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Associations between urine phthalate metabolites and thyroid function in pregnant women and the influence of iodine status

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ABSTRACT

Background: Human populations, including susceptible subpopulations such as pregnant women and their fetuses, are continuously exposed to phthalates. Phthalates may affect the thyroid hormone system, causing concern for pregnancy health, birth outcomes and child development. Few studies have investigated the joint effect of phthalates on thyroid function in pregnant women, although they are present as a mixture with highly inter-correlated compounds. Additionally, no studies have investigated if the key nutrient for thyroid health, iodine, modifies these relationships.

Methods: In this study, we examined the cross-sectional relationships between concentrations of 12 urinary phthalate metabolites and 6 plasma thyroid function biomarkers measured mid-pregnancy (~17 week gestation) in pregnant women (N = 1072), that were selected from a population-based prospective birth cohort, The Norwegian Mother, Father and Child Cohort study (MoBa). We investigated if the phthalate metabolite-thyroid function biomarker associations differed by iodine status by using a validated estimate of habitual dietary iodine intake based on a food frequency questionnaire from the 22nd gestation week. We accounted for the phthalate metabolite mixture by factor analyses, ultimately reducing the exposure into two uncorrelated factors. These factors were used as predictors in multivariable adjusted linear regression models with thyroid function biomarkers as the outcomes.

Results: Factor 1, which included high loadings for mono-*iso*-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), and monobenzyl phthalate (MBzP), was associated with increased total triiodothyronine (TT3) and free T3 index (fT3i). These associations appeared to be driven primarily by women with low iodine intake (< 150 μg/day, ~70% of our sample). Iodine intake significantly modified (*p*-interaction < 0.05) the association of factor 1 with thyroid stimulating hormone (TSH), total thyroxine (TT4) and free T4 index (fT4i), such that only among women in the high iodine intake category (≥150 μg/day, i.e. sufficient) was this factor associated with increased TSH and decreased TT4 and FT4i, respectively. In contrast, factor 2, which included high loadings for di-2-ethylhexyl phthalate metabolites (ΣDEHP) and di-*iso*-nonyl phthalate metabolites (ΣDiNP), was associated with a decrease in TT3 and fT3i, which appeared fairly uniform across iodine intake categories.

Conclusion: We find that phthalate exposure is associated with thyroid function in mid-pregnancy among Norwegian women, and that iodine intake, which is essential for thyroid health, could influence some of these relationships.

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1. Introduction

Phthalate diesters are a group of synthetic chemicals used extensively in our modern society, as plasticizers in polyvinyl chloride (PVC) products such as building material, food containers, medical products and children's toys as well as ingredients in cosmetics, personal care products and some medication and dietary supplements (Cao, 2010; Wormuth et al., 2006). Phthalates are not chemically bound to the product, and therefore can migrate or leach out to the surroundings. This has led to a ubiquitous presence in our environment (air, water and food), with a continuous release and spread into environmental compartments (Cao 2010; Katsikantami et al. 2016). Humans are exposed to a complex mixture of phthalates through inhalation, dermal contact, or ingestion of food and beverages contaminated with phthalates. As opposed to persistent environmental contaminants, phthalates are rapidly metabolized to monoesters or hydroxylated metabolites (elimination half-lives of 4–25 h) and excreted via urine (Koch and Angerer 2007; Koch et al. 2005; Koch et al. 2012). Hence, phthalate exposure is generally assessed by measuring urinary metabolites. Studies of urinary phthalate metabolites have demonstrated worldwide exposure to phthalates in human populations, including pregnant women, infants and children (Haug et al. 2018; Katsikantami et al. 2016). Phthalates have also been detected in human amniotic fluid, cord blood, and breast milk (Katsikantami et al. 2016). Norwegian women, including pregnant women, are mainly exposed to phthalates via intake of food and beverages (Giovanoulis et al. 2016; Sakhi et al. 2014), in addition to use of personal care products (Sakhi et al. 2017).

Phthalates are considered endocrine disruptors; increasing evidence from human and experimental studies suggest that they may affect the hypothalamic-pituitary-thyroid (HPT)-axis at multiple target points (Benjamin et al. 2017; Kim et al. 2019). Maternal thyroid function is an important determinant of childhood neurodevelopmental outcomes (e.g. cognitive deficits, and neurodevelopmental disorders), and is also associated with pregnancy complications and adverse birth outcomes such as pre-eclampsia, miscarriage, preterm birth and low birth weight (Blount et al. 2006; Drover et al. 2019; Ferguson et al. 2014; Korevaar et al. 2017; Medici et al. 2013; Shinohara et al. 2018). Given the many adversities that may follow altered maternal thyroid function, a better understanding of the relationship between phthalates and thyroid function is needed. Iodine is an essential nutrient required for the thyoroid gland's synthesis of thyroid hormones, as it is an integral part of thyroxine (T4) and triiodothyronine (T3), and is thus closely linked to thyroid health (Pearce et al. 2016). Iodine deficiency is highly prevalent among women of childbearing age and pregnant women in Norway (Abel et al. 2018; Dahl et al. 2018). In a recent Norwegian study, only 4% of the included pregnant women (N = 2,910) reached the World Health Organization (WHO) recommended iodine intake of 250 μg/day (WHO 2007), and low iodine status was related to altered thyroid function with particularly increased plasma levels of free T3 (Abel et al. 2018). As both thyroid disrupting chemicals, such as phthalates, and iodine deficiency might alter thyroid hormone biosynthesis and metabolism (Ghassabian and Trasande 2018; Mughal et al. 2018; Obregon et al. 2005), interactions among these factors are biologically plausible. It has been hypothesised that iodine deficiency causes the maternal thyroid gland to be more susceptible to effects of thyroid disrupting chemicals (Preau et al. 2015; Roman et al. 2013), which has been found in a few experimental and epidemiologic studies (Blount et al. 2006; Giray et al. 2010; Medda et al. 2017; Steinmaus et al. 2013). However, large scale studies of thyroid toxicants and iodine have not been conducted. More evidence is needed to understand whether key nutrient insufficiencies may enhance vulnerability to thyroid disrupting chemicals.

Although a number of previous human studies report relationships between phthalates and thyroid hormones during pregnancy (Cathey et al. 2019; Gao et al. 2017; Huang et al. 2018; Huang et al. 2007; Huang et al. 2016; Johns et al. 2016; Johns et al. 2015; Kuo et al. 2015; Romano et al. 2018; Yao et al. 2016), which is supported by findings in experimental studies (Breous et al. 2005; Du et al. 2018; Ghisari and Bonefeld-Jorgensen 2009; Ishihara et al. 2003; Kashiwagi et al. 2009; Liu et al. 2015; Shen et al. 2009; Sun et al. 2018; Wenzel et al. 2005; Ye et al. 2017; Zhai et al. 2014; Zoeller 2005), studies have been inconsistent as to the specific implicated phthalate and the shape of the phthalate-thyroid function relationship. Seven of the ten studies had small sample sizes (N < 500), and only one considered the complex mixture of inter-correlated phthalates in the population (Romano et al. 2018). Furthermore, although one study assessed the effect of adjusting for iodine (Romano et al. 2018), none of these previous human or experimental studies investigated whether iodine status modifies the relationship between phthalates and thyroid function during pregnancy.

Thus, in the present study we aimed to assess the individual and joint associations of phthalates with circulating levels of thyroid function biomarkers in pregnant women, using a large, nested case-cohort study of the Norwegian Mother, Father and Child Cohort Study (MoBa), and explore the extent to which these associations were modified by dietary iodine intake among pregnant women.

2. Methods

2.1. The Norwegian Mother, Father and child cohort study (MoBa)

MoBa is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 41% of the pregnancies. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers (Magnus et al. 2016; Magnus et al. 2006). Women were recruited to participate prior to their first ultrasound appointment (around 17 weeks' gestation). Consenting women completed two general background and health questionnaires during pregnancy (17 and 30 weeks' gestation) and a food frequency questionnaire at 22 weeks' gestation (Magnus et al. 2016). Mothers returned questionnaires at regular intervals after birth of the child. Blood samples were taken from both parents, and urine from the women at around 17 weeks' gestation, and blood were taken from mothers and children (umbilical cord) at birth (Magnus et al. 2016; Paltiel et al. 2014). MoBa is also linked to the Medical Birth Registry of Norway (MBRN), providing information on pregnancy and birth records.

2.2. Study population

This was a secondary analysis of data derived from two nested Attention-deficit/hyperactivity disorder (ADHD) case-cohort studies of MoBa (Fig. 1; Figure S1). In these studies, mothers of cases with elevated ADHD symptoms from the preschool ADHD sub-study (Overgaard et al. 2018) or childhood ADHD diagnosis identified by linkage to the Norwegian Patient Registry (NPR) (Engel et al., 2018), along with a randomly sampled subset of the eligible cohort (i.e. control sample; N = 556) (Engel et al., 2018) were included. In our study, we excluded 21 women lacking measurements of all thyroid function biomarkers in plasma or measurements of all phthalate metabolites or specific gravity in urine. Thus, the final study sample (N = 1,072) included the mothers of 534 cases and 538 MoBa controls (Fig. 1). In section 2.7 below, we describe how oversampling for ADHD (preschool and NPR sub-studies) was accounted for in statistical analyses. A more detailed description of the two ADHD case-cohort samples can be found in Supplemental information (Appendix 1; Figure S1).

2.3. Covariates

Covariates were obtained from prenatal maternal MoBa questionnaires and MBRN. Questionnaire-based estimation of dietary intake

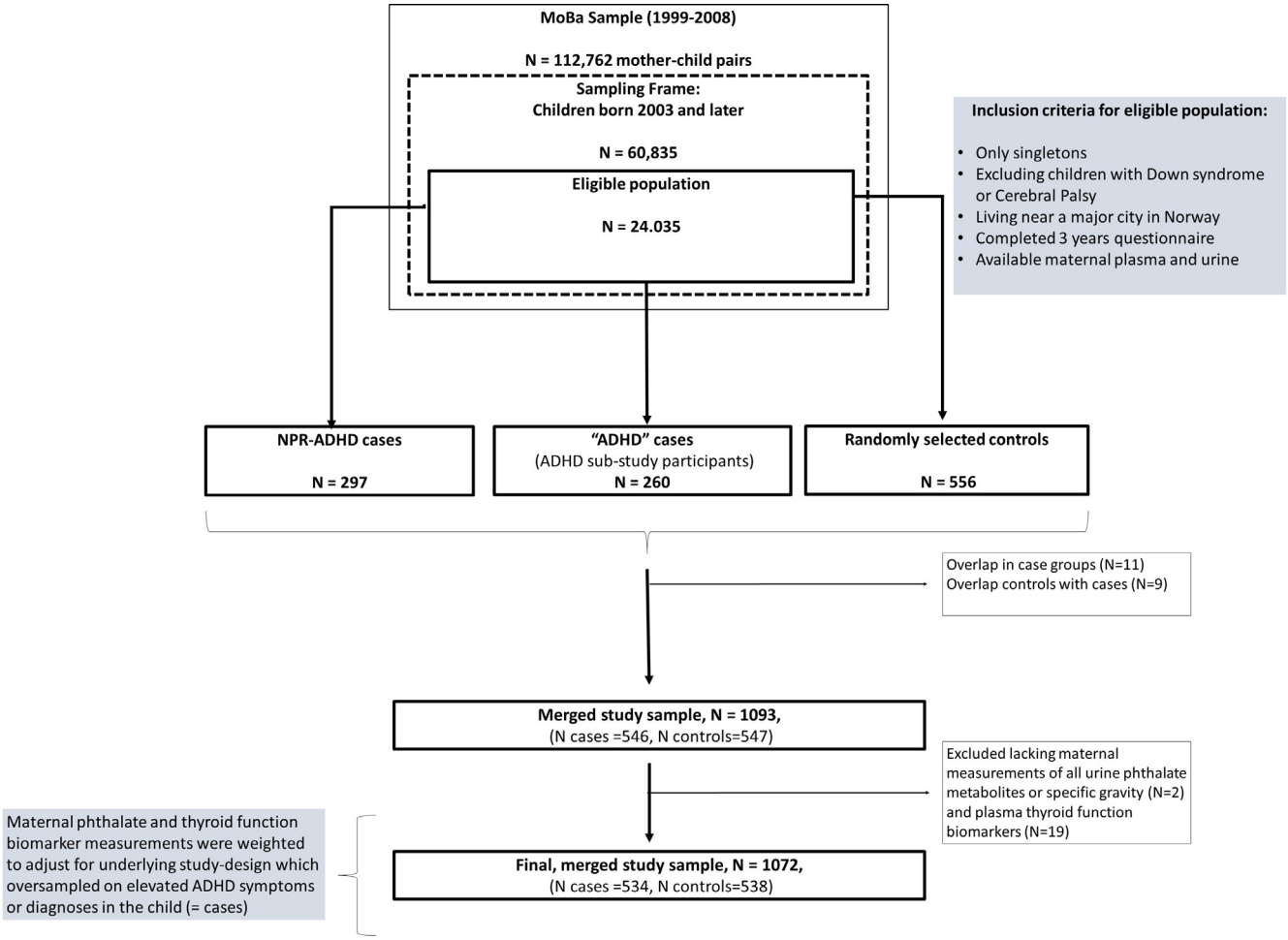

Fig. 1. Flow-chart of sampled study population from The Norwegian Mother, Father and Child Cohort Study (MoBa).

was obtained by food frequency questionnaire (FFQ) that participants completed at 22 weeks' gestation. The FFQ asks about usual dietary intake since becoming pregnant (Brantsaeter et al. 2008; Meltzer et al. 2008). Estimates of dietary iodine intake were validated against 24-hour urine iodine excretion and a 4-days weighted food diary in MoBa (Brantsaeter et al. 2008; Brantsaeter et al. 2007). Iodine intake was dichotomized as recommended by Brantsaeter et al. (2008), and we chose a cut-off at 150 μg/day, which is just below the average requirement in pregnant women (Alexander et al. 2017; Nordic Council of Ministers 2014). Given that MoBa only collected one spot-urine during pregnancy and there is substantial within and across day variability in urinary iodine excretion (Soldin 2002), habitual dietary intake is the best measure of iodine status in MoBa women (Abel et al. 2018). Maternal age at delivery, preexisting thyroid disease, year of birth of the child, infant sex and parity were obtained by linkage with MBRN. Self-reported smoking in the first or second trimester, pre-pregnancy maternal body mass index (BMI, kg/m^2), preexisting thyroid disease, intake of thyroid medication, and maternal education were obtained from the first MoBa questionnaire, which was completed at approximately 17 weeks' gestation.

2.4. Determination of thyroid function biomarkers in plasma

We measured levels of thyroid function biomarkers in plasma sampled from pregnant MoBa women at approximately week 17 of pregnancy. Blood were collected in ethylenediamin-etetraacetic acid (EDTA) sample tubes, and shipped overnight to a central biorepository and separated to plasma which was stored as 930 μl aliquots in 1 mL cryotubes at −80 °C. The plasma samples were transferred to 5 mL sampling tubes and shipped frozen on dry-ice to Arup laboratories (Salt Lake City, Utah, USA) for analyses of the following biomarkers: Total T4 (TT4), total T3 (TT3), thyroid stimulating hormone (TSH), T3-uptake and Thyroid peroxidase antibody (TPO-Ab). The analytical methods and quality have been described elsewhere (Engel et al. 2018; Villanger et al. 2017). Briefly, TT3, TT4, T3-uptake and TPO-Ab were measured using a quantitative electrochemiluminescent immunoassay, TSH was measured using chemiluminescent immunoassay and all thyroid function biomarkers were quantified on a Roche Cobas e602. The inter- and intra-assay coefficients of variation were $< 5\%$ for TT3, TT4, TSH, and T3-uptake and $< 7\%$ for TPO-Ab. T3-uptake is an indirect measure of number of unoccupied binding sites at thyroxin-binding globulin (TBG), and can be used to calculate indices of free levels of T4 and T3 in plasma. Contrary to immunoassay, quantification of free thyroid hormone concentrations by these indices will not be confounded by pregnancy related factors such as increased TBG levels (Lee et al. 2009; Thienpont et al. 2013). By using T3-uptake to calculate free T3 and T4 indices (fT3i and fT4i, respectively), we have previously demonstrated excellent reliability (Villanger et al. 2017). Thus, we calculated fT3i and fT4i based on T3-uptake using the following equation according to Dunlap (1990) (Eq. (1)):

$$\mathrm{fT4i(orfT3i)} = \mathrm{TT4(orTT3)} \times \mathrm{T3} - \mathrm{uptake(\%)/28(\%)} \quad (1)$$

where 28 was the median T3-uptake in a set of random control samples included in the analytical runs.

There were four individuals with TSH values below the limit of detection (0.01 mU/L). These values were replaced by limit of detection

divided by the square root of two (Lubin et al. 2004). Approximately 100 people were missing TPO-Ab due to levels below the limit of detection (< 0.3 mIU/L), but this variable was dichotomized at 9 mIU/L, and therefore these values were included in the reference category (< 9 mIU/L). Slight differences in sample size across thyroid biomarkers is due to the lack of sufficient peripheral blood plasma to perform all thyroid biomarker analyses for some subjects.

The participants were not fasting and time of plasma collection was variable throughout the day. Our sampling procedure is in line with clinical measurement of thyroid hormones, which does not require fasting nor a specific time of day. Also, a recent study found that time of day was not associated with a change in the reference interval for fT4 among women, or with TSH within daytime hours (when our participants' plasma was sampled) (Ehrenkranz et al. 2015). Thus, it is unlikely that the sampling procedure affected measurements of thyroid function biomarkers.

Second trimester reference ranges for TSH (0.19–4.06 mU/L) and TPO-Ab (0–9.0 mIU/L) were provided by the analytical laboratory. Internal reference ranges were calculated for TT4, TT3, fT4i, and fT3i using the 2.5–97.5th percentiles in the control population, excluding women who reported previously diagnosed thyroid disorders or taking thyroid medications, or with TPO-Ab positivity (TPO-Ab > 9 mIU/L), in accordance with the 2017 Guidelines of the American Thyroid Association (Alexander et al. 2017). Participants were grouped into normal or thyroid dysfunction (clinical and sub-clinical) subgroups in accordance with the definitions described in Lazarus et al. (2014).

2.5. Determination of phthalates metabolites in urine

Urine from pregnant MoBa women was collected as spot-urine samples at the same time as blood sampling during the routine ultrasound appointment (approximately 17 weeks' gestation). The urine sample was collected in urine containers with added preservatives to prevent bacterial growth (chlorhexidine plus ethyl paraben and sodium propionate) (UAP Vacutainers; Becton-Dickinson) (Ronningen et al. 2006). In a previous study in MoBa, this preservative did not show any impact on the measurements of phthalate metabolites (Hoppin et al. 2006). The samples were shipped overnight at ambient temperature to the MoBa Biobank in Oslo where they were aliquoted into 1 mL cryotubes and frozen at −80 °C.

Urine analyses were conducted at the Norwegian Institute of Public Health (Oslo, Norway) using on-line column switching liquid chromatography coupled with tandem mass spectrometry. For a detailed description of analytical methods, see previous studies (Engel et al. 2018; Sabaredzovic et al. 2015). Briefly, we analysed 12 phthalate metabolites in the urine samples as listed in Table S1 together with limit of quantification (LOQ) for each metabolite. The relative standard deviation for internal and external controls was below 16% for all the metabolites. Specific gravity (SG_j) for each participant (j), as a measure of urine dilution, was measured using a pocket refractometer (PAL-10S) from Atogo and used to standardise phthalate metabolite concentration (P^*_{ij}) following the approach described in Hauser et al. (2004) and in Engel et al. (2018), by the following equation (Equation (2)):

$$P^*_{ij} = P_{ij} \times (c/SG_j - 1), \quad (2)$$

where c represents the common normalizing constant which was calculated as the geometric mean of specific gravity among all participants minus 1. Additionally, the phthalate concentrations were adjusted to placement of each individual sample in the analytical batch using Ratio-G batch adjustment method with a scaled variation (Luo et al. 2010) as described in Engel et al. (2018). Due to insufficient urine volume from one subject, specific gravity could not be measured, and thus the specific gravity adjusted metabolite concentrations could not be calculated. In another subject, due to analytical interference, the urinary phthalate metabolite levels could not be obtained. Phthalates concentrations for these two subjects were omitted (Fig. 1). The specific gravity and batch adjusted phthalate metabolite concentrations were used in statistical analyses, and are presented in all tables and figures. All initial phthalate measurement were above LOQ, however, after specific gravity and batch adjustment, some of the lower concentrations fell below the analytic LOQ, explaining the minor difference in samples size for a few metabolites.

Following specific gravity and batch adjustments of all phthalate metabolites, the molar sums of di-2-ethylhexyl phthalate (DEHP) and di-*iso*-nonyl phthalate (DiNP) metabolites were computed (hereafter referred to as ΣDEHP and ΣDiNP, respectively). Each phthalate metabolite was first converted from μg/L to μmol/L by dividing it by its molecular mass. After conversion, the metabolites were then summed to produce ΣDEHP and ΣDiNP measures in μmol/L.

2.6. Ethics

The establishment and data collection in MoBa was previously based on a licence from the Norwegian Data Protection Agency and approval from The Regional Committee for Medical Research Ethics, and it is now based on regulations related to the Norwegian Health Registry Act. Parents enrolled in MoBa have given written consent for the use of this data. The linkage between NPR and MoBa identifying ADHD diagnosis cases has also been approved by NPR. Written consent has been given by parents with children participating in the MoBa ADHD sub-study. Finally, this study was approved by The Regional Committee for Medical Research Ethics (ref. nu. 2012/985-1).

2.7. Statistical analyses

The current analysis is based on version 9 of the MoBa quality assured data files. For all analyses we used R statistical software (R 2016). To achieve normally distributed residuals, the square root of the thyroid function biomarkers (TT3, TT4, TSH, fT3i and fT4i) was used in all models. Since about 100 participants were missing TPO-Ab (below limit of detection), this variable was dichotomised as TPO-Ab positivity (> 9 mIU/L) or negativity (≤9 mIU/L), where the latter category also included those with TPO-Ab below limit of detection. To be consistent with previous studies, phthalate metabolite concentrations were natural log-transformed for all statistical analyses. Due to unusual low phthalate metabolite concentrations in one participant, these lower outliers were removed before statistical modelling.

We investigated the correlation pattern of phthalates metabolites by Pearson correlation. Then we conducted factor analysis with varimax rotation of centred and scaled (to variance 1) phthalate metabolites and molar group sum concentrations. This was done in order to reduce dimensionality of the dataset into a smaller number of uncorrelated factors explaining the correlation patterns of the phthalate metabolite exposure. To assess the joint influence of the correlated phthalates on thyroid function, we performed multivariate multiple regression using scores of the chosen factors as predictors (co-adjusted) for levels of thyroid function biomarkers, mutually adjusted for phthalate factors. For TPO-Ab as the outcome, we performed multivariate multiple logistic regression using the chosen factors (co-adjusted) as predictors. Although there are many available statistical methods to assess effects of complex contaminant mixtures, the method of choice will depend on the research goal and type of contaminant mixture. We chose factor analyses because it combines highly correlated variables in a way that maximally preserves the variability present in the entire group of variables. As such it fitted with our research goal to assess joint effect of phthalates that tend to travel together by a common source. Thus, inclusion of the factor scores in a regression analysis allows us to get a better sense of the joint effect of the mixture of correlated exposures without zeroing out the effects of many components, which can be the case for other supervised methods. Based on literature-informed a priori directed acyclic graphs, these models were adjusted for binary iodine

intake estimated from the food frequency questionnaire (dichotomized ≥ 150 or < 150 μg/day), maternal age, maternal smoking during pregnancy, and parity. We also assessed whether iodine intake was an effect measure modifier of the association between the phthalate factors and thyroid function biomarkers by including interaction terms between iodine (dichotomized ≥ 150 or < 150 μg/day) and the factors. As a sensitivity analysis, we additionally adjusted for maternal pre-pregnancy BMI and year of birth of the child in the factor models. Furthermore, as sensitivity analyses, we investigated the factor models in a population restricted to MoBa participants who were randomly sampled to be in the sub-cohort and where the child did not have ADHD case status (i.e. the control sample, N = 538), to ensure that results were not driven by mothers of these cases. We investigated if the factor models with iodine interaction could be driven by thyroid disease in the participants by restricting analyses to the euthyroid population (N = 963) as a sensitivity analyses.

We further investigated the exposure–response relationships and potential non-linearity between concentrations of individual phthalate metabolites and thyroid function biomarkers, which cannot be inferred from associations with factor scores. To do so, we fit separate models for each thyroid function biomarker-phthalate combination, using individual phthalate metabolites or molar group sums as a categorical predictor, with quintile scores acting as category boundaries in multivariable adjusted regression models. Next, we modelled each thyroid function biomarker-phthalate metabolite combination using a restricted cubic spline on the phthalate (with knots at the 20th, 40th, 60th and 80th percentiles of the phthalates distribution), and we assessed whether the functional form of the relationship significantly differed from linearity using Wald test (significance at $p \leq 0.05$). Both the quintile and the restricted cubic spline models were adjusted for binary iodine intake (dichotomized ≥ 150 or < 150 ug/day), maternal age, maternal smoking during pregnancy, and parity.

For all analyses, inverse probability weights based on the child's ADHD case-status were used to account for confounding due to the original case-cohort design of the study population and thus increase generalisability of the result for the population of pregnant women in MoBa. All regression models were expressed with estimated regression coefficient (b) or odds ratio (OR) and accompanying 95% confidence intervals (CIs).

3. Results

Characteristics of women included in this study are presented in Table 1 and in Table S2 (subdivided into original case-control subgroups). Notably, only ~30% of the study population had iodine intake that can be characterised as sufficient (≥150 μg/day). Most women in our study (93%) had normal thyroid function (Table 1).

The distribution of phthalate metabolites (corrected for geometric mean of specific gravity and batch variation) in urine, and the distribution of thyroid function biomarkers in plasma are presented in Table 2. Table S3 presents the weighted phthalate metabolite and thyroid function biomarker concentrations, while Table S4 presents the weighted thyroid function biomarker results by population characteristics. Even though all initial phthalate measurement were above LOQ, after adjustment for batch and specific gravity, some of the lower concentrations fell below the analytic LOQ. Also, some subjects lacked sufficient peripheral blood plasma to perform all thyroid biomarker analyses. Thus, sample sizes vary accordingly (Table 2; Table S3). Monoethyl phthalate (MEP) was quantitatively the most dominating phthalate metabolite in the urine samples, while metabolites of DiNP had the lowest concentrations (Table 2; Table S3).

The correlation pattern among the phthalate metabolites are presented in correlation heat maps in Figure S2, and we found a clear and strong correlation structure. Metabolites originating from the same parent-phthalate were typically highly correlated (Figure S2A), such as metabolites of DEHP and those of DiNP, which also justified collapsing these single metabolites into their respective group sums on molar weight (ΣDEHP and ΣDiNP). In Figure S2B there were mainly two groups of highly correlated phthalates; metabolites mono-*iso*-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP) (r = 0.51–0.64) as one group and ΣDEHP and ΣDiNP (r = 0.41) as another. These two groups were also extracted as factor 1 and 2, respectively, in the factor analysis with high loadings in each respective factors (Fig. 2); MnBP, MBzP and MiBP had high loadings on factor 1 (0.665–0.818) while ΣDiNP and ΣDEHP had high loadings on factor 2 (0.547 and 0.752, respectively). Factors 1 and 2 explained 29.6% and 15.2% of the variation in the phthalate dataset, respectively. MEP did not correlate with any of the other metabolites (|r| = 0.01–0.19; Figure S2), and had low loadings on both factors (Fig. 2). Although the scree plot was inconclusive about the appropriate number of factors to model (see Figure S3), we chose a two-factor model because it effectively captured the main correlation structure among the phthalate metabolites.

Using the scores for factor 1 and 2 as the independent variables (co-adjusted) in a multivariable adjusted multivariate multiple regression model, predicting the levels of each thyroid function biomarker and TPO-Ab positivity (logistic regression), we found that factor 1

Table 1
Characteristics of the study population (N = 1,072).

Variable	Total N	Mean or N	SD or %
Maternal Age at Delivery (Years) (Mean, SD)	1068	30.24	4.48
Maternal Pre-Pregnancy BMI (kg/m^2) (Mean, SD)	1017	24.19	4.74
Maternal Education			
< College (N, %)	1036	359	34.65
College (N, %)	1036	406	39.19
> College (N, %)	1036	245	23.65
Other (N, %)	1036	26	2.51
Any smoking in 1st or 2nd Trimester (N, %)	1039	223	21.46
Iodine intake in pregnancy ≥ 150 μg/day (N, %)	1021	313	30.66
Primparous (N, %)	1068	544	50.94
Infant sex; Boy (N, %)	1068	609	57.02
Year of Birth			
2003–2004 (N, %)	1072	204	19.03
2005 (N, %)	1072	268	25.00
2006 (N, %)	1072	320	29.85
2007–2008 (N, %)	1072	280	26.12
Preexisting maternal thyroid condition	1072	33	3.08
Maternal thyroid medication during pregnancy	1072	21	1.96
TPO-Ab positive[1]	1072	94	8.77
Maternal thyroid conditions[2]			
Normal	1060	986	93.02
Overt Hypothyroidsm	1060	1	0.09
Subclinical Hypothyroidsm	1060	9	0.85
Hypothyroxinemia	1060	26	2.45
Over hyperthyroidsm	1060	3	0.28
Subclinical Hyperthyroidsm	1060	7	0.66
Hyperthyroxinemia	1060	28	2.64

[1] Thyroid peroxidase antibody (TPO-Ab) positive women were defined as having TPO-Ab concentrations above reference range (Alexander et al. 2017). Based on pregnancy 2nd trimester reference range (0.0–9.0 mIU/L) provided by the analytical laboratory, women with levels > 9 mIU/L were TPO-Ab positive.

[2] Clinical definition of thyroid conditions are based on measured biomarkers and laboratory based or calculated reference ranges in accordance with Lazarus et al. (2014); overt hypothyroidism includes individuals with TSH above the reference range, and fT4i below the reference range; subclinical hypothyroidism includes individuals with TSH above the reference range, fT4i within the reference range, and TPO-Ab above 9 mIU/L; hypothyroxinemia includes individuals with TSH in the reference range and fT4i below the reference range; overt hyperthyroidism includes individuals with TSH below the reference range and fT4i above the reference range; subclinical hyperthyroidism includes individuals with TSH below the reference range and fT4i in the reference range; and hyperthyroxinemia includes individuals with TSH in the reference range, and fT4i above the reference range.

Table 2
Distribution of urinary phthalate metabolites and plasma thyroid function biomarkers in pregnant women.

	N	Mean	Min	20%	40%	50%	60%	80%	Max	
Phthalate metabolites[1]										
MEP µg/L	1,071	319	0.05	29.0	68.0	105	162	425	7,532	
MiBP µg/L	1,072	29.8	0.02	9.25	14.7	18.4	22.5	38.2	562	
MnBP µg/L	1,072	96.3	0.03	10.9	16.0	19.9	24.4	37.5	70,164	
MBzP µg/L	1,072	8.88	0.00	2.46	3.88	5.06	6.37	11.5	151	
ΣDEHP µmol/L	1,072	0.43	0.00	0.17	0.22	0.25	0.28	0.41	22.4	
ΣDiNP µmol/L	1,071	0.03	0.00	0.01	0.02	0.02	0.02	0.03	1.07	
Thyroid function biomarkers										Reference range[2]
TSH (mU/L)	1,072	1.80	0.01	1.10	1.50	1.60	1.90	2.40	17.3	0.19–4.06
TT3 (ng/dL)	1,068	170	92.0	145	161	167	174	182	284	118–228
TT4 (µg/dL)	1,069	10.6	6.10	9.20	10.1	10.5	10.9	11.9	18.1	7.72–14.0
fT3i	1,060	168	108	147	160	166	171	187	286	122–219
fT4i	1,060	10.5	5.80	9.10	9.90	10.3	10.8	11.9	21.7	7.73–14.0

Note. Concentrations of phthalate metabolites and thyroid function biomarkers are expressed to three significant digits. For TSH, four missing values were replaced with limit of detection divided by the square root of 2. Differences in sample size across thyroid biomarkers is due to the lack of sufficient peripheral blood plasma to perform all thyroid biomarker analyses for some subjects. The differences in N across some phthalates is due to concentrations below limit of quantification. Thyroid stimulating hormone (TSH), Total triiodothyronine (TT3), Total thyroxine (TT4), free T4 index (fT4i), free T3 index (fT3i), Monoethyl phthalate (MEP), mono-*iso*-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), di-2-ethylhexyl phthalate (DEHP), di-*iso*-nonyl phthalate (DiNP).

[1] Adjusted for batch and specific gravity. [2] We calculated internal reference ranges for T4, T3, fT4i, fT3i using the 2.5–97.5th percentiles in our MoBa control population, excluding women who report previously diagnosed thyroid disorders, those that reported taking thyroid medications, and those with Thyroid peroxidase antibody (TPO-Ab) > 9 mIU/L (Alexander et al. 2017). For TSH and TPO-Ab (0.0–9.0 mIU/L), the reference range for 2nd trimester was provided by the ARUP laboratories.

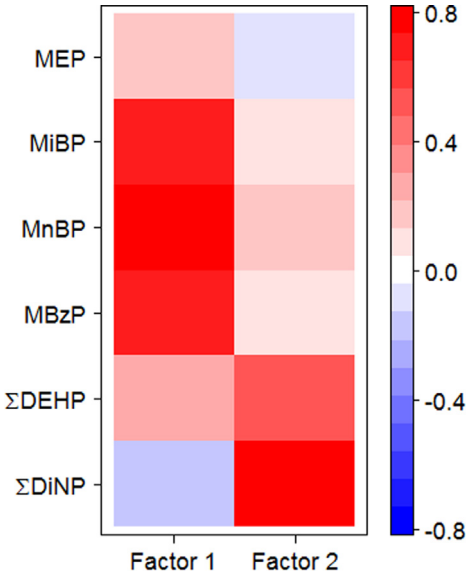

Fig. 2. Factor Loadings for phthalates. To investigate a multi-pollutant model, we estimated factor loadings using a maximum likelihood exploratory factor analysis with varimax rotation of log-transformed phthalates metabolites. The first factor accounted for 29.6% of variation, and the second factor for 15.2%.

(explained mainly by MnBP, MBzP, and MiBP) was positively associated with TT3 (b = 0.18, 95% CI 0.08, 0.27; Table 3) and fT3i (b = 0.13, 95% CI 0.05, 0.21; Table 3). In sensitivity analyses, these associations were robust when restricted to the control sample (N = 538; TT3: b = 0.17, 95% CI 0.07, 0.27; fT3i: b = 0.13, 95% CI 0.04, 0.21; Table S6) and the euthyroid population (N = 963; TT3: b = 0.18, 95% CI 0.08, 0.27; fT3i: b = 0.14, 95% CI 0.06, 0.22; Table S9) and additional adjustment for child birth year (TT3: b = 0.17, 95% CI 0.08, 0.26; fT3i: b = 0.12, 95% CI 0.04, 0.20; Table S7), but only the relationship between factor 1 and TT3 remained significant when pre-pregnancy BMI was added to the adjustment set (b = 0.10, 95% CI 0.01, 0.18; Table S8). Furthermore, the significant associations between factor 1 and TT3 and fT3i appeared to be somewhat stronger among women with low iodine intake (< 150 µg/day) (TT3: b = 0.20, 95% CI 0.09, 0.31; fT3i: b = 0.15, 95% CI 0.06, 0.25; Table 3), although the interaction with iodine intake was above our *a priori* threshold of $p \leq 0.05$ (*p*-interaction = 0.30; Table 3). Restricting analyses to the euthyroid populations did not alter the latter results (Table S9). Iodine intake significantly modified associations between factor 1 and TSH (*p*-interaction = 0.04), TT4 (*p*-interaction = 0.03), and fT4i (*p*-interaction = 0.03) with negative associations between factor 1 and fT4i (b = -0.06, 95% CI −0.11, −0.01) and TT4 (b = -0.05, 95% CI −0.10, −0.01) and positive association with TSH (b = 0.06, 95% CI −0.00, 0.12) only among women in the high iodine intake group (≥150 µg/day) (Table 3), however these relationships attenuated when restricted to the euthyroid population with only the effect modification for the association between factor 1 and TSH remaining significant (*p*-interaction = 0.05; ≥150 µg/day: b = 0.05, 95% CI −0.01, 0.11; Table S9).

Factor 2 (mainly explained by DEHP and DiNP metabolites) was negatively associated with TT3 (b = -0.11, 95% CI −0.22, −0.01; Table 3) and fT3i levels (b = -0.11, 95% CI −0.24, −0.01; Table 3). As with factor 1, the observed associations between factor 2 and TT3 and fT3i were the same when analyses were restricted to the control population (TT3: b = -0.11, 95% CI −0.22, −0.01; fT3i: b = -0.11, 95% CI −0.20, −0.01; Table S6) and additional adjustment for child birth year (TT3: b = -0.11, 95% CI −0.22, −0.01; fT3i: b = -0.11, 95% CI −0.20, −0.02; Table S7), but were attenuated somewhat by the adjustment for pre-pregnancy BMI (TT3: b = -0.06, 95% CI −0.15, 0.03; fT3i: b = -0.07, 95% CI −0.15, 0.02; Table S8) and when restricted to the euthyroid population (TT3: b = -0.09, 95% CI −0.20, 0.02; fT3i: b = -0.09, 95% CI −0.19, 0.00; Table S9). There was no significant effect modification by iodine intake for associations between factor 2 and TT3 and fT3i (*p*-interactions = 0.86 and 0.48, respectively). Moreover, the 95% CIs for these estimates crossed the null in both iodine intake strata (Table 3).

Because MEP loaded poorly on both factor 1 and factor 2, we ran a separate linear regression model with urinary MEP concentration as predictor for thyroid biomarker concentrations and TPO-Ab positivity, using same covariate adjustments set as for the factor models as well as investigating effect modification by iodine intake (Table 4). There were no evidence of associations between MEP and thyroid function biomarkers nor effect modification by iodine intake during pregnancy (Table 4).

The results of multivariable adjusted quintile models for individual

Table 3
Phthalate factors by thyroid hormone regression models and with iodine interaction terms.

	TSH			TT3			fT3i			TT4			fT4i			TPO-Ab positivity		
	b	95% CI		b	95% CI		b	95% CI		b	95% CI		b	95% CI		OR	95% CI	
Factor 1	0.01	−0.02,0.04		0.18	0.08,0.27		0.13	0.05,0.21		−0.01	−0.03,0.01		−0.02	−0.04,0.01		0.89	0.67,1.17	
Interaction			***p***			***p***			***p***			***p***			***p***				***p***
Iodine < 150	−0.02	−0.04,0.02	0.04	0.20	0.09,0.31	0.30	0.15	0.06,0.25	0.30	0.01	−0.02,0.03	0.03	−0.00	−0.03,0.02	0.03	0.87	0.62,1.21	0.79
Iodine ≥ 150	0.06	−0.00,0.12		0.10	−0.05,0.25		0.06	−0.09,0.21		−0.05	−0.10, −0.01		−0.06	−0.11, −0.01		0.94	0.58,1.52	
Factor 2	0.02	−0.02,0.05		−0.11	−0.22, −0.01		−0.11	−0.24, −0.01		0.01	−0.01,0.03		0.01	−0.01,0.04		0.80	0.80,1.38	
Interaction			***p***			***p***			***p***			***p***			***p***				***p***
Iodine < 150	0.02	−0.02,0.06	0.82	−0.11	−0.25,0.03	0.86	−0.09	−0.20,0.02	0.48	0.01	−0.02,0.04	0.86	0.02	−0.01,0.05	0.52	1.20	0.87,1.67	0.25
Iodine ≥ 150	0.01	−0.07,0.09		−0.13	−0.28,0.03		−0.17	−0.35,0.02		0.01	−0.03,0.04		−0.00	−0.05,0.05		0.83	0.49,1.43	

Note. A separate regression model was conducted for each square-root transformed thyroid hormone outcome. TPO-Ab was dichotomised at 9 mIU/L, and logistic regression give the odds ratio (OR) with 95% confidence interval (CI) of being TPO-Ab positive (> 9 mIU/L), otherwise the regression models yield estimated beta (b) with 95% CI. The following phthalates most heavily loaded on Factor 1: MiBP, MnBP, MBzP. The following phthalates most heavily loaded on Factor 2: DEHP and DINP. All phthalates were log transformed and adjusted for batch and specific gravity before computing factors. Before the factor analysis, the lower outlier in all phthalates was removed. Each regression model was adjusted for: dichotomized iodine intake, maternal age, maternal smoking and parity. Inverse probability weights were used to account for the case-control design. The statistical significance of the interaction between iodine and each thyroid hormone is presented by the p values (significance at $p \leq 0.05$).

Table 4
Urinary MEP levels by thyroid hormone regression models and with iodine interaction terms.

	TSH			TT3			fT3i			TT4			fT4i			TPO-Ab positivity		
	b	95% CI		b	95% CI		b	95% CI		b	95% CI		b	95% CI		OR	95% CI	
MEP (μg/L)	−0.00	−0.02,0.01		0.04	−0.02,0.09		0.04	−0.01,0.08		−0.01	−0.02,0.00		−0.01	−0.03,0.00		0.90	0.76,1.06	
Interaction			***p***			***p***			***p***			***p***			***p***				***p***
Iodine < 150	−0.00	−0.02,0.01	0.85	0.04	−0.02,0.10	0.77	0.04	−0.01,0.10	0.64	−0.01	−0.03,0.01	0.86	−0.01	−0.03,0.01	0.70	0.94	0.77,1.15	0.42
Iodine ≥ 150	−0.00	−0.04,0.03		0.02	−0.08,0.13		0.02	−0.09,0.12		−0.01	−0.04,0.02		−0.02	−0.05,0.02		0.82	0.61,1.09	

Note. A separate regression model was conducted for each square-root transformed thyroid hormone outcome. TPO-Ab was dichotomised at 9 mIU/L, and logistic regression give the odds ratio (OR) with 95% confidence interval (CI) of being TPO-Ab positive (> 9 mIU/L), otherwise the regression models yield estimated beta (b) with 95% CI. MEP was log transformed and adjusted for batch and specific gravity, and the lowest outlier was removed before regression analyses. The regression model was adjusted for dichotomized iodine intake, maternal age, maternal smoking and parity. Inverse probability weights were used to account for the case-control design. The statistical significance of the interaction between iodine and each thyroid hormone is presented by the *p* values (significance at $p \leq 0.05$).

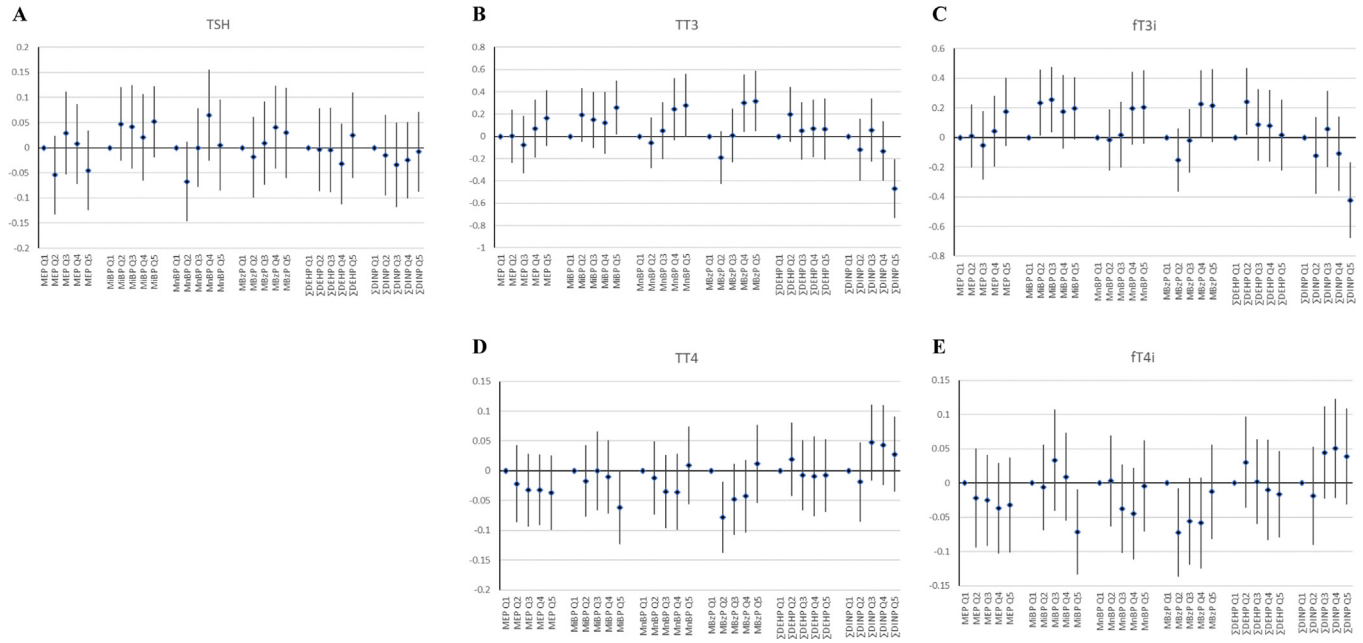

Fig. 3. Beta coefficients and 95% confidence intervals for regression models predicting thyroid hormone levels from quintile categories of each phthalate. Note. Each log-transformed phthalate by square-root transformed thyroid hormone combination was modelled using a separate linear regression. The beta coefficient and 95% confidence intervals for each phthalate quintile are represented on the vertical axis (the reference level was the first quintile). The lower outlier in all phthalates was removed before running these regression models. Each regression model was adjusted for: dichotomized iodine intake, maternal age, maternal smoking and parity. Inverse probability weights were used to account for the case-control design.

phthalate metabolite concentrations (or molar group sums) were largely in agreement with the factor models (Fig. 3; Table 3; Table S5). Also, MEP did not show any clear pattern of association with thyroid function biomarkers in quintile models similar to the findings in the regression model (Fig. 3; Table 4; Table S5). In the restricted cubic spline models the relationships between four thyroid function biomarker-phthalate combinations were significantly non-linear (Fig. 4; Table S5; Figure S4;); MBzP and fT3i (p = 0.01), MBzP and TT3 (p = 0.01), MBzP and TT4 (p = 0.05), as well as MnBP and TSH (p = 0.002). Notably, for the restricted cubic spline models the wide confidence intervals in the tails (1st and 5th quintiles), probably due to more widely distributed data points in the extremes, prevented any clear interpretation of relationships in these areas (Fig. 4). However, looking at the part of the cubic splines between the 1st and the 5th quintiles (i.e. the area between the tails), the confidence intervals are narrower and the trends more interpretable. When looking at these areas, MBzP's relationships with TT3 and fT3i, and MnBP's relationship with TSH seem to be characterised by a steep, linear increase in TT3 and fT3i with increasing MBzP or MnBP after a "threshold" concentration and then a flatting of the curve at the higher phthalate metabolite levels (Fig. 4). MBzP concentrations appear to have a U-shaped relationship with TT4; decreasing TT4 levels with increasing MBzP at lower MBzP levels and increasing TT4 with increasing MBzP at higher MBzP levels (Fig. 4).

4. Discussion

In this large, cross-sectional study of prenatal phthalate exposure and thyroid function biomarkers measured at mid-pregnancy, we found that factor 1 (mainly explained by MnBP, MiBP and MBzP) was significantly associated with increased TT3 and fT3i, and these positive associations appeared to be driven primarily by stronger associations in the iodine deficient population; whereas factor 2 (mainly explained by DEHP and DiNP metabolites) was associated with decreased TT3 and fT3i, although these latter associations attenuated somewhat in the euthyroid population. We also found significant effect measure modification by iodine intake in the relationship between factor 1 and TSH, TT4 and fT4i, in all cases suggestive of a hypothyroid pattern (higher TSH, lower TT4 and fT4i) among iodine sufficient participants, though these relationships attenuated when restricted to the euthyroid population. We did not find any evidence of associations between MEP and thyroid function biomarkers, nor effect modification by iodine intake. Our study is notable in that it accounts for co-exposure among correlated phthalate metabolites using factor analysis, and investigates effect measure modification by habitual dietary intake of iodine, a critical determinant of thyroid health.

4.1. Phthalate metabolite levels and patterns

The levels of urinary phthalate metabolites in the present study were consistent with previous results in Norwegian women (Giovanoulis et al. 2016; Haug et al. 2018; Sabaredzovic et al. 2015; Sakhi et al. 2017; Ye et al. 2009), and within ranges reported in studies of pregnant women worldwide (Haug et al. 2018; Katsikantami et al. 2016; Kim et al. 2019). The phthalate metabolites in this study loaded onto two factors that were very similar to the two principal components extracted in a recent study of urinary phthalate metabolites in Taiwanese children and adults (Huang et al. 2017). In both studies, MEP (a metabolite of diethyl phthalate, DEP) did not correlate with other metabolites. The factors we observed probably reflect metabolites with similar sources/exposure routes, physiochemical characteristics, and elimination half-life of their respective parent-compound. The metabolites that loaded on factor 1 (MiBP, MnBP, and MBzP) are metabolites of the low-molecular weight (LMW) phthalates which are used as additives in personal care products, dietary supplements, medication, printing inks, adhesives and more (Cao 2010; Wormuth et al. 2006). In Norwegian women, the use of personal care products is the most important determinant of urinary metabolites of LMW phthalates (Giovanoulis et al. 2016; Sakhi et al. 2014; Sakhi et al. 2017). The phthalates that loaded on factor 2 (DEHP and one of its replacements, DiNP) are high molecular weight (HMW) compounds mainly used in PVC consumer plastic products, and are the most prominent phthalates

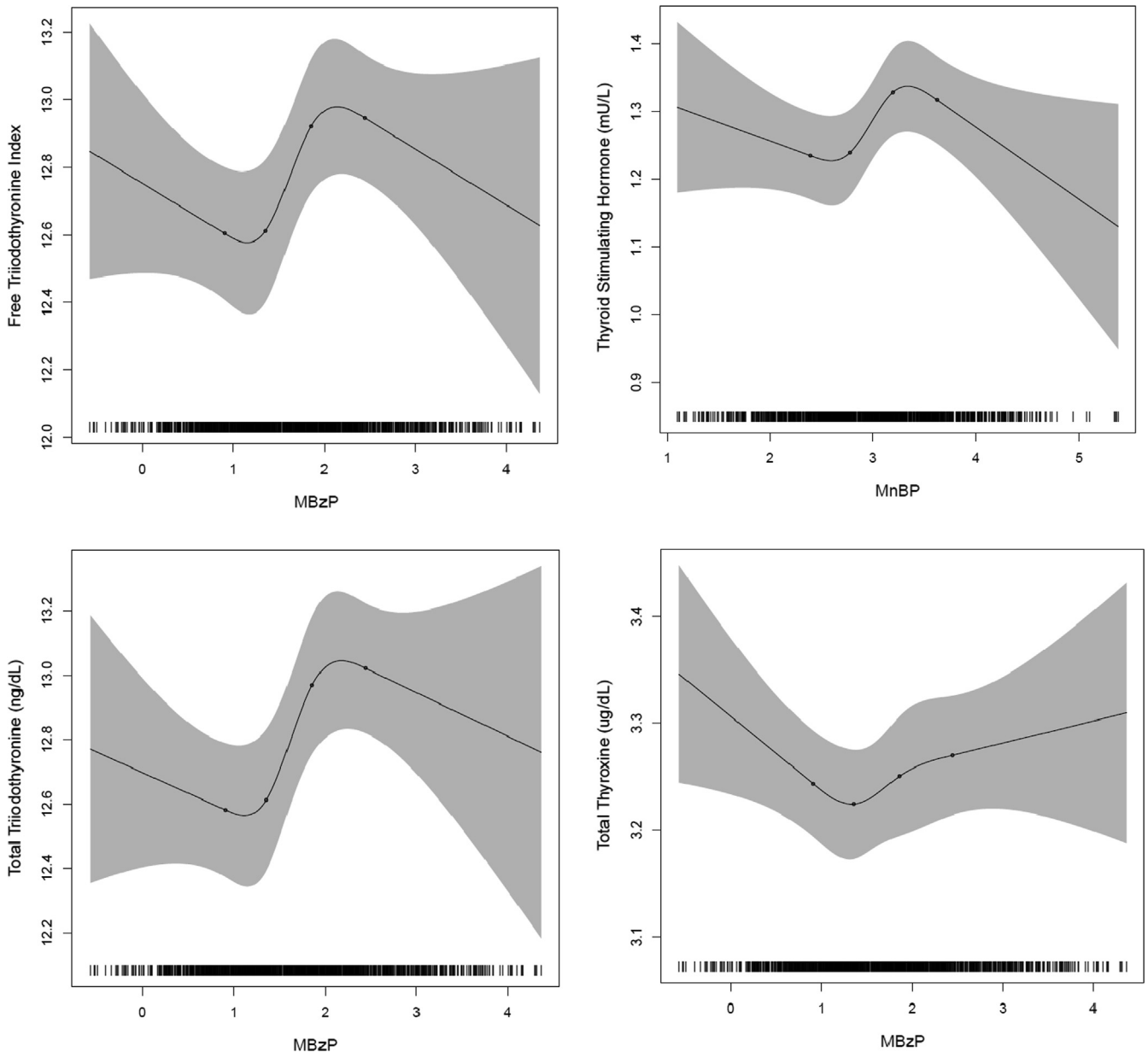

Fig. 4. Restricted cubic splines of phthalate by thyroid hormone combinations that were significantly non-linearly associated. Knots are at the 20th, 40th, 60th, and 80th quintiles for each phthalate. Shading reflects the 95% confidence intervals, and hashing along the horizontal axes represents the distribution of the phthalates. Phthalates were log-transformed, and were adjusted for batch and specific gravity. Before log transformations, MBzP and MnBP were measured in micrograms per litre. Outliers specific to each phthalate were excluded for these spline plots. The thyroid function biomarker concentrations were square-root transformed. Non-linearity was tested using Wald test (significance: $p \leq 0.05$).

found in food items in Norway (Sakhi et al. 2014).

4.2. Phthalate metabolites and thyroid function biomarkers

In previous studies of pregnant women, relationships between urinary phthalate metabolites and T4 and/or TSH have predominately been reported (Cathey et al. 2019; Huang et al. 2007; Huang et al. 2016; Johns et al. 2016; Johns et al. 2015; Kim et al. 2019; Romano et al. 2018; Yao et al. 2016) although the general picture of the phthalate metabolite relationship with thyroid function across studies is somewhat inconsistent (Table S10). We mainly observed associations with T3 biomarkers. A few previous studies have also observed associations with T3 biomarkers, though the direction of effect is variable (Cathey et al. 2019; Huang et al. 2018; Johns et al. 2016; Johns et al. 2015). The underlying biological mechanisms supporting phthalate induced thyroid dysregulation are not yet fully understood, however, emerging experimental *in vivo* and *in vitro* studies suggest that various phthalates may affect target points in the HPT-axis, such as iodine uptake in the thyroid gland, thyroid hormone synthesis, binding to thyroid hormone receptor or transport proteins in blood, biotransformation and excretion, and hypothalamic-pituitary control of thyroid hormone production (Breous et al. 2005; Du et al. 2018; Ghisari and Bonefeld-Jorgensen 2009; Ishihara et al. 2003; Kashiwagi et al. 2009; Liu et al. 2015; Shen et al. 2009; Sun et al. 2018; Wenzel et al. 2005; Ye et al. 2017; Zhai et al. 2014; Zoeller 2005). These studies indicate that phthalates may have multiple and possibly overlapping target points in the HPT-axis, sometimes acting as agonist or antagonist, and the result may not be easily predictable from a given phthalate mixture.

4.3. Influence of iodine status

In Norway, as in many other parts of the world, there is a high prevalence of mild-to-moderate iodine deficiency among pregnant women (Abel et al. 2018; Lazarus 2014; Pearce et al. 2013). In our study population, ~70% of the women had low iodine intake from food (here defined as < 150 μg/day; Table 1), which is below the estimated average requirement of 160 μg/day for pregnant women (Alexander et al. 2017; Nordic Council of Ministers 2014). We observed that the positive associations between factor 1 (mainly explained by MiBP, MnBP and MBzP) and TT3 and fT3i were stronger in women with a dietary iodine intake below 150 μg per day, although the interaction with iodine was not significant. This is in line with findings in a recent study of pregnant MoBa women, where low iodine intake (< 150 μg/day) was associated with increased plasma levels of fT3 (Abel et al, 2018), possibly explained by an autoregulatory response to lowered iodine availability where the thyroid gland will start to produce more T3 relative to T4, saving one iodine atom per molecule (Obregon et al. 2005; Pedraza et al. 2006). Experimental studies suggest that the sodium/iodine symporter (NIS), which mediates the active transport of iodine into the thyroid gland, is a target of several phthalates (Breous et al. 2005; Wenzel et al. 2005). Both thyroid hyperactivity and increased transcription (up-regulation) and expression of NIS have been reported for some phthalates, among them BBzP (of which MBzP is the metabolite) (Breous et al. 2005). For DEHP and DINP, however, there were no effects (Breous et al. 2005). DEHP have even showed an inhibitory effect on iodine transport in rats, with reduced levels of NIS and thyroglobulin, accompanied by lowered circulating concentrations of thyroid hormones (T3, T4) (Liu et al. 2015). In contrast to the positive relationships of MiBP, MnBP and MBzP (as part of factor 1) with TT3 and fT3i, we did not find evidence to suggest any influence of iodine on the negative relationship between DEHP (as part of factor 2) and TT3 and fT3i. Thus, we hypothesize that BBzP and similar LMW phthalates interfere with NIS-transport and iodine uptake in the thyroid gland, enhancing the autoregulatory response to low iodine intake. While this response ensures general euthyroidism, some tissues may become hypothyroid (Pedraza et al. 2006). An increased T3 production at the expense of T4 may thus jeopardize the availability and the critical *trans*-placental transfer and delivery of T4 from mother to fetus (de Escobar et al. 2007; Morreale de Escobar et al. 2004).

We observed a significant effect modification by iodine intake in the association of factor 1 with TSH, TT4 and fT4i, such that only among women in the high (sufficient) iodine intake group was this factor associated with increased TSH and decreased TT4 and FT4i, respectively, although these relationships attenuated when restricting the analyses to the euthyroid population. The reason why we find this only in women with sufficient iodine intake and attenuates in the euthyroid population is unclear. Overall, few studies have investigated how iodine affects the relationship between phthalates and thyroid function. A study by Mendez and Eftim (2012) did not find any relations between of urinary iodine concentrations and phthalates or thyroid function in a study of US adult men and non-pregnant women. Also, a recent study of pregnant women reported that measured urinary iodine did not alter the pattern of results of phthalate-thyroid function biomarkers when added as covariate (Romano et al. 2018). Difference in iodine status across study populations may be a contributing factor explaining the inconsistent results of phthalate-thyroid function relationships across studies of pregnant women (Table S10). Nonetheless, the steep increase in iodine requirement during pregnancy in order to increase thyroid hormone production to supply the developing fetus as well as the increased renal clearance of iodine (Alexander et al. 2017; Zimmermann 2012), can result in iodine-deficiency in pregnant sub-populations even though the general population is iodine-sufficient (Brantsaeter et al. 2013; Pearce et al. 2013). Altogether our results suggest that several of the phthalate-thyroid function biomarker relationships in pregnant women differ by habitual dietary iodine intake. Future studies should consider iodine as well as other nutritional factors that could influence thyroid health, such as selenium and iron intake, or dietary intake of cruciferous vegetables (O'Kane et al. 2018; Roman 2007).

4.4. Study limitations and strengths

This study has some limitations. First, we used a single urine sample for phthalate measurement and a single plasma sample for thyroid function biomarker measurement at mid-gestation, thus our results cannot necessarily be generalized to the full duration of pregnancy. However, phthalates are rapidly metabolized and excreted and therefore temporally linked samples may be most relevant to the investigation of thyroid disruption (Cathey et al. 2019; Morgenstern et al. 2017). Because we wanted to investigate possible underlying non-linear exposure–response relationships, and that phthalate metabolites load differently on the factors making factor splines difficult to interpret, we decided to investigate non-linearity with thyroid function biomarkers using only individual phthalate metabolite concentrations as predictors. It may be a limitation that we assumed only linearity in the factor models. Nevertheless, assuming linearity is a simplification that is made in the majority of the literature on phthalates and thyroid function (Cathey et al. 2019; Gao et al. 2017; Huang et al. 2018; Huang et al. 2007; Huang et al. 2016; Johns et al. 2016; Johns et al. 2015; Kim et al. 2019; Kuo et al. 2015; Morgenstern et al. 2017; Park et al. 2017; Romano et al. 2018; Weng et al. 2017; Yao et al. 2016). We used data from two case-cohort studies of ADHD, and there may be residual bias by the oversampling by child ADHD case status, or from factors that are strongly associated with ADHD. However, we accounted for the oversampling of ADHD case status by employing inverse probability weights to phthalate metabolites and thyroid function biomarkers, and adjusted for relevant confounders in our analysis. Also, sensitivity analyses restricted to only controls (i.e. non-cases) were in line with results employing inverse probability of selection weights. Lastly, based on the existing literature, BMI can be a consequence of, or an antecedent to, both thyroid function and phthalate exposure (Amouzegar et al. 2018; Hatch et al. 2008; Longhi and Radetti 2013; Radke et al. 2019; Stojanoska et al. 2017). Due to many uncertainties about the phthalate-BMI-thyroid function relationships, especially the directionality of relationships and thus the possibility of introducing bias due to over-adjusting or conditioning on a collider (Cole et al. 2010; Schisterman et al. 2009), we did not adjust for maternal BMI in our main analyses. Although the phthalate-thyroid function biomarker associations attenuated when we included BMI as a covariate in the factor regressions, it remains unclear whether this attenuation reflects an overall increase or decrease in bias.

Despite these limitations, our study has several strengths. Apart from being one of the largest studies of its kind, a major strength of the present study is that we investigated the phthalate-thyroid function relationship using factor analysis. This approach accounts for the correlations among phthalate metabolites, while maximally preserving the variability present in the entire group of exposures. Multicollinearity in the exposure variables often results in highly imprecise regression coefficient estimates, thereby obscuring the effects of single exposures. By using uncorrelated, mutually adjusted phthalate factors in multivariate multiple regression models in the present study, the results were more reliable and enabled us to investigate the joint influence of intercorrelated phthalates on thyroid function biomarkers. Second, our thyroid function biomarkers were previously validated for pregnant women (Villanger et al. 2017). And using free thyroid hormone indices is advantageous compared to immunoassays as it accounts for pregnancy-induced changes in levels of thyroid hormone binding proteins (Lee et al. 2009; Thienpont et al. 2013). Third, we leveraged a validated estimate of habitual iodine intake from food (Brantsaeter et al. 2008), and dichotomised this variable just below the average requirement in pregnant women (Alexander et al. 2017; Nordic Council of Ministers 2014), which enabled us to investigate effect measure modification by

sufficient and insufficient iodine intake, crucial determinants of thyroid health. Although the Norwegian population is exposed to relatively similar phthalate concentrations compared to other populations worldwide, the insufficient iodine intake in the pregnant sub-population may increase susceptibility to thyroid disruption, as some of our results indicated.

4.5. Public health implications

Our study population was sampled in 2003–2008, and since then, bans or regulations as well as classification and labelling concerning the use DEHP, BBzP and DBP and DiNP in consumer products, especially in those targeting children, have been implemented in Norway parallel to the European Union. Thus, the level and composition of population phthalate exposure is changing, underscoring the importance of continued monitoring and investigation of health effects of today's exposure levels.

Our restricted cubic spline models point to the possible existence of non-linear and even non-monotonic exposure–response relationships for some phthalate-thyroid function biomarker combinations, which is not uncommon for endocrine disrupting chemicals (Vandenberg 2014; WHO, UNEP 2013; Zoeller et al. 2012). The implication for public health is that low-dose effects are not necessarily extrapolated from high-dose effects typical of experimental studies (Lagarde et al. 2015; Vandenberg et al. 2012). Thus, future studies as well as chemical testing and health risk assessment should consider the potential for non-linear exposure–response relationships.

Characterizing phthalate exposure and the effect on thyroid function in pregnant women is particularly important because thyroid hormones (mainly T4) are transported over the placenta to supply the fetal compartment (Patel et al. 2011; Rovet and Willoughby 2010), and neurodevelopment has been shown to be sensitive to alterations in maternal thyroid hormone levels (Drover et al. 2019; Fetene et al. 2017; Haddow 1999; Moog et al. 2015; Morreale de Escobar et al. 2004; Pop et al. 2003; Rovet and Willoughby 2010; Thompson et al. 2018). Overall our results suggest that phthalate exposure affects thyroid function in a population of pregnant women with a predominant mild-to-moderate iodine deficiency. This homeostatic disturbance is thus of concern for both pregnancy health and child development.

5. Conclusion

Using a mixture approach we found that correlated phthalate exposures measured mid-pregnancy were associated with thyroid function biomarkers at the same time-point, mainly T3 markers, and that iodine status modified these associations. We also identified possible non-linear and non-monotonic associations when investigating exposure–response relationships of individual phthalate metabolites. Habitual iodine intake from food may affect some of the phthalate-thyroid function relationships in pregnant women, highlighting the importance of investigating the role of iodine in future studies as well as linking our findings to pregnancy and child health outcomes.

CRediT authorship contribution statement

Gro D. Villanger: Conceptualization, Visualization, Investigation, Writing - original draft, Writing - review & editing. **Samantha S.M. Drover:** Investigation, Writing - original draft, Writing - review & editing. **Rachel C. Nethery:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Cathrine Thomsen:** Resources, Methodology, Writing - original draft. **Amrit K. Sakhi:** Resources, Methodology, Writing - original draft. **Kristin R. Øvergaard:** Resources, Writing - original draft. **Pal Zeiner:** Resources, Writing - original draft. **Jane A. Hoppin:** Writing - original draft. **Ted Reichborn-Kjennerud:** Resources, Writing - original draft. **Heidi Aase:** Funding acquisition, Resources, Data curation, Writing - original draft. **Stephanie M. Engel:** Conceptualization, Visualization, Funding acquisition, Data curation, Writing - original draft, Writing - review & editing.

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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.2020.105509.

References

Abel, M.H., Korevaar, T., Erlund, I., Villanger, G.D., Caspersen, I.H., Arohonka, P., Alexander, J., Meltzer, H.M., Brantsaeter, A.L., 2018. Iodine intake is associated with thyroid function in mild-to moderately iodine deficient pregnant women. Thyroid. https://doi.org/10.1089/thy.2018.0305.

Alexander, E.K., Pearce, E.N., Brent, G.A., Brown, R.S., Chen, H., Dosiou, C., Grobman, W.A., Laurberg, P., Lazarus, J.H., Mandel, S.J., Peeters, R.P., Sullivan, S., 2017. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. Thyroid 27, 315–389. https://doi.org/10.1089/thy.2016.0457.

Amouzegar, A., Kazemian, E., Abdi, H., Mansournia, M.A., Bakhtiyari, M., Hosseini, M.S., Azizi, F., 2018. Association Between Thyroid Function and Development of Different Obesity Phenotypes in Euthyroid Adults: A Nine-Year Follow-Up. Thyroid 28, 458–464. https://doi.org/10.1089/thy.2017.0454.

Benjamin, S., Masai, E., Kamimura, N., Takahashi, K., Anderson, R.C., Faisal, P.A., 2017. Phthalates impact human health: Epidemiological evidences and plausible mechanism of action. J. Hazard Mater. 340, 360–383. https://doi.org/10.1016/j.jhazmat.2017.06.036.

Blount, B.C., Pirkle, J.L., Osterloh, J.D., Valentin-Blasini, L., Caldwell, K.L., 2006. Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ. Health Perspect. 114, 1865–1871. https://doi.org/10.1289/ehp.9466.

Brantsaeter, A.L., Abel, M.H., Haugen, M., Meltzer, H.M., 2013. Risk of suboptimal iodine intake in pregnant Norwegian women. Nutrients 5, 424–440. https://doi.org/10.3390/nu5020424.

Brantsaeter, A.L., Haugen, M., Alexander, J., Meltzer, H.M., 2008. Validity of a new food frequency questionnaire for pregnant women in the Norwegian Mother and Child Cohort Study (MoBa). MaternChild Nutr. 4, 28–43. https://doi.org/10.1111/j.1740-8709.2007.00103.x.

Brantsaeter, A.L., Haugen, M., Hagve, T.A., Aksnes, L., Rasmussen, S.E., Julshamn, K., Alexander, J., Meltzer, H.M., 2007. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Ann. Nutr. Metab. 51, 146–154. https://doi.org/10.1159/000103275.

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

Cao, X.-L., 2010. Phthalate Esters in Foods: Sources, Occurrence, and Analytical Methods. Compr Rev Food Sci F 9, 21–43. https://doi.org/10.1111/j.1541-4337.2009.00093.x.

Cathey, A.L., Watkins, D., Rosario, Z.Y., Vélez, C., Alshawabkeh, A.N., Cordero, J.F., Meeker, J.D., 2019. Associations of Phthalates and Phthalate Replacements With CRH and Other Hormones Among Pregnant Women in Puerto Rico. J. Endocr. Soc. 3, 1127–1149. https://doi.org/10.1210/js.2019-00010.

Cole, S.R., Platt, R.W., Schisterman, E.F., Chu, H., Westreich, D., Richardson, D., Poole, C., 2010. Illustrating bias due to conditioning on a collider. Int. J. Epidemiol. 39, 417–420. https://doi.org/10.1093/ije/dyp334.

Dahl, L., Wik Markhus, M., Sanchez, P.V.R., Moe, V., Smith, L., Meltzer, H.M., Kjellevold,

M., 2018. Iodine Deficiency in a Study Population of Norwegian Pregnant Women-Results from the Little in Norway Study (LiN). Nutrients 10, 513. https://doi.org/10.3390/nu10040513.
de Escobar, G.M., Obregon, M.J., del Rey, F.E., 2007. Iodine deficiency and brain development in the first half of pregnancy. Public Health Nutr. 10, 1554–1570. https://doi.org/10.1017/S1368980007360928.
Drover, S.S.M., Villanger, G.D., Aase, H., Skogheim, T.S., Longnecker, M.P., Zoeller, R.T., Reichborn-Kjennerud, T., Knudsen, G.P., Zeiner, P., Engel, S.M., 2019. Maternal Thyroid Function During Pregnancy or Neonatal Thyroid Function and Attention Deficit Hyperactivity Disorder: A Systematic Review. Epidemiology 30, 130–144. https://doi.org/10.1097/EDE.0000000000000937.
Du, Z., Cao, Y.F., Li, S.N., Hu, C.M., Fu, Z.W., Huang, C.T., Sun, X.Y., Liu, Y.Z., Yang, K., Fang, Z.Z., 2018. Inhibition of UDP-glucuronosyltransferases (UGTs) by phthalate monoesters. Chemosphere 197, 7–13. https://doi.org/10.1016/j.chemosphere.2018.01.010.
Dunlap, D., 1990. Chapter 140. Thyroid Function Tests. In: Walker, H.K.H.W., Hurst, J.W. (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations, 3rd edition. Butterworths, Boston.
Ehrenkranz, J., Bach, P.R., Snow, G.L., Schneider, A., Lee, J.L., Ilstrup, Sarah, Bennett, S.T., Benvenga, S., 2015. Circadian and Circannual Rhythms in Thyroid Hormones: Determining the TSH and Free T4 Reference Intervals Based Upon Time of Day, Age, and Sex. Thyroid 25, 954–961. https://doi.org/10.1089/thy.2014.0589.
Engel, S.M., Villanger, G.D., Nethery, R.C., Thomsen, C., Sakhi, A.K., Drover, S.S.M., Hoppin, J.A., Zeiner, P., Knudsen, G.P., Reichborn-Kjennerud, T., Herring, A.H., Aase, H., 2018. Prenatal Phthalates, Maternal Thyroid Function, and Risk of Attention-Deficit Hyperactivity Disorder in the Norwegian Mother and Child Cohort. Environ. Health Perspect. 126, 057004. https://doi.org/10.1289/EHP2358.
Ferguson, K.K., McElrath, T.F., Meeker, J.D., 2014. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 168, 61–67. https://doi.org/10.1001/jamapediatrics.2013.3699.
Fetene, D.M., Betts, K.S., Alati, R., 2017. MECHANISMS IN ENDOCRINOLOGY: Maternal thyroid dysfunction during pregnancy and behavioural and psychiatric disorders of children: a systematic review. Eur. J. Endocrinol. 177, R261–R273. https://doi.org/10.1530/EJE-16-0860.
Gao, H., Wu, W., Xu, Y., Jin, Z., Bao, H., Zhu, P., Su, P., Sheng, J., Hao, J., Tao, F., 2017. Effects of Prenatal Phthalate Exposure on Thyroid Hormone Concentrations Beginning at The Embryonic Stage. Sci. Rep. 7, 13106. https://doi.org/10.1038/s41598-017-13672-x.
Ghassabian, A., Trasande, L., 2018. Disruption in Thyroid Signaling Pathway: A Mechanism for the Effect of Endocrine-Disrupting Chemicals on Child Neurodevelopment. Front. Endocrinol. (Lausanne) 9, 204. https://doi.org/10.3389/fendo.2018.00204.
Ghisari, M., Bonefeld-Jorgensen, E.C., 2009. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicol. Lett. 189, 67–77. https://doi.org/10.1016/j.toxlet.2009.05.004.
Giovanoulis, G., Alves, A., Papadopoulou, E., Cousins, A.P., Schutze, A., Koch, H.M., Haug, L.S., Covaci, A., Magner, J., Voorspoels, S., 2016. Evaluation of exposure to phthalate esters and DINCH in urine and nails from a Norwegian study population. Environ. Res. 151, 80–90. https://doi.org/10.1016/j.envres.2016.07.025.
Giray, B., Caglayan, A., Erkekoglu, P., Hincal, F., 2010. Fenvalerate exposure alters thyroid hormone status in selenium- and/or iodine-deficient rats. Biol. Trace Elem. Res. 135, 233–241. https://doi.org/10.1007/s12011-009-8506-7.
Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D., Klein, R.Z., 1999. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N. Engl. J. Med. 341, 549–555. https://doi.org/10.1056/nejm199908193410801.
Hatch, E.E., Nelson, J.W., Qureshi, M.M., Weinberg, J., Moore, L.L., Singer, M., Webster, T.F., 2008. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ. Health 7, 27. https://doi.org/10.1186/1476-069X-7-27.
Haug, L.S., Sakhi, A.K., Cequier, E., Casas, M., Maitre, L., Basagana, X., Andrusaityte, S., Chalkiadaki, G., Chatzi, L., Coen, M., de Bont, J., Dedele, A., Ferrand, J., Grazuleviciene, R., Gonzalez, J.R., Gutzkow, K.B., Keun, H., McEachan, R., Meltzer, H.M., Petraviciene, I., Robinson, O., Saulnier, P.J., Slama, R., Sunyer, J., Urquiza, J., Vafeiadi, M., Wright, J., Vrijheid, M., Thomsen, C., 2018. In-utero and childhood chemical exposome in six European mother-child cohorts. Environ. Int. 121, 751–763. https://doi.org/10.1016/j.envint.2018.09.056.
Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ. Health Perspect. 112, 1734–1740.
Hoppin, J.A., Ulmer, R., Calafat, A.M., Barr, D.B., Baker, S.V., Meltzer, H.M., Ronningen, K.S., 2006. Impact of urine preservation methods and duration of storage on measured levels of environmental contaminants. J. Expo Sci. Environ. Epidemiol. 16, 39–48. https://doi.org/10.1038/sj.jea.7500435.
Huang, H.B., Kuo, P.L., Chang, J.W., Jaakkola, J.J.K., Liao, K.W., Huang, P.C., 2018. Longitudinal assessment of prenatal phthalate exposure on serum and cord thyroid hormones homeostasis during pregnancy - Tainan birth cohort study (TBCS). Sci. Total Environ. 619–620, 1058–1065. https://doi.org/10.1016/j.scitotenv.2017.11.048.
Huang, H.B., Pan, W.H., Chang, J.W., Chiang, H.C., Guo, Y.L., Jaakkola, J.J., Huang, P.C., 2017. Does exposure to phthalates influence thyroid function and growth hormone homeostasis? The Taiwan Environmental Survey for Toxicants (TEST) 2013. Environ. Res. 153, 63–72. https://doi.org/10.1016/j.envres.2016.11.014.
Huang, P.C., Kuo, P.L., Guo, Y.L., Liao, P.C., Lee, C.C., 2007. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum. Reprod. 22, 2715–2722. https://doi.org/10.1093/humrep/dem205.
Huang, P.C., Tsai, C.H., Liang, W.Y., Li, S.S., Huang, H.B., Kuo, P.L., 2016. Early Phthalates Exposure in Pregnant Women Is Associated with Alteration of Thyroid Hormones. PLoS ONE 11, e0159398. https://doi.org/10.1371/journal.pone.0159398.
Ishihara, A., Nishiyama, N., Sugiyama, S., Yamauchi, K., 2003. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. Gen. Comp. Endocrinol. 134, 36–43.
Johns, L.E., Ferguson, K.K., McElrath, T.F., Mukherjee, B., Meeker, J.D., 2016. Associations between Repeated Measures of Maternal Urinary Phthalate Metabolites and Thyroid Hormone Parameters during Pregnancy. Environ. Health Perspect. 124, 1808–1815. https://doi.org/10.1289/EHP170.
Johns, L.E., Ferguson, K.K., Soldin, O.P., Cantonwine, D.E., Rivera-Gonzalez, L.O., Del Toro, L.V., Calafat, A.M., Ye, X., Alshawabkeh, A.N., Cordero, J.F., Meeker, J.D., 2015. Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis. Reprod. Biol. Endocrinol. 13, 4. https://doi.org/10.1186/1477-7827-13-4.
Kashiwagi, K., Furuno, N., Kitamura, S., Ohta, S., Sugihara, K., Utsumi, K., Hanada, H., Taniguchi, K., Suzuki, K., Kashiwagi, A., 2009. Disruption of Thyroid Hormone Function by Environmental Pollutants. J. Health Sci. 55, 147–160. https://doi.org/10.1248/jhs.55.147.
Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.-I., Tsatsakis, A.M., Rizos, A.K., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97, 212–236. https://doi.org/10.1016/j.envint.2016.09.013.
Kim, M.J., Moon, S., Oh, B.C., Jung, D., Choi, K., Park, Y.J., 2019. Association Between Diethylhexyl Phthalate Exposure and Thyroid Function: A Meta-Analysis. Thyroid. https://doi.org/10.1089/thy.2018.0051.
Koch, H.M., Angerer, J., 2007. Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. Int. J. Hyg. Environ. Health 210, 9–19. https://doi.org/10.1016/j.ijheh.2006.11.008.
Koch, H.M., Bolt, H.M., Preuss, R., Angerer, J., 2005. New metabolites of di(2-ethylhexyl) phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch. Toxicol. 79, 367–376. https://doi.org/10.1007/s00204-004-0642-4.
Koch, H.M., Christensen, K.L., Harth, V., Lorber, M., Bruning, T., 2012. Di-n-butyl phthalate (DnBP) and diisobutyl phthalate (DiBP) metabolism in a human volunteer after single oral doses. Arch. Toxicol. 86, 1829–1839. https://doi.org/10.1007/s00204-012-0908-1.
Korevaar, T.I.M., Medici, M., Visser, T.J., Peeters, R.P., 2017. Thyroid disease in pregnancy: new insights in diagnosis and clinical management. Nat. Rev. Endocrinol. https://doi.org/10.1038/nrendo.2017.93.
Kuo, F.C., Su, S.W., Wu, C.F., Huang, M.C., Shiea, J., Chen, B.H., Chen, Y.L., Wu, M.T., 2015. Relationship of urinary phthalate metabolites with serum thyroid hormones in pregnant women and their newborns: a prospective birth cohort in Taiwan. PLoS ONE 10, e0123884. https://doi.org/10.1371/journal.pone.0123884.
Lagarde, F., Beausoleil, C., Belcher, S.M., Belzunces, L.P., Emond, C., Guerbet, M., Rousselle, C., 2015. Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. Environ. Health 14, 13. https://doi.org/10.1186/1476-069x-14-13.
Lazarus, J., Brown, R.S., Daumerie, C., Hubalewska-Dydejczyk, A., Negro, R., Vaidya, B., 2014. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur. Thyroid J. 3, 76–94. https://doi.org/10.1159/000362597.
Lazarus, J.H., 2014. Iodine status in europe in 2014. Eur. Thyroid J. 3, 3–6. https://doi.org/10.1159/000358873.
Lee, R.H., Spencer, C.A., Mestman, J.H., Miller, E.A., Petrovic, I., Braverman, L.E., Goodwin, T.M., 2009. Free T4 immunoassays are flawed during pregnancy. Am. J. Obstet. Gynecol. 200, 260.e261–260.e266. https://doi.org/10.1016/j.ajog.2008.10.042.
Liu, C., Zhao, L., Wei, L., Li, L., 2015. DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ. Sci. Pollut. Res. Int. 22, 12711–12719. https://doi.org/10.1007/s11356-015-4567-7.
Longhi, S., Radetti, G., 2013. Thyroid function and obesity. J. Clin. Res. Pediatr. Endocrinol. 5 (Suppl 1), 40–44. https://doi.org/10.4274/jcrpe.856.
Lubin, J.H., Colt, J.S., Camann, D., Davis, S., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ. Health Perspect. 112, 1691–1696. https://doi.org/10.1289/ehp.7199.
Luo, J., Schumacher, M., Scherer, A., Sanoudou, D., Megherbi, D., Davison, T., Shi, T., Tong, W., Shi, L., Hong, H., Zhao, C., Elloumi, F., Shi, W., Thomas, R., Lin, S., Tillinghast, G., Liu, G., Zhou, Y., Herman, D., Li, Y., Deng, Y., Fang, H., Bushel, P., Woods, M., Zhang, J., 2010. A comparison of batch effect removal methods for enhancement of prediction performance using MAQC-II microarray gene expression data. Pharmacogenomics J. 10, 278–291. https://doi.org/10.1038/tpj.2010.57.
Magnus, P., Birke, C., Vejrup, K., Haugan, A., Alsaker, E., Daltveit, A.K., Handal, M., Haugen, M., Hoiseth, G., Knudsen, G.P., Paltiel, L., Schreuder, P., Tambs, K., Vold, L., Stoltenberg, C., 2016. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. 45, 382–388. https://doi.org/10.1093/ije/dyw029.
Magnus, P., Irgens, L.M., Haug, K., Nystad, W., Skjaerven, R., Stoltenberg, C., 2006. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. 35, 1146–1150. https://doi.org/10.1093/ije/dyl170.
Medda, E., Santini, F., De Angelis, S., Franzellin, F., Fiumalbi, C., Perico, A., Gilardi, E., Mechi, M.T., Marsili, A., Citroni, A., Leandri, A., Mantovani, A., Vitti, P., Olivieri, A.,

2017. Iodine nutritional status and thyroid effects of exposure to ethylenebisdithiocarbamates. Environ. Res. 154, 152–159. https://doi.org/10.1016/j.envres.2016.12.019.
Medici, M., Timmermans, S., Visser, W., de Muinck Keizer-Schrama, S.M., Jaddoe, V.W., Hofman, A., Hooijkaas, H., de Rijke, Y.B., Tiemeier, H., Bongers-Schokking, J.J., Visser, T.J., Peeters, R.P., Steegers, E.A., 2013. Maternal thyroid hormone parameters during early pregnancy and birth weight: the Generation R Study. J. Clin. Endocrinol. Metab. 98, 59–66. https://doi.org/10.1210/jc.2012-2420.
Meltzer, H.M., Brantsaeter, A.L., Ydersbond, T.A., Alexander, J., Haugen, M., 2008. Methodological challenges when monitoring the diet of pregnant women in a large study: experiences from the Norwegian Mother and Child Cohort Study (MoBa). MaternChild Nutr 4, 14–27. https://doi.org/10.1111/j.1740-8709.2007.00104.x.
Mendez Jr., W., Eftim, S.E., 2012. Biomarkers of perchlorate exposure are correlated with circulating thyroid hormone levels in the 2007–2008 NHANES. Environ. Res. 118, 137–144. https://doi.org/10.1016/j.envres.2012.05.010.
Moog, N.K., Entringer, S., Heim, C., Wadhwa, P.D., Kathmann, N., Buss, C., 2015. Influence of maternal thyroid hormones during gestation on fetal brain development. Neuroscience. https://doi.org/10.1016/j.neuroscience.2015.09.070.
Morgenstern, R., Whyatt, R.M., Insel, B.J., Calafat, A.M., Liu, X., Rauh, V.A., Herbstman, J., Bradwin, G., Factor-Litvak, P., 2017. Phthalates and thyroid function in preschool age children: sex specific associations. Environ. Int. 106, 11–18. https://doi.org/10.1016/j.envint.2017.05.007.
Morreale de Escobar, G., Obregon, M.J., Escobar del Rey, F., 2004. Role of thyroid hormone during early brain development. Eur. J. Endocrinol. 151 (Suppl 3), U25–37.
Mughal, B.B., Fini, J.B., Demeneix, B.A., 2018. Thyroid-disrupting chemicals and brain development: an update. Endocr Connect 7, R160–R186. https://doi.org/10.1530/EC-18-0029.
Nordic Council of Ministers, 2014. Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. https://doi.org/10.6027/Nord2014-002.
O'Kane, S.M., Mulhern, M.S., Pourshahidi, L.K., Strain, J.J., Yeates, A.J., 2018. Micronutrients, iodine status and concentrations of thyroid hormones: a systematic review. Nutr. Rev. 76, 418–431. https://doi.org/10.1093/nutrit/nuy008.
Obregon, M.J., Escobar del Rey, F., Morreale de Escobar, G., 2005. The effects of iodine deficiency on thyroid hormone deiodination. Thyroid 15, 917–929. https://doi.org/10.1089/thy.2005.15.917.
Overgaard, K.R., Oerbeck, B., Friis, S., Pripp, A.H., Biele, G., Aase, H., Zeiner, P., 2018. Attention-Deficit/Hyperactivity Disorder in Preschoolers: The Accuracy of a Short Screener. J. Am. Acad. Child Adolesc. Psychiatry 57, 428–435. https://doi.org/10.1016/j.jaac.2018.03.008.
Paltiel, L., Haugan, A., Skjerden, T., Harbak, K., Baekken, S., Stensrud, N.K., Knudsen, G.P., Magnus, P., 2014. The biobank of the Norwegian mother and child cohort study - present status. Norsk Epidemiologi 24, 29–35.
Park, C., Choi, W., Hwang, M., Lee, Y., Kim, S., Yu, S., Lee, I., Paek, D., Choi, K., 2017. Associations between urinary phthalate metabolites and bisphenol A levels, and serum thyroid hormones among the Korean adult population-Korean National Environmental Health Survey (KoNEHS) 2012–2014. Sci. Total Environ. 584, 950–957.
Patel, J., Landers, K., Li, H., Mortimer, R.H., Richard, K., 2011. Delivery of maternal thyroid hormones to the fetus. Trends Endocrinol. Metab. 22, 164–170. https://doi.org/10.1016/j.tem.2011.02.002.
Pearce, E.N., Andersson, M., Zimmermann, M.B., 2013. Global iodine nutrition: where do we stand in 2013? Thyroid 23, 523–528. https://doi.org/10.1089/thy.2013.0128.
Pearce, E.N., Lazarus, J.H., Moreno-Reyes, R., Zimmermann, M.B., 2016. Consequences of iodine deficiency and excess in pregnant women: an overview of current knowns and unknowns. Am. J. Clin. Nutr. 104 (Suppl 3), 918S–923S. https://doi.org/10.3945/ajcn.115.110429.
Pedraza, P.E., Obregon, M.J., Escobar-Morreale, H.F., del Rey, F.E., de Escobar, G.M., 2006. Mechanisms of adaptation to iodine deficiency in rats: thyroid status is tissue specific. Its relevance for man. Endocrinology 147, 2098–2108. https://doi.org/10.1210/en.2005-1325.
Pop, V.J., Brouwers, E.P., Vader, H.L., Vulsma, T., van Baar, A.L., de Vijlder, J.J., 2003. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clin. Endocrinol. (Oxf.) 59, 282–288. https://doi.org/10.1046/j.1365-2265.2003.01822.x.
Preau, L., Fini, J.B., Morvan-Dubois, G., Demeneix, B., 2015. Thyroid hormone signaling during early neurogenesis and its significance as a vulnerable window for endocrine disruption. Biochim. Biophys. Acta, Gen. Subj. 1849, 112–121. https://doi.org/10.1016/j.bbagrm.2014.06.015.
R, T.C. R., A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2016.
Radke, E.G., Galizia, A., Thayer, K.A., Cooper, G.S., 2019. Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence. Environ. Int. 132, 104768. https://doi.org/10.1016/j.envint.2019.04.040.
Roman, G.C., 2007. Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J. Neurol. Sci. 262, 15–26. https://doi.org/10.1016/j.jns.2007.06.023.
Roman, G.C., Ghassabian, A., Bongers-Schokking, J.J., Jaddoe, V.W., Hofman, A., de Rijke, Y.B., Verhulst, F.C., Tiemeier, H., 2013. Association of gestational maternal hypothyroxinemia and increased autism risk. Ann. Neurol. 74, 733–742. https://doi.org/10.1002/ana.23976.
Romano, M.E., Eliot, M.N., Zoeller, R.T., Hoofnagle, A.N., Calafat, A.M., Karagas, M.R., Yolton, K., Chen, A., Lanphear, B.P., Braun, J.M., 2018. Maternal urinary phthalate metabolites during pregnancy and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Int. J. Hyg. Environ. Health 221, 623–631. https://doi.org/10.1016/j.ijheh.2018.03.010.
Ronningen, K.S., Paltiel, L., Meltzer, H.M., Nordhagen, R., Lie, K.K., Hovengen, R., Haugen, M., Nystad, W., Magnus, P., Hoppin, J.A., 2006. The biobank of the Norwegian Mother and Child Cohort Study: a resource for the next 100 years. Eur. J. Epidemiol. 21, 619–625. https://doi.org/10.1007/s10654-006-9041-x.
Rovet, J., Willoughby, K., 2010. Maternal Thyroid Function During Pregnancy: Effects on the Developing Fetal Brain. In: Zimmerman, A.W., Connors, S.L. (Eds.), Maternal Influences on FetalNeurodevelopment. Springer, New York.
Sabaredzovic, A., Sakhi, A.K., Brantsaeter, A.L., Thomsen, C., 2015. Determination of 12 urinary phthalate metabolites in Norwegian pregnant women by core-shell high performance liquid chromatography with on-line solid-phase extraction, column switching and tandem mass spectrometry. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1002, 343–352. https://doi.org/10.1016/j.jchromb.2015.08.040.
Sakhi, A.K., Lillegaard, I.T., Voorspoels, S., Carlsen, M.H., Loken, E.B., Brantsaeter, A.L., Haugen, M., Meltzer, H.M., Thomsen, C., 2014. Concentrations of phthalates and bisphenol A in Norwegian foods and beverages and estimated dietary exposure in adults. Environ. Int. 73C, 259–269. https://doi.org/10.1016/j.envint.2014.08.005.
Sakhi, A.K., Sabaredzovic, A., Cequier, E., Thomsen, C., 2017. Phthalate metabolites in Norwegian mothers and children: Levels, diurnal variation and use of personal care products. Sci. Total Environ. 599–600, 1984–1992. https://doi.org/10.1016/j.scitotenv.2017.05.109.
Schisterman, E.F., Cole, S.R., Platt, R.W., 2009. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 20, 488–495. https://doi.org/10.1097/EDE.0b013e3181a819a1.
Shen, O., Du, G., Sun, H., Wu, W., Jiang, Y., Song, L., Wang, X., 2009. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol. Lett. 191, 9–14. https://doi.org/10.1016/j.toxlet.2009.07.019.
Shinohara, D.R., Santos, T.D.S., de Carvalho, H.C., Lopes, L.C.B., Gunther, L.S.A., Aristides, S.M.A., Teixeira, J.J.V., Demarchi, I.G., 2018. Pregnancy Complications Associated With Maternal Hypothyroidism: A Systematic Review. Obstet. Gynecol. Surv. 73, 219–230. https://doi.org/10.1097/OGX.0000000000000547.
Soldin, O.P., 2002. Controversies in urinary iodine determinations. Clin. Biochem. 35, 575–579.
Steinmaus, C., Miller, M.D., Cushing, L., Blount, B.C., Smith, A.H., 2013. Combined effects of perchlorate, thiocyanate, and iodine on thyroid function in the National Health and Nutrition Examination Survey 2007–08. Environ. Res. 123, 17–24. https://doi.org/10.1016/j.envres.2013.01.005.
Stojanoska, M.M., Milosevic, N., Milic, N., Abenavoli, L., 2017. The influence of phthalates and bisphenol A on the obesity development and glucose metabolism disorders. Endocrine 55, 666–681. https://doi.org/10.1007/s12020-016-1158-4.
Sun, D., Zhou, L., Wang, S., Liu, T., Zhu, J., Jia, Y., Xu, J., Chen, H., Wang, Q., Xu, F., Zhang, Y., Ye, L., 2018. Effect of Di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-thyroid axis in adolescent rat. Endocr. J. 65, 261–268. https://doi.org/10.1507/endocrj.EJ17-0272.
Thienpont, L.M., Van Uytfanghe, K., Poppe, K., Velkeniers, B., 2013. Determination of free thyroid hormones. Best Pract Res Cl En 27, 689–700. https://doi.org/10.1016/j.beem.2013.05.012.
Thompson, W., Russell, G., Baragwanath, G., Matthews, J., Vaidya, B., Thompson-Coon, J., 2018. Maternal thyroid hormone insufficiency during pregnancy and risk of neurodevelopmental disorders in offspring: a systematic review and meta-analysis. Clin. Endocrinol. (Oxf.) 88, 575–584. https://doi.org/10.1111/cen.13550.
Vandenberg, L.N., 2014. Low-dose effects of hormones and endocrine disruptors. Vitam. Horm. 94, 129–165. https://doi.org/10.1016/B978-0-12-800095-3.00005-5.
Vandenberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs Jr., D.R., Lee, D.H., Shioda, T., Soto, A.M., vom Saal, F.S., Welshons, W.V., Zoeller, R.T., Myers, J.P., 2012. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. Endocr. Rev. 33, 378–455. https://doi.org/10.1210/er.2011-1050.
Villanger, G.D., Learner, E., Longnecker, M.P., Ask, H., Aase, H., Zoeller, R.T., Knudsen, G.P., Reichborn-Kjennerud, T., Zeiner, P., Engel, S.M., 2017. Effects of Sample Handling and Analytical Procedures on Thyroid Hormone Concentrations in Pregnant Women's Plasma. Epidemiology 28, 365–369. https://doi.org/10.1097/EDE.0000000000000606.
Weng, T.-I., Chen, M.-H., Lien, G.-W., Chen, P.-S., Lin, J.C.-C., Fang, C.-C., Chen, P.-C., 2017. Effects of gender on the association of urinary phthalate metabolites with thyroid hormones in children: a prospective cohort study in Taiwan. Int. J. Environ. Res. Public Health 14, 123.
Wenzel, A., Franz, C., Breous, E., Loos, U., 2005. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. Mol. Cell. Endocrinol. 244, 63–71. https://doi.org/10.1016/j.mce.2005.02.008.
WHO, World Health Organization, United Nations Children's Fund, International Council for Control of Iodine Deficiency Disorders. Assessment of Iodine Deficiency Disorders and Monitoring Their Elimination: A Guide for Programme Managers. Geneva, Switzerland.; 2007.
WHO/UNEP, The State-of-the-Science of Endocrine Disrupting Chemicals - 2012 ed^eds. Geneva: UNEP/WHO; 2013
Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbuhler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26, 803–824. https://doi.org/10.1111/j.1539-6924.2006.00770.x.
Yao, H.Y., Han, Y., Gao, H., Huang, K., Ge, X., Xu, Y.Y., Xu, Y.Q., Jin, Z.X., Sheng, J., Yan, S.Q., Zhu, P., Hao, J.H., Tao, F.B., 2016. Maternal phthalate exposure during the first trimester and serum thyroid hormones in pregnant women and their newborns. Chemosphere 157, 42–48. https://doi.org/10.1016/j.chemosphere.2016.05.023.
Ye, H., Ha, M., Yang, M., Yue, P., Xie, Z., Liu, C., 2017. Di2-ethylhexyl phthalate disrupts thyroid hormone homeostasis through activating the Ras/Akt/TRHr pathway and inducing hepatic enzymes. Sci. Rep. 7, 40153. https://doi.org/10.1038/srep40153.
Ye, X., Pierik, F.H., Angerer, J., Meltzer, H.M., Jaddoe, V.W., Tiemeier, H., Hoppin, J.A., Longnecker, M.P., 2009. Levels of metabolites of organophosphate pesticides,

phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Hyg. Environ. Health 212, 481–491. https://doi.org/10.1016/j.ijheh.2009.03.004.
Zhai, W., Huang, Z., Chen, L., Feng, C., Li, B., Li, T., 2014. Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). PLoS ONE 9, e92465. https://doi.org/10.1371/journal.pone.0092465.
Zimmermann, M.B., 2012. The effects of iodine deficiency in pregnancy and infancy. Paediatr. Perinat. Epidemiol. 26 (Suppl 1), 108–117. https://doi.org/10.1111/j.1365-3016.2012.01275.x.
Zoeller, R.T., 2005. Environmental chemicals as thyroid hormone analogues: new studies indicate that thyroid hormone receptors are targets of industrial chemicals? Mol. Cell. Endocrinol. 242, 10–15. https://doi.org/10.1016/j.mce.2005.07.006.
Zoeller, R.Thomas, Brown, T.R., Doan, L.L., Gore, A.C., Skakkebaek, N.E., Soto, A.M., Woodruff, T.J., Vom Saal, F.S., 2012. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. Endocrinology 153 (9), 4097–4110. https://doi.org/10.1210/en.2012-1422.