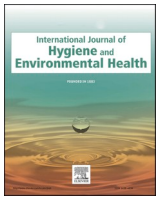
International Journal of Hygiene and Environmental Health 240 (2022) 113909

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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



Prenatal exposure to phthalates and phenols and preclinical vascular health during early adolescence

Parisa Montazeri [a,b,c], Serena Fossati [a,b,c], Charline Warembourg [a,b,c], Maribel Casas [a,b,c], Diana B.P. Clemente [d], Raquel Garcia-Esteban [a,b,c], Tim S. Nawrot [d,e], Martine Vrijheid [a,b,c,*]

[a] ISGlobal, Barcelona, Spain
[b] Universitat Pompeu Fabra (UPF), Barcelona, Spain
[c] CIBER Epidemiología y Salud Pública (CIBERESP), Spain
[d] Centre for Environmental Sciences, Hasselt University, Hasselt, Belgium
[e] Department of Public Health and Primary Care, Leuven University, Leuven, Belgium

ARTICLE INFO

Keywords:
Phenols
Phthalates
Cardiovascular diseases
Chemical exposures
Endocrine disrupting chemicals
Mixtures analysis

ABSTRACT

Background and aim: Exposure to endocrine-disrupting chemicals may increase cardiovascular risk from early life, but studies in children have shown inconsistent results, most focused on analysis of single chemicals, and none included measures of micro-vascularization as early preclinical markers. This study aimed to evaluate the association between prenatal exposure to phthalates and phenols and macro- and microvascular health during early adolescence.

Methods: Using data from a Spanish birth cohort (n = 416), prenatal exposure to eight phthalate metabolites and seven phenols (bisphenol A, four parabens, benzophenone-3, triclosan) were assessed using first and/or third trimester spot-urine concentrations. Macrovascular health (systolic and diastolic blood pressure (SBP and DBP, mmHg), pulse wave velocity (PWV, m/s)) and microvascular health (central retinal artery/vein equivalent (CRAE/CRVE, μm)), were measured at 11 years old. Linear regression models assessed associations for individual chemicals and Bayesian weighted quantile sum regression (BWQS) evaluated the overall association of the phthalate and phenol mixture with cardiovascular health.

Results: In single exposure models, bisphenol-A was associated with decreased PWV (β per doubling of exposure = −0.06; 95% CI: −0.10, −0.01). Mono-iso-butyl phthalate was associated with an increase in CRAE (β = 1.89; 95% CI: 0.34, 3.44). Methyl- and butyl-parabens were associated with a decrease in CRVE (β = −0.71; 95% CI: −1.41, −0.01) and (β = −0.96; 95% CI: −1.57, −0.35), respectively. No statistically significant associations were observed between any of the exposures and SBP or DBP. BWQS models showed no evidence of associations between the phthalate and phenol mixture and any of the outcomes.

Conclusions: Our results provide little evidence to suggest that prenatal exposure to phthalates and phenols is associated with macro- or microvascular health during early adolescence, except a few associations with certain compounds. Errors in exposure measurement and reduced variability in cardiovascular measures at this early age limit our ability to draw strong conclusions.

1. Introduction

Widespread exposure to environmental contaminants is a public health concern as each year millions of tons of plastics and other consumer products are produced around the world (Gore et al., 2015; Landrigan and Goldman, 2011). Several of these contaminants are known as endocrine disrupting chemicals (EDCs), meaning they can alter the hormonal and homeostatic systems thus interfering with natural body processes (Diamanti-Kandarakis et al., 2009). Some EDCs have long half-lives, in turn wreaking havoc on humans and animals alike for decades after production, while others are non-persistent and have shorter half-lives but are so widespread in their use that human exposure is constant making them of concern (Diamanti-Kandarakis et al., 2009). These non-persistent chemicals, including phthalates and phenols, are of concern given their widespread use in building materials, clothing, food containers, personal care products and medical devices

* Corresponding author. Barcelona Institute for Global Health - Campus MAR, Doctor Aiguader, 88, 08003, Barcelona, Spain.
E-mail address: martine.vrijheid@isglobal.org (M. Vrijheid).

https://doi.org/10.1016/j.ijheh.2021.113909
Received 9 July 2021; Received in revised form 10 November 2021; Accepted 15 December 2021
Available online 21 December 2021
1438-4639/© 2022 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

(Meeker, 2012; Wittassek et al., 2011; Wormuth et al., 2006).

Pregnant women are of particular concern as several EDCs are known to cross the placental barrier which may cause irreversible damage to the fetus resulting in increased disease susceptibility in later life (Lunder et al., 2010; Mamsen et al., 2017; Vrijheid et al., 2016). Indeed, cardiovascular morbidity can be considered a pediatric disease given that it is likely to originate during development (Heindel and Vandenberg, 2015). Cardiovascular disease risk can be traced from adulthood back to early childhood and vascular changes during childhood have been associated with arterial hypertension, coronary heart disease, diabetes mellitus and obesity in later life (Franks et al., 2010; Juonala et al., 2011; Li et al., 2016; Newman et al., 2017). Increasing evidence has suggested a relationship between early life exposure to EDCs, including phthalates and bisphenol-A (BPA), and development and progression of cardiovascular disease over the life course (Fu et al., 2020; Mariana et al., 2016; Nidens et al., 2020). However, the majority of previous work has evaluated the association between EDCs and cardiovascular risk cross-sectionally and using only blood pressure, lipid profile, and/or adiposity measurements as markers. Measures of the function of the vascular system outside of blood pressure have yet to be studied.

Examining additional vascular measures associated with cardiovascular disease would be beneficial to create a more complete picture of cardiovascular risk. In addition to blood pressure, pulse wave velocity (PWV), a measure of macrovascular health and arterial stiffness, has been associated with increased cardiovascular risk and atherosclerosis in adulthood (Cote et al., 2015; Hudson et al., 2015). Microvascular changes can be assessed through retinal vein and artery diameters, whereby venular widening is indicative of inflammation and atherosclerosis, and arterial narrowing is indicative of arterial damage (Newman et al., 2017). Such measures have been associated with cardiovascular and metabolic disease in adults (Newman et al., 2017), and related to elevated blood pressure, obesity, and type 1 diabetes during childhood (Li et al., 2016; Newman et al., 2017). To the best of our knowledge, no studies have assessed the relationship between prenatal urinary concentrations of phthalates and phenols and cardiovascular markers beyond blood pressure, such as PWV or retinal imaging.

Given that the prenatal period is a critical period of development during which exposure to EDCs may increase cardiovascular disease susceptibility in later life this study aimed to: 1) analyze the association between prenatal exposure to phenols and phthalates and measurements of macro- and microvascular function during early adolescence; 2) consider multiple chemical exposures by using a statistical approach that accounts for chemical mixtures; and 3) explore if these associations may be altered by sex and social class.

2. Methods

2.1. Study population

The participants in this study were from the INMA (INfancia y Medio Ambiente, Environment and Childhood) study, a longitudinal birth cohort study from Sabadell, Spain that included 657 women who were recruited at 10–13 weeks of gestation through regional hospitals from 2004 to 2006. The inclusion criteria were: ≥16 years of age, singleton pregnancy, intention to deliver at reference hospital, and no assisted conception or communication issues (Guxens et al., 2012). Children and their families participated in regular follow-up visits in which data was collected via questionnaires and biological samples. The present analysis was limited to mother – child pairs in which the mother gave at least one spot urine sample during pregnancy and their child participated in the 11-year follow-up with at least one cardiovascular measurement available (N = 416). This study was approved by the Ethics Review Committee and all mothers signed a written consent for themselves and their child's participation.

2.2. Measurement of phthalates and phenols

Spot urine samples were collected from pregnant women during the first and third trimesters of pregnancy in 100 mL polypropylene containers. These samples were aliquoted in 10 mL polyethylene tubes and stored at −20 °C.

2.3. Phthalates

Phthalate analyses were carried out in the Bioanalysis Research Group at the Hospital del Mar Medical Research Institute (Spain). This study uses data for eight measured phthalate metabolites: MBzP (monobenzyl phthalate), MEP (mono-ethyl phthalate), MiBP (mono-iso-butyl phthalate), MnBP (mono-n-butyl 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). Analysis was performed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) (Valvi et al., 2015). The limit of detection (LOD) values were: 0.5 μg/L for MEHHP, MEOHP, MBzP and MiBP, and 1 ng/mL for MEHP, MECPP, MEP and MnBP. Phthalate concentrations were determined in pregnant women who had urine samples available both in the first and third trimesters of pregnancy (N = 334); these concentrations were averaged for analyses.

2.3.1. Phenols

Seven phenols were analyzed in this study: MEPA (methyl paraben), ETPA (ethyl paraben), PRPA (propyl paraben), BPA, BUPA (n-butyl paraben), BP-3 (benzophenone-3), TRCS (triclosan). Total BPA (free plus conjugated) determination was carried out in the Department of Analytical Chemistry laboratory – University of Cordoba (Spain) using UPLC-MS/MS (Casas et al., 2013). The remaining phenols were analyzed at the Norwegian Institute of Public Health (NIPH) laboratory using on-line solid phase extraction (SPE) prior to UPLC-MS/MS (Sakhi et al., 2018). The LOD values were: 1 μg/L for BPA, 0.07 μg/L for BUPA, and 0.04 μg/L for all remaining phenols. BPA was measured in urine samples collected both in the first and third trimester in 331 pregnant women; these were averaged for analyses. All other phenols were measured only in the third trimester urine samples (N = 405 to 407, depending on the compound).

2.3.2. Creatinine

Creatinine concentrations were measured at the Echevarne Laboratory of Barcelona (Spain) using the Jaffé method (kinetic measurement, compensated method) with Beckman Coulter© reactive in AU5400 (IZASA®). Creatine adjusted phthalate and phenol concentrations were calculated for each trimester and then averaged. The creatinine adjusted concentrations were used in all the statistical analysis (hereafter, in μg/g creatinine).

2.3.3. Cardiovascular measurements

During the 11-year follow-up visit, cardiovascular measurements were taken by INMA nurses trained to use the appropriate device. Children were visited during school hours. Measures of blood pressure and PWV were taken to assess the macrovascular structure while images of the retina were taken to assess the microvascular structure.

2.3.4. Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken using an automated oscillometric device OMROM 705IT with brachial cuff attached. Children sat in a chair with their legs uncrossed, both feet flat on the floor, and their arm and back supported. The cuff was positioned on the child's resting arm placing the artery index marker over the brachial artery. After 5 min of relaxation, three consecutive measurements were taken with 1-min intervals. An average of the second and third measurements were used for analyses and values

are presented are in millimeter of mercury (mmHg).

2.3.5. Pulse wave velocity

PWV was recorded using VICORDER® in combination with the VICORDER® vascular diagnostic program package. To take the measurement the child laid down in the supine position using a support to raise their head and shoulders 30° above their heart level. The thigh cuff was placed on their upper right thigh as high as possible, and the neck cuff was placed after palpating the pulse of the right common carotid artery. The 80% method was used (80% of the direct linear distance measured between the carotid and femoral recording sites) to ensure the smallest possible measurement error. After the child was still and relaxed, the nurse watched the system for stable wave patterns and recorded three waveform measurements. An average of the three measurements were used for analyses and values are given in meter per second (m/sec).

2.3.6. Central Retinal Arteriolar/Venular equivalent

Images of the retina were photographed using a Canon CR2-Plus Non Mydriatic retinal camera, which provides information on retinal changes not visible with standard photography. The child sat behind the device with their chin on the chin rest and forehead pressed to the overhead bar (glasses removed if needed), and looked straight into the camera lens while the nurse aligned the child's eye using the joystick and software guides. Photos were taken from both the left and right eyes and saved in high resolution for later analysis with the IFlexis software (version 2.1.1, VITO Health, Mol, Belgium). This software calculates the Central Retinal Venular Equivalent (CRVE) and Central Retinal Arteriolar Equivalent (CRAE) using the Parr-Hubbard-Knudtson formula (Knudtson et al., 2003). Average CRVE and CRAE were calculated using the big 6 methodology; an average of the six widest arterioles and venules running through a zone between 0.5 and 1 disc diameter from the optic disc margin (De Boever et al., 2014). Processing of these measurements was carried out by one trained researcher to limit observer variability. Measurements from the right and left eye were averaged for analyses and values are denoted in micrometer (μm).

2.4. Statistical analyses

For statistical analyses concentrations of phthalates and phenols with values below the LOD were substituted by LOD/2 (% < LOD ranged from 0 to 3%). As initial exposure distributions were right skewed all concentrations were log2-transformed to normalize distributions. Pearson correlation coefficients were calculated to estimate correlations between the different chemical concentrations as well as between the cardiovascular measurements. To check linearity between log2-transformed concentrations and cardiovascular outcomes we performed generalized additive models (GAMs) using the "mgcv" package in Rstudio. If the effective degrees of freedom were equal or close to 1, the relationship was considered linear. Given that some of the GAMs showed evidence of non-linearity we modelled concentrations as both continuous and categorical (tertiles) in single exposure models. Covariates included in the models were chosen using a directed acyclic graphs (DAG) (Fig. S1) and all final models were adjusted for: maternal age at delivery (years), pre-pregnancy BMI (kg/m^2), gestational weight gain (kg), maternal smoking during pregnancy (none/until 1st trimester/until 3rd trimester), social class (high/middle, low) based on occupation following ISCO88 (Domingo-Salvany et al., 2000), parental cardiovascular history (neither parent has diagnosis/1 parent has at least one diagnosis/both parents have at least one diagnosis), child sex, gestational age, and child age at cardiovascular measurement. SBP, DBP, and PWV were additionally adjusted for child height at cardiovascular measurement.

To maximize sample size and handle missing covariate observations, multiple imputation was carried out using the multiple imputation by chained equations (MICE) using the "ice" command in STATA generating 20 datasets. The MICE approach assumes that data is missing at random, as such, additional variables that were not included in the final model were added to the predictive models for more accurate results. The imputed data was used for multiple linear regression models and results were combined using Rubin's combination rules (Marshall et al., 2009).

Associations between the chemical concentrations and the outcomes were analyzed in two steps. First, we evaluated single exposures using linear regression models. Then, we tested the association between a chemical mixture including all phthalates and phenols and the outcomes using Bayesian weighted quantile sum regression (BWQS). BWQS is an extension on WQS regression which summarizes the overall exposure to the mixture by estimating a single weighted index while accounting for the individual contribution of each concentration of the mixture using weights. BWQS is a novel approach that extends this method to overcome certain limitations of the original WQS, specifically it does not require selection of the directionality of the coefficient associated with the mixture. This allows more flexibility to the model, improving statistical power (Colicino et al., 2020). In BWQS models we used the first imputed data set as it is not currently possible to use multiple imputed data sets with the BWQS function.

Additionally, we conducted the following sensitivity analyses. To further explore the role of effect modifiers, stratification by child sex and social class was carried out for both single exposure and mixture models. In single exposure models we tested for interactions with sex and social class by inserting cross-product terms (exposure*sex or exposure*social class) into the model. To explore time windows of exposure, we ran analyses separately for first and third trimester concentrations of phthalates and BPA. Further, we ran models excluding gestational weight gain and gestational age as they could be potential mediators. BWQS mixture models were additionally divided into separate mixtures for phthalates and phenols. Lastly, because the BWQS was based on a single imputed dataset, we ran the models with the complete case dataset.

Statistical significance was defined as p-value <0.05. For interaction terms, a less stringent p-value of 0.10 was used. Regression analyses and imputation were carried out with STATA version 14 (College Station, TX). GAMs and BWQS were conducted using RStudio version 4.0.3 (RStudio Team, 2020).

3. Results

3.1. Study population

Study population characteristics are presented in Table 1. There were no differences in main characteristics between the original and imputed datasets. Children had a mean SBP and DBP of 102.1 and 60.1 mmHg, respectively, and their average PWV was 4.4 m/s (Table 1). Children's retinal measures were 181.1 μm on average for CRAE and 252.3 μm for CRVE (Table 1). Correlations between cardiovascular measures were weak or showed no correlation overall with the exceptions of SBP and DBP ($r = 0.66$), and CRAE and CRVE ($r = 0.55$) which were moderately correlated (Fig. S2a).

Phthalate metabolites and phenols were detected in almost all samples (97–100% > LOD) (Table 2). The correlations between these chemicals was strongest between the metabolites of di(2-ethylhexyl) phthalate (DEHP); MEHP, MEHHP, MEOHP and MECPP, whose correlations ranged from ($r = 0.67$ to 0.95) (Fig. S2b). Among phenols, MEPA was strongly correlated with PRPA (0.89) and moderately with BUPA (0.46), while BUPA was also moderately correlated with ETPA (0.59) (Fig. S2b).

GAMs examining the shape of the relationship between prenatal concentrations and cardiovascular outcomes were mixed (Figs. S3a–e). The strongest deviations from linearity for outcomes were observed for: SBP with MEHP; DBP with MEHP and BPA; PWV with TRCS; CRAE with MBzP, MEOHP and MECPP; and CRVE with MEHP and MEPA. (Figs. S3a–e).

Table 1
Study Population Characteristics, n = 416.

	Missing	Non-imputed data Mean (sd) or n (%)	Imputed data Mean (sd) or %
Maternal/Familial characteristics			
Maternal age (years)	1	31.9 (4.1)	31.9 (4.1)
Pre-pregnancy BMI	0	23.8 (4.5)	–
Gestational Weight Gain (kg)	12	14.0 (5.0)	14.0 (5.0)
Smoking during pregnancy	4		
None		303 (73.5)	73.6
Until 1st trimester		56 (13.6)	13.6
Until 3rd trimester		53 (12.9)	12.9
Socioeconomic Status	0		
High/middle		273 (65.6)	–
Low		143 (34.4)	–
Parental Cardiovascular History	4		
None		196 (47.6)	47.6
1 parent has 1 + diagnosis		178 (43.2)	43.1
Both parents have 1 + diagnosis		38 (9.2)	9.2
Child Characteristics			
Sex	0		
Female		197 (47.4)	–
Male		219 (52.6)	–
Gestational age at birth (weeks)	0	39.8 (1.3)	–
Age at cardio measure (years)	1	11.1 (0.5)	11.1 (0.5)
Height at cardio measure (cm)	1	146.6 (7.8)	146.6 (7.8)
Systolic blood pressure (mmHg)	2	102.1 (9.9)	–
Diastolic blood pressure (mmHg)	3	60.1 (7.7)	–
Pulse wave velocity (m/s)	19	4.4 (0.5)	–
Central retinal arteriolar equivalent (μm)	34	181.1 (12.8)	–
Central retinal venular equivalent (μm)	34	252.3 (17.2)	–

3.2. Single exposure models

Fig. 1a and b shows the associations from single exposure models using continuous measures of prenatal phthalate and phenol concentrations and cardiovascular outcome measurements in early adolescence. Regarding macrovascular outcomes, no statistically significant associations were observed between any of the chemical concentrations and SBP or DBP when using continuous exposure measures. When using categorical exposure measures, MEPA concentrations in the 2nd tertile were associated with an increase in SBP (β 2nd vs 1st tertile = 2.48; 95% CI: 0.31, 4.66) (Table S1). Continuous concentrations of phenols and phthalates were not associated with PWV, with the exception of BPA (β per doubling of exposure = −0.06; CI: −0.10, −0.01) (Fig. 1a, Table S1). When exposures were classified in tertiles, MBzP concentrations in the 2nd tertile were statistically significantly associated with an increase in PWV (β 2nd vs 1st tertile = 0.14; CI: 0.02, 0.25) (Table S1). We note that the few observed statistically significant estimates for categorical exposure variables occurred in associations that did not show departure from linearity in the GAMs.

Regarding microvascular measures, phthalates tended to be associated with increases in both CRAE and CRVE, however only the association between MiBP and CRAE reached statistical significance (β per doubling of exposure = 1.89; CI: 0.34, 3.44) (Fig. 1b, Table S1). Conversely, phenols tended to be related with decreases in retinal measures with three reaching statistical significance with CRVE: MEPA (β per doubling of exposure = −0.71; CI: −1.41, −0.01), BUPA (β per doubling of exposure = −0.96; CI: −1.57, −0.35), and BPA (β 2nd vs 1st tertile = −4.98; CI: −9.98, 0.01) (Fig. 1b, Table S1). This latter categorical association did not show departure from linearity in the GAMs.

3.3. Mixture models

In mixture models using BWQS there was no evidence of associations between the phthalates and phenols mixture and SBP (β = 0.50; Credible Interval (CrI): −1.44, 2.37), DBP (β = 0.43; CrI: −1.20, 2.09), PWV (β = −0.03; CrI: −0.12, 0.07), CRAE (β = −0.49; CrI: −3.47, 2.59) or CRVE (β = −1.33; CrI: −5.43, 2.69) (Table 3). Components of the mixture contributed about equally to the mixture (Table S2).

3.4. Sensitivity analyses

Associations between chemical concentrations and cardiovascular outcomes were mostly consistent between sexes, with a few associations found to be statistically significant only in boys or only in girls in single exposure models (Table S3). For example: the relationship between BPA and PWV was only significant in boys (β = -0.08; CI: −0.15, −0.01); and MiBP and PWV was only significant in girls (β = 2.73; CI: 0.10, 5.36) (Table S3). However, there was little evidence for interaction with these results (p interaction>0.10) (Table S3). Similarly, in the mixture models there was little evidence for differences by sex (Table 3, Table S2).

When we stratified by social class, we did not observe a consistent pattern of interaction for most exposure-outcome associations in single exposure models (Table S4). BPA was associated with decreased PWV among those of low social class (β = −0.10; CI: −0.19, −0.02, p interaction = 0.09), but not high/middle social class. MiBP and TRCS were associated with increased CRVE measures in those of low social class (β = 6.12; CI: 2.06, 10.18, p interaction = 0.02) and (β = 0.94; 95% CI: 0.01, 1.87, p interaction = 0.04), respectively. While one paraben, BUPA

Table 2
Prenatal exposure to phthalates (averaged between 1st and 3rd trim) and phenols (3rd trimester only), summary statistics in μg/g creatinine adjusted.

	n	LOD (μg/L)	n (% >LOD) 1 tr.	n (% >LOD) 3rd tr.	Min	P25	P50	P75	Max
MEHP	334	1	332 (99)	332 (99)	1.79	7.28	10.78	17.13	69.41
MEHHP	334	0.5	334 (100)	334 (100)	5.29	17.54	26.88	41.21	503.41
MEP	334	1	334 (100)	333 (99)	37.45	199.84	389.76	746.88	9379.85
MiBP	334	0.5	334 (100)	334 (100)	5.08	22.09	31.53	48.19	334.23
MnBP	334	1	331 (99)	332 (99)	5.77	20.09	30.75	47.53	835.66
MBzP	334	0.5	331 (99)	331 (99)	1.54	7.09	11.78	19.84	405.08
MEOHP	334	0.5	334 (100)	334 (100)	4.11	13.83	20.62	29.99	378.28
MECPP	334	1	334 (100)	333 (99)	7.74	27.05	38.97	58.07	718.85
MEPA	407	0.04	–	407 (100)	1.77	82.16	248.57	536.75	45927.10
ETPA	407	0.04	–	404 (99)	0.01	4.74	18.22	57.97	3753.74
PRPA	405	0.04	–	405 (100)	0.06	15.97	54.95	143.91	14132.27
BPA	331	1	331 (100)	328 (99)	5.52	0.33	1.69	2.53	69.44
BUPA	405	0.07	–	394 (97)	0.00	0.65	4.16	12.60	217.25
BP-3	407	0.04	–	407 (100)	0.12	1.15	3.24	20.00	10028.70
TRCS	407	0.04	–	407 (100)	0.31	4.08	28.36	149.03	1909.51

Abbreviations: LOD: limit of detection; min: minimum value max: maximum value; P: percentile; MEHP: Mono-2-ethylhexyl phthalate; MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate; MEP: Monoethyl phthalate; MiBP: Mono-iso-butyl phthalate; MnBP: Mono-n-butyl phthalate; MBzP: Mono benzyl phthalate; MEOHP: Mono-2-ethyl-5-oxohexyl phthalate; MECPP: Mono-2-ethyl 5-carboxypentyl phthalate; MEPA: Methyl paraben; ETPA: Ethyl paraben; PRPA: Propyl paraben; BPA: Bisphenol-A; BUPA: N-Butyl paraben; BP-3: Benzophenone-3; TRCS: Triclosan.

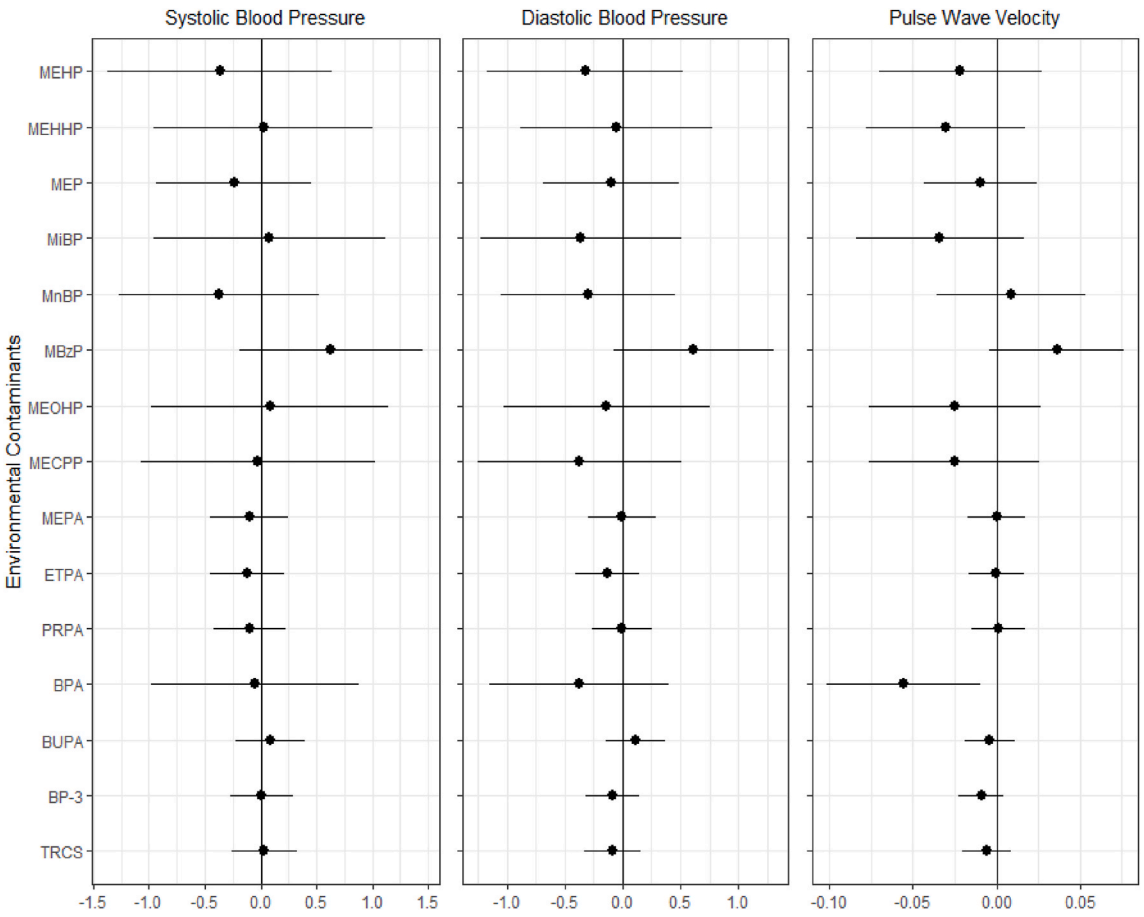


Fig. 1a. Associations (Beta and 95% CI) between individual phthalate and phenol exposures and macrovascular measurements from linear regression models. Using covariate imputed data (m = 20) and log2-transformed concentrations.
Abbreviations: MEHP: Mono-2-ethylhexyl phthalate; MEHHP: Mono-2-ethyl-5-hydroxyhexyl phthalate; MEP: Monoethyl phthalate; MiBP: Mono-iso-butyl phthalate; MnBP: Mono-n-butyl phthalate; MBzP: Mono benzyl phthalate; MEOHP: Mono-2-ethyl-5-oxohexyl phthalate; MECPP: Mono-2-ethyl 5-carboxypentyl phthalate; MEPA: Methyl paraben; ETPA: Ethyl paraben; PRPA: Propyl paraben; BPA: Bisphenol-A; BUPA: N-Butyl paraben; BP-3: Benzophenone-3; TRCS: TriclosanMacro-vascular
measurements models adjusted for child age and height at visit, child sex, mother's age at delivery, prepregnancy BMI, gestational weight gain, social class, parental cardiovascular history, smoking during pregnancy and gestational age.

was associated with decreased CRVE in those of high/middle social class (β = −1.40; CI: −2.16, −0.64, p interaction = 0.08) (Table S4). In mixture models these was no evidence for differences by social class (Table 3, Table S2).

When analyzing the separate trimester time points for phthalates and BPA, we observed similar associations as in single exposure models for MBzP and PWV in the 1st trimester (β = 0.04; CI: 0.00, 0.07) and MiBP and CRAE in the 3rd trimester (β = 1.23; CI: −0.01, 2.46) (Table S5). The observed associations between BPA and PWV and CRVE were no longer significant in models separated by trimester, however the coefficients were in a similar direction for PWV in both trimesters and CRVE in the 1st trimester. We found an association between MBzP and DBP in the 1st trimester which was not significant in single exposure models, however coefficients were similar (β = 0.59; CI: 0.02, 1.15) (Table S5). Excluding gestational weight gain and gestational age from the continuous single exposure models did not change the results (Table S6). In mixture models separately for phthalates and phenols no significant associations were observed (Tables S7–S8). Results from complete case analysis using mixture models were similar to those observed using imputed data (Table S9).

4. Discussion

In this Spanish birth cohort, we found little evidence to suggest that prenatal urinary concentrations of phthalates and phenol metabolites are associated with parameters of macro- and microvascular health during early adolescence. A few statistically significant associations were found between certain chemical exposures and measures of SBP, PWV, CRAE and CRVE. None of the exposures showed associations with DBP and there was no evidence of associations between the phthalates and phenols mixture and any of the outcomes.

Several studies have examined the relationship between exposure to phthalates during pregnancy and/or childhood and blood pressure in children. Consistent with our mostly null findings, a birth cohort in Greece found that prenatal urinary phthalate metabolites and BPA were not associated with childhood blood pressure at 4 years of age (Vafeiadi et al., 2018). In contrast other studies have found significant associations with blood pressure. Two studies observed an association between prenatal phthalate exposure and decreased SBP in girls only at 9 years old (Sol et al., 2020) and using repeated measurements at 4 and 7 years (Valvi et al., 2015). A systematic review and meta-analysis on phthalates concluded a positive association between several phthalate metabolites and both SBP and DBP in children and adolescents (Golestanzadeh et al., 2019). Regarding phenols, recent studies found a positive association between prenatal BPA and childhood SBP in girls at 2 years (Ouyang et al., 2020) and boys at 9 years (Sol et al., 2020). Similar positive associations were also observed with DBP and both sexes combined during early and late childhood (Bae et al., 2017; Warembourg et al., 2019) and

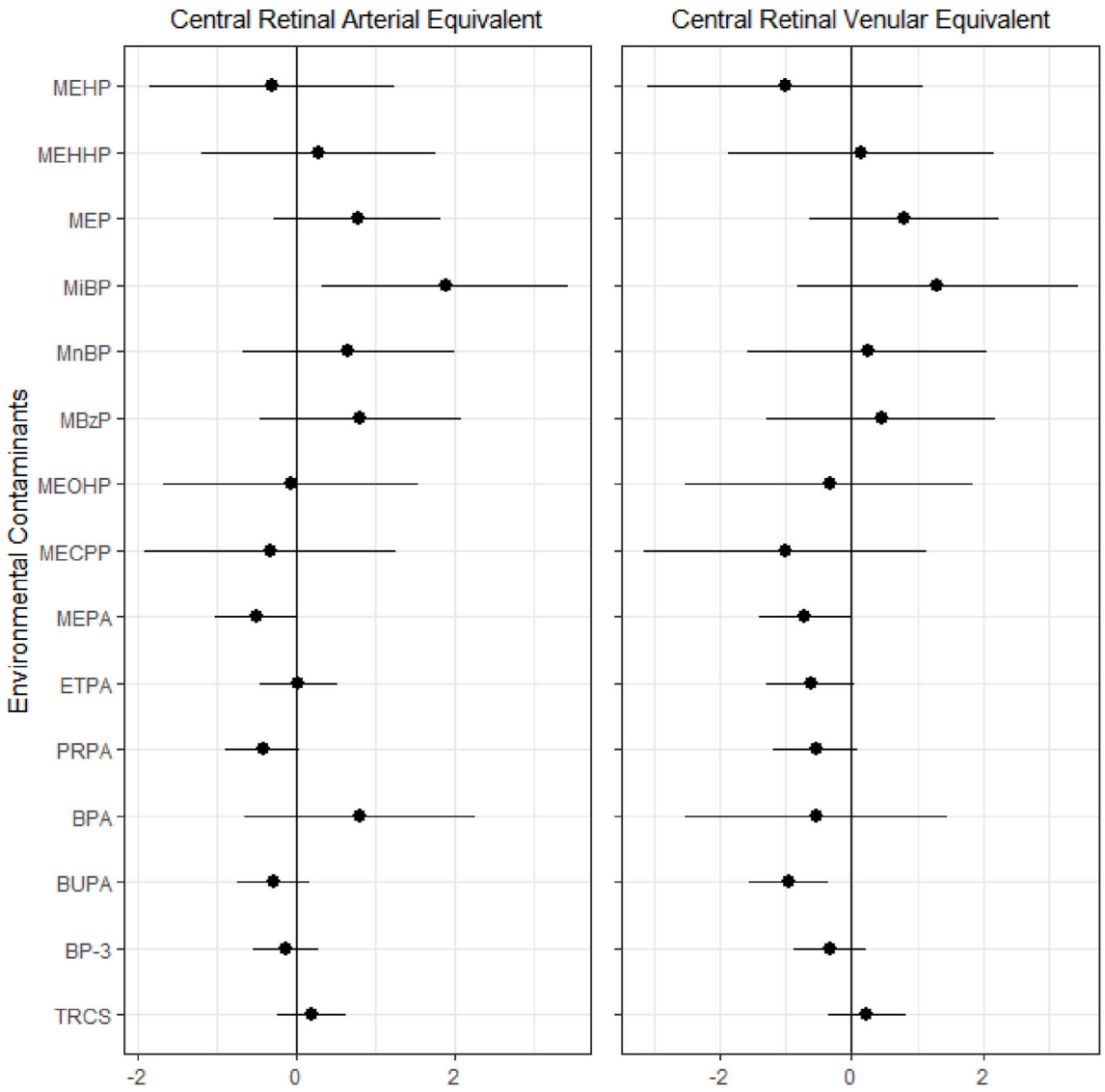


Fig. 1b. Associations (Beta and 95% CI) between individual phthalate and phenol exposures and microvascular measurements from linear regression models. Microvascular measurement models adjusted for child age at visit, child sex, mother's age at delivery, prepregnancy BMI, gestational weight gain, social class, parental cardiovascular history, smoking during pregnancy and gestational age.

girls only at 2 years (Ouyang et al., 2020). However, one study identified a negative relationship between BPA and DBP in girls at 9 years (Sol et al., 2020). While several studies found a significant association between phthalate and/or BPA exposure and blood pressure, often sex dependent, our results were mostly null, even when stratified by sex. Aside from BPA, the HELIX study analyzed prenatal BP-3 and TRCS and observed no association with blood pressure during childhood (Warembourg et al., 2019).

We found that an increase in prenatal BPA exposure was related to a decrease in PWV in children, which remained significant for boys and those of low social class in our stratified analyses. Additionally, we observed a positive association with the phthalate metabolite MBzP and PWV for those of higher social class. Two other studies in children have examined the relationship between phthalate exposure and a measure of arterial stiffness, though both were cross-sectional studies, measuring phthalate exposure and arterial stiffness at the same time point (Kataria et al., 2017; Su et al., 2019). Su et al. (2019) found higher phthalate concentrations to be associated with higher risk of increased carotid intima-media thickness in subjects 6–18 years of age (Su et al., 2019). Kataria et al. (2017), found that DEHP metabolites were associated with decreased brachial artery distensibility and they found no relevant associations between phthalates and PWV at 12 years (Kataria et al., 2017).

We did not identify any previous studies that assessed the relationship between phthalates, phenols and retinal measurements (CRAE and CRVE), so our study is the first. We observed that one phthalate metabolite, MiBP, was associated with increased CRAE, and this association was also observed for girls and those of lower social class in stratified analyses. CRAE assesses artery width, whereby narrowing is indicative of arterial damage. However, it is unclear how MiBP may mitigate arterial narrowing. Additionally, two parabens, MEPA and PRPA, were associated with reduced CRAE for boys, indicative of arterial narrowing. MiBP and TRCS were associated with an increase in CRVE for those of lower social class, which could indicate some venular widening or potential inflammation. However, the parabens BUPA, MEPA and ETPA were all found to be associated with reduced CRVE, implying the mitigation of any venular widening. These findings give an indication that phthalate and phenol compounds may play a role in altering the microvascular structure. However, these are the very first results and require replication in further studies.

While the mechanism(s) underlying the associations of phthalates and phenols are not well understood, oxidative stress, a key indicator of

Table 3
Associations between phthalates and phenols mixture and cardiovascular measurements using BWQS regression.

Mixture	Systolic Blood Pressure		Diastolic Blood Pressure		Pulse Wave Velocity		Central Retinal Arteriolar Equivalent		Central Retinal Venular Equivalent	
	n	beta 1 (BWQS index) (95% CI)	n	beta 1 (BWQS index) (95% CI)	n	beta 1 (BWQS index) (95% CI)	n	beta 1 (BWQS index) (95% CI)	n	beta 1 (BWQS index) (95% CI)
Unstratified[a]	305	0.50 (−1.44, 2.37)	304	0.43 (−1.20, 2.09)	292	−0.03 (−0.12, 0.07)	280	−0.49 (−3.47, 2.59)	280	−1.33 (−5.43, 2.69)
Social Class[b]										
High/ middle	209	0.34 (−1.72, 2.31)	209	−0.19 (−2.02, 1.68)	199	−0.04 (−0.14, 0.08)	191	−0.63 (−4.31, 3.37)	191	−2.77 (−7.80, 2.04)
Low	96	1.12 (−2.77, 5.05)	95	1.24 (−2.11, 4.85)	93	−0.01 (−0.21, 0.20)	89	1.09 (−4.74, 6.63)	89	5.11 (−3.05, 13.02)
Sex[c]										
Female	141	0.42 (−2.31, 3.08)	140	0.56 (−1.76, 3.02)	139	−0.01 (−0.15, 0.13)	127	2.36 (−2.56, 7.20)	127	0.80 (−5.69, 6.79)
Male	164	0.86 (−1.91, 3.57)	164	0.75 (−1.62, 3.13)	153	−0.02 (−0.16, 0.11)	153	−2.28 (−6.12, 1.37)	153	−1.72 (−7.27, 3.71)

Data is from the 1st set of imputed data.
[a] Models adjusted for child age at visit, child sex, mother's age at delivery, prepregnancy BMI, gestational weight gain, social class, parental cardiovascular history, smoking during pregnancy and gestational age. SBP, DBP and PWV were additionally adjusted for child height at visit.
[b] Models adjusted for the same as unstratified with the exception of social class.
[c] Models adjusted for the same as unstratified with the exception of sex.

cardiovascular disease, may play a central role (Ferguson et al., 2017). Oxidative stress in regards to cardiovascular health describes injury caused to cells from the increased formation of reactive oxygen species (ROS) which ultimately overwhelms the ability to eliminate ROS or repair ROS-induced damage (Dhalla et al., 2000). Oxidative stress from increased levels of ROS have been connected to several risk factors for cardiovascular disease including hypertension, diabetes, obesity, arrhythmia and atherosclerotic plaque formation (Pignatelli et al., 2018; Senoner and Dichtl, 2019). A study on phthalate exposure and markers of oxidative stress found a positive association between phthalate metabolites and increased body weight, risk of insulin resistance and F2-isoprostane levels (systemic oxidative stress biomarker) (Kataria et al., 2017). Additionally, BPA exposure was correlated with the expression of pro-inflammatory genes related to c-reactive protein, a marker indicative of CVD risk (Tsen et al., 2021). Also, phthalates and BPA have been related to changes in placental micro-RNA expression, DNA methylation, and genomic imprinting (Mose et al., 2007; Strakovsky and Schantz, 2018). Given that the placenta plays a vital role in fetal growth and development these changes may result in adverse health outcomes for the child. Findings from a 2020 in vitro study found that exposure to the phthalate metabolite MEHP on human endometrial microvascular endothelial cells, resulted in apoptosis and pyroptosis, forms of cell death that can lead to impairment of normal tissue and organ function (An, 2021). These few studies point to the mechanism(s) by which chemicals like phthalates and phenols may induce oxidative stress in the body thus interrupting normal development and increasing cardiovascular risk. However, further studies are needed, both longitudinal cohort studies and in vivo studies, to elucidate the mechanisms involved in the development of cardiovascular risk.

Our study has several strengths. The longitudinal nature of this study allowed us to examine prenatal exposure and later cardiovascular health in mother-child pairs. We included novel measures of vascular health, as well as phenolic compounds not previously studied in relation to cardiovascular outcomes. Additionally, while many studies have accounted for sex through stratification, ours is the first to stratify by social class. Social class is an important factor given that it plays a large role in dictating to which chemicals and in what quantity exposure occurs (Montazeri et al., 2019). As our study demonstrates, merely adjusting for social class or similar variable does not completely disentangle these complex associations. Lastly, our study included a novel statistical approach, BWQS, which complimented single exposure models by accounting for the high dimensionality among phthalates and phenols that the single exposure models alone cannot account for (Carrico et al., 2015; Colicino et al., 2020).

Our study also has some limitations. Non-persistent chemicals such as phthalates and phenols have short half-lives leading to large variability in sample concentrations relying on spot urine samples (Casas et al., 2018). This type of measurement error on the exposure variable can lead to null associations due to regression dilution bias (Hutcheon et al., 2010). One way to minimize this bias is by obtaining repeated measures to better estimate the true exposure values (Hutcheon et al., 2010). For phthalate metabolites and BPA, we had two measurements during pregnancy and averaged those to lessen this bias somewhat. For the other phenols we had only one sample during pregnancy which could lead to an attenuation bias as high as 69% (Vernet et al., 2019). New cohort studies measuring nonpersistent chemicals like phthalates and phenols should aim to collect and pool three samples per day at minimum to more effectively estimate weekly exposure (Vernet et al., 2019). Another limitation was that our sample size was small, particularly for the stratified analyses (sex and social class), limiting our ability to detect significant associations and draw firm conclusions. Additionally, it is unclear how sensitive the measured cardiovascular outcomes are during early adolescence. While, several studies have examined health effects using blood pressure, fewer have used PWV and this is the first to use retinal imaging. Studies can improve on our assessment by using these outcome measures at multiple age points to further understand at which age these measures are most appropriate. Further, although we attempted to control for factors such as sex, social class and parental cardiovascular history, we cannot exclude residual confounding by unmeasured factors related to cardiovascular measures in young adolescents. Lastly, multiple comparison is a concern in multi-pollutant studies. Rather than apply an overly conservative adjustment for multiple comparison, we complemented our single exposure models with mixture models and draw our final conclusions on the consistency between the results.

5. Conclusion

This is the first birth cohort study to evaluate prenatal exposure to urinary phthalate metabolites and phenols, and their mixture during pregnancy with novel measures of vascular health during early adolescence. Our results provide little evidence to suggest that prenatal exposure to phthalates and phenols is associated with early adolescent measures of macro- and microvascular health. Future longitudinal studies would benefit from using similar novel measures of vascular health as well as repeat urinary measurements for exposure assessment.

FUNDING

This study was funded by grants from Instituto de Salud Carlos III (Red INMA G03/176; CB06/02/0041; PI041436; PI081151 incl. FEDER funds; PI12/01890 incl. FEDER funds; CP13/00054 incl. FEDER funds; PI15/00118 incl. FEDER funds; CP16/00128 incl. FEDER funds; PI16/00118 incl. FEDER funds; PI16/00261 incl. FEDER funds; PI17/01340 incl. FEDER funds; PI18/00547 incl. FEDER funds), CIBERESP, Generalitat de Catalunya-CIRIT 1999SGR 00241, Generalitat de Catalunya-AGAUR (2009 SGR 501, 2014 SGR 822), Fundació La marató de TV3 (090430), Spanish Ministry of Economy and Competitiveness (SAF2012-32991 incl. FEDER funds), Agence Nationale de Securite Sanitaire de l'Alimentation de l'Environnement et du Travail (1262C0010; EST-2016 RF-21), EU Commission (261357, 308333, 603794 and 634453). CW held a Sara Borrell postdoctoral grant (CD18/00132) from the Instituto de Salud Carlos III. We acknowledge support from the Spanish Ministry of Science and Innovation and the State Research Agency through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program.

Declaration of competing interest

None.

Acknowledgments

We thank all of the study participants for their support with the INMA Sabadell birth cohort study. We also thank the dedicated INMA research nurses, Silvia Fochs and Núria Pey, for their work in conducting the study visits.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113909.

References

An, L., 2021. Exposure to mono (2-ethylhexyl) phthalate facilitates apoptosis and pyroptosis of human endometrial microvascular endothelial cells through NLRP3 inflammasome. J. Appl. Toxicol. 41, 755–764. https://doi.org/10.1002/jat.4106.

Bae, S., Lim, Y.H., Lee, Y.A., Shin, C.H., Oh, S.Y., Hong, Y.C., 2017. Maternal urinary bisphenol A concentration during midterm pregnancy and children's blood pressure at age 4. Hypertension 69, 367–374. https://doi.org/10.1161/HYPERTENSIONAHA.116.08281.

Carrico, C., Gennings, C., Wheeler, D.C., Factor-Litvak, P., 2015. Characterization of a weighted quantile score approach for highly correlated data in risk analysis scenarios. J. Agric. Biol. Environ. Stat. 20, 100–120. https://doi.org/10.1007/s13253-014-0180-3. Characterization.

Casas, M., Basagaña, X., Sakhi, A.K., Haug, L.S., Philippat, C., Granum, B., Manzano-Salgado, C.B., Brochot, C., Zeman, F., de Bont, J., Andrusaityte, S., Chatzi, L., Donaire-Gonzalez, D., Giorgis-Allemand, L., Gonzalez, J.R., Gracia-Lavedan, E., Grazuleviciene, R., Kampouri, M., Lyon-Caen, S., Pañella, P., Petraviciene, I., Robinson, O., Urquiza, J., Vafeiadi, M., Vernet, C., Waiblinger, D., Wright, J., Thomsen, C., Slama, R., Vrijheid, M., 2018. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ. Int. 121, 561–573. https://doi.org/10.1016/j.envint.2018.09.046.

Casas, M., Valvi, D., Luque, N., Ballesteros-Gomez, A., Carsin, A.E., Fernandez, M.F., Koch, H.M., Mendez, M.A., Sunyer, J., Rubio, S., Vrijheid, M., 2013. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ. Int. 56, 10–18. https://doi.org/10.1016/j.envint.2013.02.014.

Colicino, E., Pedretti, N.F., Busgang, S., Gennings, C., 2020. Per- and poly-fluoroalkyl substances and bone mineral density: results from the Bayesian weighted quantile sum regression. Environ. Epidemiol. 4, e092. https://doi.org/10.1101/19010710.

Cote, A.T., Phillips, A.A., Harris, K.C., Sandor, G.G.S., Panagiotopoulos, C., Devlin, A.M., 2015. Obesity and arterial stiffness in children: systematic review and meta-analysis. Arterioscler. Thromb. Vasc. Biol. 35, 1038–1044. https://doi.org/10.1161/ATVBAHA.114.305062.

De Boever, P., Louwies, T., Provost, E., Int Panis, L., Nawrot, T.S., 2014. Fundus photography as a convenient tool to study microvascular responses to cardiovascular disease risk factors in epidemiological studies. JoVE 1–9. https://doi.org/10.3791/51904.

Dhalla, N.S., Temsah, R.M., Netticadan, T., 2000. Role of oxidative stress in cardiovascular diseases. J. Hypertens. 655–673.

Diamanti-Kandarakis, E., Bourguignon, J., Giudice, L., Hauser, R., Prins, G., Soto, A., Zoeller, R., Gore, A., 2009. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr. Rev. 30, 293–342.

Domingo-Salvany, A., Regidor, E., Alonso, J., Alvarez-Dardet, C., Borrell, C., Doze, F., Gasulla, G., Rosell, M., Montasell, M., Rodríguez, J., Doz, F., 2000. Proposal for a social class measure. Aten. primaria 25, 350–363. https://doi.org/10.1016/s0212-6567(00)78518-0.

Ferguson, K.K., Chen, Y.H., Vanderweele, T.J., McElrath, T.F., Meeker, J.D., Mukherjee, B., 2017. Mediation of the relationship between maternal phthalate exposure and preterm birth by oxidative stress with repeated measurements across pregnancy. Environ. Health Perspect. 125, 488–494. https://doi.org/10.1289/EHP282.

Franks, P., Hanson, R., Knowler, W., Sievers, M., Bennett, M., Looker, H., 2010. Childhood obesity, other cardiovascular risk factors, and premature death. N. Engl. J. Med. 362, 485–493. https://doi.org/10.1056/NEJMoa0904130.Childhood.

Fu, X., Xu, J., Zhang, R., Yu, J., 2020. The association between environmental endocrine disruptors and cardiovascular diseases: a systematic review and meta-analysis. Environ. Res. 187, 109464. https://doi.org/10.1016/j.envres.2020.109464.

Golestanzadeh, M., Riahi, R., Kelishadi, R., 2019. Association of exposure to phthalates with cardiometabolic risk factors in children and adolescents: a systematic review and meta-analysis. Environ. Sci. Pollut. Res. 26, 35670–35686. https://doi.org/10.1007/s11356-019-06589-7.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. Executive summary to EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, 593–602. https://doi.org/10.1210/er.2015-1093.

Guxens, M., Ballester, F., Espada, M., Fernández, M.F., Grimalt, J.O., Ibarluzea, J., Olea, N., Rebagliato, M., Tardón, A., Torrent, M., Vioque, J., Vrijheid, M., Sunyer, J., 2012. Cohort profile: the INMA-INfancia y Medio Ambiente-(environment and childhood) project. Int. J. Epidemiol. 41, 930–940. https://doi.org/10.1093/ije/dyr054.

Heindel, J.J., Vandenberg, L.N., 2015. Developmental origins of health and disease: a paradigm for understanding disease cause and prevention. Curr. Opin. Pediatr. 27, 248–253. https://doi.org/10.1097/MOP.0000000000000191.

Hudson, L.D., Rapala, A., Khan, T., Williams, B., Viner, R.M., 2015. Evidence for contemporary arterial stiffening in obese children and adolescents using pulse wave velocity: a systematic review and meta-analysis. Atherosclerosis 241, 376–386. https://doi.org/10.1016/j.atherosclerosis.2015.05.014.

Hutcheon, J.A., Chiolero, A., Hanley, J.A., 2010. Random measurement error and regression dilution bias. BMJ c2289.

Juonala, M., Magnussen, C.G., Berenson, G.S., Venn, A., Burns, T.L., Sabin, M.A., Srinivasan, S.R., Daniels, S.R., Davis, P.H., Chen, W., Sun, C., Cheung, M., Viikari, J. S., Dwyer, T., Raitakari, O.T., 2011. Childhood adiposity, adult adiposity, and cardiovascular risk factors. N. Engl. J. Med. 365, 1876–1885. https://doi.org/10.1056/NEJMoa1010112.

Kataria, A., Levine, D., Wertenteil, S., Vento, S., Xue, J., Rajendiran, K., Kannan, K., Thurman, J.M., Morrison, D., Brody, R., Elaine Urbina, M., Attina, T., Trasande, L., Trachtman, H., 2017. Exposure to bisphenols and phthalates and association with oxidant stress, insulin resistance, and endothelial dysfunction in children. Pediatr. Res. 81, 857–864. https://doi.org/10.1093/ajcp/24.11.1259.

Knudtson, M.D., Lee, K.E., Hubbard, L.D., Wong, T.Y., Klein, R., Klein, B.E.K., 2003. Revised formulas for summarizing retinal vessel diameters. Curr. Eye Res. 27, 143–149. https://doi.org/10.1076/ceyr.27.3.143.16049.

Landrigan, P.J., Goldman, L.R., 2011. Children s vulnerability to toxic chemicals: a challenge and opportunity to strengthen health and environmental policy. Health Aff. 30, 842–850. https://doi.org/10.1377/hlthaff.20.2.225.

Li, L.J., Ikram, M.K., Wong, T.Y., 2016. Retinal vascular imaging in early life: insights into processes and risk of cardiovascular disease. J. Physiol. 594, 2175–2203. https://doi.org/10.1113/JP270947.

Lunder, S., Hovander, L., Athanassiadis, I., Bergman, A., 2010. Significantly higher polybrominated diphenyl ether levels in young U.S. children than in their mothers. Environ. Sci. Technol. 44, 5256–5262. https://doi.org/10.1021/es1009357.

Mamsen, L.S., Jönsson, B.A.G., Lindh, C.H., Olesen, R.H., Larsen, A., Ernst, E., Kelsey, T. W., Andersen, C.Y., 2017. Concentration of perfluorinated compounds and cotinine in human foetal organs, placenta, and maternal plasma. Sci. Total Environ. 596–597, 97–105. https://doi.org/10.1016/j.scitotenv.2017.04.058.

Mariana, M., Feiteiro, J., Verde, I., Cairrao, E., 2016. The effects of phthalates in the cardiovascular and reproductive systems: a review. Environ. Int. 94, 758–776. https://doi.org/10.1016/j.envint.2016.07.004.

Marshall, A., Altman, D.G., Holder, R.L., Royston, P., 2009. Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. BMC Med. Res. Methodol. 9 https://doi.org/10.1186/1471-2288-9-57.

Meeker, J., 2012. Exposure to environmental endocrine disruptors and child development. Arch. Pediatr. Adolesc. 166, E1–E7. https://doi.org/10.1001/archpediatrics.2012.241.Exposure.

Montazeri, P., Thomsen, C., Casas, M., de Bont, J., Haug, L.S., Maitre, L., Papadopoulou, E., Sakhi, A.K., Slama, R., Saulnier, P.J., Urquiza, J., Grazuleviciene, R., Andrusaityte, S., McEachan, R., Wright, J., Chatzi, L., Basagaña, X., Vrijheid, M., 2019. Socioeconomic position and exposure to multiple environmental chemical contaminants in six European mother-child cohorts. Int. J. Hyg Environ. Health 222, 864–872. https://doi.org/10.1016/j.ijheh.2019.04.002.

Mose, T., Knudsen, L.E., Hedegaard, M., Mortensen, G.K., 2007. Transplacental transfer of monomethyl phthalate and mono(2-ethylhexyl) phthalate in a human placenta

perfusion system. Int. J. Toxicol. 26, 221–229. https://doi.org/10.1080/10915810701352721.
Newman, A.R., Andrew, N.H., Casson, R.J., 2017. Review of paediatric retinal microvascular changes as a predictor of cardiovascular disease. Clin. Exp. Ophthalmol. 45, 33–44. https://doi.org/10.1111/ceo.12773.
Nidens, N., Vogel, M., Körner, A., Kiess, W., 2020. Prenatal exposure to phthalate esters and its impact on child development. Best Pract. Res. Clin. Endocrinol. Metabol. https://doi.org/10.1016/j.beem.2020.101478, 101478.
Ouyang, F., Zhang, G.-H., Du, K., Shen, L., Ma, R., Wang, Xia, Wang, Xiaobin, Zhanga, J., 2020. Maternal prenatal urinary bisphenol A level and child cardio-metabolic risk factors: a prospective cohort study. Environ. Pollut. 265 (Pt A) https://doi.org/10.1016/j.envpol.2020.115008.
Pignatelli, P., Menichelli, D., Pastori, D., Violi, F., 2018. Oxidative stress and cardiovascular disease: new insights. Kardiol. Pol. 76, 713–722. https://doi.org/10.5603/KP.a2018.0071.
RStudio Team, 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.
Sakhi, A.K., Sabaredzovic, A., Papadopoulou, E., Cequier, E., Thomsen, C., 2018. Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children. Environ. Int. 114, 242–251. https://doi.org/10.1016/j.envint.2018.02.037.
Senoner, T., Dichtl, W., 2019. Oxidative stress in cardiovascular diseases: still a therapeutic target? Nutrients 11. https://doi.org/10.3390/nu11092090.
Sol, C.M., Santos, S., Asimakopoulos, A.G., Duijts, L., Kannan, K., Trasande, L., Jaddoe, V. W.V., Group, G.R.S., City, N.Y., City, N.Y., City, N.Y., City, Y., City, N.Y., 2020. Associations of maternal phthalate and bisphenol urine concentrations during pregnancy with childhood blood pressure in a population-based prospective cohort study. Environ. Int. 138 https://doi.org/10.1016/j.envint.2020.105677.
Strakovsky, R.S., Schantz, S.L., 2018. Impacts of bisphenol A (BPA) and phthalate exposures on epigenetic outcomes in the human placenta. Environ. Epigenetics 4, 1–18. https://doi.org/10.1093/eep/dvy022.
Su, T.-C., Hwang, I.-S., Torng, P.-L., Wu, C., Lin, C.-Y., Sung, F.-C., 2019. Phthalate exposure increases subclinical atherosclerosis in young population. Environ. Pollut. 586–593. https://doi.org/10.1016/j.envpol.2019.04.006.
Tsen, C.-M., Liu, J.-H., Yang, D.-P., Chao, H.-R., Chen, J.-L., Chou, W.-C., Ho, Y.-C., Chuang, C.-Y., 2021. Study on the correlation of bisphenol A exposure, pro-inflammatory gene expression, and C-reactive protein with potential cardiovascular disease symptoms in young adults. Env. Sci Pollut Res Int. Online ahe. https://doi.org/10.1007/s11356-021-12805-0.
Vafeiadi, M., Myridakis, A., Roumeliotaki, T., Margetaki, K., Chalkiadaki, G., Dermitzaki, E., Venihaki, M., Sarri, K., Vassilaki, M., Leventakou, V., Stephanou, E. G., Kogevinas, M., Chatzi, L., 2018. Association of early life exposure to phthalates with obesity and cardiometabolic traits in childhood: sex specific associations. Front. Public Health 6, 1–11. https://doi.org/10.3389/fpubh.2018.00327.
Valvi, D., Monfort, N., Ventura, R., Casas, M., Casas, L., Sunyer, J., Vrijheid, M., 2015. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int. J. Hyg Environ. Health 218, 220–231. https://doi.org/10.1016/j.ijheh.2014.11.003.
Vernet, C., Philippat, C., Agier, L., Calafat, A.M., Ye, X., Lyon-Caen, S., Hainaut, P., Siroux, V., Schisterman, E.F., Slama, R., 2019. An empirical validation of the within-subject biospecimens pooling approach to minimize exposure misclassification in biomarker-based studies. Epidemiology 30, 756–767. https://doi.org/10.1097/EDE.0000000000001056.
Vrijheid, M., Casas, M., Gascon, M., Valvi, D., Nieuwenhuijsen, M., 2016. Environmental pollutants and child health-A review of recent concerns. Int. J. Hyg Environ. Health 219, 331–342. https://doi.org/10.1016/j.ijheh.2016.05.001.
Warembourg, C., Maitre, L., Tamayo-Uria, I., Fossati, S., Roumeliotaki, T., Aasvang, G. M., Andrusaityte, S., Casas, M., Cequier, E., Chatzi, L., Dedele, A., Gonzalez, J.R., Gražulevičienė, R., Haug, L.S., Hernandez-Ferrer, C., Heude, B., Karachaliou, M., Krog, N.H., McEachan, R., Nieuwenhuijsen, M., Petraviciene, I., Quentin, J., Robinson, O., Sakhi, A.K., Slama, R., Thomsen, C., Urquiza, J., Vafeiadi, M., West, J., Wright, J., Vrijheid, M., Basagaña, X., 2019. Early-life environmental exposures and blood pressure in children. J. Am. Coll. Cardiol. 74, 1317–1328. https://doi.org/10.1016/j.jacc.2019.06.069.
Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. Assessing exposure to phthalates – the human biomonitoring approach. Mol. Nutr. Food Res. 55, 7–31.
Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26, 803–824. https://doi.org/10.1111/j.1539-6924.2006.00770.x.