Environment International 178 (2023) 108091

Contents lists available at ScienceDirect

Environment International

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

Full length article

Association of endocrine disrupting chemicals exposure with human chorionic gonadotropin concentrations in pregnancy

Arash Derakhshan [a,b], Huan Shu [c], Maarten A.C. Broeren [d], Andreas Kortenkamp [e], Christian H. Lindh [f], Barbara Demeneix [g], Robin P. Peeters [a,b], Carl-Gustaf Bornehag [c,h], Tim I. M. Korevaar [a,b,*]

[a] Academic Center for Thyroid Diseases, Erasmus MC, Dr. Molewaterplein 15, 3051 GE Rotterdam, the Netherlands
[b] Department of Internal Medicine, Erasmus MC, Dr. Molewaterplein 15, 3051 GE Rotterdam, the Netherlands
[c] Department of Public Health, Karlstad University, Sweden
[d] Laboratory of Clinical Chemistry and Haematology, Máxima Medical Centre, Veldhoven, De Run 4600, The Netherlands
[e] Division of Environmental Sciences, College of Health, Medicine and Life Sciences, Brunel University, London, Uxbridge, UK
[f] Division of Occupational and Environmental Medicine, Lund University, Lund, Sweden
[g] Laboratoire d'Evolution des Régulations Endocriniennes, CNRS/Muséum National d'Histoire Naturelle, 57 Rue Cuvier, 75005 Paris, France
[h] Icahn School of Medicine at Mount Sinai, New York City, NY, USA

ARTICLE INFO

Keywords:
Endocrine disrupting chemicals
Pregnancy
Human chorionic gonadotropin
Placenta

ABSTRACT

Background: Human chorionic gonadotropin (hCG) is produced by the placenta and plays an essential role in the maintenance of pregnancy. Endocrine disrupting chemicals (EDCs) have the potential to interfere with functions related to the production and secretion of hCG; however associations between exposure to EDCs and hCG concentrations in humans remain to be elucidated.

Objectives: To investigate the association of urinary, serum and plasma concentrations of EDCs during pregnancy with serum hCG concentrations.

Methods: We utilized data form the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study. We investigated the association of 26 EDCs measured in early pregnancy urine or blood with serum hCG concentrations using multi-variable adjusted linear regression models per EDC and Weighted Quantile Sum (WQS) regression with repeated holdout validation for the EDCs mixture.

Results: In 2,039 included women, higher exposure to bisphenol A was associated with lower hCG (beta [95% CI]: −0.06 [−0.11 to −0.002]) while higher triclosan exposure was associated with a higher hCG (0.02 [0.003 to 0.04]). Higher exposure to several phthalates, including mono-ethyl and mono-butyl phthalates (MEP and MBP) as well as metabolites of di-2-ethylhexyl phthalate (DEHP) was associated with a lower hCG (beta [95% CI] for sum of DEHP metabolites: −0.13 [−0.19 to −0.07]). Likewise, higher exposure to several polychlorinated biphenyls (PCBs) was associated with a lower hCG. In the WQS regression, each quartile increase in the EDCs mixture was associated with −0.27 lower hCG (95% CI: −0.34 to −0.19).

Discussion: Higher exposure to several EDCs during pregnancy was associated with a lower hCG; and despite the small effect sizes, still indicating that the exposure may negatively affect production or secretion of hCG by the placenta. Our results provide the impetus for future experimental studies to investigate the placenta as a target organ for adverse effects of EDCs.

1. Introduction

Endocrine disrupting chemicals (EDCs) are chemical compounds that interfere with any feature of the endocrine system. As a result of the extensive production and use of different chemicals, human exposure to known EDCs is wide-ranging and ubiquitous. There is growing evidence that EDC exposure adversely affects human health including a loss of IQ, a higher risk of metabolic diseases, infertility, adverse pregnancy outcomes and cancer (Kahn et al., 2020). The physiology of the endocrine system changes profoundly during pregnancy in order to regulate the

* Corresponding author at: Room Na-2918, Doctor Molewaterplein 40, 3015 GD Rotterdam, the Netherlands.
E-mail address: t.korevaar@erasmusmc.nl (T.I.M. Korevaar).

https://doi.org/10.1016/j.envint.2023.108091
Received 9 February 2023; Received in revised form 7 July 2023; Accepted 10 July 2023
Available online 13 July 2023

increase in maternal metabolism and maintain a suitable environment for the developing fetus (Korevaar et al., 2017). Human chorionic gonadotropin (hCG) is a pregnancy-specific hormone that is produced almost exclusively by trophoblast cells of placenta. hCG is responsible for stimulating the secretion of progesterone by the corpus luteum, angiogenesis, placentation, maternal immune-tolerance to pregnancy and differentiation and growth of fetal organs (Cole, 2010; Heidegger and Jeschke, 2018). hCG shares the same alpha-subunit as luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone (TSH) (Nwabuobi et al., 2017). Through its weak affinity for the TSH receptor it can increase the secretion and production of thyroid hormones which regulate gestational metabolism and fetal development (Hershman, 2004). Pregnancy is a vulnerable period for potential adverse effects of exposure to EDCs considering additional risks for the developing fetus and placenta.

EDCs can disrupt the development and function of the placenta, resulting in lower placental weight, hampered steroidogenesis, oxidative stress via mechanisms such as affecting cytochromes P450 activity, signaling pathways and molecular targets (Gingrich et al., 2020; Yang et al., 2019). These could potentially mean that placental hCG production can also be affected by EDCs exposure. For example, exposure to phthalates affected the gene expression of essential human placental genes and these effects were reflected by lower secretion of β-hCG by differentiated trophoblasts exposed to a mixture of phthalates (Adibi et al., 2017). Only one small study has investigated the association of EDCs with hCG in humans, showing that a higher first-trimester concentrations of three phthalate metabolites were associated with higher hCG in women carrying a female fetus as compared to a male fetus (Adibi et al., 2015). Furthermore, the authors estimated that 25 to 78% of the association of phthalate metabolites with anogenital distance was mediated through changes in hCG concentration (Adibi et al., 2015). Despite the clear susceptibility for endocrine disruption of placental function and the aforementioned proof-of-concept study, it remains unknown which endocrine disruptors affect hCG concentration and to what extent.

Therefore, we investigated the association of exposure to various well-known EDC families during early pregnancy with serum concentration of hCG.

2. Methods

This study was embedded in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study which is an on-going prospective birth cohort focused on investigating the consequences of early life exposure to environmental toxicants, particularly EDCs, on the mothers health, pregnancy outcomes and child health and development.

Between September 2007 and March 2010 pregnant women were enrolled (median gestational week of 10 in the county of Värmland (Sweden). Informed written consent was given by participating families for biological sample collections and participation in the SELMA study. The SELMA study has been approved by the regional ethical committee, Uppsala, Sweden (2007–05-02, Dnr: 2007/062) (Bornehag et al., 2012).

2.1. Urine and blood analysis

At the first prenatal visit, first-morning void urine and non-fasting blood samples were collected from mothers. Samples were kept at −70 °C (serum) and –20 °C (urine) in a biobank at the Central Hospital in Karlstad, Sweden, and analyzed at the Laboratory of Occupational and Environmental Medicine (OEM) at Lund University, Lund, Sweden (for EDCs); and at Laboratory of Clinical Chemistry and Hematology, Máxima Medical Centre, Veldhoven, the Netherlands (for hCG). At the OEM, we quantified 24 analytes in urine, and 8 PFAS analytes and cotinine (a smoking biomarker) in serum using liquid chromatography tandem mass spectrometry (LC-MS/MS, QTRAP 5500, Sciex, Framingham, MA, USA) with procedures described by Gyllenhammar et al (Gyllenhammar et al., 2017), and Norén et al (Norén et al., 2021), respectively. Concentrations of analytes were adjusted for urinary dilution using enzymatically determined creatinine levels (adjusted analyte = [analyte]/[creatinine]). We quantified 22 persistent chlorinated compounds in plasma using gas chromatography - high triple quadrupole mass spectrometry (Agilent 7010 GC–MS/MS system (Wilmington, DE, USA), GC column DB-5MS UI (J&W Scientific, 20 m, ID 0.18 mm, 0.18 μm)) at the National Institute for Health and Welfare, Finland. The limits of detection (LOD) were 0.003–0.100 ng/mL for urinary compounds and 0.01–0.06 ng/mL for PFAS. The limits of quantitation (LOQ) for persistent chlorinated compounds were 0.005–0.040 ng/mL. The limit of detection for serum cotinine was 0.2 ng/mL.

In total, 45 analytes were measured in urine, serum, and plasma. We calculated molar sums of di-2-ethylhexyl phthalate (DEHP), di-*iso*-nonyl phthalate (DINP) and di-*iso*-decyl phthalate (DIDP) metabolites. In this study, we limited the analysis to individual compounds detectable in ≥ 75% of samples. After exclusions, 39 compounds were available for the statistical analyses, including 22 urine, 6 serum, and 11 plasma compounds (Supplemental Tables 1 and 2). Machine read values were used for urinary and serum compounds with values < LOD. Plasma compounds with values < LOQ were substituted with LOQ/$\sqrt{2}$ for analyses. Details on laboratory quality assurance/quality control (QA/QC) can be found in Supplemental Table 3.

hCG was determined in lithium–heparin plasma using an electrochemiluminescence assay with monoclonal antibodies that recognize the holo hormone, the β core fragment, the free β subunit and the "nicked" forms of hCG (Cobas® e601; Roche Diagnostics). Within-laboratory CV was 2.5% at 4.3 U/L and 2.1% at 171 U/L.

2.2. Covariates

Maternal education level, ethnicity and height were assessed using questionnaires. Data on weight were derived from the Swedish National Birth Register; however if unavailable, the self-reported weight at study enrollment from the SELMA study questionnaires were used to fill in the missing data. BMI was calculated as weight/heightˆ2. Serum cotinine levels were categorized using the following cut-offs: non-smoker (below 0.2 ng/mL), passive smoker (0.2–15 ng/mL) or active smoker (higher than 15 ng/mL) (Bernert et al., 2000; Pirkle et al., 1996).

2.3. Statistical analysis

Concentrations of hCG rapidly change throughout gestation, in order to take this into account model-based reference ranges were created using Generalized Additive Models for Location, Size and Shape (GAMLSS). This statistical method allows flexible and (semi) parametric calculations of reference ranges while considering the skewness and kurtosis of the data (Stasinopoulos and Rigby, 2008). We used 10 cubic splines for gestational age at the time of blood sampling, 3 cubic splines for sigma variation and a Box Cox *t* family distribution to achieve the best fit (according to Akaike Information Criterion and worm plots), while considering the known physiological trajectory of hCG during pregnancy. Then, gestational age specific Z-scores were derived from the model. More detailed explanation is provided in the supplemental methods. In addition, we calculated multiple of median (MoM) values of hCG by dividing the individual total hCG level with the median value of the group of participants per gestational week.

We utilized multivariable linear regression to study the association of each EDC with the gestational age specific Z-scores of hCG. We utilized directed acyclic graphs (Textor et al., 2016) to visualize the potential confounders which were selected according to biological plausibility or established associations based on previous literature (Supplemental Figs. 1 and 2). Hence, all analyses were adjusted for the following potential confounders: maternal age, gestational age at the time of

Table 1
Characteristics of the study population.

Characteristics	N = 2,039
Human chorionic gonadotropin (IU/L)	69,708 (10,718–167,618)
Gestational age (weeks)	10 (6–14)
Age	30.9 (4.9)
BMI	24.8 (4.4)
Parity, n (%)	
0	915 (45)
1	723 (35)
≥2	401 (20)
Ethnicity, n (%)	
Western	1,976 (97)
Non-Western	63 (3)
Serum Cotinine levels, n (%)	
Non-smoker: <0.2 ng/mL	1,734 (85.1)
Passive smoker: 0.2–15 ng/mL	121 (5.9)
Active smoker: >15 ng/mL	184 (9)
Education level, n (%)	
Elementary school	81 (0.5)
High school	743 (36.5)
College/University	1,215 (59.5)
Fetal sex, n (%)	
Female	956 (46.9)
Male	1,083 (53.1)

Data are median (95% range), mean (SD) or number (percentage) as appropriate. Except for hCG, the missing values are imputed.

sampling, BMI, parity, smoking status (defined according to serum cotinine concentrations), education level, ethnicity and fetal sex. In addition, for EDCs that are measured in urine, on top of the prior standardization, urinary creatinine was incorporated as a covariate in all models, to optimally adjust for urinary dilution (O'Brien et al., 2016). Restricted cubic splines with 3 knots was used to asses non-linearity.

Furthermore, to investigate the association of EDCs as a mixture with hCG, we utilized Weighted Quantile Sum (WQS) regression with repeated holdout validation (Tanner et al., 2019). We scaled the log-transformed value of chemicals for a more stable analysis. Detailed methodology of WQS regression with repeated holdout validation is described in the supplemental methods. We constructed the WQS index in both negative and positive directions, while adjusting for the covariates, and report the estimate for difference in hCG per each quartile increase in the WQS index as well as the weight of each chemical in the association. We consider the threshold of 2.56% (100%/39 chemicals) for the mean weight of the chemical to be identified as chemicals of concern, representing a larger contribution to the association than what could be expected by chance.

Based on a previous study reporting sex-specific associations of phthalates with hCG concentration we further checked for any effect modification of fetal sex by adding the interaction term of fetal sex with EDC to the regression models (Adibi et al., 2017). We used Student's *t*-test to investigate the difference between mean absolute concentrations of hCG as well as hCG Z-scores between women carrying male fetuses versus those carrying female fetuses. We used Spearman's correlation coefficients to assess the correlations between all EDCs.

Missing data of covariates were imputed using multiple imputation by chained equations (25 datasets) (Buuren et al., 2011). All statistical analyses were performed using R statistical software version 3.6.1 (packages *mice*; *rms and gamlss*; https://www.r-project.org/).

Table 2
Association of urinary EDC (metabolites) with serum hCG concentration.

Phenols	β (95% CI)	P value
BPA	−0.06 (−0.11 to −0.002)	0.04
BPS	−0.02 (−0.07 to 0.02)	0.28
BPF	−0.003 (−0.03 to 0.02)	0.81
Triclosan	0.02 (0.003 to 0.04)	0.02
Phthalates		
MEP	−0.07 (−0.11 to −0.02)	0.001
MBP	−0.15 (−0.21 to −0.08)	<0.001
MBzP	−0.07 (−0.11 to −0.02)	0.005
DEHP Molar sum	−0.13 (−0.19 to −0.07)	<0.001
MEHP	−0.10 (−0.15 to −0.05)	<0.001
MEHHP	−0.12 (−0.18 to −0.07)	<0.001
MEOHP	−0.12 (−0.18 to −0.07)	<0.001
MECPP	−0.13 (−0.18 to −0.07)	<0.001
MCMHP	−0.07 (−0.13 to −0.01)	0.013
DINP Molar sum	−0.02 (−0.06 to 0.02)	0.28
MHiNP	U-shaped*	0.007
MOiNP	U-shaped*	0.002
MCiOP	U-shaped*	0.004
MOiNCH	−0.008 (−0.04 to 0.02)	0.62
DiDP Molar sum	−0.04 (−0.09 to 0.02)	0.17
MHiDP	U-shaped*	0.02
MCiNP	0.005 (−0.04 to 0.05)	0.84
Others		
DPHP (DPP)	−0.07 (−0.11 to −0.01)	0.01
TCP	0.01 (−0.03 to 0.06)	0.59
PBA	−0.07 (−0.11 to −0.02)	0.003
2OHPH	−0.03 (−0.08 to 0.03)	0.34

Betas (SE) are calculated using a multi-variable linear regression model for natural log-transformed urinary levels of each EDC (per gram urinary creatinine) separately, adjusted for gestational age at the time of sampling, maternal age, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity and fetal sex.

* Fig. 1. P values are for the non-linear association between each compound fitted with restricted cubic splines (with 3 knots) and hCG.

3. Results

After exclusions of women with no data on EDCs or hCG, the final study population included 2,039 pregnant women (Supplemental Fig. 3). Characteristics of the study population are shown in Table 1. The median gestational age of the participants at the time of sampling was 10 weeks (95% range: 6–14 weeks). The centile curves of hCG according to gestational age of pregnancy in the study population is presented in Supplemental Fig. 4. Mean absolute concentrations of hCG was significantly higher in women carrying female fetuses compared with those carrying male fetuses; 78,251 (IU/L) vs. 71,488 (IU/L), P = 0.0001. The same was for hCG Z-scores; 0.08 in women with female fetus vs. −0.07 in women with male fetus (P = 0.0005). The Spearman correlation coefficients between all EDCs ranged from − 0.15 to 0.98 (Supplemental Fig. 5).

3.1. Phenols

Higher BPA was associated with a lower hCG (β [95% CI]: −0.06 [−0.11 to −0.002], P = 0.04) but there was no association of BPS and BPF with hCG (Table 2). However, a higher urinary triclosan was associated with a higher hCG (β [95% CI]: 0.02 [0.004 to 0.04], P = 0.02).

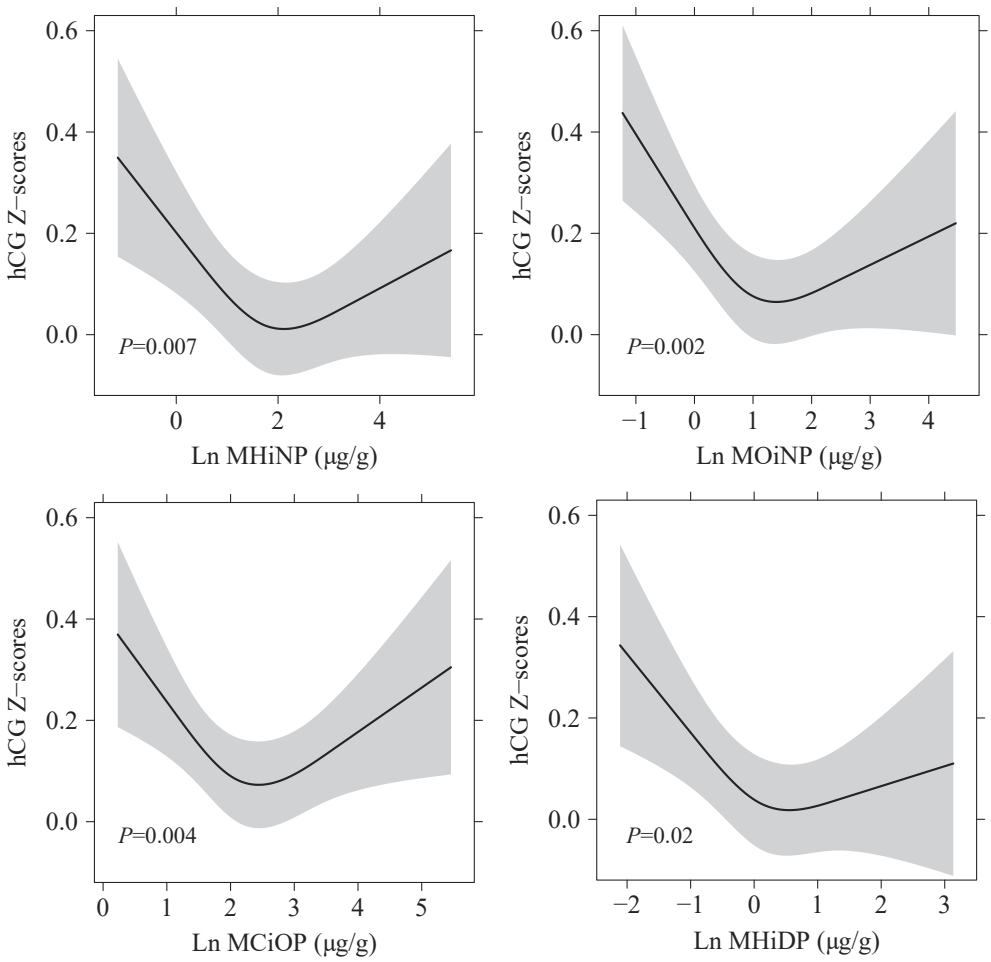

Fig. 1. Association of (natural log-transformed) MHiNP, MOiNP, MCiOP and MHiDP with serum hCG concentration.

3.2. Phthalates

Higher MEP, MBP, MBzP and all metabolites of DEHP (MEHP, MEHHP, MEOHP, MECPP and MCMHP) were associated with a lower hCG (Table 2; β [95% CI] for 1 M increase in sum of DEHP metabolites: −0.13 [-0.19 to −0.07], P < 0.001). Moreover, there was an U-shaped association of all DINP and DIDP metabolites (except for MCiNP (linear)) with hCG (Fig. 1 and Table 2).

3.3. PFAS

None of the PFAS were associated with hCG and there was no uniform direction amongst the beta estimates (Table 3).

3.4. Persistent chlorinated compounds

A higher HCB was associated with a lower hCG but there was no association of DDE with hCG (Table 3). In addition, a higher concentration of any PCBs was associated with a lower hCG; 7 out of 9 studied PCB associations were statistically significant (Table 3 and Fig. 2).

3.5. Other compounds

A higher DPHP or PBA was associated with a lower hCG but there was no association of TCP and 2OHPH with hCG (Table 2).

3.6. Sensitivity analyses with MoM of hCG and interaction with fetal sex

All results for MoM of hCG were generally the same as Z-scores of hCG (Supplemental Tables 4 and 5). Moreover, we did not find any significant interaction between EDCs and fetal sex in association with hCG (Supplemental Tables 6 and 7).

3.7. Mixture analysis

In the negative direction, each quartile increase in the EDC mixture was associated with −0.27 lower hCG Z-score (95% CI: −0.34 to −0.19) in the WQS regression model with repeated holdout validation; and MBP, MEP, PCB 183, PBA and MEHP had the largest weights in the association (Fig. 3). The WQS regression with repeated holdout validation in the positive direction did not show any association of the mixture with hCG (β [95% CI]: 0.02 [-0.05 to 0.09]).

4. Discussion

In this study, we show that exposure to BPA, phthalates, the organophosphate flame retardant DPHP, the pyrethroid pesticide PBA and persistent chlorinated pollutants (including PCBs and HCB) were associated with a lower hCG while none of the PFAS or bisphenols S and F were associated with hCG. The small effect sizes for these negative associations mean that there is minimal cross-sectional clinical relevance for the pregnant women. However, considering the vital role of the placenta in fetal development, from implantation to birth, if our observations for hCG are due to a direct effect on the placenta, we cannot

Table 3
Association of serum or plasma EDC (metabolites) with serum hCG concentration.

PFAS	β (95% CI)	P value
PFNA	−0.02 (−0.10 to 0.06)	0.66
PFDA	0.007 (−0.08 to 0.09)	0.86
PFUnDA	0.03 (−0.04 to 0.10)	0.45
PFHxS	0.05 (−0.02 to 0.12)	0.22
PFOA	−0.01 (−0.09 to 0.07)	0.82
PFOS	0.03 (−0.04 to 0.12)	0.35
Persistent chlorinated compounds		
HCB	−0.16 (−0.30 to −0.02)	0.02
DDE	−0.06 (−0.13 to 0.002)	0.06
Polychlorinated biphenyls		
PCB 99	−0.11 (−0.20 to −0.03)	0.007
PCB 118	Inverted J-shaped*	0.03
PCB 138	Inverted J-shaped*	0.03
PCB 153	Inverted J-shaped*	0.03
PCB 156	Inverted J-shaped*	0.02
PCB 170	−0.07 (−0.16 to 0.01)	0.10
PCB 180	−0.09 (−0.19 to −0.003)	0.04
PCB 183	−0.10 (−0.18 to −0.01)	0.03
PCB 187	−0.09 (−0.17 to 0.004)	0.06

Betas (SE) are calculated using a multi-variable linear regression model for natural log-transformed serum or plasma levels of each EDC separately, adjusted for gestational age at the time of sampling, maternal age, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity and fetal sex. PFAS are measured in serum. Persistent chlorinated compounds, including polychlorinated biphenyls are measured in plasma.

* Fig. 2. P values are for the non-linear association between each compound fitted with restricted cubic splines (with 3 knots) and hCG.

rule out potential (short- or long-term) adverse effects on the fetus even with low-exposure and subtle non-clinical effects of EDCs.

Placental formation, viability and function (including hormone production) can be adversely affected by EDCs via several pathways (Gingrich et al., 2020; Yang et al., 2019). BPA has been shown to reduce estrogen synthesis, induce placental cell apoptosis and interfere with enzymatic activity of cytochrome P450 in placental-related cell-lines (Gingrich et al., 2020). DEHP phthalates have been associated with lower placental weight, oxidative stress and inhibition of hCG production (Gingrich et al., 2020; Yang et al., 2019). PCBs can induce trophoblast cell apoptosis and disrupt placental invasion (Gingrich et al., 2020). Overall, the evidence from experimental studies point to toxic effects of EDCs on the placenta (Blake and Fenton, 2020; Feng et al., 2016; Gingrich et al., 2020; Szilagyi et al., 2020; Warner et al., 2021; Zhao et al., 2015; Adu-Gyamfi et al., 2022; Mannelli et al., 2014; Rajakumar et al., 2015); thus, we can speculate that such adverse effects can also further result in a reduced hCG production and secretion by the placenta.

In our study, among phenols, only BPA exposure was associated with a lower hCG while triclosan was associated with higher hCG. Although some experimental studies have shown that low dose exposure to BPA can increase the secretion of β-hCG by placental explants (Mannelli et al., 2014; Mørck et al., 2010) or increase the gene expression of hCG by human placental trophoblast cells (Rajakumar et al., 2015); but there is strong experimental evidence that BPA can reduce migration and invasion of trophoblast cells (Spagnoletti et al., 2015), impair decidualization (Nelson et al., 2020) and in general cause pathological changes in the placenta via metabolic and hormonal pathways (Adu-Gyamfi et al., 2022). We did find one human study of 137 pregnant women in which there was no association of urinary BPA with the pattern of rise in hCG concentration during the first 6 days following implantation (Chin et al., 2019). Previous data on other bisphenols is scarce and we did not find any association of BPS and BPF with hCG. Furthermore, in contrast to BPA, we did find a positive association of triclosan with hCG. However experimental data show that higher exposure of human choriocarcinoma JEG-3 cells to triclosan was associated with a decrease in β-hCG secretion while increasing the secretion of progesterone and estradiol (Honkisz et al., 2012). Moreover, pregnant rats exposed to higher doses of triclosan had lower serum levels of hCG (Feng et al., 2016). On the other hand, triclosan is a known estrogen agonist *in vivo and in vitro* (Yoon and Kwack, 2021; Kiyama and Wada-Kiyama, 2015) which might explain a positive effect on hCG production. In general for phenols, experimental evidence point towards a negative effect on placental function and further studies are needed to replicate our finding for triclosan.

We identified negative associations between low molecular weight phthalates and all DEHP metabolites and hCG while there was a U-shaped association of metabolites of DINP and DIDP with hCG. These findings are supported by results from experimental studies. It has been shown that secretion of β-hCG can decrease in primary placental cells exposed to a mixture of phthalates (Adibi et al., 2017). In addition, higher exposure to DEHP, DBP and BBP resulted in reduction of both basal and hCG-stimulated release of progesterone from cultured human luteal cells (Romani et al., 2014). Moreover, several *in vitro* studies have shown that phthalate exposure is toxic for placenta cells and can cause morphological changes in size and shape of placenta (Warner et al., 2021). Most experimental studies have focused on DEHP and its metabolites, showing several effects of exposure to DEHP on placental cells, such as induction of oxidative stress, downregulation of genes related to trophoblast development, inhibition of hCG production and disruption of progesterone feedback loop (Warner et al., 2021). Our findings are consistent with a study of 137 pregnant women, where those with a higher urinary concentrations of MBzP or the sum of DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP) had a slower rise in hCG concentration during the 6 days following implantation (Chin et al., 2019). Interestingly, higher maternal DEHP urine concentration are also associated with intra-uterine growth restriction (Zhao et al., 2015; Guo et al., 2022) which can also happen due to low β-hCG concentration (Sirikunalai et al., 2016). In a study among 541 pregnant women, higher first-trimester urinary MnBP, MBzP, and MCiOP were associated with higher first-trimester hCG in women carrying female fetuses, but a lower hCG in women carrying males (Adibi et al., 2015). Although such effects may point towards estrogen specific-pathways, we could not replicate any fetal sex-specific association between phthalates and hCG.

Experimental or human evidence on placental effect of DINP and DIDP is scarce which does not allow us to explain the observed U-shaped associations with hCG concentrations. We did not find any association of MOiNCH (metabolite of DINCH) with early pregnancy hCG concentrations. Many EDCs are known to have non-monotonic dose–response associations with endocrine endpoints but due to the multi-level and complex effects of EDCs on the endocrine system these often remain unexplained. Further studies are needed to replicate the U-shaped findings of our study before any investigation into the underlying mechanism of such association.

We did not find any association of PFAS with hCG concentrations. To the best of our knowledge this is the only human epidemiologic study investigating the association of exposure to PFAS with hCG concentration during pregnancy. Human studies have shown that PFAS measured during pregnancy are associated with low birth weight, suggesting a placental pathway (Szilagyi et al., 2020; Blake and Fenton, 2020). Experimental studies have shown that PFAS can decrease or inhibit trophoblast invasion (Szilagyi et al., 2020), decrease secretion of hCG and progesterone by syncytiotrophoblasts of human placenta (Zhang et al., 2015) and decrease placental weight (Gingrich et al., 2020; Blake and Fenton, 2020). Further studies are needed to replicate our results and investigate the potential effects of PFAS on hCG concentration in humans during later pregnancy or in high-risk pregnancies.

In our study higher exposure to several PCBs was associated with lower hCG concentrations. This could be explained by disruptive effects of PCBs on placental formation and/or trophoblast invasion including

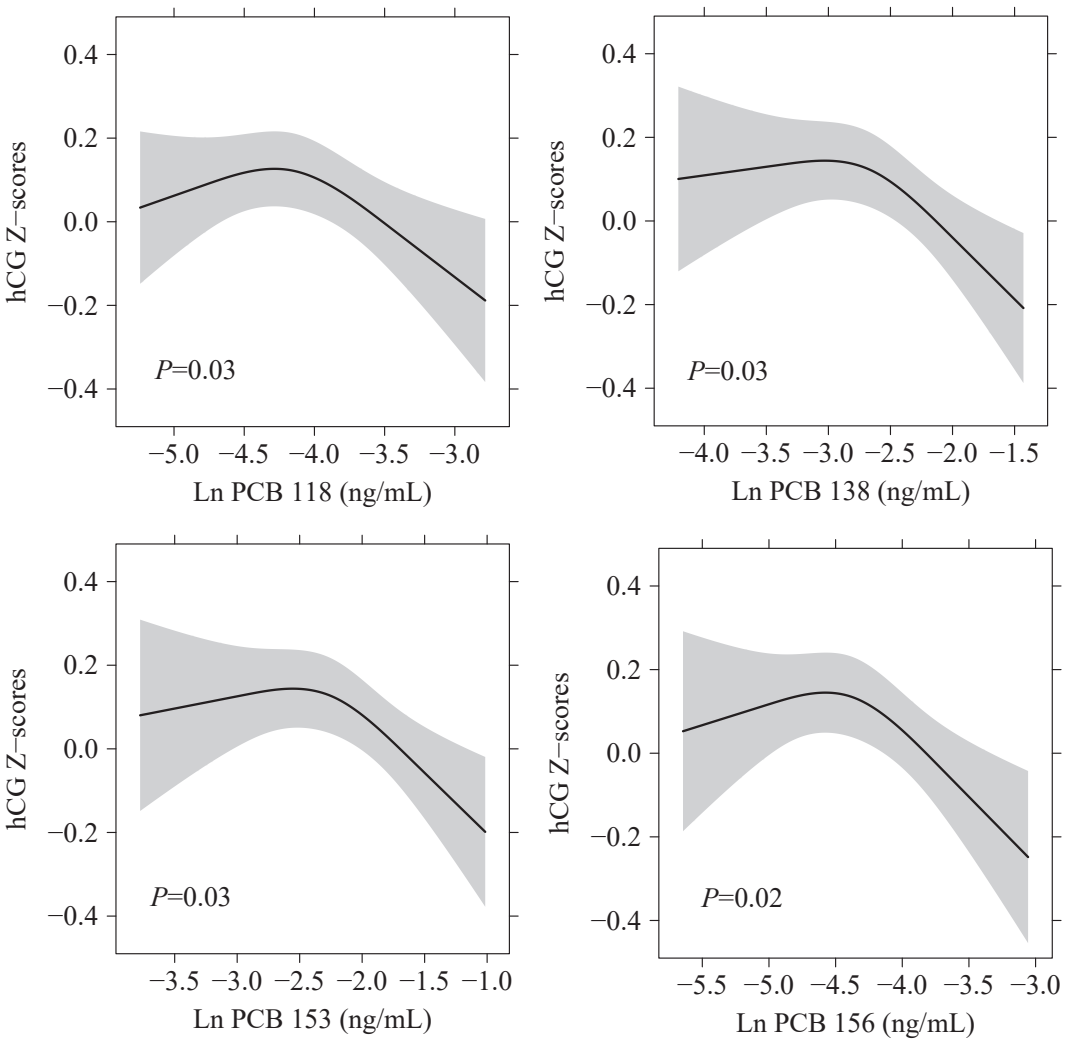

Fig. 2. Association of (natural log-transformed) PCB 118, PCB 138, PCB 153 and PCB 156 with serum hCG concentration.

the induction of apoptosis of trophoblast cells as shown in experimental studies (Gingrich et al., 2020). An alternative explanation could be that higher exposure to PCBs might increase the metabolism and clearance of hCG because PCBs are known inducers of cytochrome P450 family of enzymes (Gauger et al., 2007; Korytko et al., 1999; Machala et al., 1998; Drahushuk et al., 1997). Interestingly, higher gestational exposure to PCBs is associated with lower birth weight (Eguchi et al., 2022; Govarts et al., 2012) and a higher risk of preeclampsia (Eslami et al., 2016), outcomes for which lower hCG concentration is also a risk factor. Future studies could explore to what extend changes in hCG may mediate the association of PCB exposure with adverse pregnancy outcomes.

In this study, higher exposure to DPHP (an organophosphate flame retardant), PBA (a (pyrethroid pesticide) and HCB (fungicide) was also associated with a lower hCG concentration. Experimental data on effects of these chemicals on placenta development and hCG production is very limited. HCB has been shown to increase the induction and expression of an enzyme from cytochrome P450 family (CYP1A1) in human placental tissue (Gregoraszczuk et al., 2014) and exposure of human placental choriocarcinoma cells to DPHP can result in a decrease in progesterone secretion and an increase in hCG production (Hu et al., 2017). Because of a scarcity of data, we cannot extract enough supporting evidence for our findings from experimental or human studies; however, our findings advocate for further research on potential adverse effects of these chemicals on placenta or hCG production or metabolism, considering their well-known endocrine disruptive properties.

4.1. Mixture approach

Using WQS regression, we generally replicated the results of the single-EDC linear regression models. Based on the WQS regression, there was an overall negative association of EDCs mixture with hCG, and the chemicals with the highest weights were mostly phthalates (namely MBP and MEP). Even though utilizing a mixture approach cannot provide us with any information about the mechanisms of a mixture effect of EDCs in a complex biological setting; it provides a general quantitative and statistically calculated view of the association of a number of EDCs as a whole with hCG, while also validating the results of single-EDC regression models.

4.2. Strength and limitations

To the best of our knowledge this is the first large prospective study to investigate the association of several groups of EDCs with hCG concentration during pregnancy. However, several limitations of our study should be taken into account when interpreting the results. First, we did not have multiple measurements of EDCs that have a short half-life (such as phenols and phthalates) during pregnancy, which could improve the accuracy of exposure assessment. Moreover, considering the year of data collection, our data for chemicals with a short-half-life that have been gradually removed from the market since then might be less relevant; however the risk of the substitute chemicals cannot be ruled out. Second,

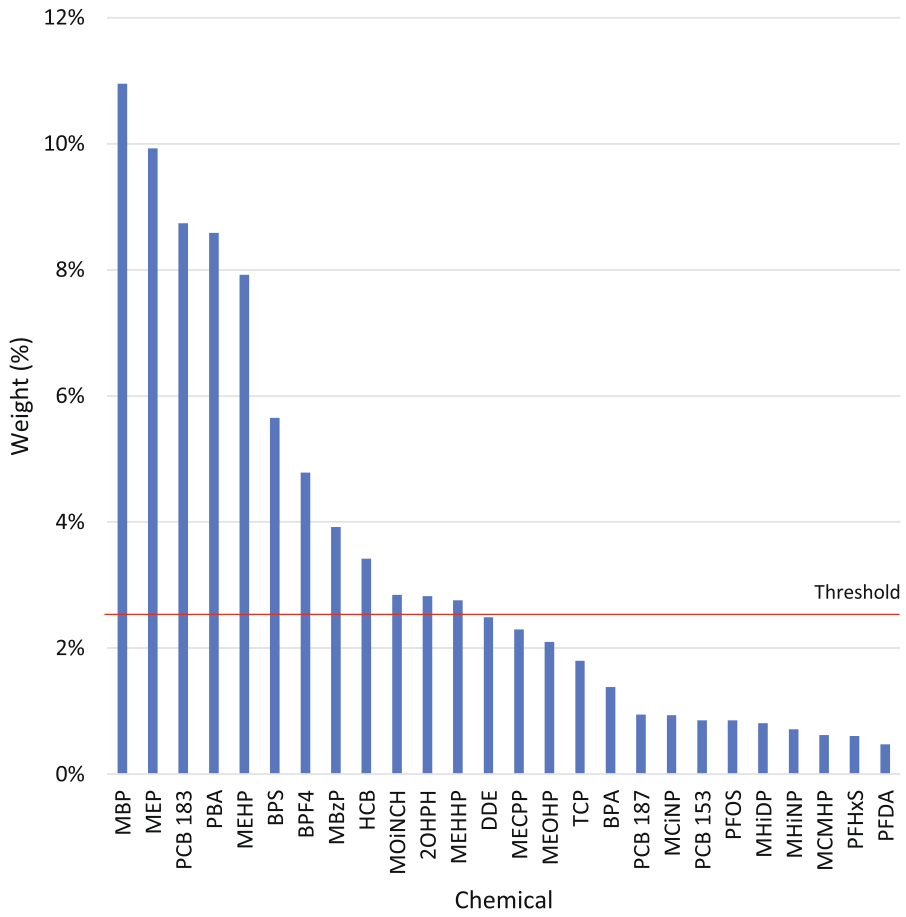

Fig. 3. Mean weights based on 100 repeated holdouts of WQS regression for the association of chemicals with lower hCG.

we did not have lipid measurements to have an optimal standardized measurement of PCBs. Third, we only had a single measurements of hCG, although hCG concentrations were measured at the same time as EDCs measurements and we standardized the measurements for gestational age in two different ways, having multiple measurements of hCG over time could have enabled us to better quantify the potential effects of EDCs on the trajectory of hCG which may be of more relevance from a biological point of view.

5. Conclusions

A higher gestational exposure to several groups of EDCs was associated with a lower hCG concentration. Combined with currently available results from experimental studies, our results suggest that that gestational exposure to EDCs could either reduce hCG production by placenta or increase hCG metabolism. Given the many physiological processes that depend on proper secretion of hCG – ranging from immune functions to optimal production of thyroid hormones – our observations warrant attention. Even though further human epidemiological studies are needed to replicate our results, our results provide an indication for further experimental studies to consider placenta as a target organ for adverse effects of EDCs.

Funding

This project has been supported by the Exchange in Endocrinology Expertise (3E) program of the European Union of Medical Specialists (UEMS), Section and Board of Endocrinology and the ATHENA project, funded under the European Union's Horizon 2020 Programme for research, technological development and demonstration, grant agreement no. 825161. The SELMA study was funded by grants from the Swedish Research Council (Formas). The sample analysis were supported by Region Skåne and the Medical Faculty at Lund University, Sweden.

CRediT authorship contribution statement

Arash Derakhshan: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Huan Shu:** Methodology, Resources, Data curation, Writing – review & editing. **Maarten A.C. Broeren:** Methodology, Resources, Data curation, Writing – review & editing. **Andreas Kortenkamp:** Project administration, Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Christian H. Lindh:** Methodology, Resources, Data curation, Writing – review & editing. **Barbara Demeneix:** Project administration, Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Robin P. Peeters:** Project administration, Supervision, Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Carl-Gustaf Bornehag:** Project administration, Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Tim I.M. Korevaar:** Supervision, Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

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

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108091.

References

Adibi, J.J., Lee, M.K., Naimi, A.I., Barrett, E., Nguyen, R.H., Sathyanarayana, S., Zhao, Y., Thiet, M.-P., Redmon, J.B., Swan, S.H., 2015. Human chorionic gonadotropin partially mediates phthalate association with male and female anogenital distance. J. Clin. Endocrinol. Metab. 100 (9), E1216–E1224.

Adibi, J.J., Zhao, Y., Zhan, L.V., Kapidzic, M., Larocque, N., Koistinen, H., Huhtaniemi, I. T., Stenman, U.-H., 2017. An investigation of the single and combined phthalate metabolite effects on human chorionic gonadotropin expression in placental cells. Environ. Health Perspect. 125 (10).

Adu-Gyamfi, E.A., Rosenfeld, C.S., Tuteja, G., 2022. The impact of bisphenol A on the placenta†. Biol. Reprod. 106(5), 826–834.

Bernert Jr., J.T., McGuffey, J.E., Morrison, M.A., Pirkle, J.L., 2000. Comparison of serum and salivary cotinine measurements by a sensitive high-performance liquid chromatography-tandem mass spectrometry method as an indicator of exposure to tobacco smoke among smokers and nonsmokers. J. Anal. Toxicol. 24 (5), 333–339.

Blake, B.E., Fenton, S.E., 2020. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects. Toxicology 443, 152565.

Bornehag, C.-G., Moniruzzaman, S., Larsson, M., Lindström, C.B., Hasselgren, M., Bodin, A., von Kobyletzkic, L.B., Carlstedt, F., Lundin, F., Nånberg, E., Jönsson, B.A. G., Sigsgaard, T., Janson, S., 2012. The SELMA study: a birth cohort study in Sweden following more than 2000 mother–child pairs. Paediatr. Perinat. Epidemiol. 26 (5), 456–467.

Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: Multivariate imputation by chained equations in R. J. Statist. Software 45(3).

Chin, H.B., Jukic, A.M., Wilcox, A.J., Weinberg, C.R., Ferguson, K.K., Calafat, A.M., McConnaughey, D.R., Baird, D.D., 2019. Association of urinary concentrations of phthalate metabolites and bisphenol A with early pregnancy endpoints. Environ. Res. 168, 254–260.

Cole, L.A., 2010. Biological functions of hCG and hCG-related molecules. Reprod. Biol. Endocrinol. 8 (1), 102.

Drahushuk, A.T., Choy, C.O., Kumar, S., McReynolds, J.H., Olson, J.R., 1997. Modulation of cytochrome P450 by 5, 5′-bis-trifluoromethyl-2, 2′-dichlorobiphenyl, a unique environmental contaminant. Toxicology 120 (3), 197–205.

Eguchi, A., Sakurai, K., Yamamoto, M., Watanabe, M., Hisada, A., Takahashi, T., Todaka, E., Mori, C., 2022. Association between Total and Individual PCB Congener Levels in Maternal Serum and Birth Weight of Newborns: Results from the Chiba Study of Mother and Child Health Using Weighted Quantile Sum Regression. Int. J. Environ. Res. Public Health 19 (2), 694.

Eslami, B., Malekafzali, H., Rastkari, N., Rashidi, B.H., Djazayeri, A., Naddafi, K., 2016. Association of serum concentrations of persistent organic pollutants (POPs) and risk of pre-eclampsia: a case-control study. J. Environ. Health Sci. Eng. 14, 17.

Feng, Y., Zhang, P., Zhang, Z., Shi, J., Jiao, Z., Shao, B., Kanellopoulos-Langevin, C., 2016. Endocrine disrupting effects of triclosan on the placenta in pregnant rats. PLoS One 11 (5), e0154758.

Gauger, K.J., Giera, S., Sharlin, D.S., Bansal, R., Iannacone, E., Zoeller, R.T., 2007. Polychlorinated biphenyls 105 and 118 form thyroid hormone receptor agonists after cytochrome P4501A1 activation in rat pituitary GH3 cells. Environ. Health Perspect. 115 (11), 1623–1630.

Gingrich, J., Ticiani, E., Veiga-Lopez, A., 2020. Placenta Disrupted: Endocrine Disrupting Chemicals and Pregnancy. Trends Endocrinol. Metab. 31 (7), 508–524.

Govarts, E., Nieuwenhuijsen, M., Schoeters, G., Ballester, F., Bloemen, K., de Boer, M., Chevrier, C., Eggesbø, M., Guxens, M., Krämer, U., Legler, J., Martínez, D., Palkovicova, L., Patelarou, E., Ranft, U., Rautio, A., Petersen, M.S., Slama, R., Stigum, H., Toft, G., Trnovec, T., Vandentorren, S., Weihe, P., Kuperus, N.W., Wilhelm, M., Wittsiepe, J., Bonde, J.P., 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ. Health Perspect. 120 (2), 162–170.

Gregoraszczuk, E.L., Ptak, A., Karpeta, A., Fiedor, E., Wróbel, A., Milewicz, T., Falandysz, J., 2014. Hexachlorobenzene and pentachlorobenzene accumulation, metabolism and effect on steroid secretion and on CYP11A1 and CYP19 expression in cultured human placental tissue. Reprod. Toxicol. 43, 102–110.

Guo, X., Sheng, Y., Liu, B., Tang, P., Liu, R., Wu, L.i., Chen, J., Huang, D., Liu, S., Qiu, X., 2022. Exposure to phthalates in early pregnancy and the risk of fetal growth restriction: a nested case-control study in a Zhuang Chinese population. Environ. Sci. Pollut. Res. Int. 29 (38), 57318–57329.

Gyllenhammar, I., Glynn, A., Jönsson, B.A.G., Lindh, C.H., Darnerud, P.O., Svensson, K., Lignell, S., 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ. Res. 153, 48–54.

Heidegger, H., Jeschke, U., 2018. Human Chorionic Gonadotropin (hCG)-An Endocrine, Regulator of Gestation and Cancer. Int. J. Mol. Sci. 19 (5), 1502.

Hershman, J.M., 2004. Physiological and pathological aspects of the effect of human chorionic gonadotropin on the thyroid. Best Pract. Res. Clin. Endocrinol. Metab. 18 (2), 249–265.

Honkisz, E., Zieba-Przybylska, D., Wojtowicz, A.K., 2012. The effect of triclosan on hormone secretion and viability of human choriocarcinoma JEG-3 cells. Reprod. Toxicol. 34 (3), 385–392.

Hu, W., Gao, F., Zhang, H., Hiromori, Y., Arakawa, S., Nagase, H., Nakanishi, T., Hu, J., 2017. Activation of Peroxisome Proliferator-Activated Receptor Gamma and Disruption of Progesterone Synthesis of 2-Ethylhexyl Diphenyl Phosphate in Human Placental Choriocarcinoma Cells: Comparison with Triphenyl Phosphate. Environ. Sci. Technol. 51 (7), 4061–4068.

Kahn, L.G., Philippat, C., Nakayama, S.F., Slama, R., Trasande, L., 2020. Endocrine-disrupting chemicals: implications for human health. Lancet Diabetes Endocrinol. 8 (8), 703–718.

Kiyama, R., Wada-Kiyama, Y., 2015. Estrogenic endocrine disruptors: Molecular mechanisms of action. Environ. Int. 83, 11–40.

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. 13 (10), 610–622.

Korytko, P.J., Casey, A.C., Bush, B., Quimby, F.W., 1999. Induction of hepatic cytochromes P450 in dogs exposed to a chronic low dose of polychlorinated biphenyls. Toxicol. Sci. 47 (1), 52–61.

Machala, M., Neča, Jiří, Drábek, P., Ulrich, R., Šabatová, V., Nezveda, K., Raszyk, J., Gajdušková, V., 1998. Effects of chronic exposure to PCBs on cytochrome P450 systems and steroidogenesis in liver and testis of bulls (Bos taurus). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 120 (1), 65–70.

Mannelli, C., Ietta, F., Carotenuto, C., Romagnoli, R., Szostek, A.Z., Wasniewski, T., et al., 2014. Bisphenol A alters β-hCG and MIF release by human placenta: An in vitro study to understand the role of endometrial cells. Mediators Inflamm. 2014;2014:635364.

Mørck, T.J., Sorda, G., Bechi, N., Rasmussen, B.S., Nielsen, J.B., Ietta, F., Rytting, E., Mathiesen, L., Paulesu, L., Knudsen, L.E., 2010. Placental transport and in vitro effects of Bisphenol A. Reprod. Toxicol. 30 (1), 131–137.

Nelson, W., Adu-Gyamfi, E.A., Czika, A., Wang, Y.X., Ding, Y.B., 2020. Bisphenol A-induced mechanistic impairment of decidualization. Mol. Reprod. Dev 87 (8), 837–842.

Norén, E., Lindh, C., Glynn, A., Rylander, L., Pineda, D., Nielsen, C., 2021. Temporal trends, 2000–2017, of perfluoroalkyl acid (PFAA) concentrations in serum of Swedish adolescents. Environ. Int. 155, 106716.

Nwabuobi, C., Arlier, S., Schatz, F., Guzeloglu-Kayisli, O., Lockwood, C.J., Kayisli, U.A., 2017. hCG: Biological Functions and Clinical Applications. Int. J. Mol. Sci. 18(10).

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

Pirkle, J.L., Flegal, K.M., Bernert, J.T., Brody, D.J., Etzel, R.A., Maurer, K.R., 1996. Exposure of the US Population to Environmental Tobacco Smoke: The Third National Health and Nutrition Examination Survey, 1988 to 1991. J. Am. Med. Assoc. 275 (16), 1233–1240.

Rajakumar, C., Guan, H., Langlois, D., Cernea, M., Yang, K., 2015. Bisphenol A disrupts gene expression in human placental trophoblast cells. Reproduct. Toxicol. 53:39-44.

Romani, F., Tropea, A., Scarinci, E., Federico, A., Dello Russo, C., Lisi, L., et al., 2014. Endocrine disruptors and human reproductive failure: The in vitro effect of phthalates on human luteal cells. Fertil. Steril. 102 (3), 831–837.

Sirikunalai, P., Wanapirak, C., Sirichotiyakul, S., Tongprasert, F., Srisupundit, K., Luewan, S., Traisrisilp, K., Tongsong, T., 2016. Associations between maternal serum free beta human chorionic gonadotropin (β-hCG) levels and adverse pregnancy outcomes. J. Obstet. Gynaecol. 36 (2), 178–182.

Spagnoletti, A., Paulesu, L., Mannelli, C., Ermini, L., Romagnoli, R., Cintorino, M., Ietta, F., 2015. Low concentrations of Bisphenol A and para-Nonylphenol affect extravillous pathway of human trophoblast cells. Mol. Cell. Endocrinol. 412, 56–64.

Stasinopoulos, D.M., Rigby, R.A., 2008. Generalized additive models for location scale and shape (GAMLSS) in R. J. Stat. Softw. 23, 1–46.

Szilagyi, J.T., Avula, V., Fry, R.C., 2020. Perfluoroalkyl Substances (PFAS) and Their Effects on the Placenta, Pregnancy, and Child Development: a Potential Mechanistic Role for Placental Peroxisome Proliferator-Activated Receptors (PPARs). Curr. Environ. Health Rep. 7 (3), 222–230.

Tanner, E.M., Bornehag, C.G., Gennings, C., 2019. Repeated holdout validation for weighted quantile sum regression. MethodsX. 6, 2855–2860.

Textor, J., van der Zander, B., Gilthorpe, M.S., Liśkiewicz, M., Ellison, G.T.H., 2016. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. Int. J. Epidemiol. 45 (6), 1887–1894.

Warner, G.R., Dettogni, R.S., Bagchi, I.C., Flaws, J.A., Graceli, J.B., 2021. Placental outcomes of phthalate exposure. Reprod. Toxicol. 103, 1–17.

Yang, C., Song, G., Lim, W., 2019. A mechanism for the effect of endocrine disrupting chemicals on placentation. Chemosphere 231, 326–336.

Yoon, K.S., Kwack, S.J., 2021. In vitro and in vivo estrogenic activity of triclosan. J. Toxic. Environ. Health A 84 (19), 800–809.

Zhang, N., Wang, W.S., Li, W.J., Liu, C., Wang, Y., Sun, K., 2015. Reduction of progesterone, estradiol and hCG secretion by perfluorooctane sulfonate via induction of apoptosis in human placental syncytiotrophoblasts. Placenta 36 (5), 575–580.

Zhao, Y., Shi, H.-j., Xie, C.-m., Chen, J., Laue, H., Zhang, Y.-h., 2015. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. Environ. Mol. Mutagen. 56 (3), 286–292.