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Exposure to a mixture of non-persistent environmental chemicals and neonatal thyroid function in a cohort with improved exposure assessment

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ABSTRACT

Background: In vitro and toxicological studies have shown that non-persistent environmental chemicals can perturb thyroid hormone homeostasis. Epidemiological studies with improved exposure assessment (i.e., repeated urine samples) are needed to evaluate effects of these compounds, individually or as a mixture, in humans. We studied the associations between prenatal exposure to non-persistent environmental chemicals and neonatal thyroid hormones.

Methods: The study population consisted of 442 mother–child pairs from the French SEPAGES mother–child cohort recruited between July 2014 and July 2017. For each participant, four parabens, five bisphenols, triclosan, triclocarban, benzophenone-3 as well as metabolites of phthalates and of di(isononyl)cyclohexane-1,2-dicarboxylate were assessed in two pools of repeated urine samples (median: 21 spot urines per pool), collected in the 2nd and 3rd trimesters of pregnancy, respectively. Thyroid stimulating hormone (TSH) and total thyroxine (T4) levels were determined in newborns from a heel-prick blood spot. Maternal iodine and selenium were assessed in urine and serum, respectively. Adjusted linear regression (uni-pollutant model) and Bayesian Kernel Machine Regression (BKMR, mixture model) were applied to study overall and sex-stratified associations between chemicals and hormone concentrations.

Results: Interaction with child sex was detected for several compounds. Triclosan, three parabens, and one phthalate metabolite (OH-MPHP) were negatively associated with T4 among girls in the uni-pollutant model. BKMR also suggested a negative association between the mixture and T4 in girls, whereas in boys the association was positive. The mixture was not linked to TSH levels, and for this hormone the uni-pollutant model revealed associations with only a few compounds.

Conclusion: Our study, based on repeated urine samples to assess exposure, showed that prenatal exposure to some phenols and phthalates disturb thyroid hormone homeostasis at birth. Furthermore, both uni-pollutant and

Abbreviations: BKMR, Bayesian Kernel Machine Regression; cx-MiNP, mono-4-methyl-7-carboxyoctyl phthalate; DEHP, Di(2-ethylhexyl) phthalate; DEP, Diethyl phthalate; DiNP, Diisononyl phthalate; DINCH, Di(isononyl)cyclohexane-1,2-dicarboxylate; LOD, limit of detection; LOQ, limit of quantification; MBzP, monobenzyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP, mono-2-ethyl-5-oxohexyl phthalate; MEP, monoethyl phthalate; MiBP, mono-*iso*-butyl phthalate; MMCHP, mono-2-carboxymethyl hexyl phthalate; MnBP, mono-n-butyl phthalate; Oh-MiNP, Mono-4-methyl-7-hydroxyoctyl phthalate; Oh-MINCH, 2-(((hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid; OH-MPHP, mono-6-hydroxy-propylheptyl phthalate; oxo-MINCH, 2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid; oxo-MiNP, mono-4-methyl-7-oxooctyl phthalate; ΣDEHP, molar sum of the five DEHP metabolites; ΣDiNP, molar sum of the three DiNP metabolites; ΣDINCH, molar sum of the two DINCH metabolites; TSH, thyroid stimulating hormone; T3, triiodothyronine; T4, thyroxine; WHO, World Health Organization.

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mixture models, suggested effect modification by child sex, while, to date underlying mechanisms for such sex-differences are not well understood.

1. Introduction

Congenital hypothyroidism can lead to mental and growth retardation if left untreated. Symptoms are usually only detectable several weeks after birth, when brain damage has already occurred (Hijman et al., 2020). Consequently, many countries now include the assessment of either thyroid stimulating hormone (TSH) or thyroxine (T4) in their routine neonatal screens. Several factors can affect neonatal thyroid hormone levels including maternal age, parity, maternal health (diabetes, hypertension, thyroid condition), sex and age of the child at blood collection, as well as mode of delivery (Hijman et al., 2020; Herbstman et al., 2008). In addition, thyroid hormone homeostasis may be disrupted by exposure to environmental contaminants. Among potential contaminants to which the general population is exposed, two chemical families are of interest: synthetic phenols and phthalates. Chemicals from these classes have been shown to inhibit the sodium–iodide symporter (NIS) (triclosan, bisphenol A, some phthalates) and the enzyme thyroperoxidase (TPO) (triclosan, bisphenol A), both of which are involved in thyroid hormone synthesis (Nakiwala et al., 2022; US EPA). Some phenols and phthalates also bind to thyroid hormone receptors (triclosan, bisphenol A, dibutyl phthalate) (Nakiwala et al., 2022; US EPA). Epidemiological studies examining the associations between prenatal exposure to such chemicals and neonatal thyroid hormone levels have assessed exposure biomarkers in cord blood (de Cock et al., 2014; Minatoya et al., 2017) or in a limited number ($\leq$3) of spot urine samples collected during pregnancy. The short half-life of phenols and phthalates (<24 h) means that these samples are unlikely to represent overall exposure during pregnancy. The resulting error in exposure measurement leads to results that are biased toward lack of effect, and weakens the statistical power (Perrier et al., 2016). Although phenols and phthalates could disrupt thyroid hormone homeostasis through shared mechanisms, most previous studies explored the effects of each chemical individually without considering mixture effect.

Here, we used both uni-pollutant and mixture models to investigate the associations between prenatal exposure to phenols and phthalates, and thyroid hormone concentrations in newborn blood spots. We relied on two pools of up to 21 urine samples per participant to assess prenatal exposure.

2. Methods

2.1. Study population

The SEPAGES (*Suivi de l'Exposition à la Pollution Atmosphérique durant la Grossesse et Effet sur la Santé*) cohort (Lyon-Caen et al., 2019) recruited 484 pregnant women from obstetrical ultrasonography practices located in Grenoble area (France) between July 2014 and 2017. Inclusion criteria were being pregnant by less than 19 gestational weeks, older than 18 years old, having a singleton pregnancy, planning to give birth in one of the four maternity clinics from Grenoble and living in the study area.

Ethical authorizations were obtained by the relevant ethical committees (CPP: Comité de Protection des Personnes Sud – Est V; CNIL: Comité Nationale de l'Informatique et des Libertés). Included women gave written consent for themselves and their children.

This analysis was restricted to the 442 mother–child pairs with a measurement of 1) neonatal TSH or T4, 2) phenols and phthalate metabolites during pregnancy and 3) with mothers who did not report taking medication for thyroid problems (Supplemental Material, Figure S1).

2.2. Collection of urine samples

Non fasting urine samples were collected at two periods of the pregnancy (gestational age (median) at the first and second urine collection weeks were 18 and 34 gestational weeks, respectively). At each period, women were asked to collect 3 spot urine samples per day (morning, mid-day and evening) over seven consecutive days. Samples were collected in polypropylene tubes and stored in the participant's freezer at −20 °C. A fieldworker picked up the tubes at the end of the collection week and brought them to the biobank. Samples were thawed over night at 4 °C, and for each women a pool of the same volume of all the spots collected over a measurement week were made. Pools were then aliquoted and stored at −80 °C. A pool of the three urines collected the last day of the first collection week was also made (daily pool, median (5th, 95th percentiles) number of samples included in the pool = 3 (2, 3)).

An aliquot of each pool was sent to the Norwegian Institute of Public Health (NIPH), that assessed phenol and phthalate metabolite concentrations (Sabaredzovic et al., 2015; Sakhi et al., 2018). A list of the chemicals assessed is available in Table 1. Phenols were analyzed using on-line column switching ultra-high performance liquid chromatography and tandem mass spectrometry (UPLC-MS-MS) as published earlier (Sakhi et al., 2018). The accuracy of the method ranged from

Table 1
Phenols and phthalates (parent compounds and metabolites) measured in pooled urine samples of the SEPAGES cohort.

Parent compounds	Metabolites
Phenols	
Methyl paraben	
Ethyl paraben	
Propyl paraben	
Butyl paraben	
Bisphenol A	
Bisphenol S	
Bisphenol F	
Bisphenol B	
Bisphenol AF	
Benzophenone-3	
Triclosan	
Triclocarban	
Phthalates	
Diethyl phthalate (DEP)	Monoethyl phthalate (MEP)
Diisobutyl phthalate (DiBP)	Mono-isobutyl phthalate (MiBP)
Dibutyl phthalate (DBP)	Mono-n-butyl phthalate (MnBP)
Butyl-benzyl phthalate (BBzP)	Mono-benzyl phthalate (MBzP)
Di(2-propylheptyl) phthalate (DPHP)	6-hydroxy-mono-propyl-heptyl phthalate (OH-MPHP)
Di(2-ethylhexyl) phthalate (DEHP)	Mono(2-ethylhexyl) phthalate (MEHP)
	Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)
	Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)
	Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)
	Mono(2-methylcarboxyhexyl) phthalate (MMCHP)
Diisononyl phthalate (DiNP)	Mono(4-methyl-7-hydroxy-octyl) phthalate (oh-MiNP)
	Mono(4-methyl-7-oxo-octyl) phthalate (oxo-MiNP)
	Mono(4-methyl-7-carboxy-heptyl) phthalate (cx-MiNP)
Non-phthalate plasticizers	
Di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH)	2-(((hydroxy-4-methyloctyl)oxy)carbonyl) cyclohexanecarboxylic acid (oh-MINCH)
	2-(((4-methyl-7-oxyooctyl)oxy)carbonyl) cyclohexanecarboxylic acid (oxo-MINCH)

75% to 120% and the precision given as relative standard deviation was below 26%. In brief, 200 µl of urine sample were taken and labelled internal standards and enzyme solution were added. After 4 h, the enzymatic reaction was stopped by adding formic acid. The samples were centrifuged, and the supernatant was injected into UPLC-MS-MS system. Both in-house control samples and standard reference materials (SRM 3673, a non-smokers urine) from National Institute of Standards and Technology (NIST) were analyzed along with each batch.

Phthalate and DINCH metabolites were analyzed using on-line column switching high performance liquid chromatography and tandem mass spectrometry (HPLC-MS-MS) as published earlier (Sabaredzovic et al., 2015). The accuracy of the method ranged from 70% to 125% and the precision given as relative standard deviation was below 25%. In brief, 300 µl of urine sample were taken and labelled internal standards and enzyme solution were added. After 1.5 h, the enzymatic reaction was stopped by adding formic acid. The samples were centrifuged, and the supernatant was injected into HPLC-MS-MS system. Both in-house control samples and standard reference materials (SRM 3673, a non-smokers urine) from National Institute of Standards and Technology (NIST) were analyzed along with each batch.

The LODs and LOQs for phenols, phthalates and DiNCH metabolites are shown in Table 2.

The sampling material was tested for any possible external contamination of phenols and we found no external contamination of phenols in the prenatal samples (Rolland et al., 2020). For phthalates and DINCH metabolites, this was not necessary as urinary metabolites were measured and not the parent compounds.

Specific gravity was also assessed in each pool using a handheld Atago PAL 10-S refractometer (Atago).

2.3. Measurements of thyroid hormones

Newborn blood spots (from Guthrie cards) were collected as part of the neonatal screening program, at a median of 3 days (5th, 95th percentiles: 2, 4) and stored at −20 °C. TSH and total T4 were assessed using immunofluorescence (automaton GSP, Perkin Elmer). No assay was available on this automaton to assess triiodothyronine (T3).

2.4. Measurements of iodine and selenium

Maternal urinary iodine, an essential element for thyroid hormones synthesis, was measured in urine (daily pools), using a quadrupole inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer NexION 2000, Waltham, MA, USA) (Allain et al., 1990).

Table 2
Distribution of phenol and phthalate metabolite concentrations (µg/L) in a pool of up to 21 urine samples collected over a week (N = 442 pregnant women from the SEPAGES cohort, 2014 – 2017).

Metabolites	LOD / LOQ	% >LOD	% >LOQ	Standardized[a] concentrations Percentiles			Measured concentrations Percentiles			Rho[b]
				25th	50th	75th	25th	50th	75th	
Phenols										
Bisphenol A	0.04 / 0.10	99.5	99.3	1.12	1.71	2.98	1.41	2.20	3.68	0.95
Bisphenol S	0.10 / 0.40	25.5	20.9	/	/	/	< LOD	< LOD	2.9	/
Bisphenol AF	0.02 / 0.05	0.0	0.0	/	/	/	< LOD	< LOD	< LOD	/
Bisphenol B	0.03 / 0.10	0.0	0.0	/	/	/	< LOD	< LOD	< LOD	/
Bisphenol F	0.07 / 0.20	1.4	1.4	/	/	/	< LOD	< LOD	< LOD	/
Triclosan	0.04 / 0.10	98.2	98.2	0.50	1.05	3.49	0.50	1.05	3.49	1.00
Triclocarban	0.04 / 0.10	0.90	0.5	/	/	/	< LOD	< LOD	< LOD	/
Benzophenone-3	0.04 / 0.10	100	98.4	0.44	0.87	2.41	0.60	1.18	3.39	0.97
Methyl paraben	0.04 / 0.10	100	100	1.63	8.05	18.7	7.07	15.6	66.2	0.97
Ethyl paraben	0.04 / 0.10	99.8	99.8	0.69	1.10	3.61	0.51	0.82	2.69	0.96
Propyl paraben	0.04 / 0.10	84.1	72.5	0.16	0.96	10.8	0.16	0.93	8.58	0.97
Butyl paraben	0.07 / 0.20	24.8	10.9	/	/	/	< LOD	< LOD	0.72	/
Phthalate metabolites										
MEP	0.20 / 0.50	100	100	13.1	24.7	43.9	14.13	24.7	47.5	0.98
MnBP	0.20 / 0.50	100	100	8.80	12.0	16.2	9.99	13.4	18.6	0.98
MiBP	0.20 / 0.50	100	100	11.9	16.7	23.9	13.37	19.2	29.9	0.89
MBzP	0.07 / 0.20	100	100	3.48	5.00	7.57	3.36	4.78	7.38	0.98
OH-MPHP	0.07 / 0.20	100	98.4	0.71	0.88	1.19	0.63	0.84	1.22	0.85
MEHP	0.20 / 0.50	100	98.2	1.56	2.32	3.61	1.51	2.24	3.46	0.98
MEHHP	0.20 / 0.50	100	100	5.51	7.52	11.1	5.39	7.39	11.4	0.98
MEOHP	0.20 / 0.50	100	100	3.93	5.54	7.92	4.09	5.70	8.33	0.98
MECPP	0.70 / 2.0	100	99.8	8.01	10.51	14.0	8.30	11.2	15.3	0.96
MMCHP	0.70 / 2.0	99.5	99.5	6.19	8.03	10.8	7.42	9.61	13.6	0.93
ΣDEHP[c]	/	/	/	0.08	0.11	0.16	0.09	0.12	0.17	0.97
oh-MiNP	0.10 / 0.25	100	100	3.35	5.74	11.0	3.35	5.74	11.0	1.00
oxo-MiNP	0.10 / 0.25	100	99.5	1.53	2.33	3.95	1.67	2.57	4.49	0.97
cx-MiNP	0.40 / 1.0	100	100	3.77	4.90	7.44	2.15	3.75	5.00	0.91
ΣDiNP[c]	/	/	/	0.01	0.01	0.02	0.01	0.01	0.01	0.88
Non-phthalate plasticizer										
oh-MINCH	0.07 / 0.20	100	100	1.30	1.89	3.33	0.99	1.60	3.07	0.89
oxo-MINCH	0.07 / 0.20	99.8	99.8	1.04	1.55	2.66	0.82	1.28	2.15	0.92
ΣDINCH[c]	/	/	/	0.01	0.01	0.02	0.00	0.01	0.01	0.88

Abbreviations: cx-MiNP: mono-4-methyl-7-carboxyoctyl phthalate, LOD: limit of detection, LOQ: limit of quantification, MBzP: monobenzyl phthalate, MECPP: mono-2-ethyl-5-carboxypentyl phthalate, MEHP: mono-2-ethylhexyl phthalate, MEHHP: mono-2-ethyl-5-hydroxyhexyl phthalate, MEOHP: mono-2-ethyl-5oxohexyl phthalate, MEP: monoethyl phthalate, MiBP: mono-*iso*-butyl phthalate, MMCHP: mono-2-carboxymethyl hexyl phthalate, MnBP: mono-n-butyl phthalate, oh-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate, oh-MINCH: 2-(((hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid, OH-MPHP: mono-6-hydroxy-propylheptyl phthalate, oxo-MINCH: 2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid, oxo-MiNP: mono-4-methyl-7-oxooctyl phthalate, ΣDEHP: molar sum of the five DEHP metabolites, ΣDiNP: molar sum of the three DiNP metabolites, ΣDINCH: molar sum of the two DINCH metabolites.

[a] Concentrations were standardized for sample transport time from participant's home to the biobank, time during which the individual samples were thawed at 4 °C during the pooling procedure or analytical batches when these variables were associated with the measured biomarker concentrations (p-values < 0.2).

[b] Spearman correlation between measured and standardized concentrations.

[c] Molar sum (µmol/L).

Selenium, a micronutrient involved in the peripheral conversion of free T4 to free T3 was measured in non-fasting maternal sera, collected by trained SEPAGES field workers during a study visit at the participants' homes (median gestational age at sampling: 19.1). We used quadrupole ICP-MS with a collision-reaction chamber with Helium (Perkin Elmer NexION 2000, Waltham, MA, USA) (Stürup et al., 2005).

2.5. Statistical analysis

Bisphenols AF, B, F and triclocarban were detected in<5% of the biospecimens and were not considered in our statistical analysis. Due to low frequencies of detection butyl paraben and bisphenol S were categorized (3 categories: not detected, detected at at least one time points, detected at the two time points). For the other biomarkers showing higher detection frequencies, we singly imputed concentrations < limit of detection (LOD) and between LOD and limit of quantification (LOQ) based on their estimated underlying distribution (Helsel, 2011).

To study the impact of between-subject variations in conditions related to urine processing (i.e. sample transport time from participant's home to the biobank, time during which the individual samples were thawed at 4 °C during the pooling procedure) and assay (analytical batches) on the measured concentrations, we estimated the associations between each exposure biomarker concentration assessed in pools (natural log-transformed) and the factors above-mentioned using adjusted linear regression. If we identified a processing/assay conditions as associated with the biomarker urine concentrations (p-value < 0.2), we then used the measured biomarker concentrations and the estimated effects of the processing/assay conditions to predict standardized concentrations (i.e., concentrations that would have been observed if all samples had been processed under the same conditions and assayed in the same analytical batch) (Mortamais et al., 2012; Philippat et al., 2012).

We used the within-subject mean of the standardized concentrations (ln-transformed) from the two urine pools as exposure variable in our statistical analysis (Mortamais et al., 2012). We used molar sums for phthalate metabolites issued from a same parent compound. TSH, but not T4, was ln-transformed to approach gaussian distribution.

2.6. Adjustment factors

Data on potential confounders were collected during clinical exams as well as thought questionnaires administered by field workers or self-completed by the parents.

Adjustment factors were chosen a *priori* and included variables that caused the outcome without being a consequence of the exposure, or that caused both the outcome and exposure. This included child age at blood collection (linear term), child sex (boy / girl), delivery mode (vaginal / caesarean), maternal age (linear and quadratic terms), maternal body mass index (BMI) before pregnancy (continuous), parity (nulliparous / parous), maternal education ($\leq$ 2 years / between 3 and 4 years / $\geq$ 5 years after high school), maternal smoking during pregnancy (yes / no), maternal urinary iodine and serum selenium concentrations (continuous). Models for T4 were additionally adjusted for analytical batch (categorical, 5 batches). Pre-term birth and birthweight were not considered in our main analysis since they are likely to lie in the causal pathway.

Missing data on covariates were imputed using multiple imputation with chained equation (R package MICE, N = 20 imputed datasets).

2.7. Associations between phenols and phthalates and thyroid hormones

We first relied on uni-pollutant models and ran one adjusted linear regression per exposure biomarker and thyroid hormone. Sex-specific effects were explored by adding an interaction term between exposure and child sex. Similarly, we included an interaction term with iodine status (dichotomized variable according to the World Health Organization (WHO) threshold for iodine deficiency in pregnant women (150 µg/L (World Health Organization, 2013)) to explore effect modification by maternal iodine levels. Stratified analysis (either on sex or iodine) was performed when the p-value for interaction was below 0.2.

To assess deviation from the linearity, we ran additional models with the exposure in tertiles. We performed tests of heterogeneity in hormone concentrations across exposure tertiles; p-values of trend tests were also estimated using categorical variables whose values corresponded to the tertile specific median biomarker levels. We considered an association to be non-monotonic in situations in which biomarkers exhibited heterogeneity in outcome across tertiles (low p-value, heterogeneity test) but with little support for a trend (high p-value, trend test). To look at potential windows of susceptibility, we ran uni-pollutant models with biomarker concentrations measured either at the first or second urine collection week.

Our main analysis was not adjusted for markers of urine dilution (e.g., specific gravity or creatinine). This choice was motivated by a previous study (Philippat and Calafat, 2020) showing that while not formally accounting for urine dilution of individual samples, biomarker concentrations assessed in equal volume pools correlate well with those assessed in a pool of all urine volume collected over 24 h or a week. In addition, for some chemicals (e.g., bisphenol A) correlations with concentrations assessed in a pool of all urine volume collected over 24 h or a week were lower when standardization for urine dilution was applied (Philippat and Calafat, 2020). However, since this study did not include all the biomarkers we assessed, we performed an analysis additionally adjusted for specific gravity, a marker of urine dilution (mean of the specific gravity measured in the 2 pooled samples). Finally we performed an analysis using the measured instead of the standardized concentrations.

We then relied on adjusted Bayesian Kernel Machine Regression (BKMR, R package: bkmr) to study the associations between the phenol and phthalate mixture and each hormone. BKMR relied on flexible function (kernel function) to regress the health outcomes (i.e., the thyroid hormone concentrations) against the mixture of urinary biomarkers (Bobb et al., 2015). BKMR models were adjusted for the same covariates than the uni-pollutant models. Categorical exposures (bisphenol S and butyl paraben) were not considered for this analysis. Each exposure biomarker concentration was standardized (i.e., divided by their standard deviation (SD)). As recommended (Bobb et al., 2015), we ran 50,000 iterations, dropped the first 25,000 and among the last 25,000, kept every fifth for inference. The overall mixture effect was given by the plot that represents the expected change in hormone concentration with concomitant increase quantiles of all exposure biomarkers, relative to when they are fixed at their 25th percentile. When this plot was suggestive of an effect of the mixture, we also plotted the estimated effect of an increase from the 25th to 75th percentiles in a single biomarker when all other biomarkers were fixed at either their 25th, 50th, or 75th percentiles. We also plotted the estimated effect of each chemical individually, to detect potential non-linear associations.

BKMR was run for the whole population and in boys and girls separately, to explore sex-specific effects. We did not explore effect modification by iodine because of the small number of women in the group with iodine concentrations above 150 µg/L.

Statistical analyses were performed using R version 4.0.4. Several packages were used including *haven* to open SAS dataset, *aod* to perform Wald test, *Tidyr, tidyverse* for data manipulation, *questionr, dplyr* to perform descriptive analysis, *stats* for regression models, *ggplot2* for graphical visualization, *Hmisc* for Spearman correlation, *mice* for multiple imputation and *bkmr* for the BKMR models.

3. Results

Average gestational age at delivery was 39.7 weeks (SD: 1.6, Table 3). Boys represented 54% of our study population. SEPAGES women tended to be highly educated (57% had pursued education for $\geq$

Table 3
Characteristics of pregnant women included in this study (N = 442, SEPAGES cohort, 2014 – 2017).

	Included women		Excluded women	
Characteristics	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**
Maternal age (years)		32.5 (3.9)		32.1 (3.2)
Weight at birth (g)		3290 (460)		3246 (390)
Child age at blood collection (days)		3.3 (0.7)		3.3 (1.7)
Gestational age at delivery		39.7 (1.6)		39.3 (1.5)
Education level				
≤ 2 years after high school	74 (17)		9 (22)	
3 to 4 years after high school	116 (26)		11 (26)	
≥ 5 years after high school	251 (57)		21 (50)	
Missing	1 (0.2)		1 (2)	
Maternal ethnicity				
European	375 (85)			
Other [a]	17 (4)			
Missing	50 (11)			
Parity				
Nulliparous	200 (45)		22 (52)	
Parous	242 (55)		20 (48)	
Missing	0 (0)		0 (0)	
Maternal pre-pregnancy BMI (kg/m^2)				
< 18.5	29 (7)		0 (0)	
18.5–25	333 (75)		31 (74)	
≥ 25	77 (17)		10 (24)	
Missing	3 (1)		1 (2)	
Child sex				
Boy	237 (54)		21 (50)	
Girl	205 (46)		17 (41)	
Missing	0 (0)		4 (9)	
Vitamin use during pregnancy				
Yes	388 (88)		29 (69)	
No	42 (9)		2 (5)	
Missing	12 (3)		11 (26)	
Smoking (first trimester)				
No	382 (86)		29 (69)	
Yes	23 (5)		0 (0)	
Missing	37 (8)		13 (31)	
Delivery mode				
Caesarean section mode	73 (17)		8 (19)	
Vaginal mode	368 (83)		30 (71)	
Missing	1 (0)		4 (10)	
Diabetes [b]				
Yes	3 (0.7)		0 (0)	
No	436 (98.6)		42 (100)	
Missing values	3 (0.7)		0 (0)	
Hypertension				
Yes	8 (2)		4 (10)	
No	434 (98)		38 (90)	
Missing values	0 (0)		0 (0)	
Thyroid disorders				
Yes	22 (5)		19 (45)	
No	416 (94)		23 (55)	
Missing values	4 (1)		0 (0)	

[a] includes: Africa (N = 2), America (N = 2), South-East Asia (N = 2), Oriental Mediterranean countries (N = 3), Other (N = 4), Do not know / do not want to answer (N = 4).
[b] type 1 or 2.

5 years after high school), had a pre-pregnancy BMI between 18.5 and 25 kg/m^2 (75%,) and did not smoke (86%). Most women (81%) had a iodine urinary concentration lower than the World Health Organization (WHO) guideline for pregnant women (World Health Organization, 2013).

Medians T4 and TSH in newborn blood spot were respectively 81.2 ng/mL and 1.8 mUI/L (Supplemental Material, Table S1). Spearman correlation coefficient between newborn T4 and TSH was low (0.1, Supplemental Material, Table S2).

Median (5th, 95th percentiles) number of urine samples in each pool was 21 (17, 21). Phenol and phthalate concentrations have been described elsewhere (Philippat et al., 2021; Rolland et al., 2020). Except for butylparaben and bisphenol S, which were detected in 25% of the samples, frequencies of detection were above 84% (Table 2).

3.1. Predictors of thyroid hormone concentrations at birth

On average, girls had higher T4 than boys. T4 concentrations tended to decrease with increased maternal education and among infants of mothers who reported smoking during pregnancy (Supplemental Material, Table S3). Significant differences were also observed across the analytical batch for this variable. TSH concentrations at birth increased with increased maternal age and was higher among children born by caesarean compared to those having a vaginal delivery (Supplemental Material, Table S3).

3.2. Uni-pollutant models

Unadjusted associations between each individual biomarkers and thyroid hormones are presented in Supplemental Material, Table S4. Below we only report results of the models adjusted for covariates

Table 4
Uni-pollutant analysis: effect estimates from adjusted linear regression of individual maternal urinary phenol, phthalate, non-phthalate plasticizer standardized concentrations (mean of the two exposure weeks, ln-transformed) and newborn TSH (ln-transformed) and total T4 concentrations.

Metabolites	T4 (N = 434)			TSH (N = 442)		
	β (95%CI)	interaction with child sex (p-values)	interaction with iodine[a] (p-values)	β (95%CI)	interaction with child sex (p-values)	interaction with iodine[a] (p-values)
Phenols						
Bisphenol A	0.86 (-1.81; 3.52)	0.04	0.61	0.00 (-0.08; 0.08)	0.37	0.83
Bisphenol S						
< LOD at 2 time points	Ref			Ref		
< LOD at one time point	2.62 (-1.56; 6.79)	0.03	0.66	−0.04 (-0.17; 0.08)	0.8	0.06
>LOD	3.13 (-3.61; 9.87)		0.59	0.13 (-0.08; 0.33)		0.87
Triclosan	−1.18 (-2.32; −0.04)	0.07	0.84	−0.03 (-0.06; 0.01)	0.16	0.35
Benzophenone-3	1.61 (0.19; 3.04)	0.67	0.89	−0.02 (-0.07; 0.02)	0.23	0.95
Methyl paraben	−1.11 (-2.45; 0.24)	0.14	0.29	−0.01 (-0.05; 0.03)	0.76	0.55
Ethyl paraben	0.35 (-1.21; 1.90)	0.78	0.79	0.02 (-0.03; 0.06)	0.85	0.68
Propyl paraben	−0.83 (-1.63; −0.03)	0.04	0.20	0.01 (-0.01; 0.03)	0.41	0.88
Butyl paraben						
< LOD at 2 time points	Ref			Ref		
< LOD at one time point	−2.54 (-7.16; 2.08)	0.2	0.50	0.08 (-0.06; 0.22)	>0.90	0.76
>LOD	−0.57 (-6.70; 5.56)		0.33	0.22 (0.03; 0.40)		0.09
Phthalate metabolites						
MEP	1.21 (-0.99; 3.41)	0.96	0.57	0.01 (-0.06; 0.07)	0.62	0.35
MnBP	−0.67 (-4.24; 2.91)	0.55	0.12	0.10 (0.00; 0.21)	0.25	0.50
MiBP	0.56 (-2.76; 3.89)	0.69	0.03	−0.01 (-0.11; 0.09)	0.81	0.44
MBzP	−0.84 (-3.62; 1.94)	0.37	0.19	0.01 (-0.08; 0.09)	0.66	0.33
OH-MPHP	−2.64 (-6.82; 1.54)	0.16	0.59	0.01 (-0.11; 0.14)	0.73	0.39
ΣDEHP	−1.61 (-5.67; 2.44)	0.43	0.27	0.07 (-0.06; 0.19)	0.27	0.24
ΣDiNP	−0.50 (-3.71; 2.71)	0.4	0.18	−0.01 (-0.11; 0.09)	0.03	0.61
Non-phthalate plasticizer						
ΣDINCH	1.13 (-1.35; 3.61)	0.27	0.31	−0.02 (-0.10; 0.06)	0.57	0.49

Abbreviations: LOD: limit of detection, MBzP: monobenzyl phthalate, MEP: monoethyl phthalate, MiBP: mono-*iso*-butyl phthalate, MnBP: mono-n-butyl phthalate, OH-MPHP: mono-6-hydroxy-propylheptyl phthalate, ΣDEHP: molar sum of the five DEHP metabolites, ΣDiNP: molar sum of the three DiNP metabolites, ΣDINCH: molar sum of the two DINCH metabolites, TSH: thyroid stimulating hormone, T4: thyroxine.
Adjustment factors: child age at blood collection and sex, delivery mode, maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal education, maternal smoking during pregnancy, maternal urinary iodine, serum selenium concentrations and batch (for T4 analysis only).
[a] Iodine was categorized as follow (<150 μg/L; ≥ 150 μg/L).

(Table 4).

Regarding T4, when studying boys and girls together, we observed a positive association between benzophenone-3 and T4 (β = 1.6; 95% Confidence Interval (CI): 0.2; 3.0) while propyl paraben as well as triclosan were negatively associated with this hormone concentration that decreased by 0.8 (95%CI: -0.1.6; −0.03) and 1.2 (95%CI: −2.3; −0.04) ng/mL for each increase by one in the ln-transformed propyl paraben and triclosan concentration, respectively. P-values for interaction with child sex was below 0.2 for the three parabens (propyl, methyl and butyl), triclosan, bisphenol A, bisphenol S and one phthalate metabolite (OH-MPHP, Table 4). Stratification for child sex was suggestive of negative associations with T4 for the three parabens, triclosan and OH-MPHP among girls but not boys (Fig. 1). Both bisphenol A and S were positively associated with T4, however this association was seen in boys for bisphenol A and girls for bisphenol S.

Our results were also suggestive of interaction with iodine status for several biomarkers including propyl paraben, MnBP, MiBP, MBzP and DiNP (Table 4). After stratification, we observed a negative association between propyl paraben and T4 among newborns of women with iodine levels below 150 μg/L (Fig. 2). However, given the low number of women with normal iodine level (N = 85) the lack of association in this group might result from the lack of power.

Only few associations were observed for TSH. When boys and girls were studied together, TSH increased with prenatal exposure to butyl paraben and MnBP (Table 4). Interaction with child sex was detected for diisononyl phthalate (ΣDiNP) and triclosan that were both negatively associated with TSH in girls (Fig. 1). ΣDiNP also tended to be positively associated with TSH in boys (Fig. 1). For this hormone, the only chemicals for which the p-value for interaction with iodine concentrations was below 0.2 were bisphenol S and butyl paraben (Table 4). After stratification, no significant association was observed for bisphenol S, while a positive association between butyl paraben and TSH was observed among women with iodine deficiency (Fig. 2).

To look at potential windows of susceptibility, we ran uni-pollutant models with biomarker concentrations measured either at the first or second urine collection week (Supplemental Material, Table S5,S6). In addition to the associations detected when we used the average concentrations, we observed associations between methyl paraben at the 2nd urine collection week and T4 (β = -1.12, 95% CI: −2.18; −0.06) and between ΣDEHP in the second urine collection week and TSH (β = 0.12, 95% CI: 0.01; 0.23).

When we relied on exposure coded in tertiles, results of the uni-pollutant analyses were not suggestive of non-linear associations except maybe for MnBP and T4 that increased in the second but not in the third exposure tertile (β: of 4.9 (95%CI 0.23; 9.51) and −0.28 (95% CI: −5.05; 4.49), respectively) (Supplemental Material, Table S7).

When we additionally adjusted our models for specific gravity or used the measured instead of the standardized concentrations, most of the associations observed in our main analysis were preserved (Supplemental Material, Table S8,S9).

3.3. Mixture model

When studying boys and girls together we did not observe clear association between the mixture and T4 or TSH (Supplemental Material, Figure S2). After stratification for child sex, T4 tended to decrease in girls and increase in boys with increased exposure to the mixture, while no clear association was observed for TSH (Fig. 3). In line with the results of the uni-pollutant analysis, BKMR suggested that both triclosan and propyl paraben were important contributors for the mixture effect observed in girls with T4 (Fig. 4). Regarding the shape of the associations between individual chemicals and hormone concentrations, as for

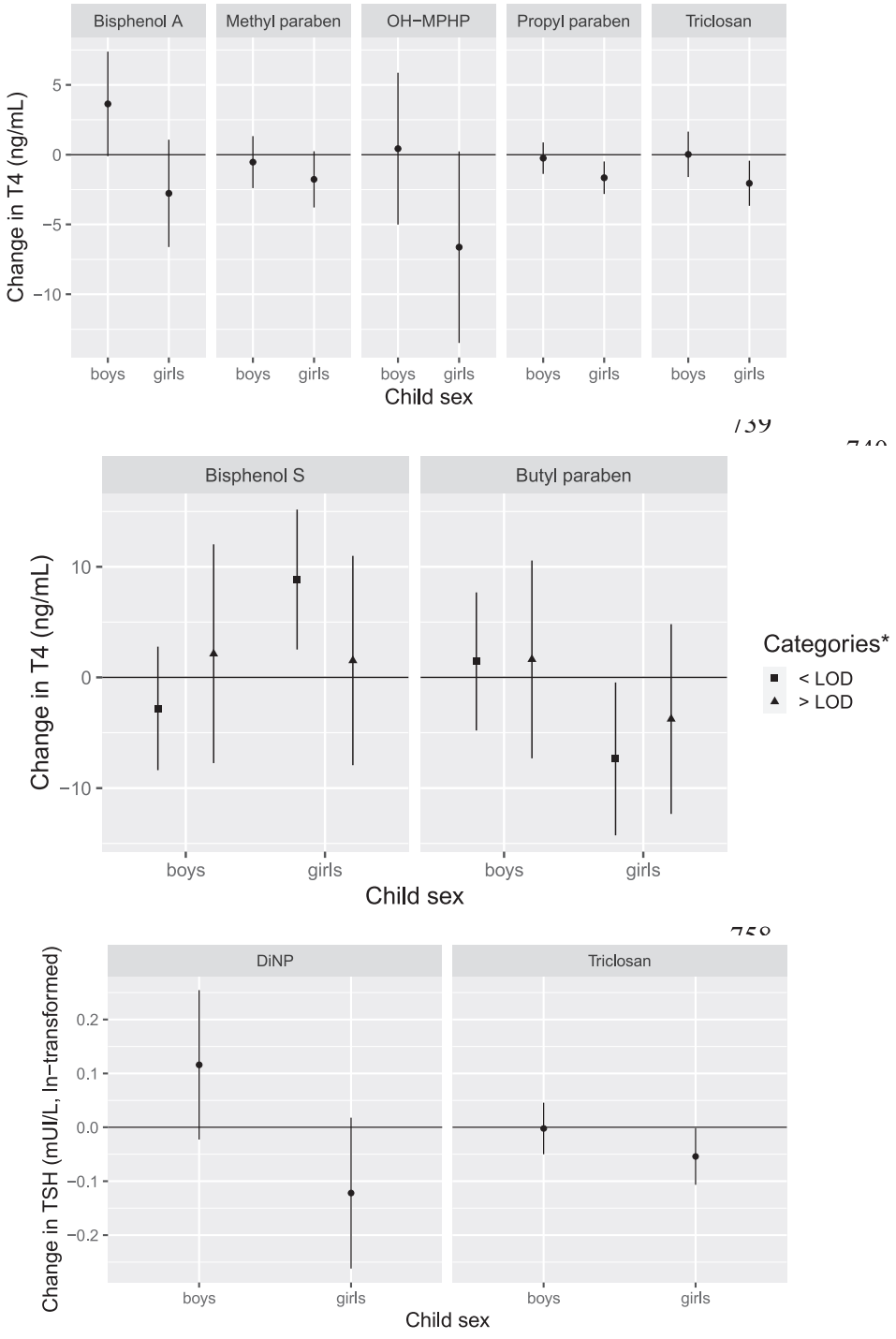


Fig. 1. Uni-pollutant analysis: effect estimates from adjusted linear regression of individual maternal urinary phenol, phthalate standardized concentrations and newborn TSH (ln-transformed) and total T4 concentrations, stratified for sex (T4: N = 231 boys and 203 girls, TSH: N = 237 boys and 205 girls). Abbreviations: DiNP: diisononyl phthalate, OH-MPHP: mono-6-hydroxy-propylheptyl phthalate, TSH: thyroid stimulating hormone, T4: thyroxine. * < LOD: detected at only one time point, > LOD: detected at the two time points, reference was: not detected at both time points. Adjustment factors: child age at blood collection and sex, delivery mode, maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal education, maternal smoking during pregnancy, maternal urinary iodine, serum selenium concentrations and batch (for T4 analysis only).

the uni-pollutant model with exposure coded in tertile, except for MnBP, all the associations seemed linear (Supplemental Material Figures S3 and S4).

4. Discussion

The aim of this study was to determine whether pre-natal exposure to synthetic phenols and phthalates was associated with thyroid hormone levels in neonates. Relying on two pools of up to 21 maternal urine samples collected during the 2nd and 3rd trimesters of pregnancy to assess exposure, we observed associations between exposure to individual compounds and thyroid hormones, mainly T4, measured in newborn heel-prick samples. Both the uni-pollutant and mixture models suggested sex-specific associations. Sexual dimorphism has been reported for the thyroid gland, which volume is usually higher in males than females due to overall higher body weight (Hegedüs et al., 1983) as well as for thyroid hormones circulating levels (Ehrenkranz et al., 2015). This is in line with our results suggesting higher T4 concentrations in girls compared to boys. While the underlying mechanisms are so far not well understood, these gender differences, in addition to the higher overall prevalence of thyroid diseases in women compared to men (Bauer et al., 2014), may have led to different sensitivity to disruptors of thyroid function by sex.

According to the uni-pollutant model, urinary concentrations to

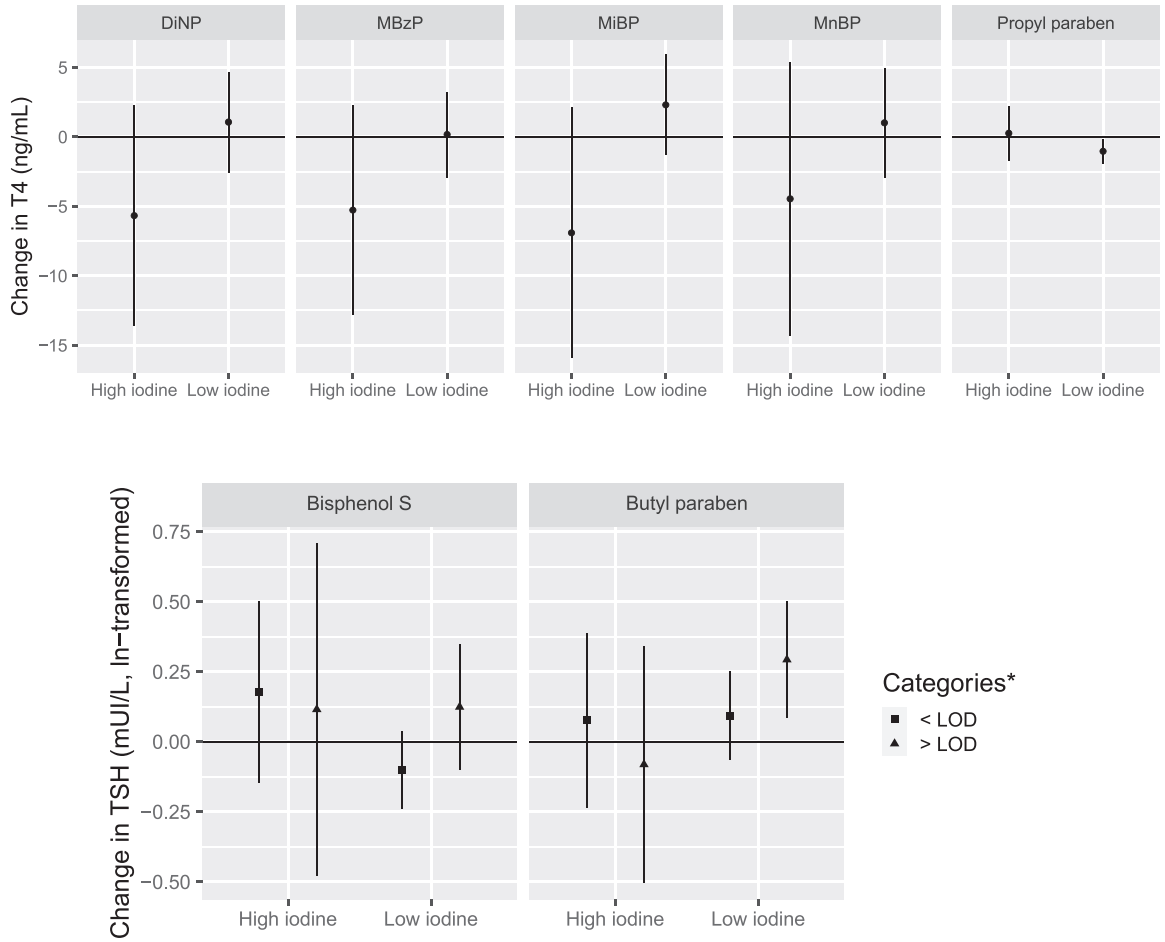


Fig. 2. Uni-pollutant analysis: effect estimates from adjusted linear regression of individual maternal urinary phenol, phthalate standardized concentrations and newborn TSH (ln-transformed) and total T4 concentrations, stratified for iodine status (T4: N = 350 and 84 in the low and high iodine group, respectively, TSH: N = 357 and 85 in the low and high iodine group, respectively). Abbreviations: DiNP: diisononyl phthalate, MBzP: monobenzyl phthalate, MiBP: mono-*iso*-butyl phthalate, MnBP: mono-n-butyl phthalate, TSH: thyroid stimulating hormone, T4: thyroxine. * < LOD: detected at only one time point, > LOD: detected at the two time points, reference was not detected at both time points. Low iodine: iodine concentration < 150 μg/L. High iodine: iodine concentration ≥ 150 μg/L. Adjustment factors: child age at blood collection and sex, delivery mode, maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal education, maternal smoking during pregnancy, maternal urinary iodine, serum selenium concentrations and batch (for T4 analysis only).

three of the four parabens measured were negatively associated with T4 concentrations in girls. Propyl paraben was also identified as a main contributor of the mixture for the association with T4 in girls. The mother's iodine status also appeared to interact with propylparaben exposure, with T4 levels in newborns born from women with iodine deficiency, but not in women with normal iodine levels, showing a negative association with this compound. However, this result should be cautiously interpreted given the small number of women in the normal iodine status group. Among the two previous epidemiological studies investigating associations between exposure to parabens and neonatal thyroid hormones, neither reported a negative association with T4 (Berger et al., 2018; Li et al., 2020). However, one assessed parabens in a spot urine sample collected on the day of delivery (Li et al., 2020), which, given the short half-life of parabens, is unlikely to be representative of prenatal exposure. The other study only assessed TSH (Berger et al., 2018), thus limiting comparability with the results presented here. Among the few studies that looked at the associations between parabens and maternal thyroid hormone levels, none reported associations with T4 (Aker et al., 2019; Berger et al., 2018), whereas negative associations with TSH were observed for methyl (Aker et al., 2019) and propyl (Berger et al., 2018) parabens. Our results suggested a negative association between several parabens and levels of T4 in girls at birth, however the limited number of corroborating studies examining these associations mean that further investigations will be necessary. *In vitro,* parabens can activate xenobiotic nuclear receptors such as the constitutive androstane receptor (CAR), and pregnane X receptor (PXR), which regulate the expression of genes encoding hepatic metabolic enzymes (Nakiwala et al., 2022; US EPA). Consequently, exposure to these compounds could increase metabolism and excretion of thyroid hormones.

Our results showed exposure to triclosan to be negatively associated with both T4 and TSH in girls. It was also identified as a significant contributor to the mixture effect for T4 levels in girls (BKMR model). Three previous studies looked at the associations between triclosan and thyroid hormones at birth (Berger et al., 2018; Guo et al., 2020; Wang et al., 2017) but did not report such negative associations. Two of them (Guo et al., 2020; Wang et al., 2017) assessed T4 levels in cord blood rather than in newborn blood spot. Due to maternal placental transfer of T4, these two sources of blood may not be directly comparable. In addition, one study collected maternal urine samples at birth (Guo et al., 2020) whereas the other assessed free T4 (Wang et al., 2017); while we measured total T4. Our observations indicated that associations existed in girls only, but two of the previous studies did not explore sex-specific effects (Berger et al., 2018; Wang et al., 2017), making confirmation difficult. Regarding studies assessing thyroid hormones during pregnancy, most did not report association for triclosan either (Aker et al.,

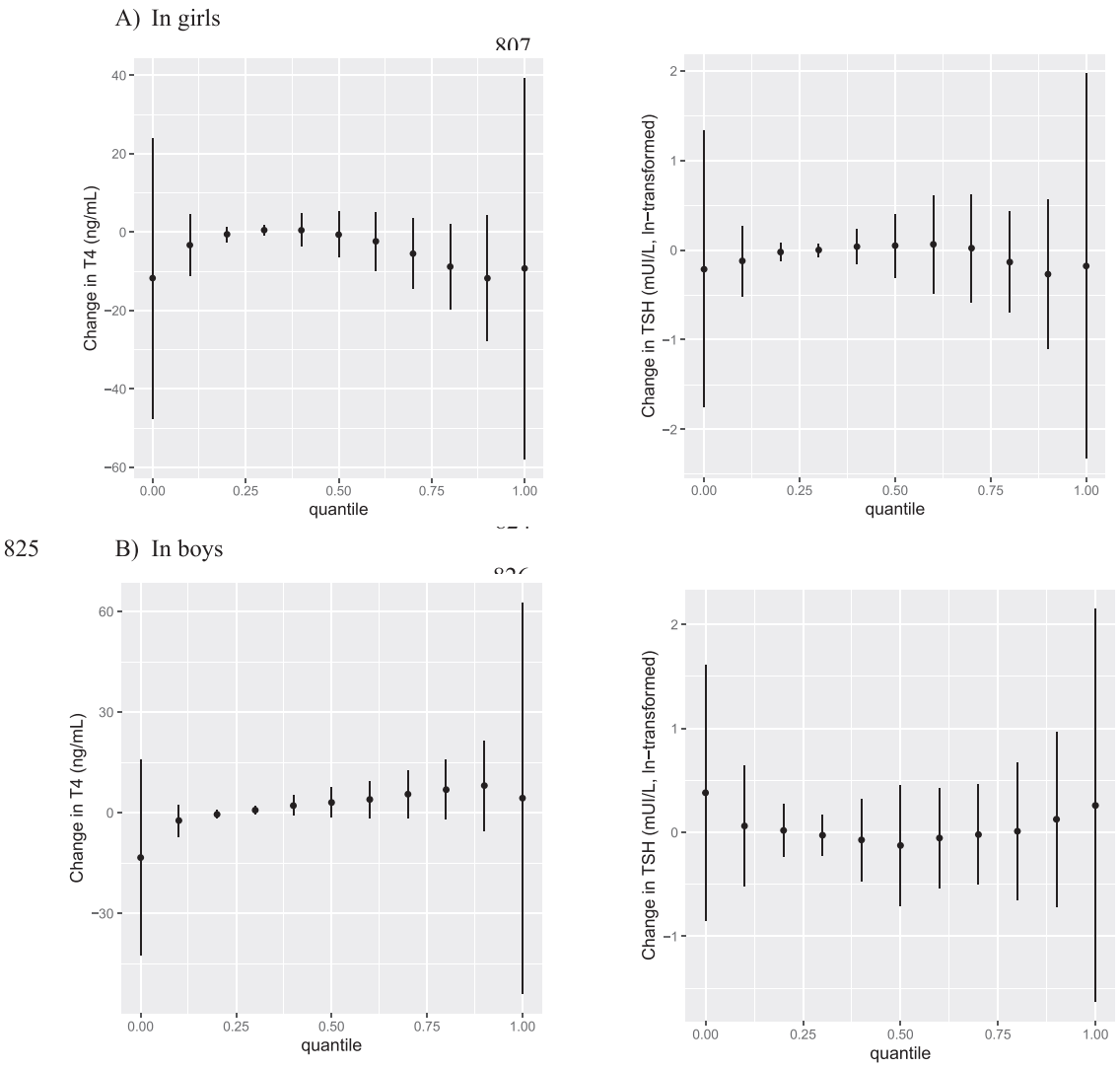


Fig. 3. Analysis stratified for child sex (A) Girls, B) Boys). Expected changes in total T4 and TSH (ln-transformed) concentrations associated with concurrently increasing quantiles of all chemicals, relative to when all concentrations are fixed at their 25th percentile. (T4: N = 231 boys and 203 girls, TSH: N = 237 boys and 205 girls). Abbreviation: TSH: thyroid stimulating hormone, T4: thyroxine. Adjustment factors: child age at blood collection and sex, delivery mode, maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal education, maternal smoking during pregnancy, maternal urinary iodine, serum selenium concentrations and batch (for T4 analysis only).

2019; Berger et al., 2018; Derakhshan et al., 2019). Our results for T4 were consistent with those from a study in rodents (Paul et al., 2012), where exposure to triclosan during gestation was associated with a 30% overall decrease in T4 in pups on post-natal day 4; no effect on TSH was observed. This study also suggested that triclosan could up-regulate hepatic catabolism of thyroid hormones. Alternatively, triclosan could affect T4 concentrations by inhibiting TPO and NIS, as shown *in vitro* (Nakiwala et al., 2022; US EPA).

The uni-pollutant model revealed a positive association between benzophenone-3 and T4 levels. The two previous studies looking at associations between this UV-filter and T4 at birth, either in cord blood (Guo et al., 2020) or newborn blood spot (Berger et al., 2018), found no such association (one study assessed benzophenone-3 in a spot urine sample collected at delivery). However, a few studies have reported negative associations between this chemical and T3 (free or total), but not T4, during pregnancy (Aker et al., 2016, 2018).

The uni-pollutant model revealed only few associations between phthalates and thyroid hormone levels. For example, MnBP was associated with increased TSH and T4 (2nd tertiles only). Associations were also found for OH-MPHP and ΣDiNP after stratification for child sex.

This is the first study to report an association between MnBP and neonatal TSH or T4. Previous studies relied on thyroid hormone concentrations measured in cord blood and either did not report any association for this metabolite (Guo et al., 2020; Kuo et al., 2015; Romano et al., 2018) or reported increased T3 (Huang et al., 2018), which was not assessed here. Several studies have reported associations between MnBP and serum T4 (either free or total) during pregnancy, but whether it increased or decreased hormone levels differed across studies (Derakhshan et al., 2019; Gao et al., 2017; Souter et al., 2020; Huang et al., 2016; Huang et al., 2017).

For ΣDiNP, our results suggested sex-specific associations with TSH (negative association in girls; positive in boys). Two previous studies reported no association (Kuo et al., 2015; Romano et al., 2018). However, these studies did not explore interaction with child sex and once again assessed TSH levels in cord blood, which may not be directly comparable to newborn blood spot due to the physiological TSH surge observed at birth. Two other studies assessing thyroid hormones during pregnancy rather than at birth reported associations for either a DiNP metabolite or a group of phthalates including DiNP metabolites, however these associations were seen with T4 (Derakhshan et al., 2021) and

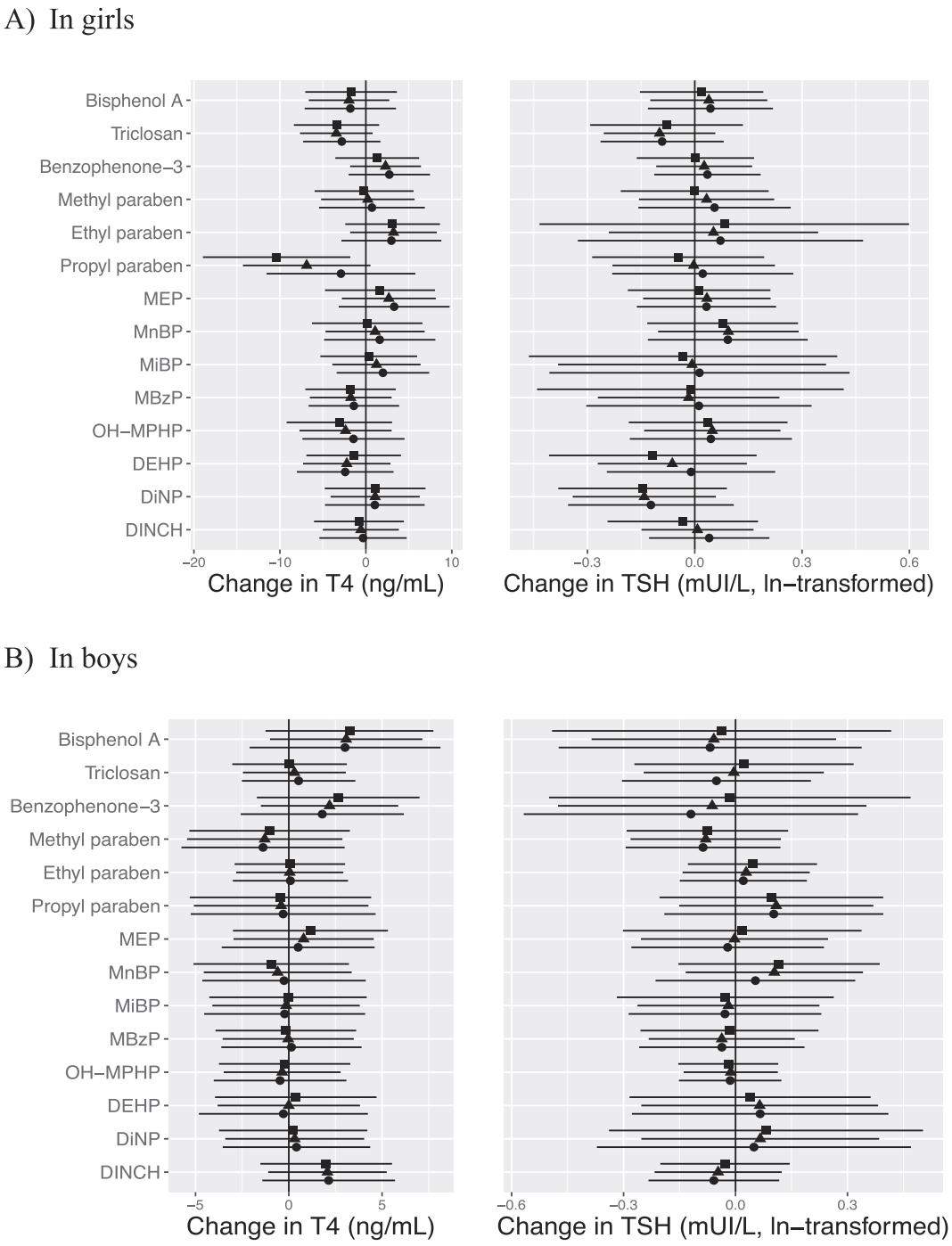


Fig. 4. Estimated effect of an increase from the 25th to 75th percentile in a single biomarker concentration on TSH (ln-transformed) and total T4 concentrations when all other biomarkers are fixed at either the 25th, 50th, or 75th percentiles, analyses stratified for child sex. Legend: circular dots and bars: all other biomarkers are fixed at their 25th percentiles, triangular: all other biomarkers are fixed at their 50th percentiles, squared: all other biomarkers are fixed at their 75th percentiles. Abbreviation: MBzP: monobenzyl phthalate, MEP: monoethyl phthalate, MiBP: mono-*iso*-butyl phthalate, MnBP: mono-n-butyl phthalate, OH-MPHP: mono-6-hydroxy-propylheptyl phthalate, ΣDEHP: molar sum of the five DEHP metabolites, ΣDiNP: molar sum of the three DiNP metabolites, ΣDINCH: molar sum of the two DINCH metabolites, TSH: thyroid stimulating hormone, T4: thyroxine. Adjustment factors: child age at blood collection and sex, delivery mode, maternal age, maternal body mass index (BMI) before pregnancy, parity, maternal education, maternal smoking during pregnancy, maternal urinary iodine, serum selenium concentrations and batch (for T4 analysis only). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

T3 (Villanger et al., 2020), not with TSH.

To our knowledge, our study is the first to investigate the association between OH-MPHP and thyroid hormone concentrations. DPHP, the parent compound of OH-MPHP, is a recently developed phthalate for which little data is available, and which merits further investigation.

4.1. Strengths and limitations

We relied on two pools of up to 21 urine samples from each mother to assess exposure. While measurement error may remain, compared to studies relying on a smaller number of samples (≤3), our urine sampling design should better represent exposure over the course of pregnancy

and thus decrease bias in the effect estimates (Perrier et al., 2016). A few studies aimed at estimating associations between a mixture of phenols and/or phthalates and thyroid hormones in early life. Among them two relied on variable selection approaches (e.g., Elastic Net) to identify the most important chemicals for an effect on thyroid hormones (Berger et al., 2018; Li et al., 2020) and only one, estimated the effect of the mixture as a whole (Romano et al., 2018). This is of interest since a toxicological study reported effects of co-exposure to DEHP and bisphenol A on relevant endpoints for the thyroid function (Tassinari et al., 2021).

Our analysis was adjusted for maternal selenium and iodine concentrations, two essential elements for thyroid hormone synthesis that were rarely considered in previous studies. However, given the low number of women with urinary iodine concentrations above 150 µg/L (N = 85), effect modifications by iodine status observed in our study should be interpreted cautiously. As most studies relying on newborn blood spots, we were not able to measure T3, which is also biologically relevant since it has a higher affinity for thyroid hormone receptors than T4. This prevented us to assess associations with the T3 to T4 ratio, an indicator of T4 deiodination into the bioactive T3 form. This ratio during pregnancy was previously associated with exposure to phenols and phthalates in our study population (Nakiwala et al., 2022). We did not record the time of blood sampling, and consequently could not account for potential diurnal variations in thyroid hormone concentrations.

5. Conclusion

Relying on two pools of up to 21 urine samples from each pregnant women to assess prenatal exposure, we highlighted several associations between phenols, and phthalate metabolites, studied individually or as a mixture, and neonatal T4 levels. Furthermore, our results suggested that these compounds can affect thyroid hormone concentrations in a sex specific manner. This requires confirmation in further studies with sufficient sample sizes to explore sex-specific effects.

CRediT authorship contribution statement

Ophélie Coiffier: Writing – original draft, Formal analysis, Visualization. **Dorothy Nakiwala:** . **Matthieu Rolland:** Methodology. **Andres Malatesta:** Formal analysis. **Sarah Lyon-Caen:** . **Benoît Chovelon:** Investigation. **Patrice Faure:** Investigation. **Anne Sophie Gauchez:** Investigation. **Dorra Guergour:** Investigation. **Amrit K. Sakhi:** Investigation. **Azemira Sabaredzovic:** Investigation. **Cathrine Thomsen:** Investigation. **Isabelle Pin:** Resources. **Rémy Slama:** Resources. **Christelle Corne:** Investigation. **Claire Philippat:** Conceptualization, Writing – original draft, Funding acquisition, Project administration.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107840.

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