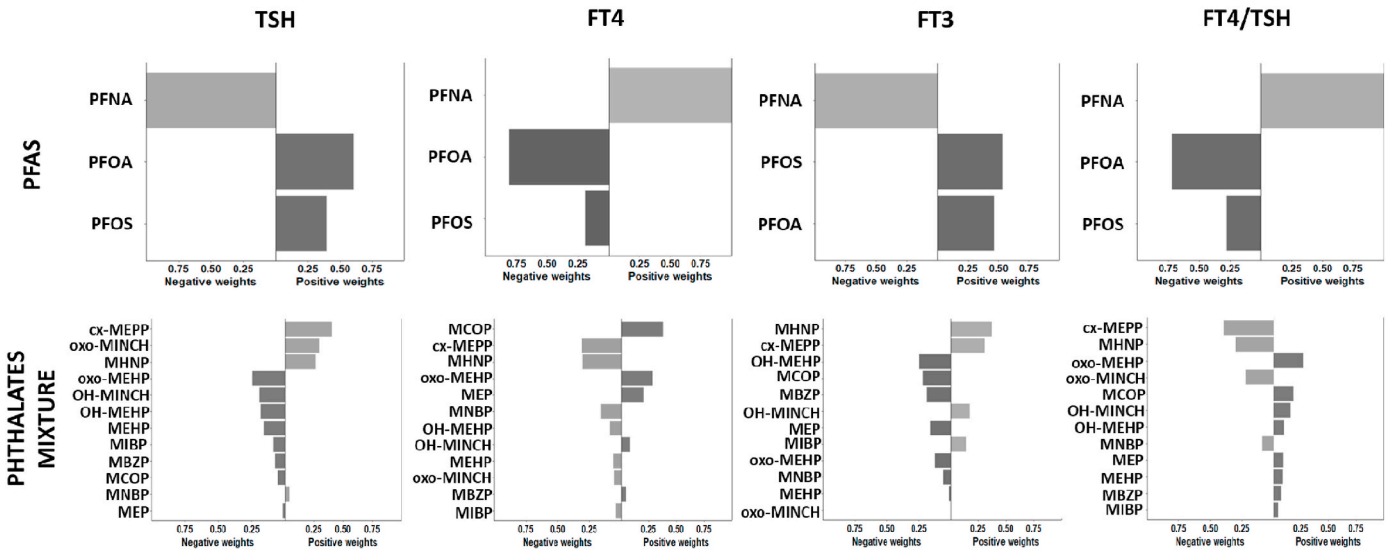

Fig. 2. G-computational regression models for the mixture effect of PFAS/phthalate metabolites on thyroid hormones among male and female adolescents (n = 733). PFAS mixture components: PFNA, PFOS, PFOA; Phthalate/DINCH metabolite mixture components: MEP, MBzP, MiBP, MnBP, MEHP, OH-MEHP, oxo-MEHP, cx-MEPP, MHNP, MCOP, OH-MINCH, and cx-MINCH. Dark bars colors refer to those components of the mixture with effects showing the same direction to the overall effect. Light bars colors refer to those compounds with effects in the opposite direction to the overall effect.

in males alone is in partial agreement with findings derived from the U. S. National Health and Nutrition Examination Survey (NHANES), which found positive associations of serum PFNA and PFOS with TSH levels in male but not female adolescents (Lewis et al., 2015). In females from NHANES, however, PFOA was associated with lower TSH (Lewis et al., 2015), whereas no association between PFOA and TSH was found in the present females. In the study of children and adolescents living near a Teflon factory, PFOA was associated with increased odds of self-reported hyperthyroidism (Lopez-Espinosa et al., 2012). Although PFOA exposure was considerably lower in the present study (median: 29 μg/L serum *vs.* 0.79 μg/L plasma), the inverse association of the PFAS mixture with the FT4/TSH ratio in the present males is consistent with the promotion of hypothyroid-inducing effects.

PFAS have been reported to interfere with thyroid function at several levels (Coperchini et al., 2021), influencing thyroid hormone biosynthesis, homeostasis, transport, and metabolism and interfering with thyroid receptors (Noyes et al., 2019). For instance, several PFAS, including PFOA, PFOS, and PFNA, can competitively bind to the human thyroid hormone transport protein transthyretin (TTR) (Weiss et al., 2009). In addition, PFOS has been shown to enhance the hepatic uptake and metabolism of T4 (Yu et al., 2009, 2011) and increase the conversion of T4 to biologically active T3 *via* upregulation of type 1 deiodinase (Yu et al., 2009). These mechanisms would explain the inverse association found between PFAS exposure and T4 levels; however, the concomitant increase in TSH that could be expected from feedback stimulation was not observed in the present study, because the association of the PFAS mixture with lower FT4 was seen in females alone and the association with higher TSH and FT4/TSH in males alone; therefore, further research is warranted.

4.2. Phthalate metabolites and thyroid hormones

The urinary concentrations of phthalate/DINCH® metabolites in the present adolescents were within the range published by other studies of European children (Govarts et al., 2023; Vogel et al., 2023; Bastiaensen et al., 2021; Fillol et al., 2021; Schwedler et al., 2020) and by investigations of the association between phthalates and thyroid function (Boas et al., 2010; Hyun Kim et al., 2018; Meeker and Ferguson, 2011). In the present study, several phthalate/DINCH® biomarkers, including MEP, MBzP, ∑DEHP, ∑DiNP, and ∑DINCH, were associated with altered levels of thyroid hormones, particularly higher FT4 and lower FT3 in females and higher FT4 and lower TSH in males. Some of the associations observed in adults, including pregnant women, are consistent or partially consistent with these findings. In this way, DEHP urinary metabolites were associated with lower FT3 in a large sample of Korean adults (Choi et al., 2020) and subfertile U.S. women (Souter et al., 2020), while MEHP and MBzP were associated with lower TSH in Chinese men (Y. X. Wang et al., 2018) and pregnant U.S. women (Romano et al., 2018), respectively. Regarding DINCH®, exposure of pregnant women to this non-phthalate plasticizer has been associated with higher maternal total T3 levels (Derakhshan et al., 2021) and unchanged newborn thyroid hormone levels (Coiffier et al., 2023), which is not consistent with the present study. Nonetheless, these results may have been influenced by between-study differences in the profile and concentrations of phthalate mixtures and in the age, sex, race, and other sociodemographic characteristics of study populations that may be associated with thyroid hormone levels.

Some cross-sectional analyses in children and adolescents may also partially support the present results. For instance, Boas et al. (2010) found that urinary ∑DEHP metabolites were inversely related to total T3 and FT3 in Danish children aged 4–9 years and that ∑DEHP was significantly associated with FT3 in the girls alone. However, they found a suggestive inverse association between the DiNP metabolite MCOP and total T3 in boys but not girls, whereas in the present study, ∑DiNP was associated with higher FT4 in both males and females. In a birth cohort study in the USA, Morgenstern et al. (2017) reported inverse associations of MEP, MnBP, MiBP, MEHP, and MEOHP measured at the age of 3 years with FT4 at the same age in the girls alone but found no association with FT3 levels. In their study of Korean children and adolescents (<19 years), Hyun Kim et al. (2018) described inverse associations of serum DEHP with TSH in the adolescents, although this association was observed in females alone. By contrast, a study of adolescents (aged 12–19 years) in the USA found a positive association between urinary DEHP metabolites and total T3 or TSH; however, results were not stratified by sex (Meeker and Ferguson, 2011). Likewise, a positive association of urinary DEHP metabolites with FT4 levels was reported in Taiwanese children (2–18 years) (Huang et al., 2020a,b) and in Chinese adolescent and young adult males (16–19 years) (Zhao et al., 2022), while MEP was not associated with any thyroid parameter in the Taiwanese study (Huang et al., 2020a,b). Moreover, the inverse association of MBzP and DEHP with TSH observed in this study is not consistent with previous findings that phthalate exposure may increase

TSH levels (Kim et al., 2019). However, these results may have been affected by differences in the design of studies and in the age and characteristics of participants.

According to the analysis of mixture effects, combined exposure to multiple phthalate metabolites may lead to a higher FT4/TSH ratio in males and lower FT3 and higher FT4 levels in females, largely driven by DEHP metabolites and the DiNP metabolite MCOP. In this line, a recent Spanish study of adults found a positive association between exposure to a mixture of phthalates and FT4, although a sex-stratified analysis was not conducted (Donat-Vargas et al., 2021). In a study of pregnant women (Villanger et al., 2020), exposure to a mixture of urinary phthalate metabolites was related to a decrease in plasma FT3 and total T3 levels, with ∑DEHP and ∑DiNP making a major contribution to the effect. Finally, Souter et al. (2020) described MEHHP as primary contributor to the association found between a mixture of DEHP metabolites and FT3 and total T3 in subfertile women.

The precise mechanisms underlying the effects of phthalate metabolites on altered thyroid function remain unknown; however, experimental studies have indicated that phthalates might affect thyroid hormone biosynthesis, biotransformation, transportation, receptor levels, and metabolism (Wu et al., 2021). Some phthalates, including BBzP, DEHP, and DiNP, induce changes in the iodide uptake of thyroid follicular cells *in vitro* by altering the transcriptional activity of the sodium-iodide symporter (NIS), with the consequent effect on T4 levels (Breous et al., 2005; Wenzel et al., 2005). BBzP was shown to block T3 cellular uptake (Shimada and Yamauchi, 2004), weakly inhibit T3 binding to TTR (Ishihara et al., 2003), and interfere with expression of the thyroid receptor beta gene. BBzP and DEHP have demonstrated competitive binding affinities for integrin $\alpha_v\beta_3$, a thyroid hormone membrane receptor (Li et al., 2020). In rats, treatment with DEHP decreased serum levels of T3, T4, FT3 and FT4 and protein and mRNA levels of TSH receptor (Sun et al., 2022). Recent animal and *in vitro* studies also showed that DEHP might competitively bind to thyroid-binding globulin (TBG) (Sheikh and Beg, 2022), increase protein and mRNA levels of thyroid transcription factor 1, NIS, and thyroid peroxidase (Dong et al., 2019), and upregulate the gene expression of TSH subunit beta, deiodinase 1, deiodinase 2, and thyroid hormone receptor alpha and beta (Horie et al., 2022). Interestingly, a study of Korean adults found a positive association between urinary DEHP metabolites and TBG levels (Choi et al., 2020), which could partly explain the present observation of an inverse association between DEHP and FT3. In the Korean study, DEHP was also associated with higher total T3 and greater peripheral deiodinase activity (Choi et al., 2020). Indeed, information on total T3 measurements, which were not available in the present study, would provide further insight into possible mechanistic pathways involved in these associations. In addition, Zhao et al. (2022) found that DEHP metabolites in adolescents were positively associated with mRNA levels of thyroglobulin, the main precursor of thyroid hormones, and thyroid transcription factor 1 and paired box gene 8, which play a role in regulating genes expressed in the thyroid. Upregulation of the expression of these genes could explain the increase in FT4 levels and decrease in TSH observed in the mixture analysis.

Our findings strongly suggest that exposure to PFAS and phthalate metabolites may disrupt thyroid hormone homeostasis in a sex-specific manner. This is plausible because thyroid hormones indirectly regulate the synthesis, secretion, and action of sex steroid hormones, which are distributed differently between girls and boys (Ren and Zhu, 2022). Indeed, disturbances in thyroid function are more prevalent among women (Hollowell et al., 2002; McGrogan et al., 2008), and serum PFOA was associated with thyroid disease in female but not male adults from NHANES (Melzer et al., 2010). Sex-specific associations have also been observed for other child outcomes related to PFAS and phthalates exposure, such as cognitive impairment (Factor-Litvak et al., 2014; Liew et al., 2018; Whyatt et al., 2012) and behavioral problems (Lenters et al., 2019; Oulhote et al., 2016). Although the mechanism of these potential sex differences is unclear, it may be related to the ability of PFAS and phthalates to disrupt sex steroid hormones (Bigambo et al., 2022; Lopez-Espinosa et al., 2016; Maisonet et al., 2015; Martinez-Arguelles et al., 2013; Sathyanarayana et al., 2008; Wang et al., 2021).

4.3. Strengths and limitations

This study has several strengths. The relatively large sample size allowed the examination of sex differences, and it was possible to analyze the effect of combined exposures to multiple PFAS/phthalate metabolites on thyroid function, which has been poorly investigated in adolescents. The quantile g-computation approach offered various advantages in the mixture analysis, including the possibility to assess individual exposure-effect relationships in opposite directions, generating an unbiased and highly interpretable estimate of the overall combined effect (Keil et al., 2020). This is the first study to examine the association between DINCH® exposure and thyroid function in adolescents. The harmonized approach of HBM4EU is an additional strength of the study. Finally, serum PFAS and urinary phthalate biomarker measurements from this sample of adolescents were within range of results obtained in other study populations, enhancing the external validity of the results.

This study also has limitations. First, PFAS/phthalate exposure and thyroid function were assessed at the same time point, preventing evaluation of the temporal relationship between PFAS/phthalates and thyroid hormones and the possibility of reverse causation. However, it appears unlikely that thyroid hormones affect serum PFAS or urinary phthalate metabolite concentrations. Second, a single urine sample was collected from participants and may not represent their average exposure to phthalate metabolites, although it may be moderately predictive of individual exposure over a few months (Hauser and Calafat, 2005; Teitelbaum, 2018). Nonetheless, it was not possible to determine whether differences in thyroid hormone levels related to exposure to environmental chemicals are transient or sustained. Third, it is unclear whether the associations observed in single-exposure models represent cause-effect relationships or result from the performance of multiple comparisons; however, the mixture analysis corroborated the effect observed in single-exposure models. Fourth, the timing of biological sample collection varied among studies, and it is not known whether PFAS/phthalate metabolites/thyroid hormone concentrations differ between fasting and non-fasting samples, particularly in relation to phthalate metabolites. This issue should be addressed in future studies by measuring repeated urine samples. Finally, information was not available on the iodine status of adolescents, which is essential for thyroid health and could influence in part the relationship between PFAS/phthalate exposure and thyroid hormones (Freire et al., 2023; Villanger et al., 2020).

5. Conclusion

This is the first study on exposure to PFAS and phthalates and thyroid function among European adolescents. Results suggest that serum PFOA and certain urinary phthalate metabolite concentrations are associated with thyroid hormones, particularly FT4 and FT3 in females and FT4 and TSH in males. However, given the cross-sectional design of the study, associations should be interpreted with caution. Further research is needed to characterize the associations of PFAS and phthalate exposure with thyroid hormone levels in young people and explore underlying pathways. Longitudinal studies are required to establish whether exposure during childhood or adolescence increases the risk of later thyroid disease, while thyroid hormone alterations observed in adolescents may also have more immediate health repercussions.

Credit author statement

Conceptualization: Carmen Freire, Andrea Rodriguez-Carrillo. Methodology: Andrea Rodriguez-Carrillo, Carmen Freire, Elena Salamanca-Fernández. Formal analysis: Andrea Rodriguez-Carrillo, Carmen

Freire. Resources: Andrea Rodriguez-Carrillo, José Vicente García-Lario, Elena Salamanca-Fernández, Mariana F. Fernández, Elly den Hond, Elly den Hond, Lucia Fábelová;, Lubica Palkovicova Murinova, Susana Pedraza-Díaz, Argelia Castaño. Writing – Original Draft: Carmen Freire, Andrea Rodriguez-Carrillo, Elena Salamanca-Fernández. Writing - Review & Editing: all co-authors. Visualization: Andrea Rodriguez-Carrillo, Carmen Freire. Supervision: Carmen Freire, Mariana F. Fernandez. Project administration: Eva Govarts, Mariana F. Fernández.

Funding

This work was supported by the European Commission Project: Human Biomonitoring for Europe "HBM4EU". Exp: 733032. The authors are also grateful to the ISCIII/FEDER for the Miguel Servet II contract granted to C. Freire (CPII21/00014), to the Fundación Ramón Areces, for the postdoctoral fellowship granted to A. Rodriguez-Carrillo, and to the "Servicio Andaluz de Salud" (SAS) for the "Refuerzo de la Actividad Investigadora" research grant granted to N. Olea (Exp. A1-0047-2021). The FLEHS IV study was conducted within the framework of the Flemish Center of Expertise on Environment and Health (FLEHS, 2016–2020) and funded by the Government of Flanders, Department of Environment & Spatial Development. PCB cohort follow-up received additional funding from the Ministry of Health of the Slovak Republic, program 07B0103. The BEA study was co-funded by the Spanish Ministry of Agriculture, Fisheries and Food and the Instituto de Salud Carlos III (SEG 1321/15). Funding for open access charge: Universidad de Granada / CBUA.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116897.

References

Albert, O., Huang, J.Y., Aleksa, K., Hales, B.F., Goodyer, C.G., Robaire, B., Chevrier, J., Chan, P., 2018. Exposure to polybrominated diphenyl ethers and phthalates in healthy men living in the greater Montreal area: a study of hormonal balance and semen quality. Environ. Int. 116, 165–175. https://doi.org/10.1016/J.ENVINT.2018.04.012.

Ballesteros, V., Costa, O., Iñiguez, C., Fletcher, T., Ballester, F., Lopez-Espinosa, M.J., 2017. Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: a systematic review of epidemiologic studies. Environ. Int. 99, 15–28. https://doi.org/10.1016/J.ENVINT.2016.10.015.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ. Health Perspect. 118 (2), 222–228. https://doi.org/10.1289/EHP.0901252.

Bastiaensen, M., Gys, C., Colles, A., Malarvannan, G., Verheyen, V., Koppen, G., Govarts, E., Bruckers, L., Morrens, B., Francken, C., Den Hond, E., Schoeters, G., Covaci, A., 2021. Biomarkers of Phthalates and Alternative Plasticizers in the Flemish Environment and Health Study (FLEHS IV): Time Trends and Exposure Assessment, 1987. Environmental Pollution, Essex, p. 276. https://doi.org/10.1016/J.ENVPOL.2021.116724. Barking.

Bergman, Å., Heindel, J.J., Jobling, S., Kidd, K.A., Thomas Zoeller, R., 2012. State of the Science of Endocrine Disrupting Chemicals 2012 Summary for Decision-Makers Inter-Organization Programme for the Sound Management of Chemicals. World Health Organization.

Bigambo, F.M., Zhang, M., Zhang, J., Yang, X., Yu, Q., Wu, D., Wang, X., Xia, Y., 2022. Exposure to a mixture of personal care product and plasticizing chemicals in relation to reproductive hormones and menarche timing among 12-19 years old girls in NHANES 2013-2016. Food Chem. Toxicol. : An International Journal Published for the British Industrial Biological Research Association 170. https://doi.org/10.1016/J.FCT.2022.113463.

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.(Barking, Essex : 1987) 242 (Pt A), 894–904. https://doi.org/10.1016/J.ENVPOL.2018.07.042.

Boas, M., Frederiksen, H., Feldt-Rasmussen, U., Skakkebæk, N.E., Hegedüs, L., Hilsted, L., Juul, A., Main, K.M., 2010. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ. Health Perspect. 118 (10), 1458–1464. https://doi.org/10.1289/EHP.0901331.

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: Global Access Sci. Source 19 (1), 1–28. https://doi.org/10.1186/S12940-020-00647-1/TABLES/10.

Breous, E., Wenzel, A., Loos, U., 2005. The promoter of the human sodium/iodide symporter responds to certain phthalate plasticisers. Mol. Cell. Endocrinol. 244 (1–2), 75–78. https://doi.org/10.1016/J.MCE.2005.06.009.

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.

Caron-Beaudoin, É., Ayotte, P., Laouan Sidi, E.A., Gros-Louis McHugh, N., Lemire, M., 2019. 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.

Centers for Disease Control and Prevention. (n.d.). National Environmental Public Health Tracking Network..

Choi, S., Kim, M.J., Park, Y.J., Kim, S., Choi, K., Cheon, G.J., Cho, Y.H., Jeon, H.L., Yoo, J., Park, J., 2020. Thyroxine-binding globulin, peripheral deiodinase activity, and thyroid autoantibody status in association of phthalates and phenolic compounds with thyroid hormones in adult population. Environ. Int. 140 https://doi.org/10.1016/J.ENVINT.2020.105783.

Coiffier, O., Nakiwala, D., Rolland, M., Malatesta, A., Lyon-Caen, S., Chovelon, B., Faure, P., Sophie Gauchez, A., Guergour, D., Sakhi, A.K., Sabaredzovic, A., Thomsen, C., Pin, I., Slama, R., Corne, C., Philippat, C., 2023. Exposure to a mixture of non-persistent environmental chemicals and neonatal thyroid function in a cohort with improved exposure assessment. Environ. Int. 173, 107840 https://doi.org/10.1016/j.envint.2023.107840.

Coltro, L., Pitta, J.B., da Costa, P.A., Fávaro Perez, M.Â., de Araújo, V.A., Rodrigues, R., 2014. Migration of conventional and new plasticizers from PVC films into food simulants: a comparative study. Food Control 44, 118–129. https://doi.org/10.1016/j.foodcont.2014.03.058.

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, 1077. https://doi.org/10.3389/FENDO.2020.612320/BIBTEX.

Derakhshan, A., Shu, H., Broeren, M.A.C., Lindh, C.H., Peeters, R.P., Kortenkamp, A., Demeneix, B., Bornehag, C.G., Korevaar, T.I.M., 2021. Association of phthalate exposure with thyroid function during pregnancy. Environ. Int. 157 https://doi.org/10.1016/J.ENVINT.2021.106795.

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L.C., Hauser, R., Prins, G.S., Soto, A.M., Zoeller, R.T., Gore, A.C., 2009. Endocrine-disrupting Chemicals: an Endocrine Society Scientific Statement. Academic.Oup.Com. https://doi.org/10.1210/er.2009-0002.

Domingo, J.J., Ericson-Jogsten, I., Perelló, G.P., Nadal, M., Van Bavel, B., Ka, A., 2012. Human exposure to perfluorinated compounds in Catalonia, Spain: contribution of drinking water and fish and shellfish. ACS Publications 60 (17), 4408–4415. https://doi.org/10.1021/jf300355c.

Donat-Vargas, C., Perez-Carrascosa, F., Gomez-Peña, C., Mustieles, V., Salcedo-Bellido, I., Frederiksen, H., Åkesson, A., Arrebola, J.P., 2021. Associations of serum phthalate metabolites with thyroid hormones in GraMo cohort, Southern Spain. Environ. Pollut.(Barking, Essex : 1987) 287, 117606. https://doi.org/10.1016/j.envpol.2021.117606.

Dong, J., Cong, Z., You, M., Fu, Y., Wang, Y., Wang, Y., Fu, H., Wei, L., Chen, J., 2019. Effects of perinatal di (2-ethylhexyl) phthalate exposure on thyroid function in rat offspring. Environ. Toxicol. Pharmacol. 67, 53–60. https://doi.org/10.1016/J.ETAP.2019.01.012.

ECHA, 2009. Data on Manufacture, Import, Export, - Google Académico.

Esteban López, M., Göen, T., Mol, H., Nübler, S., Haji-Abbas-Zarrabi, K., Koch, H.M., Kasper-Sonnenberg, M., Dvorakova, D., Hajslova, J., Antignac, J.P., Vaccher, V., Elbers, I., Thomsen, C., Vorkamp, K., Pedraza – Díaz, S., Kolossa-Gehring, M., Castaño, A., 2021. The European human biomonitoring platform - design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. Int. J. Hyg Environ. Health 234, 113740. https://doi.org/10.1016/J.IJHEH.2021.113740.

Fábelová, L., Beneito, A., Casas, M., Colles, A., Dalsager, L., Den Hond, E., Dereumeaux, C., Ferguson, K., Gilles, L., Govarts, E., Irizar, A., Lopez Espinosa, M.J., Montazeri, P., Morrens, B., Patayová, H., Rausová, K., Richterová, D., Rodriguez Martin, L., Santa-Marina, L., Palkovičová Murínová, 2023. PFAS levels and exposure determinants in sensitive population groups. Chemosphere 313, 137530. https://doi.org/10.1016/J.CHEMOSPHERE.2022.137530.

Factor-Litvak, P., Insel, B., Calafat, A.M., Liu, X., Perera, F., Rauh, V.A., Whyatt, R.M., 2014. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. PLoS One 9 (12). https://doi.org/10.1371/JOURNAL.PONE.0114003.

Fernández, M.F., Olea, N., Mustieles, V., Rodriguez-Carrillo, A., Olivas, A., Suárez, B., Reina, I., D'Cruz, S.C., Legoff, L., Smagulova, F., David, A., Bláha, L., Blahova, L., 2021. Guidelines for the validation of biomarkers of effect: implementing quality assurance , trueness , precision , and accuracy as well as the availability of quality control measures by qualified laboratories. Deliverable Report 14 (7). WP14 Effect Biomarkers.

Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, É., Marchand, P., Le Bizec, B., Bouchart, V., Le Gléau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014-2016: results from the Esteban study. Environ. Int. 147 https://doi.org/10.1016/J.ENVINT.2020.106340.

Freire, C., Vela-Soria, F., Castiello, F., Salamanca-Fernández, E., Quesada-Jiménez, R., López-Alados, M.C., Fernández, M., Olea, N., 2023. Exposure to perfluoroalkyl substances (PFAS) and association with thyroid hormones in adolescent males. Int. J. Hyg Environ. Health, 114219. https://doi.org/10.1016/J.IJHEH.2023.114219.

Fromme, H., Tittlemier, S.A., Völkel, W., Wilhelm, M., Twardella, D., 2009. Perfluorinated compounds - exposure assessment for the general population in western countries. Int. J. Hyg Environ. Health 212 (3), 239–270. https://doi.org/10.1016/j.ijheh.2008.04.007.

Gilles, L., Govarts, E., Martin, L.R., Andersson, A.-M., Appenzeller, B.M.R., Barbone, F., Castaño, A., Coertjens, D., Hond, E. Den, Dzhedzheia, V., Eržen, I., López, M.E., Fábelová, L., Fillol, C., Franken, C., Frederiksen, H., Gabriel, C., Haug, L.S., Horvat, M., Schoeters, G., 2022. Harmonization of human biomonitoring studies in Europe: characteristics of the HBM4EU-aligned studies participants. Int. J. Environ. Res. Publ. Health 19 (11), 6787. https://doi.org/10.3390/IJERPH19116787, 2022, Vol. 19, Page 6787.

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castaño, A., Esteban López, M., Rodriguez Martin, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L.E., Kolossa-Gehring, M., Schoeters, G., 2021. HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity – the HBM4EU survey. Int. J. Hyg Environ. Health 237, 113809. https://doi.org/10.1016/J.IJHEH.2021.113809.

Govarts, E., Gilles, L., Rodriguez Martin, L., Santonen, T., Apel, P., Alvito, P., Anastasi, E., Andersen, H.R., Andersson, A.-M., Andryskova, L., Antignac, J.-P., Appenzeller, B., Barbone, F., Barnett-Itzhaki, Z., Barouki, R., Berman, T., Bil, W., Borges, T., Buekers, J., et al., 2023. Harmonized human biomonitoring in European children, teenagers and adults: EU-wide exposure data of 11 chemical substance groups from the HBM4EU Aligned Studies (2014–2021). Int. J. Hyg Environ. Health 249, 114119. https://doi.org/10.1016/J.IJHEH.2023.114119.

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.

Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62 (11), 806–818. https://doi.org/10.1136/oem.2004.017590.

HBM4EU - science and policy for a healthy future. (n.d.).

Health Canada, 2019. Fifth Report on Human Biomonitoring of Environmental Chemicals in Canada.

Health Canada, 2021. Sixth Report on Human Biomonitoring of Environmental Chemicals in Canada.

Hollowell, J.G., Staehling, N.W., Dana Flanders, W., Harry Hannon, W., Gunter, E.W., Spencer, C.A., Braverman, L.E., 2002. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): national health and nutrition examination survey (NHANES III). J. Clin. Endocrinol. Metabol. 87 (2), 489–499. https://doi.org/10.1210/JCEM.87.2.8182.

Horie, Y., Nomura, M., Ramaswamy, B.R., Harino, H., Yap, C.K., Okamura, H., 2022. Thyroid Hormone Disruption by Bis-(2-Ethylhexyl) Phthalate (DEHP) and Bis-(2-Ethylhexyl) Adipate (DEHA) in Japanese Medaka Oryzias latipes. Aquatic Toxicology, Amsterdam, Netherlands), p. 252.

Huang, P.-C., Waits, A., Chen, H.-C., Chang, W.-T., Jaakkola, J.J.K., Huang, H.-B., 2020a. Mediating role of oxidative/nitrosative stress biomarkers in the associations between phthalate exposure and thyroid function in Taiwanese adults. Environ. Int. 140, 105751 https://doi.org/10.1016/j.envint.2020.105751.

Huang, P.C., Chang, W.H., Wu, M.T., Chen, M.L., Wang, I.J., Shih, S.F., Hsiung, C.A., Liao, K.W., 2020b. Characterization of Phthalate Exposure in Relation to Serum Thyroid and Growth Hormones, and Estimated Daily Intake Levels in Children Exposed to Phthalate-Tainted Products: A Longitudinal Cohort Study, 1987. Environmental Pollution, Essex, p. 264. Barking.

Huang, P.C., Kuo, P.L., Guo, Y.L., Liao, P.C., Lee, C.C., 2007. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum. Reprod. (Oxf.) 22 (10), 2715–2722. https://doi.org/10.1093/HUMREP/DEM205.

Hyun Kim, D., Min Choi, S., Soo Lim, D., Roh, T., Jun Kwack, S., Yoon, S., Kook Kim, M., Sil Yoon, K., Sik Kim, H., Wook Kim, D., Lee, B.M., 2018. Risk assessment of endocrine disrupting phthalates and hormonal alterations in children and adolescents. J. Toxicol. Environ. Health, Part A 81 (21), 1150–1164. https://doi.org/10.1080/15287394.2018.1543231.

Ishihara, A., Nishiyama, N., Sugiyama, S.I., Yamauchi, K., 2003. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. Gen. Comp. Endocrinol. 134 (1), 36–43. https://doi.org/10.1016/S0016-6480(03)00197-7.

Jensen, A.A., Leffers, H., 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl. 31 (2), 161–169. https://doi.org/10.1111/J.1365-2605.2008.00870.X.

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., Aldous, 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.

Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999-2008. Environ. Sci. Technol. 45 (19), 8037–8045. https://doi.org/10.1021/ES1043613.

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) https://doi.org/10.1289/EHP5838.

Kim, M.J., Moon, S., Oh, B.C., Jung, D., Choi, K., Park, Y.J., 2019. Association between diethylhexyl phthalate exposure and thyroid function: a meta-analysis. Thyroid : Official Journal of the American Thyroid Association 29 (2), 183–192. https://doi.org/10.1089/THY.2018.0051.

Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture. Phil. Trans. Biol. Sci. 364 (1526), 2063–2078. https://doi.org/10.1098/RSTB.2008.0208.

Kuo, F.C., Su, S.W., Wu, C.F., Huang, M.C., Shiea, J., Chen, B.H., Chen, Y.L., Wu, M.T., 2015. Relationship of urinary phthalate metabolites with serum thyroid hormones in pregnant women and their newborns: a prospective birth cohort in Taiwan. PLoS One 10 (6). https://doi.org/10.1371/JOURNAL.PONE.0123884.

Lenters, V., Iszatt, N., Forns, J., Čechová, E., Kočan, A., Legler, J., Leonards, P., Stigum, H., Eggesbø, M., 2019. 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. 125, 33–42. https://doi.org/10.1016/j.envint.2019.01.020.

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.

Li, J., Liu, H., Zuo, R., Yang, J., Li, N., 2020. Competitive binding assays for measuring the binding affinity of thyroid-disrupting chemicals for integrin αvβ3. Chemosphere 249. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126034.

Liew, Z., Ritz, B., Bach, C.C., Asarnow, R.F., Bech, B.H., Nohr, E.A., Bossi, R., Henriksen, T.B., Bonefeld-Jørgensen, E.C., Olsen, J., 2018. Prenatal exposure to perfluoroalkyl substances and IQ scores at age 5; a study in the Danish national birth cohort. Environ. Health Perspect. 126 (6) https://doi.org/10.1289/EHP2754.

Lin, C.Y., Wen, L.L., Lin, L.Y., Wen, T.W., Lien, G.W., Hsu, S.H.J., 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.

Lopez-Espinosa, M.J., Mondal, D., Armstrong, B.G., Eskenazi, B., Fletcher, T., 2016. Perfluoroalkyl substances, sex hormones, and insulin-like growth factor-1 at 6-9 Years of age: a cross-sectional analysis within the C8 health project. Environ. Health Perspect. 124 (8), 1269–1275. https://doi.org/10.1289/EHP.1509869.

Maisonet, M., Calafat, A.M., Marcus, M., Jaakkola, J.J.K., Lashen, H., 2015. Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 Years of age in female ALSPAC study participants. Environ. Health Perspect. 123 (12), 1325–1330. https://doi.org/10.1289/EHP.1408847.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.J., Ballester, F., Martinez, D., Ibarluzea, J., Santa-Marina, L., Vioque, J., Sunyer, J., Vrijheid, M., 2016. Variability of perfluoroalkyl substance concentrations in pregnant women by socio-demographic and dietary factors in a Spanish birth cohort. Environ. Int. 92 (93), 357–365. https://doi.org/10.1016/j.envint.2016.04.004.

Martinez-Arguelles, D.B., McIntosh, M., Rohlicek, C.V., Culty, M., Zirkin, B.R., Papadopoulos, V., 2013. Maternal in utero exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate affects the blood pressure of adult male offspring. Toxicol. Appl. Pharmacol. 266 (1), 95–100. https://doi.org/10.1016/J.TAAP.2012.10.027.

McGrogan, A., Seaman, H.E., Wright, J.W., De Vries, C.S., 2008. The incidence of autoimmune thyroid disease: a systematic review of the literature. Clin. Endocrinol. 69 (5), 687–696. https://doi.org/10.1111/J.1365-2265.2008.03338.X.

Meeker, J.D., Calafat, A.M., Hauser, R., 2007. Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. Environ. Health Perspect. 115 (7), 1029–1034. https://doi.org/10.1289/ehp.9852.

Meeker, J.D., Ferguson, K.K., 2011. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. Adults and adolescents from the national health and nutrition examination survey (NHANES) 2007–2008. Environ. Health Perspect. 119 (10), 1396–1402. https://doi.org/10.1289/ehp.1103582.

Melzer, D., Rice, N.E., Lewis, C., Henley, W.E., Galloway, T.S., 2010. Association of urinary bisphenol A concentration with heart disease: evidence from NHANES 2003/06. PLoS One 5 (1). https://doi.org/10.1371/journal.pone.0008673.

Miller, M.D., Crofton, K.M., Rice, D.C., Zoeller, R.T., 2009. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environ. Health Perspect. 117 (7), 1033–1041. https://doi.org/10.1289/EHP.0800247.

Mol, H.G.J., Elbers, I., Pälmke, C., Bury, D., Göen, T., López, M.E., Nübler, S., Vaccher, V., Antignac, J.P., Dvořáková, D., Hajšlová, J., Sakhi, A.K., Thomsen, C., Vorkamp, K., Castaño, A., Koch, H.M., 2022. Proficiency and interlaboratory variability in the determination of phthalate and DINCH biomarkers in human urine: results from the HBM4EU project. Toxics 10 (2), 57. https://doi.org/10.3390/TOXICS10020057/S1.

Morgenstern, R., Whyatt, R.M., Insel, B.J., Calafat, A.M., Liu, X., Rauh, V.A., Herbstman, J., Bradwin, G., Factor-Litvak, P., 2017. Phthalates and thyroid function in preschool age children: sex specific associations. Environ. Int. 106, 11–18. https://doi.org/10.1016/J.ENVINT.2017.05.007.

Nakiwala, D., Noyes, P.D., Faure, P., Chovelon, B., Corne, C., Gauchez, A.S., Guergour, D., Lyon-Caen, S., Sakhi, A.K., Sabaredzovic, A., Thomsen, C., Pin, I., Slama, R., Philippat, C., 2022. Phenol and phthalate effects on thyroid hormone levels during pregnancy: relying on in vitro assays and adverse outcome pathways to inform an epidemiological analysis. Environ. Health Perspect. 130 (11) https://doi.org/10.1289/EHP10239.

Noyes, P.D., Friedman, K.P., Browne, P., Haselman, J.T., Gilbert, M.E., Hornung, M.W., Barone, S., Crofton, K.M., Laws, S.C., Stoker, T.E., Simmons, S.O., Tietge, J.E., Degitz, S.J., 2019. Evaluating chemicals for thyroid disruption: opportunities and challenges with in vitro testing and adverse outcome pathway approaches. Environ. Health Perspect. 127 (9) https://doi.org/10.1289/EHP5297.

Nübler, S., Esteban López, M., Castaño, A., Mol, H.G.J., Haji-Abbas-Zarrabi, K., Schäfer, M., Müller, J., Hajslova, J., Dvorakova, D., Antignac, J.P., Koch, H.M., Haug, L.S., Vorkamp, K., Göen, T., 2022. Interlaboratory Comparison Investigations (ICIs) and External Quality Assurance Schemes (EQUASs) for human biomonitoring of perfluoroalkyl substances (PFASs) in serum as part of the quality assurance programme under HBM4EU. Sci. Total Environ. 847, 157481 https://doi.org/10.1016/J.SCITOTENV.2022.157481.

O'Brien, K.M., Upson, K., Cook, N.R., Weinberg, C.R., 2016. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ. Health Perspect. 124 (2), 220–227. https://doi.org/10.1289/EHP.1509693.

O'Connor, J.C., Frame, S.R., Ladics, G.S., 2002. Evaluation of a 15-day screening assay using intact male rats for identifying steroid biosynthesis inhibitors and thyroid modulators. Toxicol. Sci. 69 (1), 79–91. https://doi.org/10.1093/TOXSCI/69.1.79.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froelich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115 (9), 1298–1305. https://doi.org/10.1289/EHP.10009.

Oulhote, Y., Steuerwald, U., Debes, F., Weihe, P., Grandjean, P., 2016. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ. Int. 97, 237–245. https://doi.org/10.1016/J.ENVINT.2016.09.015.

Perfluoroalkyl chemicals (PFASs) - ECHA. (n.d.).

Preston, E.V., Webster, T.F., Claus Henn, B., McClean, M.D., Gennings, C., Oken, E., Rifas-Shiman, S.L., Pearce, E.N., Calafat, A.M., Fleisch, A.F., Sagiv, S.K., 2020. Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the Project Viva Cohort: a mixtures approach. Environ. Int. 139 https://doi.org/10.1016/J.ENVINT.2020.105728.

Ren, B., Zhu, Y., 2022. A new perspective on thyroid hormones: crosstalk with reproductive hormones in females. Int. J. Mol. Sci. 23 (5) https://doi.org/10.3390/IJMS23052708.

Renner, R., 2001. Growing concern over perfluorinated chemicals. Environ. Sci. Technol. 35 (7) https://doi.org/10.1021/ES012317K.

Rice, D., Barone, S., 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. 108 (Suppl. 3), 511–533. https://doi.org/10.1289/ehp.00108s3511.

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.

Romano, M.E., Eliot, M.N., Zoeller, R.T., Hoofnagle, A.N., Calafat, A.M., Karagas, M.R., Yolton, K., Chen, A., Lanphear, B.P., Braun, J.M., 2018. Maternal urinary phthalate metabolites during pregnancy and thyroid hormone concentrations in maternal and cord sera: the HOME Study. Int. J. Hyg Environ. Health 221 (4), 623–631. https://doi.org/10.1016/J.IJHEH.2018.03.010.

Sathyanarayana, S., Calafat, A.M., Liu, F., Swan, S.H., 2008. Maternal and infant urinary phthalate metabolite concentrations: are they related? Environ. Res. 108 (3), 413–418. https://doi.org/10.1016/J.ENVRES.2008.07.002.

Schug, T.T., Blawas, A.M., Gray, K., Heindel, J.J., Lawler, C.P., 2015. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology 156 (6), 1941–1951. https://doi.org/10.1210/en.2014-1734.

Schwedler, G., Conrad, A., Rucic, E., Koch, H.M., Leng, G., Schulz, C., Schmied-Tobies, M.I.H., Kolossa-Gehring, M., 2020. Hexamoll® DINCH and DPHP metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014-2017. Int. J. Hyg Environ. Health 229. https://doi.org/10.1016/J.IJHEH.2019.09.004.

Sheikh, I.A., Beg, M.A., 2022. Structural binding perspectives of common plasticizers and a flame retardant, BDE-153, against thyroxine-binding globulin: potential for endocrine disruption. J. Appl. Toxicol. : J. Anal. Toxicol. 42 (5), 841–851. https://doi.org/10.1002/JAT.4261.

Shimada, N., Yamauchi, K., 2004. Characteristics of 3,5,3'-triiodothyronine (T3)-uptake system of tadpole red blood cells: effect of endocrine-disrupting chemicals on cellular T3 response. J. Endocrinol. 183 (3), 627–637. https://doi.org/10.1677/JOE.1.05893.

Souter, I., Bellavia, A., Williams, P.L., Korevaar, T.I.M., Meeker, J.D., Braun, J.M., de Poortere, R.A., Broeren, M.A., Ford, J.B., Calafat, A.M., Chavarro, J.E., Hauser, R., Mínguez-Alarcón, L., 2020. Urinary concentrations of phthalate metabolite mixtures in relation to serum biomarkers of thyroid function and autoimmunity among women from a fertility center. Environ. Health Perspect. 128 (6), 1–8. https://doi.org/10.1289/EHP6740.

Stockholm Convention - Home page. (n.d.).

Sun, D., Zhou, L., Wang, S., Liu, T., Zhu, J., Jia, Y., Xu, J., Chen, H., Wang, Q., Xu, F., Zhang, Y., Ye, L., 2022. Effect of Di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-thyroid axis in adolescent rat. Endocr. J. 69 (2), 217–224. https://doi.org/10.1507/ENDOCRJ.EJ17-0272R.

Tahboub, R., Arafah, B.M., 2009. Sex steroids and the thyroid. Best practice & research. Clin. Endocrinol. Metabol. 23 (6), 769–780. https://doi.org/10.1016/J.BEEM.2009.06.005.

Teitelbaum, D., 2018. Moving towards therapists becoming better educators: a commentary on changes in the content of pediatric physical therapy for infants: a quantitative, observational study. Phys. Occup. Ther. Pediatr. 38 (5), 489–492. https://doi.org/10.1080/01942638.2018.1500845.

Textor, J., Hardt, J., 2011. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 22 (5), 745. https://doi.org/10.1097/EDE.0B013E318225C2BE.

Vandenberg, L.N., Chahoud, I., Heindel, J.J., Padmanabhan, V., Paumgartten, F.J.R., Schoenfelder, G., 2012. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Ciência Saúde Coletiva 17 (2), 407–434.

Vestergren, R., Cousins, I.T., Trudel, D., Wormuth, M., Scheringer, M., 2008. Estimating the contribution of precursor compounds in consumer exposure to PFOS and PFOA. Chemosphere 73 (10), 1617–1624. https://doi.org/10.1016/j.chemosphere.2008.08.011.

Villanger, G.D., Drover, S.S.M., Nethery, R.C., Thomsen, C., Sakhi, A.K., Øvergaard, K.R., Zeiner, P., Hoppin, J.A., Reichborn-Kjennerud, T., Aase, H., Engel, S.M., 2020. Associations between urine phthalate metabolites and thyroid function in pregnant women and the influence of iodine status. Environ. Int. 137 https://doi.org/10.1016/J.ENVINT.2020.105509.

Vogel, N., Schmidt, P., Lange, R., Gerofke, A., Sakhi, A.K., Haug, L.S., Jensen, T.K., Frederiksen, H., Szigeti, T., Csákó, Z., Murinova, L.P., Sidlovska, M., Janasik, B., Wasowicz, W., Tratnik, J.S., Mazej, D., Gabriel, C., Karakitsios, S., Barbone, F., et al., 2023. Current exposure to phthalates and DINCH in European children and adolescents – results from the HBM4EU Aligned Studies 2014 to 2021. Int. J. Hyg Environ. Health 249, 114101. https://doi.org/10.1016/J.IJHEH.2022.114101.

Wang, F., Zhen, Q., Li, K., Wen, X., 2018. Association of socioeconomic status and health-related behavior with elderly health in China. PLoS One 13 (9), e0204237. https://doi.org/10.1371/journal.pone.0204237.

Wang, Y., Aimuzi, R., Nian, M., Zhang, Y., Luo, K., Zhang, J., 2021. Perfluoroalkyl substances and sex hormones in postmenopausal women: NHANES 2013–2016. Environ. Int. 149, 106408 https://doi.org/10.1016/J.ENVINT.2021.106408.

Wang, Y.X., Zhou, B., Chen, Y.J., Liu, C., Huang, L.L., Liao, J.Q., Hu, X.J., Lu, W.Q., Zeng, Q., Pan, A., 2018. Thyroid function, phthalate exposure and semen quality: exploring associations and mediation effects in reproductive-aged men. Environ. Int. 116, 278–285. https://doi.org/10.1016/J.ENVINT.2018.04.031.

Weiss, J.M., Andersson, P.L., Lamoree, M.H., Leonards, P.E.G., Van Leeuwen, S.P.J., Hamers, T., 2009. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol. Sci. : An Official Journal of the Society of Toxicology 109 (2), 206–216. https://doi.org/10.1093/TOXSCI/KFP055.

Wenzel, A., Franz, C., Breous, E., Loos, U., 2005. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. Mol. Cell. Endocrinol. 244 (1–2), 63–71. https://doi.org/10.1016/J.MCE.2005.02.008.

WHO, 2022. WHO AnthroPlus Software. Retrieved January 3, 2023, from. https://www.who.int/tools/growth-reference-data-for-5to19-years/application-tools.

Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., Diaz, D., Quinn, J., Adibi, J., Perera, F.P., Factor-Litvak, P., 2012. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ. Health Perspect. 120 (2), 290–295. https://doi.org/10.1289/EHP.1103705.

Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. Assessing exposure to phthalates - the human biomonitoring approach. Mol. Nutr. Food Res. 55 (1), 7–31. https://doi.org/10.1002/mnfr.201000121.

Wu, H., Zhang, W., Zhang, Y., Kang, Z., Miao, X., Na, X., 2021. Novel insights into di-(2-ethylhexyl)phthalate activation: implications for the hypothalamus-pituitary-thyroid axis. Mol. Med. Rep. 23 (4), 290. https://doi.org/10.3892/mmr.2021.11930.

Yu, W.G., Liu, W., Jin, Y.H., Liu, X.H., Wang, F.Q., Liu, L., Nakayam, 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.

Yue, B., Ning, S., Miao, H., Fang, C., Li, J., Zhang, L., Bao, Y., Fan, S., Zhao, Y., Wu, Y., 2023. Human exposure to a mixture of endocrine disruptors and serum levels of thyroid hormones: a cross-sectional study. J. Environ. Sci. (China) 125, 641–649. https://doi.org/10.1016/J.JES.2022.01.017.

Zhao, Y.G., Wong, C.K.C., Wong, M.H., 2012. Environmental contamination, human exposure and body loadings of perfluorooctane sulfonate (PFOS), focusing on Asian countries. Chemosphere 89 (4), 355–368. https://doi.org/10.1016/j.chemosphere.2012.05.043.

Zhao, Y., Song, X., Ding, S., Qi, W., Zhang, Y., Xu, Q., Zhao, T., Zhang, X., Li, X., Wu, F., Ye, L., 2022. The associations of urinary DEHP metabolite levels, serum thyroid hormones, and thyroid-related genes among the adolescent students from China: a cross-sectional study. Environ. Sci. Pollut. Res. Int. 29 (13), 19081–19097. https://doi.org/10.1007/S11356-021-16909-5.