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Review article

Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: A systematic review of epidemiologic studies

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ABSTRACT

Introduction: Thyroid hormones (THs) are especially important for brain maturation and development during the fetal period and childhood. Several epidemiological studies have assessed the possible association between exposure to perfluoroalkyl substances (PFAS) and thyroid outcomes during the early stages of life. We aimed to review this evidence.

Methods: We conducted a systematic review in compliance with the PRISMA Statement (search conducted in PubMed and Embase, as well as in the citations of the selected articles). We chose studies if they dealt with thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxin (T4), or thyroid dysfunctions, and perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) or perfluorononanoic acid (PFNA) measured in the blood of pregnant women and/or children up to 19 years old.

Results: We included in this review three cross-sectional, one case-control, and six cohort studies (publication: 2011–2015), focusing on prenatal life ($n = 7$), childhood ($n = 2$) or both periods ($n = 1$). We observed a high degree of heterogeneity across studies in terms of sampling time (different gestational weeks, at birth, or childhood), outcomes, adjustment for potential confounders, and statistical approach. We found some evidence of a positive association between PFHxS and PFOS exposure and TSH levels measured in maternal blood, and PFNA and TSH levels measured in the blood of boys aged ≥11 years.

Conclusion: Although there is a small number of studies with comparable data, we found some consistency of a positive association between maternal or teenage male exposure to some PFAS and TSH levels based on the current literature. However, further studies are required to confirm these possible relationships.

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1. Introduction

Perfluoroalkyl substances (PFAS) are synthetic chemicals with unique properties, such as insolubility in both organic solvents and water, and the ability to repel oils and water. They have been manufactured for industrial applications since the 1940s, and they are found and used in many common industrial and consumer products such as fire-fighting foams, alkaline cleaners, floor polishes, photographic films, shampoos, ant insecticides, soil- and stain-resistant coatings for fabrics, carpets and leather, as well as in grease- and oil-resistant coatings for paper products, among others (OCDE, 2005; Renner, 2001; WHO, 2013). In the general population, the main routes of exposure to these substances are *via* food, food packaging and drinking water (Domingo et al., 2012; Vestergren et al., 2008). Nearly all individuals, including pregnant women and children, in the many populations studied worldwide (Fromme et al., 2009; Kato et al., 2011; Manzano-Salgado et al., 2016; Mondal et al., 2012; Mørck et al., 2015; Zhao et al., 2012), showed measurable blood concentrations of four PFAS (perfluorohexane sulfonate [PFHxS], perfluorooctanoic [PFOA], perfluorooctane sulfonate [PFOS], and perfluorononanoic acid [PFNA]). Although there have been phase-out agreements regarding the production of certain PFAS by the industry in U.S. and Europe (WHO, 2013), due to their ubiquitous presence, long half-life in humans (Bartell et al., 2010; Olsen et al., 2007), and a tendency for bioaccumulation and biomagnification (WHO, 2013), exposure to this class of compounds will persist for many years, thereby making them a potential threat to humans.

Concern about exposure to PFAS has increased after the publication of recent studies showing that these chemicals have endocrine-disrupting properties and, among their possible health effects, PFAS may have the ability to impair thyroid function (Jensen and Leffers, 2008). Our review focuses on PFAS and thyroid disruption during the prenatal and childhood periods because thyroid hormones (THs) are especially important during brain maturation and the development of the fetus and children (Dussault and Ruel, 1987). THs are involved in the processes of dendritic and axonal growth, synaptogenesis, neurogenesis, and myelination during intrauterine life (Bernal, 2007). After birth, they are still essential, since some of these neurodevelopmental processes, such as myelination, are not completed until adolescence (Rice and Barone, 2000; Schug et al., 2015) and they also play a role in the behavior and cognitive functions of the young and adolescent brain (Anderson, 2001). In fact, disorders involving TH availability, even subclinical maternal hypothyroidism (Haddow et al., 1999) or subtle changes in TH homeostasis during the first years of life (Freire et al., 2010; Julvez et al., 2013) may lead to delays in child neuropsychological development. Additionally, TH deficiency during infancy, childhood and puberty causes growth delay and precocious puberty in both sexes, and hirsutism in females (Papi et al., 2007). Finally, postnatal alterations of TH levels are also correlated with a variety of adverse effects in the pulmonary (Krude et al., 2002; Mendelson and Boggaram, 1991) and cardiovascular (Asvold et al., 2007; Biondi et al., 2005; Osman et al., 2002) systems. Therefore, the possible effects of PFAS on thyroid function during fetal and child life is a matter of public concern.

Prompted by the worldwide exposure to four PFAS (PFHxS, PFOA, PFOS, and PFNA) and the essential role of the thyroid system in the development and normal functioning of the body, we aimed to assess the evidence of associations between exposure to PFAS and thyroid function in pregnant women and children up to 19 years old.

2. Methods

We developed a protocol and performed a systematic review in accordance with the general principles recommended in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher et al., 2010).

2.1. Eligibility criteria and search strategy

Studies selected for the review were those carried out in populations of pregnant women or children up to 19 years old, written in English or Spanish, and published before the end of December 2015. In this first selection of articles, we did not impose any restrictions on outcomes if at least one of the four chemicals (PFHxS, PFOA, PFOS, PFNA) or the generic PFC, PFAA, and PFAS were included (more details about search syntax can be found in Supplementary data). We did not include other PFAS

in our review since the current published literature on their possible association with thyroid function is still too scarce. We also located some relevant studies after reading citations from the selected articles.

2.2. Study selection criteria

We used the PICOS framework (Liberati et al., 2009) to establish the criteria for selecting the studies to be included in this review, since it offers a structured approach for framing questions using five components, as follows. *Participants*: Pregnant women and children up to 19 years old. Studies on general or occupational populations were also eligible if results were stratified by sex (specifying the pregnant status of women) or age of the children, and only those results were included in this review. *Exposures*: Studies on direct measurement of PFAS in a biological matrix or indirect exposure estimations. C*omparators*: Continuous PFAS levels or groups categorized according to individual PFAS levels. *Outcomes*: TH levels (thyroid-stimulating hormone [TSH], total triiodothyronine [TT3], free T3 [FT3], thyroxin [T4], and free T4 [FT4]) or thyroid dysfunctions. *Study design*: Cross-sectional, case-control, and cohort studies.

Two reviewers screened the titles and abstracts independently. Resolution in case of disagreement was achieved by discussion and consultation with a third reviewer. Data were abstracted by one reviewer and checked by the other two, and all of them also assessed the methodological quality of the articles that were finally included.

2.3. Assessment of the methodological quality of the articles

A validated tool to evaluate the methodological quality of observational studies is still lacking. As in some previous systematic reviews (Olmos et al., 2008; Ricci-Cabello et al., 2010; González-Alzaga et al., 2014; Rodríguez-Barranco et al., 2013), we assessed the methodological quality of the studies using the nine items included in the Methods section of the STROBE statement checklist (Strengthening the Reporting of Observational Studies in Epidemiology Statement) (von Elm et al., 2014). Studies with low scores according to the STROBE checklist were excluded from the review. For more information about the STROBE checklist items used, see Supplementary data, Table S1.

2.4. Data extraction and synthesis

Tables 1–5 and Fig. 1 summarize the data extracted from the selected articles, which include information on design, location, sample size, date, outcomes, PFAS, matrices, time of sample collection, statistical analyses, covariates included in the models, and main findings (estimates of the fully adjusted models are presented in tables). For all the studies reviewed, the summarized information was based on results presented in the original papers except for Tables 2 and 4 and Fig. 1, since some data (sample size, percentiles, minimums and/or maximums) not included in the articles were provided by authors (see footnotes for further information). Publicly available data from the 2011–2012 National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of non-institutionalized U.S. residents (NHANES, 2015), was used to replicate the same database that was used in Lewis et al. (2015). In that study the authors reported sex-stratified PFAS concentrations in boys ($n = 158$) and girls ($n = 145$) aged 12–19 years, while we present these values for all children ($n = 303$) in Table 2 and Fig. 1.

3. Results

The database search identified 380 citations, and we identified another 16 citations from additional searches in other sources. A total of 319 citations were excluded at the title/abstract stage (unrelated to study topic or duplicates), leaving 77 articles for examination of the full text. Of these, 66 were later excluded because they did not meet the inclusion criteria. Hence, altogether, we identified eleven eligible studies from the searches (PRISMA flow diagram, see Supplementary data, Figure S1). One of them (Inoue et al., 2004) was a very preliminary and rather descriptive study on the association between PFAS and THs and achieved a low quality score according to the STROBE checklist. Therefore, this article was not included in the review. All included studies ($n = 10$) achieved a high score according to the STROBE checklist (Table 1).

Various design features (*i.e.*, choice of population, outcomes, covariates, and analysis type) could all be important in generating differences in results between studies, and these attributes are summarized in the sections that follow. The articles have been grouped by study populations and PFAS examined, and within each group the differences in design features generally seem to be limited, the most noticeable discrepancies being found in the mean exposures by study for some PFAS and the inclusion of some of the confounders.

3.1. Study population

A description of the epidemiologic studies is shown in Table 1. The studies were conducted in Asia, Europe, and North America. Sample size varied from around 40 to >10,000 participants and the participation rate, when provided (Lin et al., 2013; Lopez-Espinosa et al., 2012b; Wang et al., 2013; Webster et al., 2011), ranged between 7.6 and 95% (data not shown). The design of the studies was either cross-sectional ($n = 3$), case-control ($n = 1$), or cohort ($n = 6$), although some of the latter also performed cross-sectional analyses. Chan et al. (2011) conducted a case-control study among women with or without hypothyroxinemia, Lin et al. (2013) focused on adolescents and young adults with abnormal urinalysis during childhood, and Lopez-Espinosa et al. (2012b) conducted a study in pregnant mothers and children from a community living near a fluoropolymer manufacturing facility. The remaining studies focused on pregnant women and children from general populations. Most authors adjusted the statistical analyses for maternal or child age ($n = 9$). The other variables of adjustment present a greater variation among studies (Table 1).

3.2. PFAS

3.2.1. Blood compartments

Seven out of the ten studies used serum for contaminant analysis, and the rest used plasma (Tables 1 and 2).

3.2.2. Time of sampling and analysis

During pregnancy, a total of six studies determined PFAS concentrations in the second ($n = 4$) or third ($n = 2$) trimester, and one of these studies also measured these chemicals in cord serum. In addition, one study measured PFAS only in cord plasma, another used modeled maternal serum concentrations during the first trimester of pregnancy in addition to serum measures in children aged 1–17 years, and two studies measured these contaminants in the plasma or serum of children aged 12–19 years (Tables 1 and 2). In all the studies, PFAS were measured using liquid chromatography separation coupled with mass spectrometry (LC/MS). The ranges of the limits of detection (LODs) or quantification (LOQs) were 0.05–0.5, 0.03–1.5, 0.04–0.5 and 0.04–0.75 ng/mL, for PFHxS, PFOA, PFOS, and PFNA, respectively (data not shown).

3.2.3. PFAS concentrations

PFOA and PFOS were measured in all the studies, while PFHxS and PFNA were determined in seven of them. PFOA levels were higher in the population living in the vicinity of a Teflon manufacturing facility (child serum median: 29.3 ng/mL, modeled *in utero* serum: 11.5 ng/mL) (Lopez-Espinosa et al., 2012b) compared to the rest of the populations (median range: 0.89–4.3 ng/mL). PFOS was the

Table 1
Description of epidemiological studies on prenatal and child PFAS exposure and thyroid function.

Study	Design	Location (Date)	Population (n), age	PFAS	PFAS matrix	TH	TH matrix	Statistical analysis	Adjusted variables	MQ
Lewis et al. (2015)	C-S	U.S. (2011-12)	NHANES children (303), range: 12-19 yrs	PFHxS PFOA PFOS PFNA	Child serum	TSH TT4 FT4 TT3 FT3	Child serum	Multiple linear regression models between log2(PFAS) and log2(THs). Sensitivity analysis with categorized PFAS into quartiles	Child age, BMI, poverty income ratio, race/ ethnicity, and serum cotinine	H
Berg et al., 2015	C(P)	Norway (2007-09)	Pregnant women (391), median (range): 32 (18-43) yrs	PFHxS PFOA PFOS PFNA	Maternal serum (2nd T)	TSH TT4 FT4 TT3 FT3	Maternal serum (2nd T, 3 days, and 6 wks after birth)	Mixed effects models between PFAS (in quartiles) and mean effect of three repeated measurements of THs (log-transformed)	Parity, maternal age, BMI, and thyroxin binding capacity	H
de Cock et al., 2014	C(P)	Zwolle, Netherlands (2011-13)	Pregnant women (83), mean (range): 32.6 (23-40) yrs; neonates (83)	PFOA PFOS	Cord plasma	TT4	Infant blood (4-7 days of age)	Multiple linear regression models between PFAS (in quartiles) and TT4 stratified by sex (by means of an interaction term included in models)	Maternal age, GWG, BMI, smoking, alcohol intake, thyroid problem, thyroid medication, parity, gestational age, and birth weight	H
Wang et al., 2014	C(P)	Taiwan (2000-01)	Pregnant women (285), mean: 28.8 yrs; neonates (116)	PFHxS PFOA PFOS PFNA	Maternal serum (3rd T)	TSH TT4 FT4 TT3	Maternal serum (3rd T) Cord serum	Multiple linear regression models between PFAS and THs	Maternal age, education, previous children, neonatal sex, and type of delivery	H
Webster et al., 2014	C(P)	Vancouver, Canada (2007-08)	Pregnant women (152), mean (range): 34 (25-43) yrs	PFHxS PFOA PFOS PFNA	Maternal serum (2nd T)	TSH TT4 FT4	Maternal serum (twice in 2nd T)	Mixed effects linear regression models between PFAS and THs with a random intercept for subject. Interaction between TPOAb status and PFAS also included in models	Gestational age at blow draw, time of day of sampling, and TPOAb status	H
Lin et al., 2013	C-S	Taipei, Taiwan (2006-08)	Children (212), range: 12-19 yrs	PFOA PFOS PFNA	Child plasma	TSH FT4	Child serum	Multiple linear regression models between categorized[a] PFAS and ln(TSH) or FT4 stratified by sex	Child age, sex, smoking and drinking status	H
Wang et al., 2013	C-S	Norway (2003-04)	Pregnant women (903), mean (range): 30 (18-44) yrs	PFHxS PFOA PFOS PFNA	Maternal plasma (17-18 gws)	TSH	Maternal plasma (17-18 gws)	Multiple linear regression models between PFAS (continuous or quartiles) and ln(TSH) Logistic regression with dichotomized[b] TSH as the outcome	Maternal age, HDL levels, total seafood intake, parity, inter-pregnancy interval, and gestational age at blood draw	H
Lopez-Espinosa et al., 2012b	C(R)	Mid-Ohio Valley, U.S. (1987-06)	Pregnant women (4,713), mean: 26 yrs; children (10,725), range: 1-17 yrs	PFOA PFOS PFNA	Modeled serum levels *in utero* (1st T) Child serum	TSH TT4	Child serum (1-17 yrs)	Multiple linear regression models between ln(PFAS) or PFAS in quartiles and ln(TSH) or TT4. Same analyses stratified by sex and age groups. Logistic regression models with reported thyroid disease[c] and subclinical[d] hypo/hyperthyroidism as the outcomes	Child age, sex, and month of sampling	H
Chan et al., 2011	C-C	Alberta, Canada (2005-06)	Pregnant women (271), mean (range): 31 (20-45) yrs	PFHxS PFOA PFOS	Maternal serum (15-16 gws)	TSH FT4	Maternal serum (15-16 gws)	Adjusted conditional logistic regression models between ln(PFAS) in maternal serum and hypothyroxinemia[e]	Maternal age, weight, race, and gestational age at blood draw	H
Kim et al., 2011	C(P)	Seoul, Gumi, Cheongju South Korea (2008-09)	Pregnant women (44), mean (range): 32 (22-44) yrs; newborns (43)	PFHxS PFOS PFOA[f]	Maternal serum (3rd T) Cord serum	TSH TT4 TT3	Cord serum	Pearson correlations between ln(PFAS) and ln(THs) with- and without adjustment for influential covariates	Maternal age, BMI, and gestational age at blood draw	H

BMI: body mass index; C-C: case-control; C(P): cohort (prospective); C(R): cohort (retrospective); C-S: cross-sectional; FT3: free triiodothyronine; FT4: free thyroxin; GWG: gestational weight gain; gws: gestational weeks; H: high; HDL: high density lipoprotein; ln: natural log transformed; MQ: methodological quality; n: sample size; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; T: trimester; TH: thyroid hormone; TPOAb: thyroid peroxidase antibody (dichotomized according to clinical guidelines, high: ≥9 IU/mL versus normal: <9 IU/mL); TSH: thyroid-stimulating hormone; TT4: total thyroxin; TT3: total triiodothyronine; wks: weeks; yrs: years.

Some authors included adults in their articles (Lewis et al. and Lin et al.), this information has not been discussed in this review.

[a] Cut-off values: 50th, 75th and 90th percentiles for PFOA; 25th, 50th and 75th percentiles for PFOS, and 60th and 90th percentiles for PFNA.

[b] TSH above the 95th percentile (7.5 μIU/mL).

[c] For three categories of self-reported disease: any thyroid disease, hypothyroidism, and thyroid disease plus thyroid medication.

[d] TT4 within the normal reference range (4.5–12 μg/dL) and TSH >5.97, >4.84, >4.5 μIU/mL in children <6, 6–10, 11-17 years old for subclinical hypothyroidism and TSH <0.7, <0.6, <0.45 μIU/mL in children <6, 6–10, 11-17 years old for subclinical hyperthyroidism. Children who self-reported thyroid disease and/or thyroid medication were excluded.

[e] Hypothyroxinemia: defined by authors as normal maternal TSH levels with no evidence of hyperthyroidism (0.15-≤4 mU/L) and maternal FT4 levels in the lowest 10th percentile (≤8.8 pmol/L) of the sample. Controls: normal TSH (0.15-≤4 mU/L), and FT4 between the 50th and 90th percentiles (12–14.1 pmol/L).

[f] Kim also measured PFNA but associations with THs were not studied and, therefore, it has not been included in this review. In this article, all samples were collected during the 3rd trimester of pregnancy except seven of them collected at 20-25 weeks of gestation.

most commonly detected (>80–100%), with a greater variation in its concentrations (median range: 1.3–20 ng/mL). Less variation was observed for PFHxS (median range: 0.3–1.1 ng/mL) and PFNA (0.4–1.5 ng/mL) among studies, and concentrations were lower compared to the other PFAS (Table 2 and Fig. 1).

3.3. TH outcomes

3.3.1. Blood compartments

Eight, one and one studies used serum, plasma and blood for hormone analysis, respectively (Tables 1 and S2).

Table 2
Summary of PFAS concentrations (ng/mL).

Study	Detected levels[a]. Statistics	Matrix	PFHxS	PFOA	PFOS	PFNA
Maternal PFAS						
Berg et al., 2015	%>LOD	Maternal serum	99	100	>80	100
	Median (P25, P75)		0.44 (0.28, 0.66)	1.53 (0.99, 2.16)	8.03 (5.76, 11.01)	0.56 (0.43, 0.78)
Webster et al., 2014	%>LOD	Maternal serum	84	99	100	62
	Median (P25, P75)		1.0 (0.7, 1.7)	1.7 (1.0, 2.4)	4.8 (3.2, 6.5)	0.6 (<0.5, 0.8)
Wang et al., 2014	%>LOQ	Maternal serum	78	87	100	96
	Median (P25, P75)		0.81 (0.30, 1.35)	2.39 (1.54, 3.40)	12.73 (9.65, 17.48)	1.51 (0.85, 2.51)
Wang et al., 2013	%>LOQ	Maternal plasma	99	100	100	99
	Median (P25, P75)		0.60 (0.43, 0.84)	2.15 (1.57, 2.95)	12.81 (10.13, 16.49)	0.39 (0.28, 0.51)
Lopez-Espinosa et al., 2012b	%>LOD	Maternal serum (modeled *in utero*)	-	100	-	-
	Median (P25, P75)		-	11.5 (5.36, 37.2)	-	-
Chan et al., 2011	%>LOD	Maternal serum in cases	94	89	99	-
	Median (P25, P75)		0.99 (0.58, 2.28)	1.63 (0.74, 2.61)	7.75 (5.19, 11.44)	-
		Maternal serum in controls	93	93	100	-
			0.94 (0.54, 1.96)	1.50 (0.89, 2.55)	8.22 (5.19, 11.91)	-
Kim et al., 2011	%>LOD	Maternal serum	100	100	100	-
	Median (P25, P75)		0.55 (0.46, 0.85)	1.46 (1.15, 1.91)	2.93 (2.08, 4.36)	-
Cord PFAS						
de Cock et al., 2014	%>LOQ	Cord plasma	-	100	100	-
	Median (P25, P75)[b]		-	0.89 (0.59, 1.18)	1.6 (1.0, 2.08)	-
Kim et al., 2011	%>LOD	Cord serum	100	100	100	-
	Median (P25, P75)		0.34 (0.27, 0.51)	1.15 (0.95, 1.86)	1.26 (0.81, 1.82)	-
Child PFAS						
Lewis et al., 2015	%>LOD	Child serum	99	100	100	100
	Median (P25, P75)		1.05 (0.66, 1.92)	1.71 (1.32, 2.35)	4.19 (2.71, 6.22)	0.75 (0.53, 1.16)
Lin et al., 2013	%>LOD	Child plasma[c]	-	61	98	42
	Median (P25, P75)		-	4.29 (0.75, 6.78)	8.0 (4.97, 11.43)	0.38 (0.38, 3.13)
Lopez-Espinosa et al., 2012b	%>LOD	Child serum	-	100	99.8	99
	Median (P25, P75)		-	29.3 (13.1, 67.7)	20.0 (14.5, 27.8)	1.50 (1.20, 2.00)

- Not studied; LOD: limit of detection; LOQ: limit of quantification; P: percentile; PFAS: perfluoralkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate.
Some data not included in the articles was provided by authors (percentiles: Berg et al. and de Cock et al.; %>LOD, median and percentiles for children aged 12-19 years: Lin et al.). Database used in Lewis et al. has been replicated using publicly-available data from NHANES, 2011–2012 (NHANES, 2015), since Lewis et al. reported sex-stratified PFAS concentrations and we present data for both girls and boys.
[a] Imputation of non-detected concentrations: based on expected values assuming a log-normal distribution in Wang et al. (2014); LOD/√2 in Berg et al., Webster et al., Kim et al., and Lewis et al.; LOQ/√2 in de Cock et al. and Wang et al. (2013); and LOD/2 in Lin et al., Lopez-Espinosa et al., and Chan et al.
[b] n = 64.
[c] Matrix taken from the "Material and Methods" section of the article.

3.3.2. Time of sampling and analysis

During pregnancy, THs were measured in the second ($n = 4$) or third ($n = 1$) trimester, as well as in cord serum ($n = 2$). Berg et al. (2015) collected two additional blood samples at 3 days and 6 weeks after delivery and Webster et al. (2014) measured hormones twice during the second trimester of pregnancy. Also after birth, THs were measured in newborn blood ($n = 1$) or child serum ($n = 3$) (Table 1). All studies used different types of immunoassays to determine TH levels (data not shown).

Four of the studies on TH levels excluded participants who reported any thyroid disease and/or were taking thyroid medication (Lopez-Espinosa et al., 2012b; Wang et al., 2013, 2014; Webster et al., 2014), one of them (Webster et al., 2014) also excluded women with other endocrine alterations, such as diabetes mellitus, which is known to affect TH levels (data not shown), and another article (de Cock et al., 2014) adjusted models for data on thyroid gland problems and thyroid medication use (Table 1).

3.3.3. TH levels

TSH was assessed in nine studies, TT4 in seven, FT4 in six, TT3 in four, and FT3 in two (Tables 1 and S2). Median ranges of TSH and TT4 levels were 0.65–6.65 mIU/L and 107.7–145 nmol/L, respectively, in both the prenatal and at–birth studies. Respective data during childhood were 1.4–1.83 mIU/L and 95.2–99.1 nmol/L, and de Cock et al. (2014) found mean levels of TT4 of 85.6 and 89.6 mIU/L in infant boys and girls, respectively. Few studies measured other hormones (FT4, TT3 and FT3) during these two periods to compare results. In the only study measuring maternal levels during pregnancy and after birth (Berg et al., 2015), levels of TSH, TT4, and TT3 decreased after birth, while levels of the free hormones (FT4 and FT3) increased (Table S2).

3.3.4. Thyroid dysfunction outcomes

The outcome investigated by Chan et al. (2011) was hypothyroxinemia in pregnant women. Lin et al. (2013) and Wang et al. (2013) investigated dichotomized TSH levels as the outcome, but the former did not stratify by age and the results are not included in this review. Webster et al. (2014) assessed the effect of PFAS in women with high thyroid peroxidase antibody (TPOAb) levels, which is a marker of autoimmune hypothyroidism, including the interaction between TPOAb status and PFAS in the models. Berg et al. (2015) studied the association with subclinical hypothyroidism based on TH levels. Lopez-Espinosa et al. (2012b) investigated the association with self-reported thyroid disease, thyroid medication plus thyroid disease, hypothyroidism, and subclinical hypo/hyperthyroidism based on TH levels (Table 1).

3.4. Analysis of the relation between PFAS and THs

Seven studies examined the association between prenatal PFAS exposure and TH levels measured during the prenatal, neonatal or child periods (Table 3) and three cross-sectional studies between child PFAS and TH levels (Table 4).

Table 3
Summary of associations between prenatal PFAS exposure (ng/mL) and THs measured at different lifestages.

Study	Expression of results	TH matrix (units)	n	Associations between PFAS and THs			
				PFHxS	PFOA	PFOS	PFNA
Maternal PFAS							
Berg et al., 2015	Estimated mean differences in THs[a] (95% CI) across PFAS Qs	Maternal TSH (mLU/L)	375	NS	NS	↑: Q2: 0.18 (0.06, 0.31)[a] ↑*: Q3: 0.26 (0.13, 0.40) ↑*: Q4: 0.35 (0.21, 0.50)	NS
Webster et al. (2014)	β (95% CI) per IQR[b] ng/mL increase in PFAS	Maternal TSH (mIU/L)	151	↑: 0.01 (-0.05, 0.07)	↑: 0.1 (-0.05, 0.3)	↑: 0.1 (-0.03, 0.2)	↑*: 0.2 (0.01, 0.3)
		Maternal TT4 (nmol/L)	151	↓: -0.8 (-2, 0.6)	↓: -2 (-6, 2)	↓: -2 (-5, 1)	↓: -2 (-5, 2)
		Maternal FT4 (pmol/L)	150	↓: -0.02 (-0.1, 0.07)	↓: -0.06 (-0.3, 0.2)	↑: 0.03 (-0.2, 0.2)	↓: -0.03 (-0.2, 0.2)
Wang et al., 2014	β (95% CI) per 1 ng/mL increase in PFAS	Maternal TSH (μIU/mL)	283	↑*: 0.105 (0.002, 0.207)	↑: 0.011 (-0.057, 0.078)	↓: -0.005 (-0.024, 0.013)	↑: 0.033 (-0.046, 0.112)
		Maternal TT4 (μg/dL)	274	↓: -0.130 (-0.316, 0.057)	↑: 0.011 (-0.108, 0.130)	↑: 0.019 (-0.016, 0.053)	↓*: -0.189 (-0.333, -0.046)
		Maternal FT4 (ng/dL)	285	↓: -0.010 (-0.023, 0.003)	↓: -0.003 (-0.012, 0.005)	↑: 0.001 (-0.002, 0.003)	↓*: -0.019 (-0.028, -0.009)
		Maternal TT3 (μg/dL)	276	↓: -0.002 (-0.005, 0.001)	↓: -0.000 (-0.002, 0.009)	↑: 0.000 (-0.002, 0.001)	↓: -0.001 (-0.003, 0.002)
		Cord TSH (μIU/mL)	114	↑: 0.493 (-1.449, 2.434)	↓: -0.498 (-1.464, 0.468)	↓: -0.083 (-0.292, 0.127)	↓: -0.361 (-0.955, 0.234)
		Cord TT4 (μg/dL)	116	↑: 0.002 (-0.495, 0.500)	↑: 0.128 (-0.094, 0.350)	↑: 0.032 (-0.024, 0.087)	↓*: -0.213 (-0.384, -0.042)
		Cord FT4 (ng/dL)	92	↓: -0.030 (-0.098, 0.039)	↓: -0.029 (-0.062, 0.004)	↑: 0.001 (-0.006, 0.008)	↑: 0.001 (-0.021, 0.023)
		Cord TT3 (μg/dL)	112	↓: -0.001 (-0.007, 0.004)	↓: -0.001 (-0.004, 0.001)	↑: 0.000 (-0.000, 0.001)	↓*: -0.002 (-0.004, -0.001)
Wang et al., 2013	β (95% CI) per 1 ng/mL increase in PFAS	Maternal lnTSH (μIU/mL)	903	↑: 0.013 (-0.043, 0.070)	↓: -0.0001 (-0.045, 0.44)	↑*: 0.008 (0.001, 0.016)	↑: 0.165 (-0.023, 0.353)
Lopez-Espinosa et al., 2012b	% change (95% CI) in TH per IQR[b] ng/mL increase in modeled *in utero* PFOA	Child TSH (μIU/mL)	476 (1-5 yrs)	-	↓: -3.4 (-8.8, 2.4)	-	-
			1,405 (6-10 yrs)	-	↓: -1.5 (-4.9, 2.1)	-	-
			2,741 (11-17 yrs)	-	↑: 0.1 (-2.2, 2.5)	-	-
			4,622 (1-17 yrs)	-	↓: -0.5 (-2.4, 1.5)	-	-
		Child TT4 (μg/dL)	484 (1-5 yrs)	-	↑*: 2.0 (0.1, 3.9)	-	-
			1,410 (6-10 yrs)	-	↑: 0.9 (-0.3, 2.1)	-	-
			2,744 (11-17 yrs)	-	↓: -0.7 (-1.5, 0.2)	-	-
			4,638 (1-17 yrs)	-	↓: -0.1 (-0.8, 0.6)	-	-
Kim et al., 2011	Pearson correlation coefficient (r)	Cord TSH (μIU/mL)	29	↑: 0.091	↑*: 0.443	↑: 0.109	-
		Cord TT4 (μg/dL)	33	↑: 0.030	↓: -0.071	↓: -0.181	-
		Cord TT3 (ng/dL)	32	↓: -0.261	↓: -0.238	↓*: -0.414	-
Cord PFAS							
de Cock et al., 2014	β (95% CI) of each PFAS Q compared to Q1, by sex	Newborn TT4 (nmol/L)	52	-	↑:Q2b: 7.9 (-18.04, 33.92)	↓:Q2b: -7.9 (-31.56, 15.74)	-
			52		↓:Q3b: -2.1 (-20.94, 16.78)	↓:Q3b: -16.5 (-40.32, 7.34)	
			52		↑:Q4b: 6.2 (-16.08, 28.50)	↓:Q4b: -9.6 (-32.57, 13.31)	
			52		↓:Q2g: -5.9 (-26.75, 14.94)	↓:Q2g: -1.3 (-30.45, 27.94)	
			52		↑:Q3g: 11.8 (-19.08, 42.72)	↑:Q3g: 4.5 (-25.95, 34.92)	
			52		↑*:Q4g: 38.6 (13.34, 63.83	↑:Q4g: 15.9 (-10.67, 42.40)	
Kim et al., 2011	Pearson correlation coefficient (r)	Cord TSH (μIU/mL)	31	↓: -0.069	↑: 0.089	↓: -0.088	-
		Cord TT4 (μg/dL)	35	↓: -0.111	↓: -0.157	↓: -0.048	-
		Cord TT3 (ng/dL)	34	↓: -0.178	↓: -0.240	↓: -0.157	-

↑ Positive association; ↓ negative association; * $p<0.05$; - not studied; b: boys; CI: confidence interval; FT4: free thyroxin; g: girls; IQR: interquartile range; ln: natural log transformed; n: sample size; NS: non-significant; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; Q: quartile; TH: thyroid hormone; TSH: thyroid-stimulating hormone; TT4: total thyroxin; TT3: total triiodothyronine; yrs: years.
Some data not included in the articles were provided by authors (sample size of each model: Lopez-Espinosa et al.)

[a] Results for TSH but not for T3 or T4 are presented in Berg et al. $p<0.05$ for Q3 and Q4. Q1 is the reference group.

[b] IQRs (in ng/mL): 1.0(PFHxS), 0.4(PFNA), 1.4(PFOA) and 3.3(PFOS) in Webster et al.; 47.1, 38.5, 23.1, and 31.8 for 1-5, 6-10, 11-17, and 1-17 yrs groups (modeled *in utero* PFOA) in Lopez-Espinosa et al.

Table 4
Summary of associations between child PFAS exposure (ng/mL) and THs.

Study	Expression of results	TH (units)	n	Associations between PFAS and THs			
				PFHxS	PFOA	PFOS	PFNA
Lewis et al. (2015)	% change (95% CI) in TH per doubling in PFAS concentration	TSH (μIU/mL)	158 boys (12-19 yrs)	↑: 6.2 (-1.5, 14.5)	↑: 9.6 (-7.1, 29.4)	↑*: 12.3 (0.7, 25.2)	↑*: 16.3 (4.0, 30.2)
		TT4 (μg/dL)[a]		↓: -2.1 (-4.4, 0.2)	↓: -1.6 (-6.6, 3.6)	↓: -1.1 (-4.4, 2.3)	↑: 0.6 (-2.9, 4.3)
		FT4 (ng/dL)		↓: -1.5 (-3.5, 0.5)	↓: -0.6 (-4.9, 4.0)	↑: 0.6 (-2.4, 3.6)	↑: 2.6 (-0.5, 5.8)
		TT3 (ng/dL)		↑: 0.2 (-1.9, 2.4)	↓: -1.9 (-6.4, 2.9)	↓: -1.6 (-4.7, 1.5)	↓: -1.1 (-4.2, 2.2)
		FT3 (pg/mL)		↑: 0.0 (-1.4, 1.4)	↑: 0.8 (-2.2, 3.9)	↓: -0.3 (-2.3, 1.7)	↑: 0.3 (-1.8, 2.4)
		TSH (μIU/mL)	145 girls (12-19 yrs)	↓: -4.1 (-11.8, 4.3)	↓*: -16.6 (-28.6, -2.6)	↓: -6.6 (-16.0, 3.8)	↑: 4.2 (-8.4, 18.5)
		TT4 (μg/mL)		↓: -0.3 (-2.8, 2.1)	↑: 4.1 (-0.6, 8.9)	↓: -0.3 (-3.4, 2.8)	↓: -3.2 (-6.7, 0.5)
		FT4 (ng/dL)		↑: 0.2 (-2.1, 2.6)	↑: 2.1 (-2.2, 6.7)	↑: 0.1 (-2.8, 3.1)	↓: -2.6 (-6.0, 8.9)
		TT3 (ng/dL)		↓: -1.3 (-3.7, 1.1)	↑: 0.3 (-4.2, 5.1)	↓: -2.3 (-5.2, 0.8)	↓: -1.6 (-5.3, 2.2)
		FT3 (pg/mL)		↓: -0.8 (-2.6, 1.1)	↑: 1.1 (-2.4, 4.7)	↓: -1.1 (-3.3, 1.3)	↓: -1.9 (-4.7, 0.9)
Lin et al. (2013)	p for trend[b]	lnTSH (μIU/L)	65 boys (12-19 yrs)	-	p>0.05	p>0.05	p>0.05
		FT4 (ng/dL)		-	p>0.05	p>0.05	p>0.05
		lnTSH (μIU/L)	144 girls (12-19 yrs)	-	p>0.05	p>0.05	p>0.05
		FT4 (ng/dL)		-	p>0.05	p>0.05	p>0.05
Lopez-Espinosa et al., 2012b	% change (95% CI) in TH per IQR[c] ng/mL increase in PFAS	TSH (μIU/mL)	3,328 boys (11-17 yrs)	-	↑: 1.6 (-1.1, 4.3)	↑: 1.1 (-1.0, 3.2)	↑*: 2.0 (0.0, 4.0)
		TT4 (μg/dL)	3,334 boys (11-17 yrs)	-	↑ : 0.5 (-0.5, 1.4)	↑* : 1.2 (0.5, 2.0)	↑* : 1.9 (1.2, 2.6)
		TSH (μIU/mL)	3,062 girls (11-17 yrs)	-	↑: 2.4 (-0.7, 5.7)	↑: 0.8 (-1.9, 3.5)	↑: 0.2 (-2.0, 2.4)
		TT4 (μg/dL)	3,064 girls (11-17 yrs)	-	↓ : -0.9 (-2.0, 0.2)	↑* : 1.1 (0.1, 2.0)	↑: 0.5 (-0.3, 1.3)
		TSH (μIU/mL)	6,390 children (11-17 yrs)	-	↑: 2.0 (-0.1, 4.1)	↑: 0.9 (-0.8, 2.7)	↑: 1.1 (-0.5, 2.8)
		TT4 (μg/dL)	6,398 children (11-17 yrs)	-	↓ : -0.3 (-1.1, 0.4)	↑* : 1.2 (0.6, 1.9)	↑* : 1.3 (0.7, 1.9)
		TSH (μIU/mL)	1,586 boys (6-10 yrs)	-	↓: -1.1 (-4.4, 2.3)	↓: -1.7 (-4.5, 1.2)	↓: -0.9 (-3.3, 1.6)
		TT4 (μg/dL)	1,597 boys (6-10 yrs)	-	↑: 0.1 (-1.1, 1.3)	↑: 0.4 (-0.7, 1.4)	↑: 0.5 (-0.4, 1.4)
		TSH (μIU/mL)	1,475 girls (6-10 yrs)	-	↑: 2.2 (-1.7, 6.3)	↑: 2.1 (-1.7, 5.7)	↑: 1.0 (-2.4, 4.4)
		TT4 (μg/dL)	1,480 girls (6-10 yrs)	-	↑: 1.9 (0.6, 3.2)	↑*: 1.5 (0.4, 2.7)	↑*: 1.4 (0.3, 2.5)
		TSH (μIU/mL)	3,061 children (6-10 yrs)	-	↑: 0.5 (-2.0, 3.1)	↑: 0.0 (-2.2, 2.3)	↑: 0.0 (-2.1, 2.1)
		TT4 (μg/dL)	3,077 children (6-10 yrs)	-	↑: 0.9 (0.0, 1.8)	↑*: 0.9 (0.2, 1.7)	↑*: 1.0 (0.3, 1.7)
		TSH (μIU/mL)	471 boys (1-5 yrs)	-	↓: -1.1 (-6.6, 4.7)	↑: 1.4 (-4.3, 7.5)	↓: -0.7 (-6.0, 4.8)
		TT4 (μg/dL)	487 boys (1-5 yrs)	-	↑: 1.3 (-0.7, 3.3)	↑: 0.4 (-1.7, 2.5)	↑: 1.1 (-0.8, 3.0)
		TSH (μIU/mL)	500 girls (1-5 yrs)	-	↓*: -7.7 (-13.2, -1.7)	↑: 4.7 (-0.9, 10.5)	↑: 1.5 (-3.8, 7.1)
		TT4 (μg/dL)	510 girls (1-5 yrs)	-	↓: -0.1 (-2.2, 2.0)	↑: 1.2 (-0.6, 3.0)	↑: 1.1 (-0.7, 2.9)
		TSH (μIU/mL)	971 children (1-5 yrs)	-	↓*: -4.3 (-8.2, -0.3)	↑: 3.1 (-0.9, 7.3)	↑: 0.2 (-3.5, 4.1)
		TT4 (μg/dL)	997 children (1-5 yrs)	-	↑: 0.7 (-0.7, 2.1)	↑: 0.8 (-0.6, 2.2)	↑: 1.1 (-0.2, 2.4)
		TSH (μIU/mL)	5,385 boys (1-17 yrs)	-	↑: 0.7 (-1.3, 2.7)	↑: 0.4 (-1.2, 2.1)	↑: 1.0 (-0.7, 2.6)
		TT4 (μg/dL)	5,418 boys (1-17 yrs)	-	↑: 0.4 (-0.3, 1.1)	↑*: 0.9 (0.3, 1.5)	↑*: 1.4 (0.9, 2.0)
		TSH (μIU/mL)	5,037 girls (1-17 yrs)	-	↑: 1.3 (-1.0, 3.8)	↑: 1.6 (-0.5, 3.6)	↑: 0.5 (-1.4, 2.5)
		TT4 (μg/dL)	5,054 girls (1-17 yrs)	-	↑: 0.0 (-0.8, 0.7)	↑*: 1.2 (0.5, 1.9)	↑*: 0.9 (0.2, 1.5)
		TSH (μIU/mL)	10,422 children (1-17 yrs)	-	↑: 1.0 (-0.5, 2.7)	↑: 1.0 (-0.3, 2.3)	↑: 0.8 (-0.4, 2.0)
		TT4 (μg/dL)	10,472 children (1-17 yrs)	-	↑: 0.1 (-0.5, 0.6)	↑*: 1.1 (0.6, 1.5)	↑*: 1.1 (0.7, 1.5)

↑ Positive association; ↓ negative association; * p<0.05; - not studied; CI: confidence interval; FT3: free triiodothyronine; FT4: free thyroxin; IQR: interquartile range; ln: natural log transformed; n: sample size; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; TH: thyroid hormone; TSH: thyroid-stimulating hormone; TT4: total thyroxin; TT3: total triiodothyronine; yrs: years. Some data not included in the articles were provided by authors (sample size of each model: Lopez-Espinosa et al.)

[a] Units for TT4 in Lewis et al. taken from: http://wwwn.cdc.gov/Nchs/Nhanes/2011-2012/THYROD_G.htm#LBXTT4.

[b] Authors reported mean and standard error of natural log transformed TSH and FT4 across categories of PFAS concentrations in linear regression models. Although associations were not significant, mean levels of TSH increased across tertiles of PFNA in boys.

[c] For the 1-5, 6-10, 11-17, and 1-17 years groups: 67.2, 63.4, 50.5, and 54.6 ng/mL (for PFOA), 12.9, 14.9, 12.6, and 13.3 ng/mL (for PFOS); and 0.8, 1.0, 0.8, and 0.8 ng/mL (for PFNA) in all children; 69.9, 63.1, 58.5, and 60.6 ng/mL (for PFOA), 13.0, 15.3, 12.9, and 13.7 ng/mL (for PFOS); and 0.8, 0.9, 0.7, and 0.8 ng/mL (for PFNA) in boys; and 64.6, 59.7, 41.2, and 48.4 ng/mL (for PFOA), 12.7, 14.1, 11.9, and 12.6 ng/mL (for PFOS); and 0.8, 1.1, 0.6, and 0.8 ng/mL (for PFNA) in girls.

Table 5
Summary of associations between PFAS (ng/mL) and thyroid dysfunctions.

Study	Expression of results	Thyroid outcome	n	Associations between PFAS and thyroid dysfunction			
				PFHxS	PFOA	PFOS	PFNA
Maternal PFAS							
Berg et al., 2015	Number of women with subclinical hypothyroidism[a] in each PFAS quartile	Subclinical hypothyroidism[a]		-	-	Q1: n = 12 Q2: n = 16 Q3: n = 24 Q4: n = 30	-
Webster et al. (2014)	% change (95% CI) in THs per IQR[b] ng/mL increase in PFAS compared to the median[c] THs, in women with high TPOAb[d]	Maternal TSH (μIU/L)	14	2 (-45, 48)	54 (8, 100)*	69 (15, 123)*	46 (8, 85)*
		Maternal TT4 (nmol/L)	14	-6 (-17, 5)	-6 (-17, 5)	-7 (-21, 6)	-2 (-11, 6)
		Maternal FT4 (pmol/L)	14	-5 (-15, 4)	-4 (-14, 5)	-7 (-18, 3)	-3 (-11, 4)
Wang et al. (2013)	OR (95% CI)	TSH dichotomized[e]	5%[c]	NS	NS	NS	NS
Lopez-Espinosa et al., 2012b	OR (95% CI) of thyroid disease in children per IQR[b] increase in modeled *in utero* PFOA	Reported thyroid disease	27	-	1.47 (0.95, 2.27)	-	-
		Reported hypothyroidism	20	-	1.61 (0.96, 2.63)	-	-
		Subclinical hypothyroidism[f]	155	-	0.94 (0.76, 1.16)	-	-
		Subclinical hyperthyroidism[f]	31	-	1.10 (0.69, 1.74)	-	-
Chan et al. (2011)	OR (95% CI)	Hypothyroxinemia[g]	271	1.12 (0.89, 1.41)	0.94 (0.74, 1.18)	0.88 (0.63, 1.24)	-
Child PFAS							
Lopez-Espinosa et al., 2012b	OR (95% CI) of thyroid disease in children per IQR[b] increase in PFAS	Reported thyroid disease	61	-	1.44 (1.02, 2.03)*	0.80 (0.62, 1.08)	1.05 (0.78, 1.41)
		Reported hypothyroidism	39	-	1.54 (1.00, 2.37)*	0.91 (0.63, 1.31)	1.11 (0.77, 1.60)
		Subclinical hypothyroidism[f]	365	-	0.98 (0.86, 1.15)	0.99 (0.86, 1.13)	0.99 (0.88, 1.12)
		Subclinical hyperthyroidism[f]	78	-	0.81 (0.58, 1.15)	0.80 (0.62, 1.02)	0.78 (0.61,1.01)

* $p<0.05$; - not studied; CI: Confidence interval; FT4: free thyroxin; IQR: interquartile range; n: sample size of participants with thyroid dysfunction; NS: non-significant; OR: odds ratio; PFAS: perfluoroalkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; TH: thyroid hormone; TPOAb: thyroid peroxidase antibody (dichotomized according to clinical guidelines, high: ≥9 IU/mL versus normal: <9 IU/mL); TSH: thyroid stimulating-hormone; TT4: total thyroxin.

[a] Subclinical hypothyroidism: depleted supply of T4/FT4 and T3/FT3.

[b] IQRs (in ng/mL): 1.0(PFHxS), 0.4(PFNA), 1.4(PFOA) and 3.3(PFOS) in Webster et al.; 31.8 (modeled in utero PFOA), 54.6 (child PFOA), 13.3 (child PFOS), and 0.8 (child PFNA) for children aged 1-17 years in Lopez-Espinosa et al.

[c] Medians in the whole study: 1.3 mIU/L at 15 weeks (TSH), 9.5 pmol/L at 15 weeks (FT4), and 126.4 nmol/L at 15 weeks (TT4).

[d] Webster et al. assessed the effect of PFAS in women with TPOAb levels including the interaction between TPOAb status and PFAS in the models. For PFHxS, PFOA, PFOS, and PFNA: p interaction = 0.97, 0.05, 0.03, 0.05 (for TSH); 0.30, 0.37, 0.40, 0.82 (for TT4); and 0.23, 0.40, 0.15, 0.37 (for FT4).

[e] TSH >95th percentile (7.5 μIU/mL) or below.

[f] TT4 within the normal reference range (4.5–12 μg/dL) and TSH >5.97, >4.84, >4.5 μIU/mL in children <6, 6–10, 11-17 years of age for subclinical hypothyroidism and TSH <0.7, <0.6, <0.45 μIU/mL in children <6, 6–10, 11-17 years of age for subclinical hyperthyroidism. Children who self-reported thyroid disease and/or thyroid medication were excluded.

[g] Hypothyroxinemia: defined by authors as normal maternal TSH levels with no evidence of hyperthyroidism (0.15-≤4 mU/L) and maternal FT4 levels in the lowest 10th percentile (≤8.8 pmol/L) of the sample.

3.4.1. PFHxS and THs

Six papers analyzed the association between levels of PFHxS and THs, five focusing on the prenatal (Table 3) and one on the postnatal (Table 4) windows of exposure to this contaminant, respectively. During pregnancy, Wang et al. (2014) found statistically significant associations between maternal PFHxS and TSH and Webster et al. (2014) and Wang et al. (2013) found non-significant increases in maternal TSH associated to maternal PFHxS. No significant associations with T3 or T4 were reported in any study.

3.4.2. PFOA and THs

Nine papers analyzed the association with this contaminant. Three out of the seven studies focusing on prenatal PFOA exposure found statistically significant associations with either maternal or child hormones measured at different lifestages (Table 3). Specifically, significant positive associations between maternal PFOA and cord TSH (Kim et al., 2011), PFOA modeled *in utero* and TT4 in children <6 years (Lopez-Espinosa et al., 2012b), and cord PFOA and female newborn TT4 (de Cock et al., 2014) were reported. Lopez-Espinosa et al. (2012b) also stratified results by sex, finding similar but not significant results for the association between PFOA and TT4 (% change [95% CI]: 1.7% [−1.2, 4.6%] and 2.1% [−0.5, 4.8%] in TT4 associated with a sex-specific IQR increment in modeled *in utero* PFOA [18 to 88 ng/mL for boys and 14 to 78 for girls]) in boys and girls aged <6 years (data not shown). During childhood (Table 4), two out of three studies reported statistically significant associations between child PFOA and TSH but at different ages during infancy. Only in the group of children aged 1–5 years, Lopez-Espinosa et al. (2012b) reported child serum PFOA to be inversely associated with child TSH, this being significant in girls but not in boys and Lewis et al. (2015) reported a significant inverse association between PFOA and TSH in girls aged 12–19 years. The association in the group of girls aged 11–17 years, although not significant, was positive in the much larger study of Lopez-Espinosa et al. (2012b).

3.4.3. PFOS and THs

Nine papers analyzed the association with this contaminant. During gestation (Table 3), Berg et al. (2015) and Wang et al. (2013) found a positive significant association between maternal PFOS and TSH, Webster et al. (2014) a non-significant positive association and Wang et al. (2014) a negative but close to null association. In addition, Kim et al. (2011) found a significant negative correlation between maternal PFOS and cord TT3. Regarding child PFOS exposure (Table 4), Lopez-Espinosa et al. (2012b) reported a positive association between PFOS and TT4 in children aged 1–17 years. In this paper, this association remained significant in boys aged 11–17 years and 6–17 year-old girls when analyses were stratified by age groups and sex. Lewis et al. (2015) reported a significant positive association between PFOS and TSH in boys aged 12–19 years.

3.4.4. PFNA and THs

Seven papers analyzed the association between PFNA and THs, four of them focused on the prenatal and three on the postnatal periods of exposure to this contaminant. During pregnancy (Table 3), maternal PFNA concentrations were positively associated with maternal TSH in three out of four studies (Wang et al., 2013, 2014; Webster et al., 2014), but statistical significance was only reached in one of them (Webster et al., 2014). PFNA was also inversely

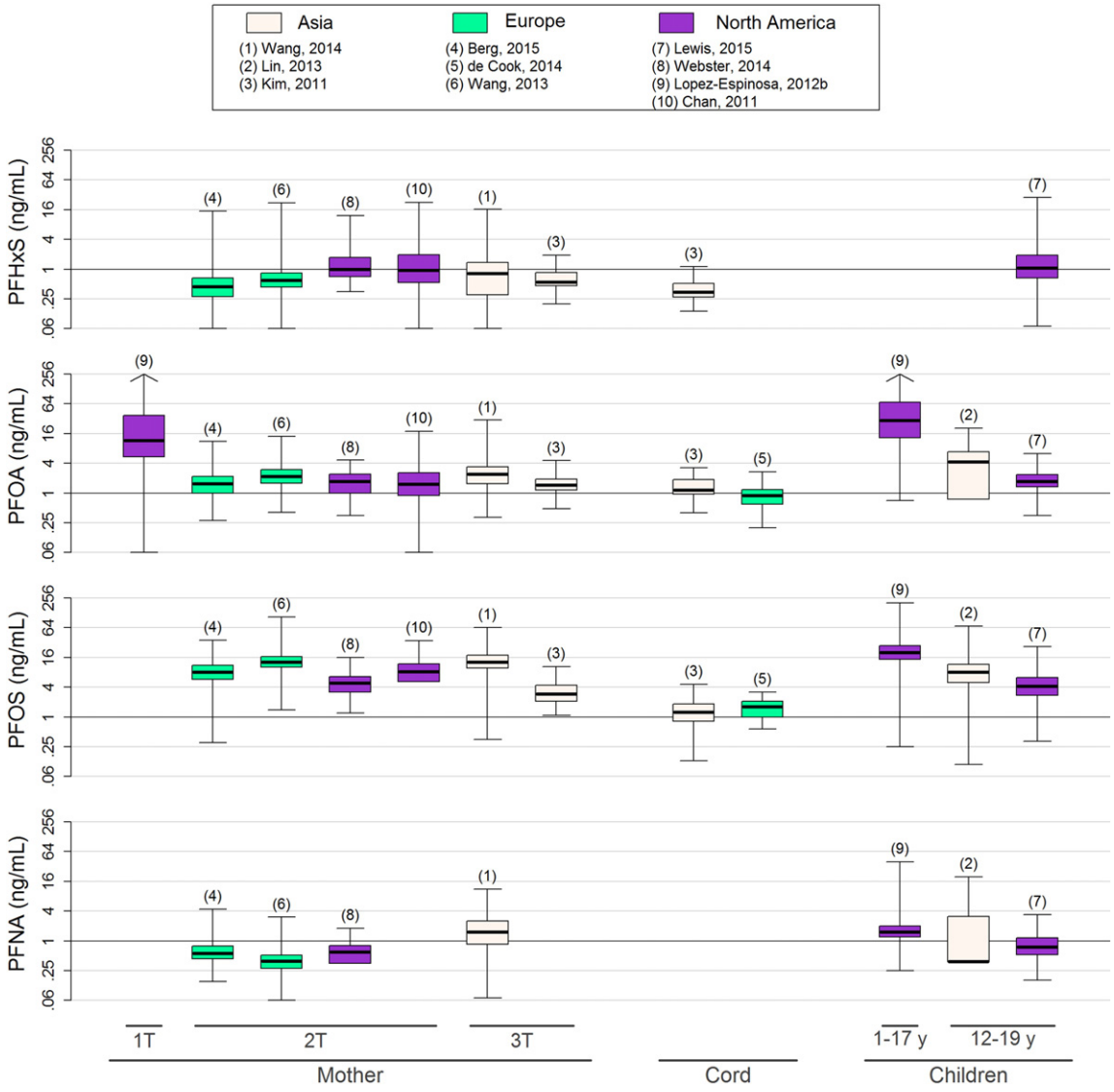

Fig. 1. Box-plot of PFAS concentrations (ng/mL) in the different studies. PFAS: perfluoroalkyl substances; PFHxS: perfluorohexane sulfonate; PFNA: perfluorononanoic acid; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulfonate; T: trimester; y: years. Some data not included in the articles were provided by authors (percentiles: Berg et al. and de Cock et al.; minimums and/or maximums: Lopez-Espinosa et al., Kim et al., and Wang et al. [2013 and 2014]; and descriptive data for children aged 12–19 years: Lin et al.). For Lewis et al., data for all children ($n = 303$) have been calculated using the publicly-available data from NHANES, 2011–2012 (NHANES, 2015), since Lewis et al. reported sex-stratified PFAS concentrations. Descriptive data for controls but not cases were represented for Chan et al.

associated with TT4, FT4, or TT3 in one study (Wang et al., 2014). Regarding child exposure (Table 4), Lopez-Espinosa et al. (2012b) reported an increase in TT4 concentrations associated to serum PFNA exposure in children aged 1–17 years. Sex-subgroup analyses revealed that the association reached statistical significance in 11–17 year-old boys and girls aged 6–10 years. TSH levels also significantly increased with PFNA concentrations (Lewis et al., 2015; Lopez-Espinosa et al., 2012b) and mean levels of TSH increased non-significantly across PFNA tertiles (Lin et al., 2013) in three studies of boys aged 11–19 years.

3.4.5. Direction of the associations

Tables 3 and 4 also show an arrow indicating the direction of the association to allow evaluation of whether there is a tendency for effect estimates to run in one direction, even if the effect is not statistically significant or close to null. For the studies using maternal levels of contaminants and hormones ($n = 4$), a clearer consistent direction in the association was found between maternal PFHxS, PFOS, or PFNA and TSH (positive) or T4 (negative). During childhood, a consistent direction of the association (positive) was found for PFNA and TSH in boys aged 11–19 years ($n = 3$ studies). T3 was not studied sufficiently to draw a conclusion.

3.5. Analysis of the relation between PFAS and thyroid dysfunctions

The number of studies on thyroid dysfunction is low ($n = 5$) and few studies found statistically significant associations (Table 5). During pregnancy, an increased proportion of women with subclinical hypothyroidism for each PFOS quartile was reported (Berg et al., 2015), and PFOA, PFOS, and PFNA were positively and statistically significantly associated with TSH levels in pregnant women with high TPOAb ($n = 14$) (Webster et al., 2014). During infancy, a positive significant association was found between reporting thyroid disease, mostly hypothyroidism, and serum PFOA concentrations in one study. However, PFOA concentrations were not associated with subclinical hypo/hyperthyroidism based on individual TH levels (Lopez-Espinosa et al., 2012b).

3.6. Dose-response relationship

To investigate the relation between PFAS and THs, different approaches have been used: log transforming, or not, of the exposure and outcome variables and categorizing exposure into percentiles. Only two studies fitted a linear relationship between PFAS and THs (Wang et al., 2014; Webster et al., 2014). Wang et al. (2014) evaluated the possibility of a non-monotonic relationship between each PFAS and each outcome by means of generalized additive models and concluded there was no evidence of a departure from linearity. Finally, a linear relationship was compared to ln-transformation of the THs with similar results (Wang et al., 2014). Categorization of PFAS was conducted in six articles (Berg et al., 2015; de Cock et al., 2014; Lewis et al., 2015; Lin et al., 2013; Lopez-Espinosa et al., 2012b; Wang et al., 2013), but only one (Lin et al., 2013) carried out a test for trend ($p > 0.05$ in all cases). The relationship was apparently monotonic for maternal PFOS and TSH in Berg et al. (2015) (Table 3) and for child PFOS (% change [95% CI]: Q2: 0.8% [−0.3, 1.8%], Q3: 0.9% [−0.2, 1.9%], Q4: 2.3% [1.2, 3.3%]) and PFNA (Q2: 0.8% [−0.3, 1.8%], Q3: 1.7% [0.7, 2.8%], Q4: 2.7% [1.7, 3.8%]) in relation to child TT4 (Lopez-Espinosa et al., 2012b). Finally, some studies reported significant positive associations between PFOS and lnTSH (Berg et al., 2015; Lopez-Espinosa et al., 2012b; Wang et al., 2013), PFOA and TT4 (de Cock et al., 2014), and PFOS or PFNA and TT4 (Lopez-Espinosa et al., 2012b) when comparing the third and/or fourth quartiles with the first one.

Some of the six studies using PFAS categorization considered at least one other approach to assess the dose-response relationship. Lewis et al. (2015) considered a linear relationship between $\log_2$-PFAS and $\log_2$-THs, but also carried out a sensitivity analysis with PFAS in quartiles to explore potential non-linearity of the relationships, and results were similar. In de Cock et al. (2014), none of the contaminants showed a linear relationship with T4, and a PFAS categorization in quartiles was used in the final statistical models. Lastly, Lopez-Espinosa et al. (2012b) conducted regression models between lnPFAS or quartiles of PFAS and lnTSH or TT4.

4. Discussion

The literature ($n = 77$ articles retrieved) showed growing interest in the role played by exposure to PFAS in thyroid outcomes. However, we found very few relevant papers ($n = 10$) on this specific subject when it comes to pregnant women, newborns, and children up to 19 years old. In sum, there were insufficient numbers of studies in each population group to make comparisons except in two cases: mothers ($n = 4$) and 11–19-year-old children ($n = 3$). In both cases, no consistent associations between four PFAS and THs or thyroid dysfunctions were found except for TSH levels. There was some evidence of a positive association between PFHxS and PFOS exposure and levels of TSH measured in the blood of mothers, as well as PFNA and TSH levels measured in the blood of teenage boys. Differences in the expression of the results and/or effect estimates, as well as the treatment of the outcome and exposure variables (*e.g.*, log transforming or not of data, continuous or categorical PFAS, *etc.*), prevented us from combining effect estimates in a meta-analysis. Therefore, due to the small number of studies with comparable data, further studies are warranted to confirm the possible relationships outlined above.

In order to draw our conclusion, we have assessed the evidence of a possible association between PFAS and thyroid function impairment by assessing the exposure and outcomes, and, by using the Bradford-Hill Criteria of consistency and coherence, strength of the association, temporality, biological gradient, and biological plausibility (Hill, 1965). The specific elements of the analysis for drawing our conclusion are explained in the following subsections.

4.1. PFAS assessment

There are no differences in the method of chemical analysis employed to determine PFAS (LC/MS in all studies) and studies did not differ markedly in the LOD or LOQ in spite of being measured in different laboratories worldwide. However, there are differences in how concentrations below these limits were handled: replacement by LOD or LOQ/$\sqrt{2}$, LOD/2, or imputation based on expected data. Nevertheless, the percentages of samples <LOD or LOQ were small in most of the studies, except for PFNA in some cases.

The articles also differ in the blood compartments used to determine PFAS concentrations. Across-compartment comparisons have previously been assessed in two studies, showing a 1:1 PFAS concentration ratio between plasma and serum in pregnant women (Manzano-Salgado et al., 2015) and workers occupationally exposed to PFAS (Ehresman et al., 2007). Therefore, determination in either compartment seems to be a good proxy with which to estimate PFAS body burden, and these differences might not influence the comparability of the exposure results.

4.2. Hormone assessment

The studies do differ in the THs measured to assess effects, since, except for TSH, which was measured in all but one article, the rest of the hormones were determined in a lower number of studies. Therefore, not all studies had information available on free THs, which reflect the levels of biologically active hormones that are available to the tissues and might have yielded more comprehensive information concerning the thyroid regulatory system.

Differences in the methods used to analyze THs might also be important. Studies used different types of immunoassay methods for hormone determination. However, some animal studies have criticized the use of these techniques for the assessment of FT4. These researchers hypothesized that the reduction in FT4 in the presence of PFOS could have been due to negative bias in analog techniques, resulting from competitive displacement of FT4 and the labeled FT4 analog from serum and assay binding proteins in the presence of this contaminant (Chang et al., 2007; Luebker et al., 2005). This concern prompted a study of potential bias from the presence of PFAS in a human population with typical U.S. serum PFOS concentrations but higher PFOA concentrations due to their proximity to a Teflon factory (Lopez-Espinosa et al., 2012a). Such bias from the use of an analog with respect to dialysis methods in experimental studies (Chang et al., 2007; Luebker et al., 2005) was not observed in this human population (Lopez-Espinosa et al., 2012a). According to the authors, possible differences in the results between animal and human studies could be due to the differences in levels of exposure to PFAS (higher in rats than in humans) and also the inter-species differences in the principal proteins that bind T4 (in rats: albumin and transthyretin [TTR], and in humans: thyroxine-binding globulin [TBG], while albumin and TTR play comparatively less important roles), and their interaction with PFAS (Lopez-Espinosa et al., 2012a).

4.3. Consistency and coherence

Comparisons across studies were hampered by the differences in the lifestages considered: prenatal life, childhood, or both periods. Maternal THs were measured during the second or third trimester of pregnancy and after giving birth, and child THs were determined at birth, during the first days of life, and throughout childhood. Divergences also exist in sampling time of PFAS (maternal, cord, or child samples).

Bearing in mind the sample size and the direction, magnitude, and significance of the associations, there is some consistency of a positive association between maternal exposure to some PFAS and maternal TSH levels. The evidence seems to be stronger for PFOS and PFHxS, the direction of the association being positive in all studies except in one case (but the association was close to null). In all cases, PFAS and THs were measured in either the second or third trimester of pregnancy except for Berg et al. (2015), which measured THs during the second trimester and twice after birth. The same group has recently published a

multipollutant approach on exposure to maternal PFAS and other persistent organic pollutants and alterations in TH levels in the same population, but this time relating exposure to these contaminants with hormones measured in samples collected in the second trimester of pregnancy (Berg et al., 2016, not included in this review since the date of publication is later than December 2015) and results are coherent with those reported in the first article (Berg et al., 2015).

All the cross-sectional studies on infancy examined children in similar age ranges, between 11 and 19 years old, thus enabling us to compare them. Consistency in direction (positive in the three studies) and statistically significant associations (in two cases) were found for PFNA and TSH in boys aged ≥11 years. In girls, there was little evidence of any association.

Concerning longitudinal studies on prenatal PFAS exposure and cord or child THs levels, there were few articles with comparable data, and their results showed little or no evidence of an effect.

Some studies also addressed the question of whether there was any health effect associated with hormonal changes, which is essential for understanding the clinical implication of the observed results. For that reason, several thyroid diseases were investigated. The evidence of an association is not clear, due to the few studies focused on thyroid dysfunctions, the small number of cases with these types of dysfunctions, the discrepancy in the outcomes studied, differences in lifestages studied, and the few studies with statistically significant associations. However, coherence was found in a study which reported an increased proportion of mothers with subclinical hypothyroidism for each PFOS quartile and an inverse relationship between PFOS and TSH (Berg et al., 2015). In addition, higher PFAS concentrations were found in pregnant women with additional thyroid stressors (high TPOAb) (Webster et al., 2014). These findings are coherent with results showing an association with some PFAS found in U.S. adults with low iodine and high TPOAb, both of which are stressors to the thyroid system (Webster et al., 2015). During childhood, although higher odds of self-reported thyroid disease, mostly hypothyroidism, associated to increased PFOA concentrations were reported in one study, these results were not consistent with an association between PFOA and TH levels for all children combined or with the associations for the categories of sub-clinical hypo/hyperthyroidism which were created using hormonal levels at the time of the survey (Lopez-Espinosa et al., 2012b).

4.4. Strength of the association

Several aspects make it difficult to assess the strength of the association across studies. There is a substantial variation among the studies in the estimates of the association (regression coefficients, % change, estimated mean differences, Pearson correlation coefficient, and p for trend). Although PFAS concentrations were measured in the same units (ng/mL), contaminants were not treated in the same way (continuous or categorical) in the statistical analyses, which also hampers comparison among studies.

Another important issue when discussing the strength of association is the control for confounding variables, and there was heterogeneity across studies in this respect. Several variables known to influence thyroid status and PFAS, such as BMI, are not addressed in all the studies. Some studies adjusted models for this variable (Berg et al., 2015; de Cock et al., 2014; Kim et al., 2011; Lewis et al., 2015), others checked whether it was a possible confounder but finally it was not included (Lopez-Espinosa et al., 2012b; Wang et al., 2013, 2014) and the rest did not include it in the statistical analysis. The adjustment of models for BMI is under debate, since BMI might be causally "downstream" of both exposure (PFAS) and outcome (THs) variables (Webster et al., 2014). Most studies did not measure other important biomarkers which might affect TH levels, such as iodine status or thyroid antibodies. Some studies (Lopez-Espinosa et al., 2012b; Wang et al., 2013, 2014; Webster et al., 2014) excluded people with thyroid diseases or thyroid treatments, as they receive medication prescribed to adjust hormones to normal levels. However, some studies did not make such exclusions and the medications could thus obscure the association, if present, for those individuals.

Study populations are likely to be exposed to multiple chemical contaminants at the same time and, therefore, multipollutant analyses including other PFAS were conducted in some of the studies reviewed (Berg et al., 2015; Chan et al., 2011; Lin et al., 2013; Lopez-Espinosa et al., 2012b). The magnitude of the associations was similar across studies except for Berg et al. (2015), where the associations between PFHxS or PFOA and TSH were no longer significant after including PFOS, but results were not reported in the article and have not been discussed in this review. While the combined thyroid effects of chemical mixtures are certainly possible, since other chemical substances with endocrine-disrupting properties and with similar sources of exposure such as diet have been associated with alterations of TH levels in some previous studies (Boas et al., 2012), it is important to mention the low correlation between human serum levels of PFAS and other chemicals measured in blood or urine, such as polybrominated diphenyl ethers, organochlorine compounds, polychlorinated biphenyls, bisphenols, and phtalates (Fisher et al., 2016; Robinson et al., 2015). This low correlation gives some reassurance that PFAS, and not other TH disruptors, are likely to be driving the associations seen in these studies.

Regardless of all the above limitations, significant changes in TH levels due to PFAS exposure seem to be, in general, small (except a β of 38.6 nmol/L in female newborn TT4 when comparing the highest and the lowest cord PFOA quartiles in de Cock et al. (2014)). Nevertheless, it is important to take into account that associations of a small magnitude can also be important in critical windows of exposure, such as gestation or childhood, when even small shifts in THs could have irreversible consequences in brain development (de Escobar et al., 2004).

4.5. Temporality

Studies varied in the epidemiological design: cross-sectional, case-control, or cohort. Cross-sectional studies are unable to establish a temporal sequence due to the simultaneous measurement of exposure and outcome. For example, there is some literature showing that THs can affect kidney function (Chonchol et al., 2008), and excretion rates and serum PFAS levels can depend on kidney function (Watkins et al., 2013). Therefore, this factor associated with THs could be affecting the cross-sectional relationship. In addition, effects may occur some time after exposure and thus they could not be observed at the time of the cross-sectional study.

Most of the longitudinal studies reported a single exposure measure. When using biomarkers, reliance on an exposure measurement at only one time-point is not ideal. However, PFAS have a long half-life (Bartell et al., 2010; Olsen et al., 2007), and published intraclass coefficients between the first and third trimester measurements are quite high (0.64–0.83) (Fisher et al., 2016). Thus, these measurements can be considered as being reasonably representative of serum levels during pregnancy, even if they do not allow the characterization of cumulative exposure, exposure trajectory over time, or windows of susceptibility to the contaminants. When information was provided, maternal serum concentrations were slightly higher than cord serum (Kim et al., 2011).

Concerning THs, it is preferable to compare effects across studies at the same lifestage, since TH levels change quickly during the different trimesters of pregnancy (Kapelari et al., 2008) and infancy (Hume et al., 2004), and, therefore, differences across lifestages are to be expected.

4.6. Biological gradient (dose-response relationship)

The shape of a possible dose-response relationship is not yet known. Some studies have fitted untransformed and others log-transformed data for both PFAS and THs. For PFHxS and PFNA, the range of exposures

is quite narrow and pretty similar among all studies reporting serum levels. Therefore, they are uninformative for exploring the shape of any relationship over a wider range. For PFOA and PFOS, the range of the median values is wider, although the data we have are too sparse to reach conclusions about the overall shape of the relationship, since the assessment of whether the relationship varied by exposure range would require more studies within each study type (maternal, cord, and child) and the results with stronger associations are not consistently in the high or low serum level studies.

4.7. Biological plausibility

Interactions between the hypothalamic–pituitary–thyroid axis can be inhibited or stimulated by natural physiological responses or by exposure to chemical pollutants with endocrine disrupting properties, such as PFAS (Jensen and Leffers, 2008). Although further investigation is warranted, it has been proposed that PFAS may interfere with thyroid homeostasis through various mechanisms, including regulation of hepatic glucuronidation enzymes and deiodinases in the thyroid gland, as reported in studies of exposed rat tissues (Yu et al., 2009), by competition with T4 for binding to protein TTR as seen in studies of exposed rat tissues (Weiss et al., 2009), by altering the expression of genes involved in TH signaling, as reported in salmon embryos and larvae (Spachmo and Arukwe, 2012), or by altering the function of nuclear hormone receptors, as reported in zebrafish embryos (Du et al., 2013).

Leaving aside the inter-species diversity due to differences in modes of action and the generally high exposure in the experimental studies, some animal evidence on the interference of these substances with the thyroid system exists. For example, decreased T3 and T4 levels after short-term or long-term PFOS/PFOA exposure were found in animal studies (Boas et al., 2012). Experimental studies on PFHxS and PFNA exposure are scarcer, although both altered TH function in *in vitro* tests (Long et al., 2013), PFHxS reduced plasma TH levels in a concentration-dependent manner in an *in ovo* study (Cassone et al., 2012), and long-term PFNA exposure raised T3 levels in zebrafish (Liu et al., 2011).

According to the existing scientific understanding of the functioning of the hypothalamic–pituitary–thyroid axis, TSH levels should be inversely proportional to T4 and T3 levels at the same lifestage. TSH regulates the synthesis and secretion of THs by the thyroid gland. In turn, THs negatively influence TSH secretion from the anterior pituitary gland through a negative feedback loop (Dietrich et al., 2012). However, this relationship between these hormone levels was not observed consistently in the epidemiological studies reviewed, since an increase in TSH was not always associated with a reduction in T4 and/or T3 levels or *vice versa*, when data were available.

5. Conclusion

In conclusion, heterogeneity was found across studies in terms of study design, study setting, timing of PFAS exposure assessment, timing and type of thyroid-related outcome assessment, adjustment for potential confounders, and statistical approach. As a consequence, there were insufficient numbers of comparable studies in each population group except for two cases: mothers and 11–19-year-old children. Based on the current literature, we found some consistency of a positive association between PFHxS and PFOS in relation to TSH levels measured in maternal blood and PFNA and TSH levels measured in the blood of boys aged ≥11 years. However, further studies are warranted to confirm these possible relationships. Future studies should measure FT4 as well as TSH in order to yield more comprehensive information concerning any effects on the functioning of the hypothalamic-pituitary-thyroid axis. They should preferably be longitudinal, and should include, if possible, repeated measures of PFAS and thyroid outcomes in order to identify any periods of extra vulnerability.

Declaration of interests

There is no conflict of interest in this review. The authors' affiliation is as shown on the cover page. The authors have sole responsibility for the writing and content of the paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.envint.2016.10.015.

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