MÁSTER UNIVERSITARIO EN ZOONOSIS Y UNA SOLA SALUD

Exposure to Endocrine-Disrupting Chemicals (EDCs) during pregnancy and blood pressure

Trabajo de Fin de Master de Chiara Seminati

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Abstract

Exposure to endocrine-disrupting chemicals (EDCs) has been largely studied in the last years and it has been related to different negative effects on human health. Among them alteration of fetal growth, deficiencies of neuronal development in children born from chronically exposed mothers, alterations of the male and female sexual apparatus have been described. Only few studies have investigated the association between exposure during pregnancy and change in blood pressure in pregnant women, although cardiovascular diseases (CVDs) are one of the most public health concern. In this study we have investigated the association between several EDCs biomarkers belonging to phthalates (10 metabolites), phenols (7 metabolites) and organophosphate pesticides (6 metabolites), and alterations of blood pressure (BP) in pregnant women. Women included in the study were 153 and were volunteer participant in Human Early-Life Exposome (HELIX) project. Association with urine concentration and blood pressure was investigated using a multivariates exposure model to assess the association between each compound and BP in studied women. Significant negative association was observed between systolic BP and exposure to MEP and MiBP (Phthalates) and to DEP and DETP (Organophosphate Pesticides) and between diastolic BP and exposure to MBzP (phthalates) and TRCS (phenol) during pregnancy. Our results are inconsistent with previous study that investigated similar associations and require replication.

Introduction

Cardiovascular diseases (CVDs) are a major public health concern since they are one of the first causes of death worldwide (WHO, Fact sheet on noncommunicable diseases 2016). High blood pressure (BP) during pregnancy may lead to develop high BP or other CVDs later in life, like microalbuminuria or kidney disease (Oishi M et al., 2017). Also, it can induce adverse effects on the foetus such as congenital heart defects, perinatal death, preterm birth, and child growth restriction (Ramakrishnan A et al., 2015). If high BP continues after 20 weeks of pregnancy, preeclampsia and other complications can develop (Smyth A et al., 2017). Several possible causes of high BP during pregnancy have been studied and they include overweight/obesity, smoking, low physical activity, drinking alcohol, first pregnancy, age over 40, and familial predisposition. Recently, environmental factors such as air pollution (Pedersen M et al., 2014) and exposure to chemicals present in the environment have also been proposed to contribute to the risk of gestational hypertension (Leow MK, 2015).

Endocrine-disrupting chemicals

Endocrine-disrupting chemicals (EDCs) are natural and synthetic chemicals that have the capacity to disrupt the endocrine system by competing with the endogenous hormones through binding to the receptor and hormone transport protein, or by interfering in the hormones metabolism or synthesis (Figure 1) (Casals-Casas C and Dersvergne B; 2011; Tabb MM and Blumberg B, 2006; Talsness CE et al., 2009; Wetherill YB et al, 20007; Braun JM, 2017). EDCs include a wide range of chemical compounds including pesticides, compounds used in the plastic industry and others used in consumer products. The persistence of these compounds in the human body is mainly due to their ability to accumulate in lipid tissues: organochlorine pesticides for example, are lipophilic and can persist in our body for decades. On the contrary, other EDCs have a short biological half-life and are excreted from our body in few days. Until today, most studies in animals and humans have been focused on the effects of persistent EDCs on BP alterations (Henríquez-Hernández LA et al, 2014; La Merrill MA et al., 2016); few studies have assessed the effects of non-persistent EDCs.

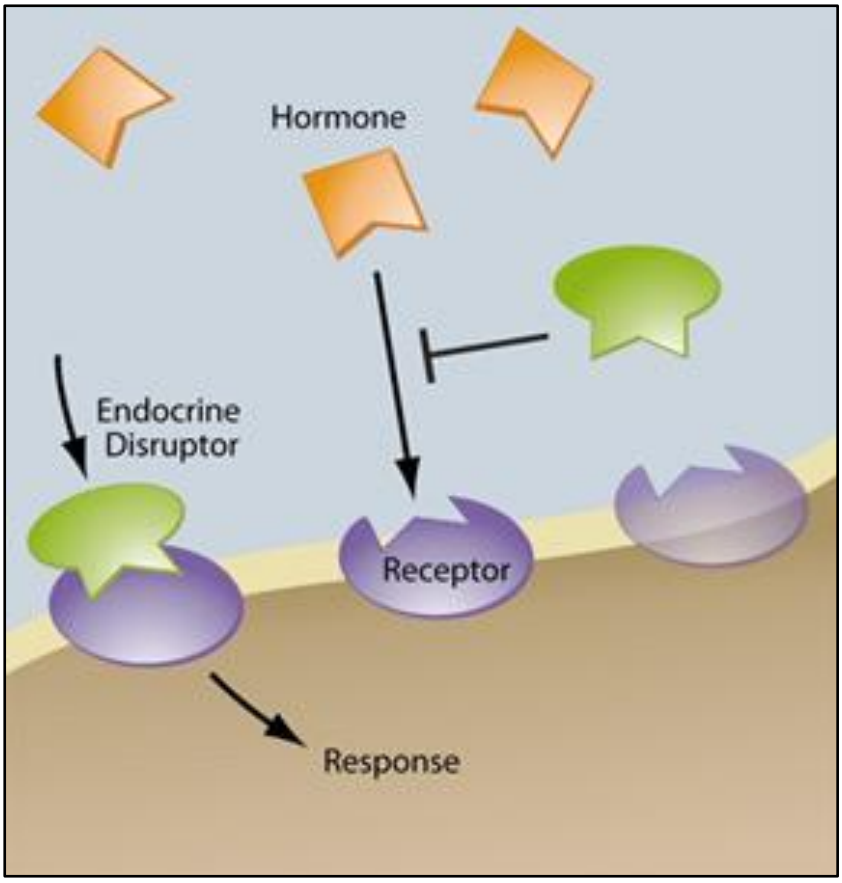


Figure 1. EDCs General Mechanism of action

Phenols, phthalates, and organophosphate pesticides

In this study we will focus on three important non-persistent EDCs: phthalates, phenols, and organophosphate (OP) pesticides.

Phthalates are widely used since the 1930s to confer flexibility, pliability and elasticity to the rigid polymers used for plastic production. They can be classified in two main groups: low molecular weight (LMW) phthalates, mainly used in cosmetic product, and high molecular weight (HMW) phthalates, used as plasticizers to impart flexibility in food packaging or medical devices. Phthalates are not covalently bound to the plastic and consequently they can be easily absorbed and detected in body fluids such as urine, blood, saliva, amniotic fluid, breast milk and cord blood (Latini G et al., 2003; Main KM et al., 2006; Mankidy R et al., 2013; Silva MJ et al., 2004a; Silva MJ et al, 2005; Silva et al., 2004b). The primary routes of phthalates exposure include consumption of

contaminated food, dermal contact to personal care products, and parenteral medication; phthalates can also be detected in dust, air, and water samples. In 1999, the European Union banned the use of some phthalates in the manufacture of toys and childcare articles (European Commission 2005). In the United States, environmental and public health organizations have conducted numerous campaigns to reduce their use in consumer products and, consequently, concentrations in the general population of some phthalates have started to decline (Zota et al. 2014). Although the health effects of exposure to phthalates are still unclear, they have the potential to alter the hormone system.

Phenols are organic compounds (acidic alcohols derived from benzene) released in the environment as products of petroleum refining, resin manufacturing, tanning, textile dyeing, etc. The most studied compound of this group has been bisphenol A (BPA). BPA is a high-volume-production monomer used since 1950s in the fabrication of polycarbonate plastics (plastic bottles, food containers, optical disks) and epoxy resins (water pipe lining, inner coating of cans, thermal papers). Due to the increased use of plastics all over the world, BPA is one of the most common environmental chemical which humans are exposed to. Dietary intake is thought to be the primary source of exposure to BPA, although it has also been detected in environmental samples including water, air and house dust (Vandenberg et al., 2007). After ingestion, BPA is quickly removed by the kidneys and excreted in urine in less than 6 hours (Dekant W and Völkel W, 2008; Völkel W et al., 2002). However, it has been described that continuous exposure to BPA may cause the deposit of BPA metabolites in lipid-rich tissues (Stahlhut RW et al., 2009). BPA has been described as weak EDCs, although the potential impact of BPA exposure on human health is not still completely clear (Bruan JM, 2017; Pergialiotis V et al., 2017). In 2011, the European Union banned the use of BPA in infant feeding bottles (European Union, 2011) and nowadays there are intensive debates about the need to ban BPA from other food contact materials, like for example currency bills (Liao C and Kannan K, 2011). For this reason, in the last years BPA has been substituted by synthetic bisphenols analogues, like bisphenol F (BPF) and bisphenol S (BPS). These BPA analogues can also have endocrine-disrupting activity.

Since organochlorine pesticides were banned in the 1970s after the Stockholm Convention (http://chm.pops.int/), OP pesticides have become the most widely used pesticides available today and they are used in agriculture, in home gardening and in veterinary practice. Even though these compounds do not persist in the environment for a long time, humans are constantly exposed to low concentrations present in the food and in the environment (Barr DB et al., 2004). Long-term neurobehavioral and neuropsychological consequences have been described associated with exposure to low-medium levels of OP pesticides (Mangas I et al., 2016).

Health effects of EDCs

During the last decade, a number of animal studies have suggested that exposure to EDCs can alter human health. More specifically, exposure to phenols, especially BPA, have been also evidenced the hormonal activity of this chemical, specifically alteration of development and sexual maturation in male and female and brain and cognitive abnormalities (Richter CA et al., 2007). Several animal studies assessing exposure to different phthalates in male have shown a variety of alterations that has been described as “Phthalates Syndrome”, being diethylhexyl phthalates (DEPH) the most potent (Kay VR et al., 2014), a set of symptoms ranging from reduced anogenital distance, hypospadias, cryptorchidism, and malformations in the epididymis, vas deferens, seminal vesicles and prostate in mice (Arcadi FA et al., 1998; Li LH et al., 1998). Other studies have shown association between phthalates exposure and development of metabolic disorder in rodents, such as changes in serum levels of insulin, blood glucose, glycogen, cortisol and alterations of levels of thyroid hormones (Gayathri NS et al., 2004; Heudorf U et al., 2007). Regarding OP pesticides toxicity, studies in animals have provided information about the neurotoxicity like changes in the nervous system development and alterations in sensory, motor and cognitive cerebral function of rodents associated with early exposure to chlorpyrifos (Maurissen JP et al., 2000b; Maurissen JP et al., 2000a; Rice D and Barone S Jr et al., 2000). Other studies evidenced the relationship between prenatal exposure even to low concentrations of chlorpyrifos and organogenesis, behavioural changes, hyperactivity and memory deficit (Tian Y et al., 2005) in mice.

Studies in humans have shown that continuous exposure to phthalates in humans can bring to alterations in the development of male sexual apparatus (Swan SH et al., 2015; Sharpe RM 2005) and also damage in the respiratory system that may lead to asthma (Bornehag CG and Nanberg E., 2010). On the other hand, BPA exposure investigated in small population, has been associated to obesity in women, recurrent miscarriages and sterility (Lang IA et al., 2008; Sugiura-Ogasawara M et al., 2005; Takeuchi T et al., 2004). Studies mainly focused on maternal outcomes have found associations between time to pregnancy (only in males) and exposure to phthalates but not to BPA (Vélez MP et al., 2015; Buck Louis GM et al., 2014). On the other hand, exposure to BPA has been associated with miscarriage risk in the first trimester (Lathi RB et al., 2014; Sugiura-Ogasawara M et a., 2005) and to preeclampsia in pregnant women just before delivery (Leclerc F et al., 2014). Other studies have found no association between exposure to phthalates metabolites and BPA and maternal blood glucose (Robledo CA et al., 2015; Robledo C et al., 2013). At the same time, other observations did not show any relationship between exposure to di(2-ethylhexyl) phthalate triglycerides and fatty acid levels (Jia X et al., 2015).

Endocrine-disrupting chemicals and blood pressure

Animal studies that have particularly investigated the association between exposure to EDCs and changes in BP found out evidences that, for example, continuous exposure to BPA impacts cardiac structure/function, protein expression, and epigenetic DNA methylation marks in males and females (Patel et al., 2013). Also, other studies provided evidences that maternal exposure to di-(2-ethylhexyl) phthalate (DEHP) has a lasting effect on the physiological functions of the vascular system (Lee KI et al, 2016).

Studies in humans have mainly focused on the effects of non-persistent EDCs in children, adolescents, and adults. Cross-sectional analyses of the National Health and Nutrition Examination Survey for example, suggested that exposure to phthalates during childhood and adolescence was associated with higher systolic BP (Trasande L et al., 2013) and low-grade albuminuria, a marker of vascular dysfunction associated with chronic kidney and CVD risks (Trasande L et al., 2014). Additionally, a cross-sectional study of older adults in Sweden found that increased serum concentrations of

monobenzyl phthalate (MBzP) were associated with several cardiovascular risk factors (Wiberg et al 2014). A study in Spain however, showed that prenatal exposure to phthalates was associated with lower systolic BP in girls but not in boys (Valvi et al., 2015). Exposure to OP pesticides have been associated with higher glucose plasma levels, higher triglycerides and high-density lipoprotein, and lower lower-density lipoprotein in adults (Ranjbar et al., 2015). OP pesticides have been also associated with higher body mass index (BMI) and BP in children, only in children carrying the HDL-associated enzyme paraoxonase 1 (PON1) R-allele (Andersen HR et al., 2012).

Very few studies have investigated the association between exposure to EDCs and BP in pregnant women (Werner et al 2015, Leclerc et al 2014, Jia et al 2015). A study within the HOME study in US (Werner et al. 2015), found that higher urinary concentrations of mono-benzyl phthalate (MBzP) at <20 weeks of pregnancy were associated with increased diastolic BP and risk of pregnancy-induced hypertensive diseases. Leclerc et al (2014), observed that women with preeclampsia had higher levels of BPA in placental tissue compared to women without preeclampsia. Finally, Jia et al (2015) did not find an association between prenatal exposure to phthalates and triglycerides and fatty acid levels; in this study, they did not assess BP. We should consider that Werner et al (2015) only used two spot urine samples collected during pregnancy to determine phthalates concentrations and due to the high variability of these compounds misclassification of exposure may be high. Also, this study used BP measures derived from medical records without detailed information on the quality controls used and without knowing whether the patient had been previously exposed to tobacco smoke that can alter BP measures. They also did not have information on pre-pregnancy health status which may have contributed to the risk of increased BP. Therefore, there is a need to study the potential effects of exposure to non-persistent EDCs on BP during pregnancy in different population settings and with a more accurate exposure and outcome assessment.

Potential mechanisms of action

The mechanisms underlying the association between exposure to non-persistent EDCs and BP are poorly understood. Some mechanisms have been suggested, particularly for phthalates. First, some studies describe that phthalates may cause a pro-inflammatory

response and increased oxidative stress (Werner EF et al., 2015); we should consider that oxidative stress is a key player in the pathogenesis of hypertension. Another possible mechanism is related to the ability of phthalates to act on human metabolism as antagonist of thyroid hormones, androgens, and cortisol and may activate peroxisome proliferator-activated (PPAR) receptors (Boas M et al., 2012; Monsalve FA et al., 2013). PPAR is a nuclear receptor superfamily with a key role in adipogenesis, lipid accumulation, and insulin resistance (Taxviq C et al., 2012). All the biological mechanisms mentioned above are directly involved in controlling the cardiac function and consequently BP. Animal studies have also underlined other potential mechanisms of effect of exposure to phthalates on BP, through direct influence on kidney (Lee KI et al., 2016) and adrenal glands (Martinez-Arguelles DB et al., 2012; Martinez-Arguelles DB et al., 2014). Finally, epigenetic changes have been suggested as another potential mechanism of EDCs exposure and alteration of BP (Baccarelli A et al., 2009).

Objective

The aim of this study was to assess the association between exposure to phthalates, phenols, and OP pesticides and BP in pregnant women by using repeated measurements of urinary EDCs concentrations in two weeks during pregnancy.

Materials and methods

Study population

A total of 156 pregnant women were recruited within the framework of the Human Early-Life Exposome (HELIX) project, a collaborative research project with the aim to understand how the different environmental exposures (described globally as the *exposome*) that mothers and children are exposed to, can influence the health, growth and development of children. They conducted an intensive follow-up during one week in the second trimester (<20weeks) and one week in the third trimester of pregnancy (<32 weeks) (Vrijheid et al 2014). For the present study we included a total of 153 pregnant women with information on EDCs and BP in both weeks. The study included

around 50 women from each of the three European regions under study: 52 from Barcelona (Spain), 46 from Grenoble (France) and 55 from Oslo (Norway). Criteria for inclusion were singleton pregnancy, age ≥18 years at the time they got pregnant, first visit to be conducted before week 20 of pregnancy and residence in the area under study. All women included in the study were volunteers. The study was approved by the Ethic Committees of each country and all participants gave their written informed consent.

Urine collection and pooling procedure

Women collected 3 urines per day during one week in the second trimester and one week in the third trimester. Urines collected were first morning, midday (when possible) and last nighttime void. Urines were collected in 70 ml polypropylene containers and stored in a domestic freezer (typically -20ºC). At the end of each monitoring week, all samples were transported to each study centre, using cool box ice packs to prevent thawing, and stored in a -80ºC freezer. Urines were defrosted overnight at 4ºC and then placed at room temperature for 30 min prior to aliquoting. Urines from each subject were processed at the same time. Each pregnant woman collected 21 urines per week; in total, 6,426 urine samples were collected. We pooled all the urines collected in a week by taking 0.5 ml from each aliquot. In all settings, collection and processing of the samples were performed in a completely harmonized way, using the same protocols and equipment.

Determination of phenols, phthalates, and OP pesticides

Concentrations of phenols, phthalates, and OP pesticides were determined in the two weekly pools of 21 urines each. We analyzed a total of 10 phthalate metabolites originating from 6 different phthalates (monoethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl 5-carboxypentyl phthalate (MECPP), mono-4-methyl-7-hydroxyoctyl phthalate (ohMiNP) and mono-4-methyl-7-oxooctyl phthalate (oxoMiNP); 7 phenols (BPA, triclosan, 5 parabens (methyl-, ethyl-, propyl-, butyl paraben), and benzophenone-3; 6 non-specific OP pesticide

metabolites: dimethyl phosphate, diethyl phosphate, dimethyl thiophosphate, diethyl thiophosphate, dimethyl dithiophosphate and diethyl dithiophosphate, cotinine, and creatinine. Samples were analyzed at the Department of Environmental Exposure and Epidemiology at the Norwegian Institute of Public Health (NIPH), in Norway following the methods described in detail elsewhere (Thompsen et al in preparation). Briefly, phenols concentrations were quantified using ultra performance liquid chromatography - tandem mass spectrometer system (UPLC-MS/MS) (Thomsen et al, under preparation), phthalate metabolites using liquid chromatography coupled with mass spectrometry system (LC-MS/MS) (Sabaredzovic et al., 2015), and OPs pesticides using the ultra-performance liquid chromatography-time-of-flight system (UPLC-TOF) (Cequier et al., 2016). Creatinine was measured at Fürst Medisinsk Laboratorium (Norway) by using AU680 Chemistry System form Beckman Coulter using DRI® Creatinine-Detect® Test. Limits of detection (LODs) for each analyte are listed in Table 2. Concentrations of phenols, phthalates, OP pesticides, and cotinine were divided by urinary creatinine concentration to control for urine dilution (concentration are expressed in micrograms per gram of creatinine). For all methods, we included internal quality control samples in each batch and results were evaluated according to method specific criteria. Further, NIPH participates in at least one inter-laboratory comparison for phenols and phthalate metabolites during the period when HELIX samples were analysed (Thompsen et al., under preparation).

Blood pressure measurements

BP measurement was performed at the end of each week by using the OMRON 705-CPII automated oscillometry. Cuff bladder was chosen according to the physical characteristic of each participant, in order to obtain the most precise measurement of BP and avoid possible over or underestimations. Women were previously informed about the way BP would be measured, especially because it was very important for the correct measurement to remain quiet and did not fell stressed. Measurements were developed using the right arm and with the woman in a sitting position, according to the following steps: after 5 minutes of rest, 2 consecutives measurements were taken with two minutes interval between them. If the difference between the first two measurements was greater than 5 mmHg, then a third one was performed. In each

measurement systolic and diastolic BP were recorded. For our study we have created a variable that represents the average of the three systolic and diastolic BP measurements of each woman.

Covariates

Information on socio-demographic and lifestyle characteristics was obtained by questionnaires completed by women and by face-to-face interviews conducted by trained interviewers at the beginning of the first week. Each pregnant women provided information on education, social class, ethnicity, weight and height, parity, health history with special focus on diseases that could interfere with BP (diabetes type I and II, heart disease, renal and suprarenal disease, alteration of blood coagulation, alteration of thyroid gland), marital status, employment status, smoking habits during pregnancy (active and passive), and complications of current pregnancy (hypertension, preeclampsia, eclampsia, gestational diabetes). At the beginning of the second week, women provided information of changes in the civil and employment status, onset of pathologies related to pregnancy, weight gained between trimesters, and changes in smoking habits (active and passive). During the two weeks women recorded time of urination of all voids and filled in diaries with information about diet habits (black coffee, tea, sweet beverages, and salty snacks).

Statistical analysis

Urinary concentrations of phthalates, phenols, and OP pesticides below the LOD were assigned a value of half the LOD. Because of the short biological half-lives of these compounds (Koch HM and Calafat AM, 2009), we used the average of creatinine-adjusted concentrations determined in 12 and 32 weeks of pregnancy to provide a better estimation of exposure during pregnancy. Because of the approximate log-normal distribution of urinary concentrations, average creatinine-adjusted concentrations of pollutants were $\log_2$ transformed. To determine the variables included

in the multivariate models, we used direct acyclic graphs (DAGs) (Shrier I and Platt Rw., 2008). Covariates were included in the DAGs if they were described to be associated with EDCs or BP in previous literature. Based on the DAGs, the final multivariate models were adjusted for ethnicity (European Caucasian; Asiatic; South America; Others) marital status (cohabitant;married;single), maternal education (Primary; Secundary; University), employment status (employed;unemployed), BMI (Underweight; Overweight;Normal), smoking during pregnancy (never smoked; non smoker but previously smoked; smoker), second-hand tobacco exposure (yes;no); previous deliveries (none;one;two;four), chronic diseases (yes;no), and maternal diet. As chronic clinical status we considered heart condition, blood coagulation problems and thyroid dysfunctions, because other pathologies were not present in the study population. As maternal diet, we included consumption of black coffee, tea, sweet beverages, and salty snacks. The association between each EDC and BP was examined using linear mixed models because our data involved 2 exposures and 2 outcome measurements for each woman. First, we only adjusted our models for cohort (Minimally-adjusted Model). Then, we adjusted our model for all covariates: maternal education, smoking during pregnancy, employment status, marital status, chronic diseases, ethnicity and maternal diet (Fully-adjusted Model). Results are expressed as change in mmHg of systolic or diastolic BP for doubling of EDC concentration.

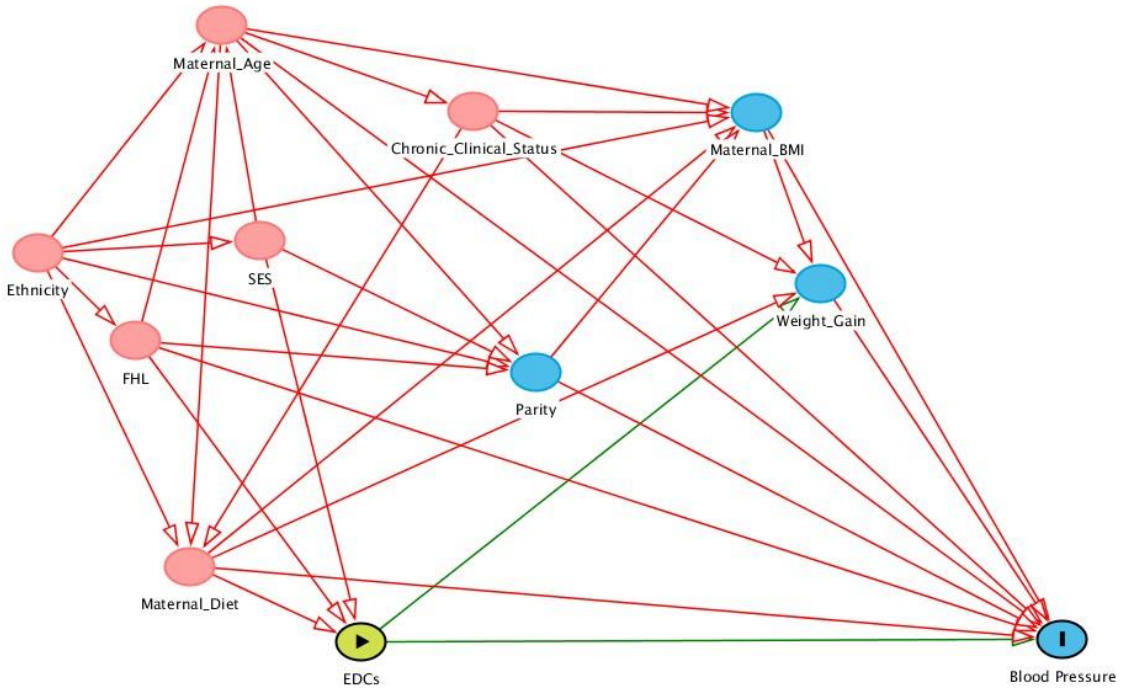
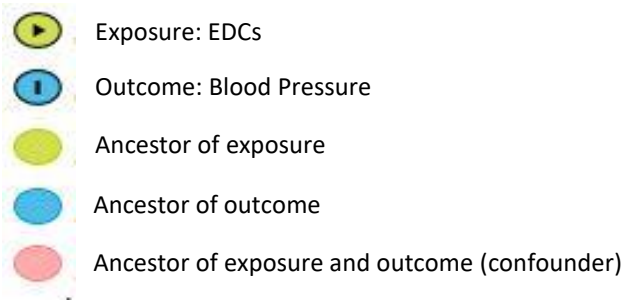


Fig 2. DAG illustration.
Legend: SES: Social Economic Status: Education, Employment Status
FHL: Family Health Lifestyle: Smoking (Active/Passive), Marital Status.



Results

Study Population and Exposure Characteristics.

Complete details of the characteristics of the study population are given in Table 1. Women included in the study were more likely to be Caucasian (86.9%), married (63.7%), well-educated (University studies, 88.2%) and employed (87.0%). Almost half of women (40.0%) were non-smokers, but 50.3% of women reported to had previously

smoked in some point in their lives although they quit when they got pregnant. Fourteen percent of women were overweight or obese at the moment of the study begin. Most of women (56.2%) enrolled in the study were in their first pregnancy. In relation to the chronic diseases that could alter BP, we finally included in the study heart, blood coagulation, and thyroid diseases. The characteristics of the study population in each cohort were similar to the general population (Table 1).

	All N=153	Barcelona n=52	Grenoble n=46	Oslo n=55
Ethnicity				
European Caucasian	133 (86.9%)	44 (84.6%)	37 (80.4%)	52 (94.5%)
Asiatic	2 (1.3%)	0	1 (2.2%)	1 (1.8%)
South America	9 (5.9%)	7 (13.5%)	1 (2.2%)	1 (1.8%)
Mixed	3 (0.6%)	1 (1.9%)	1 (2.2%)	1 (1.8%)
Missing	6 (5.2%)	0	6 (13.0%)	0
Marital Status				
Cohabitant	36 (26.7%)	13 (25.0%)	0	23 (41.8%)
Married	93 (63.7%)	22 (42.3%)	39 (84.8%)	32 (58.2%)
Single	17 (11.6%)	17 (32.7%)	0	0
Missing	7 (4.6%)	0	7 (15.2%)	0
Education				
Primary	3 (1.9%)	3 (5.8%)	0	0
Secundary	15 (9.8%)	6 (11.5%)	6 (13.0%)	3 (5.4%)
University	135 (88.2%)	43 (82.7%)	40 (87.0%)	52 (94.5%)
Missing, N	0	0	0	0
Employment Status				
Employed	133 (87.0%)	39 (75.0%)	41 (89.1%)	53 (96.4%)
Unemployed	16 (10.4%)	13 (25.0%)	1 (2.2%)	2 (3.6%)
Missing, N	4 (2.6%)	0	4 (8.7%)	0
BMI				
Underweight	2 (1.4%)	1 (2.1%)	0	1 (1.8%)
Normal	123 (84.2%)	40 (83.3%)	38 (86.4%)	45 (83.3%)
Overweight	16 (11.0%)	5 (10.4%)	4 (9.1%)	7 (13.0%)
Obese	5 (3.4%)	2 (4.2%)	2 (4.5%)	1 (1.8%)
Missing, N	7 (4.6%)	4 (7.7%)	2 (4.3%)	1 (1.8%)
Smoking during pregnancy				
Non-smoker	61 (40.0%)	24 (46.1%)	11 (24.0%)	26 (47.2%)
Non smoker but previously smoker	77 (50.3%)	25 (48.1%)	27 (58.7%)	25 (45.4%)
Smoker	6 (3.9%)	2 (3.8%)	4 (8.7%)	0

Missing, N	9 (5.9%)	1 (1.9%)	4 (8.7%)	4 (7.2%)
Second-hand-tobacco exposure				
Yes	14 (14.0%)	8 (15.4%)	6 (13.1%)	0
No	139 (90.8%)	44 (84.6%)	40 (86.9%)	55 (100%)
Missing, N	0	0	0	0
Previous deliveries				
No	86 (56.2%)	32 (61.5%)	21 (46.6%)	33 (60.0%)
One	49 (32.0%)	16 (30.7%)	16 (34.8%)	17 (30.9%)
Two	17 (11.1%)	4 (7.7%)	9 (19.6%)	4 (7.3%)
Four	1 (0.7%)	0	0	1 (1.8%)
Missing, N	0	0	0	0
Chronic disease (*)				
Yes	19 (12.6%)	7 (13.5%)	8 (17.4%)	4 (7.3%)
No	132 (90.1%)	45 (86.5%)	36 (78.2%)	51 (92.7%)
Missing	2 (1.3%)	0	2 (3.8%)	0

Table 1. Characteristics of the study population

(*) Chronic disease include: heart, blood coagulation, and thyroid diseases.

Concerning the dietary habits, most of women showed a high consumption of black coffee (34.6%) or sweet beverages (30.7%) (at least one a day or several in a week). Most women in our study population had a medium consumption of salty snacks (few units in a week) (28.7%).

	Diet			
	Black Coffee	**Tea**	**Salty Snacks**	**Caffeinated Drinks**
Never	31 (20.2%)	29 (19.0%)	7 (4.6%)	31 (20.3%)
Medium (few units a week)	23 (15.1%)	44 (28.7%)	97 (63.4%)	29 (18.9%)
High (one a day or several in a week)	53 (34.6%)	34 (22.2%)	13 (8.5%)	47 (30.7%)
Missing, N	46 (30.1%)	46 (30.1%)	36 (23.5%)	46 (30.1%)
All	153	153	153	153

Table 2. Dietary Habits of the study population

Distribution of biomarkers concentrations in urine

The distribution of phthalates, phenols and OPs pesticides in urine samples is shown in Table 3. Phthalates were detected in all urine samples (0% <LOD), phenols were detected in most of urine samples (0-3.3% <LOD), while some of the OP pesticides were variably detected (for example DEP detection was <LOD was 2.6%, while DEDTP was 99.3%). Cotinine was detected in less than 13% of samples. Of the ten phthalates studied, MEP, MiBP, and MnBP presented the highest concentrations whereas OXO-MiNP and MEHP the lowest. Of the seven phenols, MEPA presented the highest concentrations, approximately ten orders of magnitude higher than all the other phenols. Finally, for the six OP pesticides, DMP, DMTP, and DEP showed the highest concentrations whereas DMDTP and DEDTP the lowest.

		<LOD		QOR				Percentiles			
Biomarker	**LOD (µg/L)**	**N**	**%**	**N**	**%**	**GM**	**Min**	**p25**	**p50**	**p75**	**Max**
Phthalates											
MEP	0.15	0	0.0	11	3.6	49.3	2.93	23.36	48.08	93.13	1376.24
MiBP	0.15	0	0.0	0	0.0	26.3	6.92	18.16	26.18	34.64	296.21
MnBP	0.15	0	0.0	0	0.0	16.4	5.47	11.74	15.65	22.56	95.67
MBzP	0.06	0	0.0	3	1.0	4.4	1.14	2.39	4.02	7.09	115.02
MEHP	0.15	0	0.0	10	3.3	3.2	0.74	1.99	3.02	5.00	145.46
MEHHP	0.12	0	0.0	1	0.3	9.7	2.85	6.60	8.92	12.28	398.14
MEOHP	0.12	0	0.0	0	0.0	6.4	2.45	4.50	5.82	7.97	210.82
MECPP	0.61	0	0.0	0	0.0	16.2	7.30	11.72	14.62	20.20	391.45
OH-MiNP	0.06	0	0.0	0	0.0	6.5	1.16	3.31	5.40	11.40	427.76
OXO-MiNP	0.06	0	0.0	0	0.0	3.8	0.55	1.96	3.04	6.27	98.76
Phenols											
MEPA	0,03	0	0.0	1	0.3	47.4	1.99	12.67	44.24	125.28	11044.95
ETPA	0,03	1	0.3	1	0.3	2.5	0.23	0.60	1.29	8.51	307.87
PRPA	0,03	3	1.0	2	0.7	7.2	0.01	1.85	7.73	27.21	2454.44
BPA	0,03	7	2.3	3	1.0	3.5	0.67	2.12	3.41	5.34	45.82
BUPA	0,06	10	3.3	1	0.3	0.2	0.01	0.06	0.12	0.56	29.43
OXBE	0,03	0	0.0	0	0.0	8.2	0.13	2.29	6.54	24.61	7688.08
TRCS	0,03	0	0.0	0	0.0	2	0.10	0.36	0.96	5.19	907.87
OPs pesticides											
DMP	0,36	50	16.3	0	0.0	4.5	0.85	3.09	4.14	6.12	24.26
DMTP	0,06	25	8.2	0	0.0	4.9	0.29	2.96	4.60	7.39	57.78
DMDTP	0,06	231	75.5	25	8.2	1	0.15	0.33	0.96	2.79	11.77
DEP	0,09	8	2.6	0	0.0	3.8	0.80	2.36	3.66	5.60	70.04
DETP	0,13	93	30.4	10	3.3	2.2	0.19	1.28	2.11	3.30	68.39
DEDTP	0,05	304	99.3	0	0.0	0.2	0.17	0.17	0.18	0.19	0.19

Table 3. Distribution of urinary concentrations (µg/g creatinine)[a] of phthalates, phenols, OP pesticides, and cotinine in pregnant women (n=6,426 urines)[b]

[a] A total of 21 urines in two periods were collected: 21urines*2periods*153 women= 6,426

Abbreviations: GM: geometric mean; LOD: limit of detection; QOR: quantifiable out of range; Min: minimum; Max: maximum; MEP: Monoethyl phthalate; MiBP: Mono-iso-butyl phthalate; MnBP: Mono-n-butyl phthalate; MBzP: Mono benzyl phthalate; MEHP: Mono-2-ethylhexyl phthalate; MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP: Mono-2-ethyl-5-oxohexyl phthalate; MECPP: Mono-2-ethyl 5-carboxypentyl phthalate; OH-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate; OXO-MiNP: Mono-4-methyl-7-oxooctyl phthalate; MEPA: Methyl-paraben; ETPA: Ethyl-paraben; PRPA: Propyl-paraben; BPA: Bisphenol A; BUPA: Butyl-paraben; OXBE: Oxybenzone or Benzophenone-3; TRCS: Triclosan; DMP: Dimethyl phosphate; DMTP: Dimethyl thiophosphate; DMDTP: Dimethyl dithiophosphate; DEP: Diethyl phosphate; DETP: Diethyl thiophosphate; DEDTP: Diethyl dithiophosphate.

Blood Pressure

The distribution of BP is shown in Table 4. The mean value for systolic BP was 101 mmHg (64-139 mmHg) and for diastolic BP was 64 mmHg (31-97 mmHg). These measurements belong to normal range of values for pregnant women (http://www.who.int/features/qa/82/en/).

Variable	Mean	St. Dev	Min	Max
Systolic 1	106.4855	12.62175	61	148
Diastolic 1	65.82372	9.327087	38	103
Pulse1	76.42395	11.81066	50	113
Systolic 2	105.0353	11.62941	65	137
Diastolic 2	64.46474	8.995106	17	92
Pulse2	76.99344	11.74734	49	121
Systolic 3	101.7317	12.58156	65	133
Diastolic 3	63.73743	9.398978	39	96
Pulse 3	77.58382	11.89294	50	114
Systolic average	101	-	64	139
Diastolic average	64	-	31	97
Pulse average	83	-	50	116

Table 4. Distribution of BP measurements in mmHg in the study population

Associations of EDCs and BP

The association between urine biomarkers EDCs concentrations and BP are show in Table 5.

	Sistolic BP						Diastolic BP					
	Minimally-adjusted model[a]			Fully-adjusted model[b]			Minimally-adjusted model[a]			Fully-adjusted model[b]		
	N	β (95% CI)	P value	N	β (95% CI)	P value	N	β (95% CI)	P value	N	β (95% CI)	P value
Phthalates												
MEP	152	**-0.01 (-0.01, 0.00)**	**0.043**	84	**-0.01 (-0.01, 0.00)**	**0.027**	152	-0.00 (-0.01, 0.00)	0.229	84	0.00 (-0.01, 0.00)	0.079
MiBP	153	**-0.04 (-0.08, -0.01)**	**0.021**	85	**-0.12 (-0.22, -0.01)**	**0.029**	153	-0.02 (-0.05, 0.13)	0.238	85	-0.01 (-0.20, 0.00)	0.055
MnBP	153	-0.04 (-0.13, 0.05)	0.420	85	-0.05 (-0.16, 0.06)	0.335	153	-0.01 (-0.09, 0.06)	0.706	85	0.02 (-0.02, 0.13)	0.726
MBzP	153	0.04 (-0.07, 0.15)	0.464	85	0.16 (-0.27, 0.58)	0.466	153	0.04 (-0.04, 0.14)	0.333	85	-0.50 (-0.92, -0.08)	0.020
MHEP	152	0.03 (-0.08, 0.14)	0.584	84	-0.25 (-0.62, 0.12)	0.183	152	0.00 (-0.01, 0.09)	0.987	84	-0.14 (-0.51, 0.22)	0.431
MEHHP	153	0.00 (-0.04, 0.04)	0.899	85	-0.13 (-0.27, 0.01)	0.077	153	0.00 (-0.03, 0.03)	0.301	85	-0.09 (-0.23, 0.05)	0.213
MEOHP	153	0.00 (-0.07, 0.07)	0.909	85	-0.23 (-0.48, 0.02)	0.071	153	0.00 (-0.06, 0.06)	0.935	85	-0.19 (-0.44, 0.06)	0.138
MECPP	153	0.00 (-0.04, 0.04)	0.935	85	-0.08 (-0.2, 0.04)	0.175	153	0.00 (-0.03, 0.03)	0.932	85	-0.68 (-0.18, 0.05)	0.260
OH-MiNP	153	-0.02 (-0.06, 0.01)	0.152	85	-0.01 (-0.04, 0.02)	0.580	153	0.00 (-0.02, 0.03)	0.810	85	0.00 (-0.03, 0.04)	0.840
OXO-MiNP	153	-0.03 (-0.12, 0.07)	0.557	85	-0.01 (-0.12, 0.1)	0.889	153	0.04 (-0.04, 0.12)	0.315	85	0.06 (-0.04, 0.18)	0.218
Phenols												
MEPA	153	0.00 (0.00, 0.00)	0.781	85	0.00 (0.00, 0.00)	0.357	153	0.00 (-0.01, 0.00)	0.836	85	0.00 (0.00, 0.00)	0.532
ETPA	153	-0.01 (-0.05, 0.02)	0.473	85	-0.02 (-0.06, 0.01)	0.237	153	-0.02 (-0.05, 0.01)	0.895	85	-0.01 (-0.04, 0.02)	0.620
PRPA	153	0.00 (-0,01, 0.00)	0.469	85	0.00 (-0.01, 0.00)	0.158	153	0.00 (0.00, 0.00)	0.624	85	0.00 (0.00, 0.00)	0.823
BPA	153	-0.07 (-0.27, 0.13)	0.499	85	-0.08 (-0.3, 0.14)	0.466	153	-0.13 (-0.31, 0.04)	0.139	85	-0.18 (-0.40, 0.03)	0.120
BUPA	153	-0.06 (-0.36, 0.24)	0.678	85	-0.17 (-0.48, 0.14)	0.288	153	-0.16 (-0.41, 0.09)	0.218	85	-0.22 (-0.53, 0.09)	0.163
OXBE	153	0.00 (0.00, 0.00)	0.156	85	0.00 (0.00, 0.00)	0.06	153	0.00 (0.00, 0.00)	0.530	85	0.00 (0.00, 0.00)	0.457
TRCS	153	0.00 (-0.01, 0.01)	0.664	85	0.00 (-0.01, 0.01)	0.969	153	0.00 (-0.01, 0.00)	0.266	85	-0.01 (-0.02, -0.00)	0.020
OP Pesticides												
DMP	153	-0.1 (-0.32, 0.12)	0.362	85	-0.07 (-0.35, 0.2)	0.600	153	-0.12 (0.31, 0.07)	0.229	85	0.01 (-0.27, 0.28)	0.966
DMTP	153	-0.06 (-0.21, 0.08)	0.400	85	-0.04 (-0.27, 0.2)	0.766	153	-0.05 (-0.18, 0.07)	0.377	85	0.02 (-0.22, 0.25)	0.887
DMDTP	147	-0.43 (-1.17, 0.32)	0.260	81	-0.72 (-1.93, 0.5)	0.247	147	-0.42 (-1.09, 0.24)	0.207	81	-0.25 (-1.47, 0.97)	0.691
DEP	153	-0.07 (-0.23, 0.1)	0.413	85	**-0.21 (-0.42, -0.01)**	**0.044**	153	-0.08 (-0.23, 0.06)	0.260	85	-0.08 (-0.30, 0.13)	0.457
DETP	152	**-0.27 (-0.49, -0.05)**	**0.016**	85	**-0.33 (-0.57, -0.08)**	**0.009**	152	0.00 (-0.19, 0.19)	0.993	85	0.12 (-0.14, 0.36)	0.390
DEDTP	153	NA		85	NA		153	NA	NA	85	NA	

Table 5. Adjusted association between urinary EDCs Biomarkers levels (μg/g creatinine) and Blood Pressure in pregnant women

[a]Adjusted for cohort

[b]Adjusted for maternal education, smoking during pregnancy, employment status, marital status, chronic diseases, ethnicity and maternal diet.

Abbreviations: CI: confidence interval; NA: non-applicable; MEP: Monoethyl phthalate; MiBP: Mono-iso-butyl phthalate; MnBP: Mono-n-butyl phthalate; MBzP: Mono benzyl phthalate; MEHP: Mono-2-ethylhexyl phthalate; MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP: Mono-2-ethyl-5-oxohexyl phthalate; MECPP: Mono-2-ethyl 5-carboxypentyl phthalate; OH-MiNP: Mono-4-methyl-7-hydroxyoctyl phthalate; OXO-MiNP: Mono-4-methyl-7-oxooctyl phthalate; MEPA: Methyl-paraben; ETPA: Ethyl-paraben; PRPA: Propyl-paraben; BPA: Bisphenol A; BUPA: Butyl-paraben; OXBE: Oxybenzone or Benzophenone-3; TRCS: Triclosan; DMP: Dimethyl phosphate; DMTP: Dimethyl thiophosphate; DMDTP: Dimethyl dithiophosphate; DEP: Diethyl phosphate; DETP: Diethyl thiophosphate; DEDTP: Diethyl dithiophosphate.

Overall we observed that exposure during pregnancy to the majority of phthalates, phenols, and OP pesticides was associated with a decrease or no change in systolic and diastolic BP; only few associations were statistically significant (Table 5). Since results did not significantly change after adjusting for socioeconomic, lifestyle, and dietary factors, we only report results of the fully adjusted model. For phthalates, we observed a reduction of 0.01 (95% confidence interval (CI): -0.01, 0.00) and 0.12 (95% CI: -0.22, -0.01) mmHg of systolic BP for each doubling of concentration of maternal urinary MEP and MiBP, respectively. For phthalates and diastolic pressure, we observed a reduction of 0.50 (95% confidence interval (CI): -0.92, -0.08) mmHg of diastolic BP. Also, we observed border line statically significant values for MEP (95% confidence interval (CI): -0.01, 0.00) and MiBP (95% confidence interval (CI): -0.20, 0.00). Non-significant associations were observed between exposure to phenols and systolic BP. For phenols and diastolic BP, we observed a reduction of 0.01 (95% confidence interval (CI): -0.01, 0.00) for TRCS. Regarding OP pesticides, we observed a reduction of 0.33 (95% CI: -0.57, -0.08) mmHg of systolic BP for each doubling of concentration of maternal urinary DETP (Table 5). No association was observed between maternal urinary OP pesticides and diastolic BP.

Discussion

In this population of pregnant women from three European countries with a widespread exposure to phthalates and phenols and low exposure to OP pesticides, we observed few associations between these compounds and maternal BP. We only observed negative associations between exposure to phthalates MEP and MiBP and the OP pesticide DETP and systolic BP. We also report negative association between phthalate MBzP and phenol TRCS and diastolic BP.

In our study concentrations of these EDCs in urine were similar to others assessing presence of phthalates (Werner et al., 2015; Valvi et al., 2015; Agay-Shay et al., 2015; Arbuckle et al., 2014) and BPA (Vafeiadi et al., 2016; Agay-Shay et al., 2015; Arbuckle et al., 2014) in pregnant women; although the majority of these studies evaluated the effects of early life exposure (maternal exposure) to these compounds on child health, in some cases including cardiovascular diseases and blood pressure.

Only one study has previously investigated the association between exposure to EDCs and BP in pregnant women (Werner et al 2015); and they only studied phthalates concentrations during pregnancy. As far as our knowledge, our study represents the first that evaluates the association between a wide range of EDCs (including phthalates, phenols, and OPs pesticides) and maternal BP. Our findings are quite different in relation to those reported by Werner et al 2015. In 369 women they found that higher urinary concentrations of MBzP in early-mid pregnancy were associated with increased diastolic BP and risk of pregnancy-induced hypertensive diseases. No other associations were observed in relation to other phthalates including MEP. We did find association between MBzP concentrations and diastolic BP but it seems that exposure to these phthalates reduces diastolic BP. In our results, we also did find an association between MEP and systolic BP. We have to consider that in the previously mentioned study BP was not the primary outcome of interest when the study was planned, and used BP measured derived from the medical records. Also, they did not consider some of the previous clinical characteristics of the women included (pre-pregnancy health status). Moreover, MBzP levels doubled the concentrations of our population (Werner median:

9 µg/g creatinine; our study median: 4 µg/g). They only collected two spot urine samples during pregnancy and hence, misclassification of the exposure cannot be ruled out. Finally, we should also take into account differences between the study populations.

Other studies have investigated the association of prenatal exposure to phthalates and BPA with triglycerides and fatty acid levels (Jia et al 2015) or preeclampsia (Leclerc et at, 2014), respectively. While Jia et al (2015) did find any association between phthalates concentrations and triglycerides and fatty acid levels, Leclerc et al (2014) found that women with preeclampsia had higher levels of BPA in placental tissue compared to women without preeclampsia. In our study we did not find any association between BPA and BP. However, we should consider that BPA placental levels in women with preeclampsia were 3 times higher than in controls (without preeclampsia), similar to the BPA urinary concentrations of our study. Results are also contradictory among those studies assessing the effects of exposure to non-persistent EDCs and BP in other study populations (children, adolescents, and adults); however, most of them found that exposure to phthalates or OP pesticides were associated with increased BP or CVD risk (Trasande L et al., 2013, Trasande L et al., 2014, Wiberg et al 2014, Valvi et al., 2015, Ranjbar et al., 2015, Andersen HR et al., 2012). Effects of EDCs exposure on BP have been described in laboratory studies. Lee KI et al. (2015) for example described that long-term exposure to DEHP in pregnant mice could led to increased BP in the offspring. Further studies in pregnant women are needed to disentangle whether exposure to phthalates, phenols, and OP pesticides can have an effect on BP.

The main strengths of our study rely on the repeated measurements of urinary EDCs concentrations and BP during pregnancy. Also, our population involve three different European populations and results did not differ across them (data not shown). In spite of that, this study has some limitations. First, although we used the average of two weekly pools of 21 urines each to characterize EDCs exposure during pregnancy, the high within-person and within-and between-day variability of some these compounds means that exposure misclassification cannot be ruled out. Such misclassification is likely to be random with respect to our outcomes, and is thus more likely to have led to an attenuation of associations (Pollack et al., 2013). This is particularly important for those

compounds with a very low reproducibility (high variability) such as BPA or DETP (Braun et al., 2012; Casas et al under preparation) during pregnancy. Phthalates on the contrary, have relatively good between-trimester reproducibility because the intraclass-correlation coefficient (ICC) of these two weekly pools for MEP and MiBP is 0.58 (Casas et al, under preparation). The ICC is a measure of reproducibility, calculated by dividing the between-subject variability by the sum of the between- and within-subject variability. Values range from 0, indicating no reproducibility, to 1, indicating perfect reproducibility (Rosner B., 2000). Therefore, we expect that misclassification of exposure would be higher in the case of DETP associations than of MEP and MiBP. Additionally, the majority of participants show normal BP values and this may have attenuated associations between EDCS exposure and our outcome of interest. Second, we also should consider that, other covariates not included in this study may have influenced our results. For example, we could not include maternal age because this variable was not available at the moment of the study. Also, several studies have suggested an association between BP fluctuations and season or ambient temperature (Quin et al., 2016); unfortunately, these data were not available in our study, although in the future they could be extrapolated using the dates of when BP measurements were taken and the data of meteo archives of the geographic area of origin of the women. We also did not take into account the physical activity of the participants. It is known that the regular of practice physical activity can help to control the development of hypertension (Diaz KM and Shimbo D., 2013), especially in among normotensives and prehypertensives persons. The inclusion of physical activity as covariate in our study could have helped us to give more explanations about the results. Although we should consider if exposition to EDCs could be also be associated to physical activity and in that case try to clarify the possible confounding effect. In the same way, other parameters of stress that could have an influence on development of hypertension development (Choxi AA et al., 2017) such as chronic exposure to noise and few hours of sleep should also be considered. Finally, we performed quite a large number of comparisons between exposures and outcomes (23 exposure x 2 outcomes = 46 comparisons), which may have led to spurious findings.

Conclusions

- This study is the first that combine repeated measurements of a wide range of EDCs and blood pressure in pregnant women.

- We observed few associations between exposure to EDCs during pregnancy and BP

- We only observed negative associations between MEP and MiBP phthalates and DETP OP pesticide and systolic and diastolic blood pressure.

- Our results are inconsistent with previous study that investigated similar associations.

- These findings require replication.

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