Environmental Pollution 238 (2018) 389–396

Contents lists available at ScienceDirect

Environmental Pollution

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

Association between organohalogenated pollutants in cord blood and thyroid function in newborns and mothers from Belgian population☆

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ARTICLE INFO

Article history:
Received 18 September 2017
Received in revised form
15 March 2018
Accepted 16 March 2018

Keywords:
Newborns
Perfluorinated compounds
Organochlorine pesticides
Polychlorinated biphenyls
Thyroid

ABSTRACT

The last decades have seen the increasing prevalence of thyroid disorders. These augmentations could be the consequence of the increasing contamination of the environment by chemicals that may disrupt the thyroid function. Indeed, *in vitro* studies have shown that many chemicals contaminating our environment and highlighted in human serum, are able to interfere with the thyroid function. Given the crucial importance of thyroid hormones on neurodevelopment in fetus and newborns, the influence of these pollutants on newborn thyroid homeostasis is a major health concern. Unfortunately, the overall evidence for a deleterious influence of environmental pollutants on thyroid remains poorly studied. Therefore, we assessed the contamination by polychlorinated biphenyls (PCBs), organochlorine pesticides and perfluorinated compounds (PFC) in 221 cord blood samples collected in Belgium between 2013 and 2016. Our results showed that compared to previous studies performed on newborns recruited in Belgium during the two last decades, the present pollutant contamination is declining. Multivariate statistical analyses pointed out a decrease of thyroid stimulating hormone (TSH) level in male newborns with detectable level of 4,4′- dichlorodiphenyldichloroethylene (4,4′-DDE) in comparison with those with no detectable level ($p = 0.025$). We also highlighted a negative association between perfluorononanoic acid (PFNA) concentration and TSH in male newborns ($p = 0.018$). Logistic regression showed increased odds ratio for presentation of hypothyroid in mother for each one unit augmentation of log natural concentration of PFOA (OR = 2.30, [1.18–4.5]) and PFOS (OR = 2.03 [1.08–3.83]). Our findings showed that the residual contamination by PFCs and organochlorine pollutants in cord blood are correlated with thyroid hormone in the newborns and the risk of hypothyroid in mothers.

1. Introduction

Since decades, the volume of materials produced by chemical industry grows exponentially. A significant part of these chemicals reaches the environment and contaminates the living organisms. In parallel of this growing pollution, the incidence of several endocrine-related diseases is also increasing. Among them, thyroid disorders are no exception since the incidence of such diseases has been observed to increase while the mean age at presentation of Hashimoto's thyroiditis is declining (Barry et al., 2016; Benvenga et al., 2015; Leese et al., 2008). This interesting parallel have led many scientists to investigate the potential links between endocrine-related disorders and environmental pollution.

In laboratories, many studies demonstrated that several pollutants so called endocrine disruptors (ED) are able to interfere with the thyroid system and to alter the levels of thyroid hormones

Abbreviations: ED, endocrine disruptor; TH, thyroid hormone; T_4, thyroxine; T_3, triiodothyronine; PCB, polychlorinated biphenyl; TTR, transthyretin; TSH, thyroid stimulating hormone; HCB, hexachlorobenzene; DDT, dichlorodiphenyltrichloroethane; PFC, perfluorinated compound; PFOS, perfluorooctane sulfonate; BMI, body mass index; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid; PFUdA, perfluoroundecanoic acid; PFHpA, perfluoroheptanoic acid; ESI, electronic negative ionization; RT, retention time; CV, cone voltage; CE, collision energy; β-HCH, β-hexachlorohexane; 4,4′-DDE, 4,4′-dichlorodiphenyldichloroethylene; DF, detection frequency.

☆ This paper has been recommended for acceptance by David Carpenter.

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https://doi.org/10.1016/j.envpol.2018.03.058
0269-7491/

(THs) (Boas et al., 2012). Because of their structural resemblance to thyroxine (T_4) and triiodothyronine (T_3), interactions between polychlorinated biphenyls (PCBs) and the thyroid system have been investigated for many years: *in vitro* studies have shown that PCBs are able to bind to transthyretin (TTR), a protein responsible of THs transport in the blood (Chauhan et al., 2000; Marchesini et al., 2008). Fritsche et al. demonstrated that PCB 118 mimics the T_3 action and stimulates the neural differentiation via the TH pathway (Fritsche et al., 2005). In orally exposed rats, PCBs were observed to decrease T_4 but didn't induce any change in the thyroid stimulating hormone (TSH) level (Hallgren et al., 2001). Organochlorine pesticides were also examined concerning their potential thyroid-disrupting effects: studies led on laboratory animals have shown that hexachlorobenzene (HCB) would induce decrease of the circulating level of T_4 (Alvarez et al., 2005) while a reduction of levels of T_4, T_3 and TSH was observed after a chronic exposition to dichlorodiphenyltrichloroethane (DDT) (Liu et al., 2015). More recent pollutants such like perfluorinated compounds (PFCs) have also demonstrated their abilities to interfere with the thyroid system. For instance, Weiss et al. showed that, similarly to PCBs, PFCs are able to bind on TTR (Weiss et al., 2009). They also seemed to interfere with the TH metabolism since hepatic glucuronidation enzymes mRNA and deiodinases mRNA in the thyroid (two enzymes responsible of the metabolization of THs) were demonstrated to be up-regulated by a long term exposure to perfluorooctane sulfonate (PFOS) in rat (Yu et al., 2009). This alteration of the metabolism was accompanied by a reduction of the T_4 levels in the exposed animals (Yu et al., 2009).

During pregnancy and early childhood, the THs play a critical role in the development of the fetus and the newborn, especially for the neurodevelopment (Evans et al., 1999; Howdeshell, 2002). Thereby, thyroid deficiency during this critical period may irreversibly impair the neurodevelopment in children. Therefore, the potential interferences of some pollutants on the thyroid system, especially during pregnancy and early childhood should be extensively investigated. Unfortunately, the current number of epidemiologic studies assessing the association between pollutant contamination and the thyroid system homeostasis in human fetus and newborns remains insufficient and the results of these studies are not consistent (El Majidi et al., 2014).

In Belgium, although declining due to their ban, the contamination of newborns by PCBs, organochlorine pesticides and PFCs remains significant (Schoeters et al., 2017) and the influence of these residual contaminations on the newborn thyroid homeostasis is an important concern. Thereby, the objectives of this study were to assess the exposure levels of some PCBs, some organochlorine pesticides and PFCs of Belgian newborns through their measurement in cord blood on one hand and on the other hand, to investigate potential link between the ED's contaminations and thyroid disorders in these newborns and their mothers.

2. Material and methods

2.1. Study participants

Between August 2013 and March 2016, women presenting for delivery at the obstetric service of the University Hospital of Liege (Belgium) were asked to participate to a study on neonatal asphyxia biomarkers as first intention and thyroid problems as second intention. A total of 281 participants gave written informed consent, and umbilical cord blood samples were collected, centrifugated and stored at −80 °C immediately after delivery. The present population (n = 214) was selected from this cohort. The exclusion criteria were: insufficient serum volume available (<0.5 mL), absence of TSH level record (measured during the neonatal screening performed 3 days after the birth), and congenital hypothyroidism diagnosed for the newborn (TSH >20 mUI/L).

The maternal information and newborn's characteristics were collected through the medical records and included newborn's weight, gestational age, age of the mother at delivery, parity, pre-pregnancy body mass index (BMI) of the mother, tobacco habits and hypothyroidism (according to levothyroxine treatment reported in the medical records during pregnancy). The demographic characteristics are gathered in Table 1.

2.2. Chemicals and reagents

PFOS, perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA) and perfluoroheptanoic acid (PFHpA) standards were purchased from Wellington Laboratories, Inc (Ontario, Canada). Labeled PFCs ($^{13}C_4$-PFOS, $^{13}C_4$-PFOA, ^{18}O-PFHxS, $^{13}C_5$-PFNA, $^{13}C_2$-PFDA, $^{13}C_2$-PFUdA and $^{13}C_4$-PFHpA) were used as internal standard and were also purchased from Wellington Laboratories. Oasis WAX SPE cartridges (3 cc, 60 mg, 30 μm) were purchased from Waters (Milford, MA, USA). Water (LC-MS grade), acetonitrile (LC-MS grade) and methanol (HPLC grade) were purchased from J. T. Baker (Deventer, The Netherlands). Formic acid (98–100%, analytical grade) ammoniac (25%, analytical grade) and acetate ammonium (analytical grade) were bought from Merck (Darmstadt, Germany). Fetal bovine serum was purchased from Sigma-Aldrich Co (S^t Louis, MO, USA).

2.3. Analyses

The serum specimens were analyzed for the determination of 7 PFCs: PFOS, PFOA, PFHxS, PFNA, PFDA, PFHpA and PFUdA. The analytical procedure used was based on the method previously described by Kärrman et al. (2007). Twenty microliters of an internal standard solution at 1 μg/mL and 2 mL of formic acid/water mixture (1:1) were added to 1 mL of serum sample, sonicated for 15 min and then centrifugated at 3000 rpm for 5 min. Then the sample was loaded on the Oasis WAX column previously washed and conditioned with 2 mL of methanol followed by 2 mL of water. The cartridge was then washed with 1 mL formic acid 2% in water followed by 1.5 mL of methanol. Finally, PFCs were eluted with 2 × 2 mL of ammonium hydroxide 2% in methanol. The sample was further evaporated until dryness under a gentle stream of nitrogen at 30 °C and residue was reconstituted in 80 μL of a mixture 2 mM ammonium acetate in acetonitrile/2 mM ammonium acetate in

Table 1
Demographic characteristics of the sample subjects.

	No	Mean	Range	SD	%
Pre-pregnancy body mass index		24,2	[16,4–48,9]	5,0	
Parity		0,9	[0,0–6,0]	1,1	
Primipare	98				46,0%
Multipare	115				54,0%
Age, years		29,2	[18,0–42,0]	4,9	
Gestational age, days		277	[242–293]	8	
Birth weight, g		3307	[2225–5015]	438	
Newborn gender					
Male	113				52,8%
Female	101				47,2%
Mother hypothyroid					
Yes	37				17,3%
No	177				82,7%
Smoking status					
Smoker	40				18,7%
Non-smoker	174				81,3%

water (2:8) before the transfer to an injection vial. The chromatographical separation was performed using an Acquity Ultra Performance UHPLC system (Waters, Milford, MA, USA) equipped with a Kinetex F5 Core-Shell (2.1 × 100 mm, 1.7 μm) from Phenomenex (Torrance, CA, USA) maintained at 40 °C, the mobile phases consisted of 2 mM ammonium acetate in water (A) and 2 mM ammonium acetate in acetonitrile (B). The injection volume was 10 μL and a constant flow of 0.4 mL/min was applied using the following gradient: the initial condition of 78% A was maintained during 0.5 min, linearly decreased to 60% A in 10.5 min, then, linearly decreased to 0% in 0.2 min and held for 1.5 min. Finally, the gradient returned to the initial condition (78% A) in 0.3 min and held for 3 min. A Quattro Premier XE mass spectrometer (Waters) was used for the identification and quantification of the analytes. The mass spectrometer operated in negative electrospray ionization (ESI) at 1 kV, the source and desolvatation temperatures were set at 140 °C and 400 °C, respectively. Nitrogen was used as cone and desolvatation gas at a flow of 50 L/h and 800 L/h, respectively. Argon was used as collision gas at 0.1 L/min. Retention time (RT), cone voltage (CV), MRM transitions, collision energy (CE), dwell times for each PFC were reported in Table 2.

Four organochlorine pesticides, namely β-hexachlorohexane (β-HCH), HCB, *trans*-nonachlor and 4,4′-dichlorodiphenyldichloroethylene (4,4′-DDE) and 4 PCBs (−118, −138, −153, −180) were determined using the method already described elsewhere (Pirard et al., 2018). Briefly, the serum samples were liquid-liquid extracted using a hexane-acetone mixture, purified on Bond Elut Certify cartridges and evaporated to 500 μL before being transferred to GC vials containing nonane as keeper and let slowly evaporated at room temperature. The extracts were then analyzed by GC-MS operating in electron negative capture ionization (ENCI), using a 30 m SGE HT8 column. All GC-MS parameters were previously detailed (Pirard et al., 2018).

TSH level in dry blood spot collected 3 days after the delivery were determined by enzyme-linked immunosorbent assay using a commercial kit Zentech (Liège, Belgium).

2.4. Quality assurance

The analytical methods were validated according the total error approach in order to meet the ISO17025 and the guidelines of the French Society of Pharmaceutical Science and Technique (Hubert et al., 2007a,b, 2004) The limit of quantification (LOQ) was determined during the validation process and was defined as the smallest concentration measured with a maximal incertitude not exceeding 40%. The LOQ for the different compounds are reported in Table 3.

Table 2
Retention times (RT), MRM transitions (quantifier in bold), cone voltages (CV), collision energy (CE) and dwell times for PFC analysis.

Compound	RT (min)	MRM (*m/z*)	CV (V)	CE (eV)	Dwell time (s)
PFHpA	3,06	**362,8 → 318,9**	10	10	0,2
		362,8 → 168,9	10	15	0,2
PFHxS	4,77	**398,7 → 80**	45	35	0,1
		398,7 → 99	45	30	0,1
PFOA	5,05	**412,7 → 368,9**	14	10	0,1
		412,7 → 168,9	14	20	0,1
PFNA	7,1	**462,7 → 418,9**	10	10	0,2
		462,7 → 219	10	15	0,2
PFOS	8,71	**498,7 → 80**	50	50	0,04
		498,7 → 99	50	35	0,04
PFDA	9,03	**512,7 → 468,8**	13	10	0,1
		512,7 → 219	13	20	0,1
PFUdA	10,89	**562,7 → 518,8**	15	10	0,1
		562,7 → 268,9	15	20	0,1

Table 3
Pollutant concentrations in cord blood (ng/mL).

	%>LOQ	LOQ	Mean	SD	Median	Max
Hexachlorobenzene	0,0%	0,05	<LOQ		<LOQ	<LOQ
β-Hexachlorohexane	0,5%	0,05	<LOQ		<LOQ	0,061
trans-Nonachlor	0,0%	0,06	<LOQ		<LOQ	<LOQ
4,4′-DDE	24,1%	0,41	<LOQ		<LOQ	1,26
PCB 118	2,6%	0,17	<LOQ		<LOQ	0,21
PCB 138	18,8%	0,15	<LOQ		<LOQ	0,7
PCB 153	43,5%	0,07	<LOQ		<LOQ	0,19
PCB 180	61,8%	0,05	0,051	0,02	0,054	0,18
PFHpA	7,3%	0,05	<LOQ		<LOQ	0,69
PFHxS	54,4%	0,15	0,18	0,13	0,16	0,94
PFOA	94,3%	0,25	0,80	0,52	0,68	3,40
PFNA	75,1%	0,10	0,18	0,12	0,15	0,68
PFOS	71,0%	0,50	0,88	0,79	0,73	9,21
PFDA	8,8%	0,15	<LOQ		<LOQ	0,36
PFUdA	4,1%	0,10	<LOQ		<LOQ	0,39
TSH (mUI/L)			5,41	3,08	4,85	16,6

For PFCs analyses, each sequence included 27 real samples, 8 matrix-matched calibration standards made in fetal bovine serum, 1 reagent blank, 1 matrix blank, 1 home-made QC (serum at 1.5 ng/mL for PFOA and PFOS and 0.3 ng/mL for the other PFCs) and 2 different level QC obtained during the German External Quality Assessment Scheme for analyses in biological materials (G-EQUAS, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine of the University Erlangen-Nuremberg, Erlangen, Germany).

For organochlorine and PCB analysis, each sequence consisted in 30 real samples, 1 matrix blank, 2 home-made QC (serum from anonymous donor spiked at 0.2 and 2 ng/mL) and 2 different QC obtained during the AMAP (Arctic Monitoring and Assessment Program) ring test for persistent organic pollutants in human serum organized by the Institut National de Sante Publique du Quebec (INSPQ, Sainte-Anne-de-Bellevue, Quebec). The quantification was carried out using an 8 point calibration curve.

2.5. Statistical analyses

The statistical analyses were performed using Statistica 13 (Dell Software, France) and Excel 2007 (Microsoft, Redmond, WA).

The multivariate analyses assessing the associations between TSH levels and pollutant concentrations were performed on chemicals showing a detection frequency (DF) above 70%, measurements below the LOQ were replaced by LOQ × DF (Ali et al., 2013; Dirtu et al., 2010). The chemicals presenting DF lower than 70% but above 20% (PFHxS, 4,4′-DDE and PCB 153) were used as dichotomized variable (detected vs. non-detected) for statistical analyses. A natural log transformation was applied to pollutant concentrations because of their skewed distribution. TSH levels presented also a skewed distribution and were thus log transformed following the addition of 1. The covariates considered in the regression models included maternal age (continuously), maternal pre-pregnancy BMI (continuously), parity (continuously), gestational age (in days, continuously), newborn's weight (in grams, continuously), tobacco status during the pregnancy (smoker vs. non-smoker) and hypothyroidism during the pregnancy (yes vs. no). The univariate regressions were performed for each of these covariates and the covariates were included into the final models if they were roughly related to the TSH levels ($p < 0.20$). Parity and gestational age were included into these final models. The analyses were stratified by newborn sex.

Logistic regressions were performed to assess odds ratios (OR) of hypothyroid in the mother by quartile of chemicals

concentration for compounds with DF above 70% or by contamination status (detected vs. non-detected) if DF ranging between 20 and 70%. The logistic regression models were adjusted for age and tobacco use.

Statistical significance was set at $p < 0.05$.

3. Results

The characteristics of the population were gathered in Table 1. Two hundred and fourteen mother-newborn pairs were included into our study. Nearly half of the mothers (46%) were primipare. The mean pre-pregnancy BMI was 24.2 kg/m^2 and BMI values range from 16.4 to 48.9 kg/m^2. The gestational age ranged from 34 weeks to 42 weeks. Males were a little bit more represented than females (52.8% vs. 47.2%) and the birth weight range from 2225 g to 5015 g. Approximately half of the mothers was under 30 years (50.9%) and nearly one fifth smoked during pregnancy (18.7%) or presented hypothyroid (17.3%).

The pollutant concentrations in cord blood were reported in Table 3. HCB and *trans*-nonachlor were never detected while β-HCH and PCB 118 were detected in only 1 (0.5%) and 5 (2.6%) samples respectively. 4,4′-DDE was highlighted in 24.1% of samples with a maximum concentration of 1.26 ng/mL. PCB 180 was the only organochlorine pollutant detected in more than half of the samples with a median concentration of 0.054 ng/mL since PCB 138 and PCB 153 were positively detected in 18.8 and 43.5% of the samples respectively. The PFC contamination seems to be more elevated: PFOA, PFNA and PFOS were detected in more than 70% of the samples (94.3, 75.1 and 71.0% respectively) and the median concentrations measured were 0.68, 0.15 and 0.73 ng/mL respectively. PFHxS were highlighted in 54.4% of the samples with a median concentration of 0.16 ng/mL while PFHpA, PFDA and PFUdA were less frequently detected in cord blood samples (in 7.3, 8.8 and 4.1% respectively).

The results of the multivariate regressions and logistic regressions were presented in Tables 4 and 5 respectively. A significant reduction of the TSH level was observed in boys having detectable level of 4,4′-DDE ($p = 0.025$). In the same way, also for boys only, an increase of the contamination by PFNA was significantly correlated with a decrease of the TSH levels ($p = 0.018$). No statistically significant correlation between pollutant levels and TSH concentrations was highlighted for girls. The logistic regressions showed that the risk to present hypothyroid is significantly increased for mothers in the third quartile of contamination by PFOS and non-significantly in the fourth quartile (OR = 3.22, 95% confident interval (95% CI): [1.08–10.92] and OR = 2.95, 95% CI: [0.98–10.07], respectively) compared to mothers in the first quartiles. The risk is also increased for women in the second and the fourth quartiles of contamination by PFOA (OR = 4.42, 95% CI: [1.23–21.14] and OR = 5.62, 95% CI: [1.64–26.11], respectively) relative to the first quartile.

Table 5
Odds ratio (OR) of logistic regression (adjusted for maternal age and tobacco use) of hypothyroid in mother by pollutant levels in cord blood. Significance level at p < 0,05 indicated in bold.

	OR hypothyroid	% CI
PFNA		
Q1 (<LOQ)	1	
Q2 (0,10–0,15)	1,78	[0,60–5,71]
Q3 (0,15–0,22)	1,86	[0,64–5,95]
Q4 (0,23–0,68)	1,17	[0,37–3,92]
PFOA		
Q1 (<LOQ-0,44)	1	
Q2 (0,44–0,68)	**4,42**	**[1,23–21,14]**
Q3 (0,68–0,97)	3,22	[0,88–15,38]
Q4 (0,98–3,40)	**5,62**	**[1,64–26,11]**
PFOS		
Q1 (<LOQ)	1	
Q2 (0,52–0,71)	1,76	[0,49–6,56]
Q3 (0,73–1,01)	**3,22**	**[1,08–10,92]**
Q4 (1,01–9,21)	2,95	[0,98–10,07]
PFHxS (Detected vs non-detected)	1,92	[0,87–4,25]
4,4′-DDE (Detected vs non-detected)	0,82	[0,32–2,06]
PCB 153 (Detected vs non-detected)	0,76	[0,35–1,66]

4. Discussion

Our results showed that the organochlorine pollutant contamination in newborns is declining since several years in Belgium. Indeed, Covaci et al., 2002 measured median concentrations of 0.49 ng/mL, 0.07 ng/mL and 0.145 ng/mL for 4,4′-DDE, HCB and PCB 153 respectively in cord blood samples collected in 1999 in Flanders (Northern part of Belgium) while the median concentrations measured in our population were below the LOQ for each of these compounds (<0.41 ng/mL for 4,4′-DDE, <0.05 ng/mL for HCB and <0.07 ng/mL for PCB 153). This confirmed the results published by Schoeters et al. (2017) who reported a decline of the contamination by PCBs, HCB and 4,4′-DDE in newborns, but also in adolescents and adults recruited in Flanders between 2002 and 2014. For PFOS, the data published on Belgian newborns similarly suggested a decreasing trend (Fig. 1) from 5.1 ng/mL in cord blood in 2002–2005 (Roosens et al., 2010) to 1.10 ng/mL in 2013–2014 and finally to 0.73 ng/mL in the present study (2013–2016). No clear trend was observed for PFOA (Fig. 1): the median concentration in cord blood in 2002–2005 reported by Roosens et al. (2010) was similar to the median concentration observed in the present study (0.68 ng/mL) while the concentrations reported by Schoeters et al. (2017), in 2007–2008 (geometric mean = 1.51 ng/mL) and 2013–2014 (geometric mean = 1.19 ng/mL) were sensibly higher. There is a few years gap between the phase out of PFOS and the phase out of PFOA (Wang et al., 2014). This gap probably explains the difference between the trends observed for PFOS and PFOA. Moreover, PFOA is thought to be present in more items than PFOS

Table 4
Sex stratified regression coefficients (β) of multivariate regression (adjusted for parity and gestationnal age) of ln(TSH+1) by pollutant levels in cord blood. Significance level at p < 0,05 indicated in bold.

	All		Boys		Girls	
	β	p-value	β	p-value	β	p-value
LnPFOA	−0,094	0196	−0,073	0316	−0,112	0419
LnPFNA	−0,129	0064	**−0,166**	**0018**	−0,074	0564
LnPFOS	−0,028	0679	−0,042	0536	−0,019	0883
PFHxS (Detected vs non-detected)	0,011	0894	−0,057	0529	0,094	0510
4,4′-DDE (Detected vs non-detected)	−0,153	0107	**−0,236**	**0025**	−0,053	0748
PCB 153 (Detected vs non-detected)	0,065	0416	0,079	0378	0,034	0803

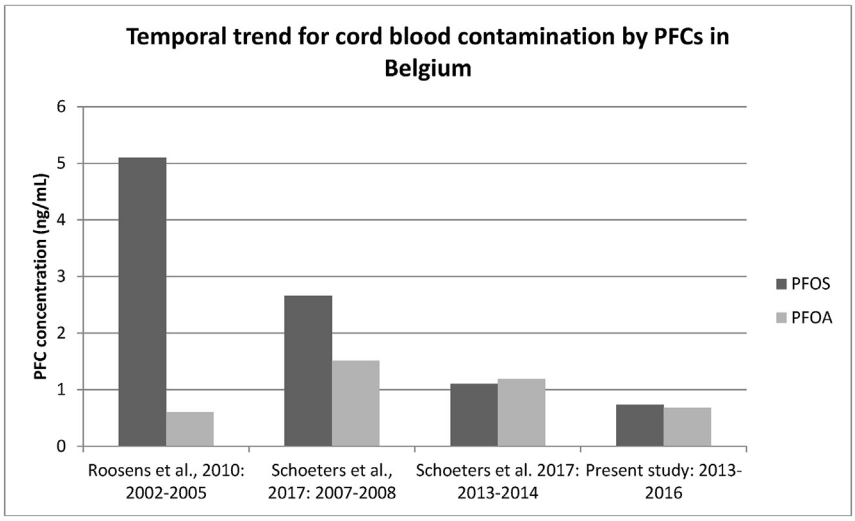

Fig. 1. Temporal trend for cord blood contamination by PFCs in Belgium.

making more difficult and slower the reduction of human exposure. Nevertheless we expect that a diminution of the PFOA contamination will be observed in the coming years.

Despite this declining contamination, a negative association was highlighted between 4,4′-DDE contamination in the cord blood and TSH level measured 3 days after the delivery in male newborns. These findings were not in accordance with others studies (Alvarez-Pedrerol et al., 2008; Darnerud et al., 2010; Lopez-Espinosa et al., 2010; Ribas-Fitó et al., 2003) which didn't observe any association between 4,4′-DDE and TSH levels. However, in these previous studies, the statistical analyses were not stratified by sex, and our model applied on both male and female newborns didn't show any significant correlation and it is well known that thyroid function is influenced by sexual hormones (Tahboub and Arafah, 2009). It is also important to note that the 4,4′-DDE contamination level in our population was much lower than those reported in the previous studies, and several authors suggested that, for endocrine disruptors, the effects observed at low dose could differ from those observed at higher levels (Langer et al., 2007; Vandenberg et al., 2012). Given the very low DF for the other organochlorine pesticides in our population, the statistical analyses could not be performed on these pollutants thereby we could not confirm the results of Alvarez-Pedrerol et al. (2008), Lopez-Espinosa et al., 2010 and Ribas-Fitó et al., 2003 which reported a positive association between TSH level of the newborn and the β-HCH contamination measured in the cord blood.

Using PCB 153 in our analyses as surrogate for the PCB contamination, no association could be highlighted between this class of pollutants and the TSH level, in accordance with the findings of Darnerud et al. (2010), Lopez-Espinosa et al., 2010 and Ribas-Fitó et al., 2003. On the other hand, Alvarez-Pedrerol et al., 2008 showed a positive association between PCB 153 concentration in cord blood and TSH level measured 3 days after delivery. This discrepancy may be explained by the higher PCB 153 level measured by Alvarez-Pedrerol (0.70 ng/mL) compared to present median level (<0.07 ng/mL). Moreover some potential confounding factors may explain discrepancies between studies, for instance, iodine or iron status which were rarely assessed although they are expected to be strong determinants of the thyroid function (Eftekhari et al., 2007). Results of the different studies assessing the association between organochlorine chemicals exposition and TSH level in newborns are summarized in Table 6.

Table 6
Summary of the studies assessing the link between organochlorine chemicals exposition and TSH level in newborns.

Chemicals	Study	Effects on TSH in newborns
4,4′-DDE	Alvarez-Pedrerol et al., 2008	0
	Darnerud et al., 2010	0
	Lopez-Espinosa et al., 2010	0
	Ribas-Fitó et al., 2003	0
	Our study	↓
β-HCH	Alvarez-Pedrerol et al., 2008	↑
	Lopez-Espinosa et al., 2010	↑
	Ribas-Fitó et al., 2003	↑
	Our study	not assessed
PCB 153	Alvarez-Pedrerol et al., 2008	↑
	Darnerud et al., 2010	0
	Lopez-Espinosa et al., 2010	0
	Ribas-Fitó et al., 2003	0
	Our study	0

0, no significant association; ↓ significant negative association; ↑ significant positive association.

The association between organochlorine contamination in cord blood and TH levels in newborn was previously assessed in Belgium by Maervoet et al. (2007) who didn't find any association between organochlorine pollutants and TSH level, however in this study, the TH levels were measured in cord blood. The comparison between these previous results and ours could be inappropriate since the TH concentration measured at birth is greatly fluctuating due to the stress of the delivery, and TH levels determined after a delay of few days may better reflect the basal thyroid function of the newborn.

Different mechanisms may explain the associations observed in the present study between TSH and PFNA levels on one hand, and 4,4′-DDE levels on the other hand. For instance, PFNA can compete with T_4 for the binding to the TTR (Weiss et al., 2009), inducing in the human organism an increase of the free T_4 level in the blood, and by negative feedback, a reduction of the TSH level produced by the pituitary gland. On the other hand, the simultaneous administration of 4,4′-DDE and PCB 153 in rat was demonstrated to disrupt the hypothalamus-pituitary-thyroid axis especially by increasing the expression of thyroid hormone receptor in the hypothalamus. This consequently leads to the reduction of the TSH level produced by the pituitary (Liu et al., 2015). Nevertheless, it is impossible to affirm that these mechanisms observed *in vitro* or in

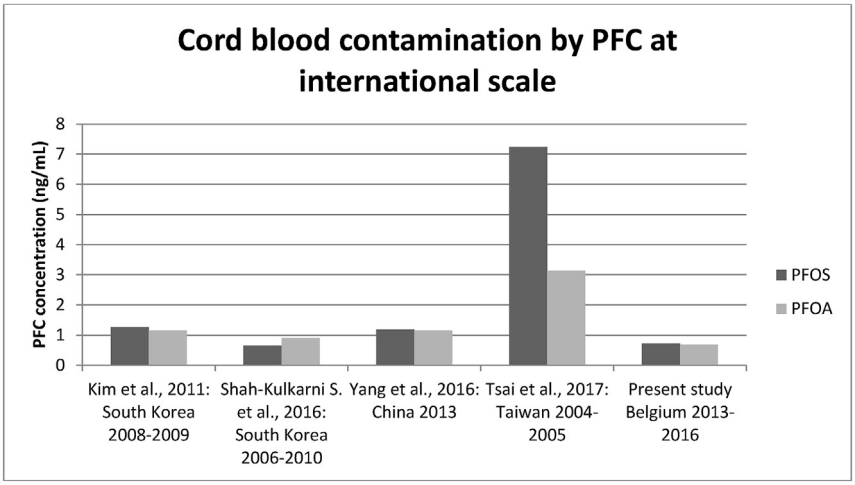

Fig. 2. Cord blood contamination by PFC at international scale.

laboratory animals exposed to relatively high level of pollutants are really effective in humans exposed to environmental contamination.

We also evaluated the influence of PFCs on the thyroid function and to the best of our knowledge, this is the first time that association between PFC levels in cord blood and TSH level measured 3 days after delivery was assessed. Several authors have already investigated the correlation between PFC contamination and TH levels determined in the cord blood with contradictory results (Kim et al., 2011; Shah-Kulkarni et al., 2016; Tsai et al., 2017; Yang et al., 2016). Our results highlighted a statistically significant negative correlation between PFNA level and TSH but only in male newborns. If Shah-Kulkarni et al., 2016 also found a significant negative association between PFNA level and TSH, this was only for female newborns. On the other hand, Kim et al., 2011 and Yang et al., 2016 didn't find any correlation between PFC contamination in cord blood and TSH level while Tsai et al., 2017 found positive correlation with PFOS levels. But once again, the comparison between our results and those of previous studies may be inappropriate since the TSH were not determined in the same conditions. Note that the PFC concentrations measured in these studies are similar to those of the present study except for Tsai et al., 2017, (Fig. 2).

We assessed the relation between the PFC contamination in the cord blood and the risk for the mother to present thyroid disorders. Since the PFC concentration in cord blood and in mother serum is known to be well correlated (Yang et al., 2016), we thus considered that cord blood contamination could be a good approximation to estimate the mother exposure. We pointed out an augmentation of the hypothyroid prevalence correlated with an increasing contamination of the mother by PFOA and PFOS. Note that the hypothyroidism prevalence observed in our population is surprisingly high compared to the general population (Spencer et al., 2015) without reasonable explanation. Therefore the presence of a bias that may interfere with our results could not be excluded. Nevertheless, our results are corroborated by those of Berg et al. (2015) which reported in Norway, a higher proportion of pregnant women classified with subclinical hypothyroid associated with increasing PFOS contamination. They also reported a positive association between PFOA concentration and TSH level but this association disappeared when PFOS were included as covariate in the model. Two studies carried out in USA are also in accordance with our results: Wen et al., 2013 pointed out higher odds ratio of subclinical hypothyroidism with increasing levels of PFOA, PFOS but also PFHxS measured in adult women, while Melzer et al., 2010 highlighted that for PFOA only, the most exposed adult women are more likely to present hypothyroid than women belonging to the first and second quartile, no association were highlighted for PFOS in this study. Contrariwise, a case-control study performed by Chan et al. (2011) on Canadian pregnant women didn't observe any statistically significant difference in the PFC contamination between women presenting hypothyroxinemia and those with normal thyroid function. Given the cross-sectional nature of our investigation, we cannot exclude that inverse causation explains our findings: THs are well known to control metabolization processes in human organism and could thus influence the levels of PFCs instead of the contrary.

Regarding studies performed *in vitro* or on laboratory animals (reviewed in Boas et al., 2012), it is well established that some pollutants are able to disrupt some aspect of the thyroid system. However the pollutant concentrations used in laboratory are high in regards to those measured in the environment and in human serum. Moreover the results provided by human epidemiological studies are less consistent. Thereby, we cannot firmly affirm that the actual environmental contamination by pollutants able to disturb the thyroid function (*in vitro* or in laboratory animals), has a negative influence on the human thyroid health. Well designed epidemiological studies are thus still required to establish (or infirm) undoubtedly the link between environmental pollution and the rise of thyroid disorders prevalence in human.

5. Conclusion

Since two decades, the contamination of newborns by organochlorine pollutants and PFCs are declining in Belgium. However, the consequences of the actual residual contamination on newborn health are still a major concern since we highlighted correlations between some pollutants and the TSH levels in newborn or the risk for the mother to present a thyroid disorder. THs are crucial during pregnancy and early childhood for the development (especially the neurodevelopment) of newborns and our results pointed out the importance to achieve extensive research regarding the pollutant influence on thyroid homeostasis in newborns.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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