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Determinants of phthalate exposure and risk assessment in children from Poland

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ABSTRACT

Phthalates are a group of widely used chemicals and humans are exposed to them in their daily life. Some phthalates may affect the hormonal balance in both children and adults. The aim of this study was to assess the phthalate exposure and its determinants among children at age of 7 years from the Polish Mother and Child Cohort Study (REPRO_PL). 250 urine samples collected in 2014–2015 were analysed for 21 metabolites of 11 parent phthalates using on-line high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). This represents the most extensive set of phthalate metabolites ever determined for Poland. Ten metabolites were quantifiable in 100% of the samples, another eight in > 90%. The highest median concentrations were found for the primary monoester metabolites of di-iso-butyl (MiBP, 72.4 μg/l), di-n-butyl (MnBP, 56.3 μg/l) and diethyl (MEP, 42.0 μg/l) phthalate, followed by the sum of di-2-ethylhexyl (ΣDEHP, 89.3 μg/l) and di-iso-nonyl (ΣDiNP, 21.9 μg/l) phthalate metabolites. Metabolite concentrations were higher in children at 7 years than in the same children at age 2 or in their mothers during pregnancy. Generally, phthalate exposures in this study were much higher than exposures reported in other European populations. Multivariate regression models showed that body mass index, place of residence, breastfeeding duration, socio-economic status and parental education were associated with the metabolite levels in the 7-year old children. Daily intake and hazard index calculations revealed that a small percentage of children (around 3–10%) exceeded the tolerable daily intakes established by international institutions such as EFSA and U.S. EPA indicating that these children might be at risk of anti-androgenic effects from the individual and cumulative exposure to phthalates. Thus, further monitoring of this population, by educational programs and follow-up interventions, is required.

1. Introduction

Endocrine disrupting chemicals (EDCs) represent a growing public health concern. This is the case for several phthalates, a group of widely used chemicals that affect the hormonal balance in both children and adults. Previous studies have shown that prenatal and early child exposures to phthalates may pose an increased health risk, including negative impact on pregnancy outcomes, children's health (*i.e.* respiratory diseases and obesity) and neurodevelopment (Katsikantami et al., 2016; Benjamin et al., 2017). Common phthalates have been used as plasticizers, stabilizers, adhesives, industrial solvents and in personal care products, such as shampoos, lotions, cosmetics, insect repellents and even in medications (NRC, 2008). Human exposure to the low-molecular weight (LMW) phthalates dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl-benzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP) and di-n-butyl phthalate (DnBP) and the high-molecular weight phthalates (HMW) di(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP) and di-iso-decyl phthalate (DiDP) has been well documented in an increasing number of human biomonitoring (HBM) studies. Other minor or less investigated phthalates include di-cyclohexyl phthalate (DCHP), dipentyl phthalate (DnPeP) and di-n-octyl phthalate (DnOP).

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Humans are exposed to phthalates *via* inhalation, ingestion and dermal absorption, often in a complex combination of various exposure sources and routes (Koch and Calafat, 2009; Wittassek et al., 2011). Phthalates are quickly metabolised and their metabolites (monoesters and oxidised monoesters) are excreted in urine. These metabolites are considered relevant indicators of phthalate exposure and are successfully used as biomarkers of exposure in many HBM (Koch and Calafat, 2009; Wittassek et al., 2011). Taking into account that several phthalates are classified as endocrine disruptors and reproductive toxicants, the exposure of children is of particular concern (Swan et al., 2005) and thus several phthalates have been strictly regulated in the EU (Regulation EC No 726/2004 of the European Parliament and the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals, REACH).

Although recent HBM data indicates a general decrease in exposure to many phthalates (due to regulatory measures and market changes) (Koch et al., 2017; Gyllenhammar et al., 2017; Zota et al., 2014) it has also been well established that children have higher exposures to most phthalates than adults, probably due to differences in their body size and metabolism, as well as specific behaviors such as hand-to-mouth and floor contact (Den Hond et al., 2015; Frederiksen et al., 2014; Hartmann et al., 2015; Kasper-Sonnenberg et al., 2012; Katsikantami et al., 2016; Larsson et al., 2014; Martínez et al., 2018). In addition, marked differences in phthalate exposures all over the world including considerable differences within 17 European countries (participating in the DEMOCOPHES study) have been observed (Den Hond et al., 2015). In this harmonized HBM programme most phthalate metabolite levels found in Polish children were significantly above the levels in children from other European countries. Specifically for MnBP and MiBP, the levels observed in Poland were almost twice as high as those found in the second highest country, Spain; DEHP metabolites ranked among the highest of the EU countries (Den Hond et al., 2015). Next to these country specific characteristics, socio-demographic and residential characteristics, lifestyles and dietary habits were recognized to be significantly associated with exposures to several phthalates (Den Hond et al., 2015; Liao et al., 2018; Schwedler et al., 2017; Correia-Sá et al., 2018; Černá et al., 2015).

The aim of the present study was to evaluate the levels and socio-demographic factors of phthalate exposure in children at age 7 years from the prospective Polish Mother and Child cohort (REPRO_PL). The study also includes an HBM-based estimation of daily intakes and comparisons with Tolerable Daily Intake values (TDI) set out by the European Food Safety Authority (EFSA) and Reference Doses (RfD) set out by the U.S. EPA. Furthermore, in order to evaluate the cumulative phthalate exposure, Hazard Indices based on potency estimates of anti-androgenicity (PEAA) of the individual phthalates have been calculated as established by the Chronic Hazard Advisory Panel (CHAP) on phthalates and phthalate alternatives (CHAP, 2014; Lioy et al., 2015). Finally, a comparison of the metabolite concentrations determined during prenatal period (mothers in 3rd trimester of pregnancy), at age of 2 and at age of 7 years was performed.

2. Methodology

2.1. Study population and sampling

The study is based on the Polish Mother and Child Cohort (REPRO_PL), a prospective birth cohort established in 2007 in Poland (with the recruitment of the women in the 1st trimester of pregnancy over a 4 year period). The details regarding the cohort methodology have been published previously (Polańska et al., 2009, 2011, 2016a). Briefly, the study comprised three phases covering prenatal period (phase I: 2007–2011), child examination at age of 1 and 2 years (phase II: 2008–2013) and child examination at age of 7 years (phase III: 2014–2019). From 407 children followed-up until the age of 7 years between the period 2014–15, 250 children (61%) provided complete data for the studied variables and a urine sample for phthalates measurement (Polańska et al., 2016a). Written informed consent was obtained from the parents of each child before the study, which was approved by the Ethical Committee of the Nofer Institute of Occupational Medicine, Lodz, Poland (Decision No. 22/2014).

2.2. Analysis of phthalate metabolites

2.2.1. Phthalate exposure during prenatal period and at age of two years

The detailed description of the methodology of assessment of phthalate metabolites in the urine collected from mothers during the 3rd trimester of pregnancy and from children at age of 2 years has been published previously (Polańska et al., 2014, 2016b). Briefly, 11 phthalate metabolites representing exposures to LMW phthalates (MEP, MBzP, MiBP, MnBP and OH-MnBP) and HMW phthalates (MEHP, OH-MEHP, oxo-MEHP, OH-MiNP, oxo-MiNP and MnOP) were analysed in the spot urine samples using high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) method.

2.2.2. Phthalate exposure at age of 7 years

For the children at the age of 7 an extended spectrum of 21 metabolites was analysed in the spot urine samples representing exposure to 11 phthalates including seven LMW (DMP, DEP, BBzP, DCHP, DnPeP, DiBP, DnBP) and four HMW phthalates (DEHP, DiNP, DiDP, DnOP). The parent phthalates, their metabolites analysed, and abbreviations are shown in Table S1. The analytical procedure has been described in detail elsewhere (Koch et al., 2003, 2017).

Briefly, 300 µl of urine were transferred into 1.8 ml vials. 10 µl of isotopically-labeled standards and 100 µl of 1 M ammonium acetate (at pH 6.0–6.4) were added. To hydrolyze possible glucuronide conjugated metabolites, 6 µl of β-glucuronidase from *E. coli* strain K-12 (arylsulfatase free), diluted 1:1 in ammonium acetate buffer, was used. The samples were mixed and incubated for 3 h at 37 °C. Then, 10 µl of acetic acid were added to adjust ph, and samples were frozen at −18 °C overnight. The samples were then thawed and equilibrated at room temperature and centrifuged at 1900 ×*g* for 10 min, and 10 µl supernatant were injected into an Agilent Technology LC 1260 system coupled with an AB Sciex TripleQuad 4500 tandem mass spectrometer. A Capcell PAK 5 u C18 MG-II column for clean-up and enrichment and an Atlantis d C18 (2.1 × 10 mm; 3 µm) for chromatographic separation were used, the two column assembly was operated in back-flush mode. Detection was performed in negative ionization mode and quantification by isotope dilution with deuterium labeled internal standards. Quality control materials were included in each batch of samples. Limits of quantification ranged between 0.2 and 1 µg/l, depending on the metabolite (Table 2).

2.3. Analysis of creatinine

Creatinine concentrations in urine were measured using the Jaffe static method with a working range of 0.05–5.00 g creatinine/l.

2.4. Covariates

The following socio-demographic information was obtained by questionnaire filled out by mothers at child examination: place of residence (rural, urban); number of siblings (none, 1, 2 or more); socio-economic status (SES) of the family (very poor, poor, good, very good); parental educational level (years of completed education: ≤9, 10–12, > 12); parental occupational activity (yes, no); child sex and age (exact age based on date of examination and date of birth). Parental age was calculated for date at child birth. Data concerning breast-feeding (no: < 2 weeks, short: 2 weeks–6 months, long: > 6 months) was collected after delivery and at follow-up examinations. Information on children's passive smoking at age of 7 years was extracted from cotinine levels in urine as described by Lupsa et al. (2015). A cut off value

at 2.1 ng/ml for child environmental tobacco smoke exposure was selected. Child height and weight was measured at age of 7 years by trained staff based on standard protocol (Polańska et al., 2016a). Body mass index (BMI) categories (underweight boys < 13.95 kg/m^2, girls < 13.80 kg/m^2; recommended weight boys 13.96–18.64 kg/m^2, girls 13.81–18.19 kg/m^2; overweight/obesity boys > 18.65 kg/m^2, girls > 18.20 kg/m^2) were based on Polish reference data BMI z-scores at age 7 (Kułaga et al., 2015).

2.5. Daily intake and cumulative risk assessment

Individual daily phthalate intakes (DI) were calculated from urinary metabolites levels according to the following equation, taken from Koch et al. (2011):

$$DI = \frac{UEsum * CEsmoothed}{Fue * bw} * MW \quad (1)$$

where DI is the daily phthalate intake (in μg/kg bw/day); UE_{sum} is the (summed) molar urinary excretion of the respective metabolite(s) (in μmol per gram creatinine); $CE_{smoothed}$ are child individual body height and sex based reference values for urinary creatinine excretion (in g/day), taken from Remer et al. (2002); Fue is the factor of urinary excretion for each phthalate and its urinary metabolite(s) (Table S3); bw is the body weight for each child (in kg); and MW is the molecular weight of the parent phthalate. The creatinine based estimate models for each phthalate were reported by Koch et al. (2007) and Wittassek et al. (2007).

In addition to DI, hazard quotient (HQ) and hazard index (HI) exposure levels of the population were calculated for five phthalates (BBzP, DiBP, DnBP, DEHP and DiNP), based on the approach described by the Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives (CHAP, 2014; Lioy et al., 2015). The framework of CHAP calculations includes three different cases, as follows: (i) a health benchmark value used in a cumulative risk assessment for mixtures of phthalates and other endocrine disruptors (Kortenkamp and Faust, 2010); (ii) a more recent mixture study of phthalates in rats enabling a direct comparison of potencies (Hannas et al., 2011); and (iii) *de novo* literature review of reproductive and developmental endpoints focused on reliable no observed adverse effect levels (NOAELS) and Points of departure (PODs). For each of the three scenarios, specific PEAA values were used, and are shown in Table S4. The HQ was calculated as the quotient between the estimated daily intake and the respective PEAA, while HI was the summed individual HQs.

2.6. Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018) and ggplot package (Wickham, 2009). For descriptive analysis, medians and geometric mean (GM) of the studied compounds with 95% confidence intervals (CI) were used. Percentile 95 and maximum values were also reported in the tables. Statistical differences between covariates were tested for significance using the Chi-square test. Non-parametric Kruskal-Wallis test was used for assessing the differences on phthalate concentrations at each follow-up time (between mothers and children at 2 and 7 years of age). Spearman's correlation coefficient (rho) was used to assess the correlations between phthalate concentrations in children (age 2 and 7 years) and their mothers (3rd trimester of pregnancy). The sums of the metabolites of DiBP, DnBP, DEHP, DiNP and DiDP were calculated and reported in Tables and Figures as summation (Σ). Multivariate linear regression analyses were used to assess the association of socio-demographic covariates with phthalate concentrations. Before inclusion in the models, phthalate concentrations and BMI were transformed into the natural logarithm. All the variables, including the categorical ones, were standardized (centred at zero and scaled to two standard deviations) (Gelman, 2008). The final model included the following covariates: sex, BMI, place of residence, breastfeeding duration, urinary cotinine levels, SES, paternal and maternal educational level and occupational status. The final model was selected by both AIC (Akaike Information Criteria) and BIC (Bayesian Information Criteria).

Table 1
Characteristics of the study population according to participation in the Phthalate metabolite analysis in Poland, 2014–15.

	Children included[a] n = 250	Children not included n = 157	p-Value[b]
	N (%)	N (%)	
Sex of the child			0.4
Female	134 (54)	77 (49)	
Male	116 (46)	80 (51)	
Child age at examination[c]	7.2 ± 0.23	7.5 ± 1.1	< 0.05
BMI			0.2
Underweight	15 (6)	16 (10)	
Recommended weight	192 (77)	118 (75)	
Overweight/obese	43 (17)	23 (15)	
Place of residence at 7 yr			0.4
Urban	216 (86)	130 (83)	
Rural	34 (14)	27 (17)	
Urinary cotinine levels at 7 yr			0.4
< 2.1 ng/ml	148 (60)	118 (75)	
> 2.1 ng/ml	99 (40)	39 (25)	
Maternal age at delivery			0.9
< 30 years	155 (62)	96 (61)	
> 30 years	95 (38)	61 (39)	
Parity at child's birth			0.8
None	138 (55)	83 (56)	
One	80 (32)	44 (29)	
Two or more	32 (13)	22 (15)	
Number of siblings at 7 yr			0.4
None	78 (31)	51 (32)	
One	149 (60)	85 (54)	
Two or more	22 (9)	21 (13)	
Breastfeeding[d]			NA
No (< 2 weeks)	20 (10)	NA	
Short (2 weeks–6 months)	62 (32)	NA	
Long (> 6 months)	113 (58)	NA	
Socio-economic status of the family at 7 yr			0.7
Most affluent (very good)	50 (20)	38 (24)	
Affluent (good)	195 (78)	116 (74)	
Least affluent (poor or very poor)	5 (2)	3 (2)	
Maternal educational level at 7 yr (years of completed education)			0.3
≤ 9	5 (2)	8 (5)	
10–12	83 (33)	34 (22)	
> 12	162 (65)	115 (73)	
Maternal occupational status at 7 yr			1
No	28 (11)	18 (11)	
Yes	219 (89)	139 (89)	
Paternal educational level at 7 yr (years of completed education)			0.09
≤ 9	10 (4)	6 (5)	
10–12	135 (54)	50 (42)	
> 12	103 (42)	63 (53)	
Paternal occupational status at 7 yr			NA
No	5 (2)	2 (2)	
Yes	227 (98)	117 (98)	

[a] Phthalate metabolites concentrations available.
[b] p-Value from Chi-square *t*-test.
[c] Mean ± SD.
[d] Most of the children not included in the study at 7 years of age were not evaluated at age 1 or 2, hence no question about breastfeeding status or duration was asked. Therefore, for this variable, data was not available, and shown as NA.

3. Results and discussion

3.1. Socio-demographic characteristics of the studied population

The characteristics of the population are described in Table 1. Except for age at examination (7.2 ± 0.23 years *vs.* 7.5 ± 1.1 years; $p < 0.05$), no differences were found between the subset of children included and not included in phthalate metabolites analyses.

Fifty-four percent of the children were girls and about 86% of the total population was residing in urban areas (Table 1). Concerning the maternal characteristics of the studied children, roughly two thirds of the mothers were younger than 30 years of age, more than half of the mothers (55%) had no previous descendants, and 13% were multiparous. A very low percentage of mothers (2%) attended only primary school, one third had a secondary school degree and 65% had a university degree. Among them, a high percentage (89%) was working. Similar percentages were found among fathers, although they showed lower educational level but higher rates of occupational status. The socio-economic status encompassed a large spectrum of cases in the affluent and most affluent levels (78% and 20%, respectively) while only 2% of the families were classified at the least affluent status (poor or very poor level). Only 10% of the children did not receive maternal breastfeeding (or < 2 weeks), 32% had short breastfeeding and 58% of the children were breastfed for > 6 months. About 40% of the children had cotinine levels in urine above 2.1 ng/ml indicating to some environmental tobacco smoke exposure. Six percent of the children were classified as underweight and 17% as overweight or obese.

3.2. Phthalate metabolite concentrations in children at age of 7 years

A total of 21 metabolites have been analysed, which represent the exposure to 11 parent phthalates, including 7 LMW and 4 HMW phthalates. The concentrations of phthalate metabolites are shown in Table 2 (in μg/l) and in Table S2 (creatinine adjusted values). Ten of the metabolites analysed were quantifiable in 100% of all samples, another eight metabolites were quantifiable in > 90% of the samples. Only three metabolites (MCHP, MnPeP and MnOP) were found in < 10% of the samples, hence they were not included in further analyses.

The most abundant individual metabolites belong to the LMW phthalates, with MiBP as the most abundant metabolite and a median concentration of 72.4 μg/l, followed by MnBP and MEP, with median concentrations of 56.3 μg/l and 42.0 μg/l, respectively. Secondary metabolites of DiBP (a LMW phthalate) and DEHP (from the HMW group) were also found in high concentrations, with median values ranging between 21.5 μg/l and 34.2 μg/l (cx-MEPP, OH-MiBP, OH-MEHP and oxo-MEHP). The sum of the DEHP metabolites cumulated to a median concentration of 89.3 μg/l, followed by the summed DiNP metabolites (21.9 μg/l) and the summed DiDP metabolites (3.8 μg/l).

Phthalate concentrations show a wide variability in the world and even in European children's population (Table 3). The levels observed in the current assessment are similar to those found in DEMOCOPHES study performed in Poland among children 6–11 years of age for MEP (42.9 μg/l *vs.* 46.9 μg/l), but lower for MBzP, MnBP, MiBP and ΣDEHP (Den Hond et al., 2015). In general, the levels of Polish children were similar to those observed in Eastern European countries, such as Slovak or Czech Republics (Černá et al., 2015; Den Hond et al., 2015; Table 3). However, in comparison with the rest of 17 countries involved in the DEMOCOPHES study, the European average reported by Den Hond et al. (2015) or the North American children studied within the U.S. (2015–16 NHANES report; CDC, 2019) or in Canada (2009–2011 Canadian Health Measures Survey Cycle 2; Haines et al., 2017), the levels of Polish children ranked among the highest (Table 3). For instance, a recently published study on Portuguese children (with median age of 10 years) evaluating the same set of phthalate metabolites and using the same analytical protocol has found lower exposure levels than in the present study (Correia-Sá et al., 2018). Specifically, median concentrations of ΣDiBP, ΣDnBP, ΣDEHP and ΣDiNP were much higher in Poland than in Portugal (Correia-Sá et al., 2018). For ΣDiDP, however, the concentrations were similar in both populations, and for DEP, the levels were even higher in Portugal than in the present Polish study (Correia-Sá et al., 2018).

Table 2
Concentrations of phthalate metabolites in urine of seven year-old children (in μg/l) in the REPRO_PL cohort (n = 250).

Parent phthalate	Metabolite	LQ[a]	DF[b] (%)	GM[c]	Median	P95	Range
LMW[d]							
DMP	MMP	1	97.6	5.1	4.5	25.3	0.50–10,500
DEP	MEP	0.5	100	42.9	42.0	206	2.7–1820
BBzP	MBzP	0.2	99.6	5.5	5.5	37.9	0.10–262.0
DCHP	MCHP	0.2	8.8	0.11	0.10	0.3	0.10–2.6
DnPeP	MnPeP	0.2	6.4	0.11	0.10	0.3	0.10–13.1
DiBP	MiBP	1	100	76.2	72.4	271	2.0–1270
	OH-MiBP	0.25	100	27.9	27.0	113.4	1.1–351.0
	ΣDiBP	–	–	104.9	97.3	370.5	3.1–1621.0
DnBP	MnBP	1	100	55.0	56.3	201	2.9–8430
	OH-MnBP	0.25	100	7.0	7.3	29.2	0.40–998.0
	ΣDnBP	–	–	62.1	62.6	226.6	3.5–9428.0
HMW[e]							
DEHP	MEHP	0.5	95.2	2.7	2.9	10.4	0.25–625.0
	OH-MEHP	0.2	100	27.1	28.1	90.9	1.1–4700
	oxo-MEHP	0.2	100	19.9	21.5	64.6	0.80–3000
	cx-MEPP	0.2	100	31.4	34.2	110	1.8–3350
	ΣDEHP	–	–	82.1	89.3	266.5	4.0–11,675
DnOP	MnOP	0.2	0.4	0.10	0.10	0.10	0.10–1.8
DiNP	OH-MiNP	0.2	100	9.5	10.5	33.1	0.30–2430
	oxo-MiNP	0.2	98.8	3.1	3.4	12.1	0.10–1570
	cx-MiNP	0.2	100	7.6	7.8	26.4	0.50–1340
	ΣDiNP	–	–	20.7	21.9	58.6	1.2–5340.0
DiDP	OH-MiDP	0.2	96.8	1.8	1.9	7.4	0.10–727.0
	oxo-MiDP	0.2	90.8	0.89	0.90	6.2	0.10–896.0
	cx-MiDP	0.2	96.4	0.91	0.90	3.2	0.10–77.3
	ΣDiDP	–	–	3.8	3.8	16.0	0.30–1700.3
Various	MCPP*	0.5	95.2	2.2	2.3	7.3	0.25–142.0

Phthalates and their abbreviations are presented in Table S1.
[a] LQ: Limit of Quantification
[b] DF: Detection Frequency (in percentage).
[c] GM: Geometric Mean.
[d] LMW: Low Molecular Weight Phthalate.
[e] HMW: High Molecular Weight Phthalate.
* MCPP is a metabolite of various LMW (DnBP) and HMW (DnOP, DiNP, DiDP) phthalates.

In summary, the current study on 7-year old children from Poland confirms previous findings from DEMOCOPHES study. In this regard, children exposures' to several phthalates are higher in Poland, as well as in some other Eastern European countries, than the European average or the U.S., taking into account similar sampling years (Table 3).

3.3. Comparison of maternal (3rd trimester of pregnancy) and children (at age of 2 and 7 years) concentrations

The data concerning the levels of 11 phthalate metabolites in mothers (3rd trimester of pregnancy) and children (at 2 years of age) has been published previously (Polańska et al., 2014, 2016b). Fig. 1 shows the concentrations of ten phthalate metabolites in children at age 2 and 7, as well as in their mothers during pregnancy. Median metabolite levels were found to be higher in 7-year old children than in the same children at age 2 or in their mothers. The differences observed at each follow-up time (mothers, children at age 2 and at age 7) were statistically significant for all the compounds (Kruskal-Wallis test's p-values were < 0.001).

The correlations between phthalate concentrations in paired samples of 7-year old children and their mothers (n = 110) are shown in Fig. 2, and between children at age 2 and 7 (n = 101) in Fig. 3. In

Table 3
Geometric mean phthalate metabolite concentrations (in ug/l) found in urine of children from different studies.

Location	Year	n	Age	MEP	MBzP	MiBP	MnBP	ΣDEHP	Reference
Poland	2014–15	250	7	42.9	5.5	76.2	55.0	82.1 (50.1[a])	This study
Poland	2011–12	115	6–11	46.9	9.3	108.3	90.4	76.4[a]	Den Hond et al., 2015
Czech Republic	2011–12	120	6–11	34.4	9.1	–	–	71.1[a]	Den Hond et al., 2015
Slovak Republic	2011–12	127	6–11	37.5	7.9	–	–	82.7[a]	Den Hond et al., 2015
Germany	2011–12	120	6–11	23.1	6.6	41.4	46.4	39.5[a]	Den Hond et al., 2015
Germany	2009–10	465	8–10	25.8	7.0	48.9	47.0	77.0	Kasper-Sonnenberg et al., 2014
Europe	2011–12	1816	6–11	34.4	7.1	45.4	34.8	47.6[a]	Den Hond et al., 2015
Portugal	2014–15	112	4–18	58.3	2.3	16.8	12.8	37.3	Correia-Sá et al., 2018
U.S.	2015–16	415	6–11	24.5	10.7	11.2	14.4	30.8[b]	CDC, 2019
Canada	2009–11	516	6–11	29.0	19.0	22.0	36.0	41.7[b]	Haines et al., 2017

[a] Sum DEHP is MEHP + OH-MEHP + oxo-MEHP.
[b] Sum of individual GM.

general, the concentrations were not significantly correlated, except for MEP and MiBP between mothers and 7-year old children, with coefficients of 0.24 (p-value = 0.01) and 0.26 (p-value = 0.006), respectively (Fig. 2).

The present study showed that phthalate concentrations in 7-year old children were higher than those measured in the same children at age 2, and also in their mothers during pregnancy (Fig. 1). Previous studies have also identified divergences in phthalate levels between children and adults (Kasper-Sonnenberg et al., 2012; Cutanda et al., 2015; Černá et al., 2015; Hartmann et al., 2015; Schwedler et al., 2017), and even between different ages among children (Kasper-Sonnenberg et al., 2012; Den Hond et al., 2015). In addition, the previous studies have also identified higher MEP levels among adults, including pregnant women and senior citizens, in relation to children, alike the present study (Kasper-Sonnenberg et al., 2012; Černá et al., 2015; Hartmann et al., 2015). Although the trends are consistent with the aforementioned studies, recent reports have found a decreasing trend in the concentrations of phthalate metabolites in European and North American countries (Koch et al., 2017; Haines et al., 2017). In this sense, the present study reveals an unexpected pattern of exposure,

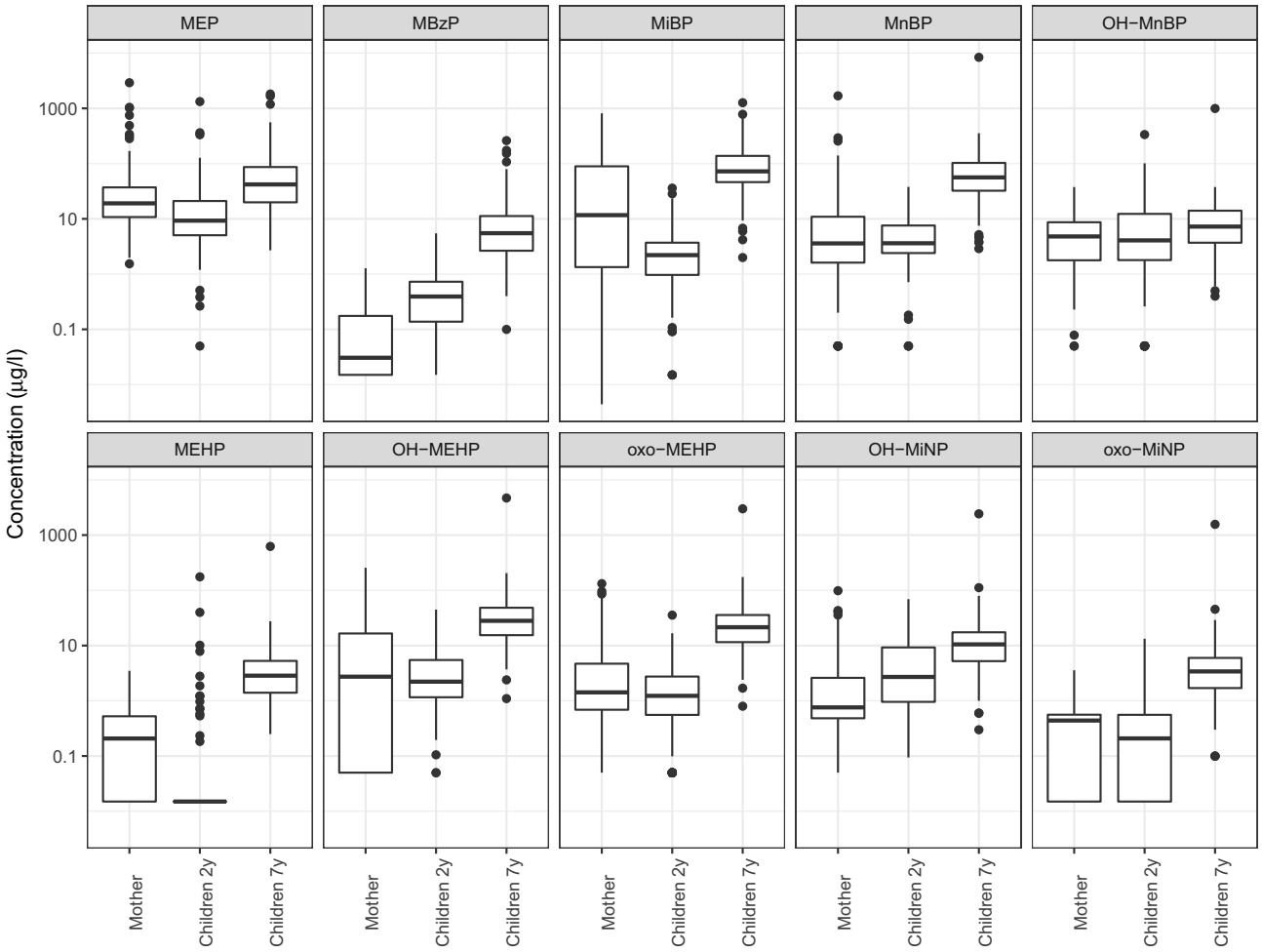


Fig. 1. Boxplot of phthalate metabolite concentrations (µg/l) in pregnant women (n = 110), children at 2 years of age (n = 101) and children at 7 years of age (n = 250) in the REPRO_PL cohort. The y-axis is shown in logarithmic scale.

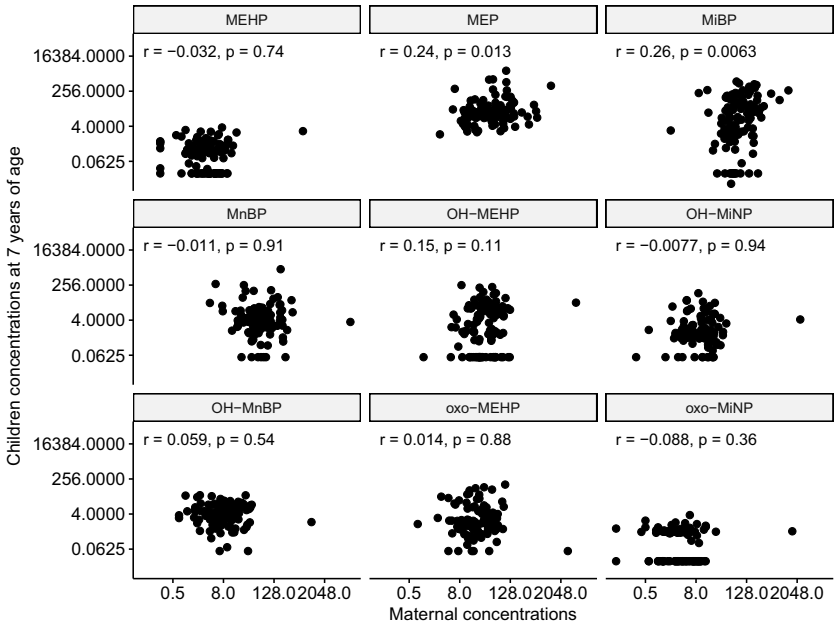


Fig. 2. Correlation plots between paired pregnant women and 7-year old children from the REPRO_PL cohort (n = 110). The axes are shown in logarithmic scale.

since most phthalate metabolite concentrations in samples collected in 2014 (those belonging to 7 year old children) are generally higher than those collected in 2009 (2-year old children) or 2007 (pregnant women). Den Hond et al. (2015) found higher levels in younger children, although this study is focused on children between 6 and 11 years of age, and therefore did not include those at lower ages (*e.g.* 2 year-old children).

Previous assessments performed at the REPRO_PL birth cohort found statistically significant correlations between pregnant women and children at age of 2 years among HMW phthalates, but not for LMW phthalates (Polańska et al., 2014, 2016b). Current analyses have found only statistically significant correlations among MEP and MiBP between pregnant women and their children at age 7 (Fig. 2). The measurements between samples are, however, separated by several years, hence market situation and uses have probably changed substantially.

3.4. Socio-demographic determinants of phthalate exposure (7-year old children)

Results of univariate analyses for the 7-year old children are shown in Fig. 4 and Fig. S1. The concentrations of certain phthalate metabolites were significantly higher in overweight and obese children (*e.g.* MBzP, ΣDnBP, ΣDiDP), in children belonging to the least affluent socio-economic status (*e.g.* MMP, ΣDnBP), and in those whose mothers or fathers had a low educational level (*e.g.* MEP, MBzP, ΣDiBP, ΣDnBP). Higher urinary cotinine levels in children were also related to higher phthalate concentrations, but the associations were only significant for some phthalates (*e.g.* ΣDiBP, ΣDnBP, ΣDiNP, MCPP) (Fig. 4). On the other hand, breastfeeding duration was negatively associated with phthalate metabolites concentrations at age 7. The tendency is more evident for HMW phthalates, and differences between children who

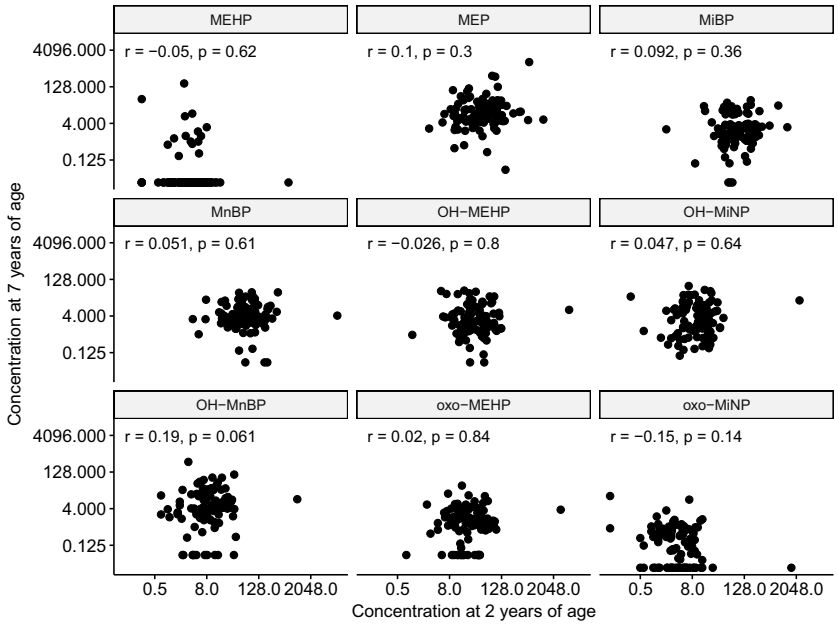


Fig. 3. Correlation plots between paired samples of children at 2 and 7 years of age from the REPRO_PL cohort (n = 101). The axes are shown in logarithmic scale.

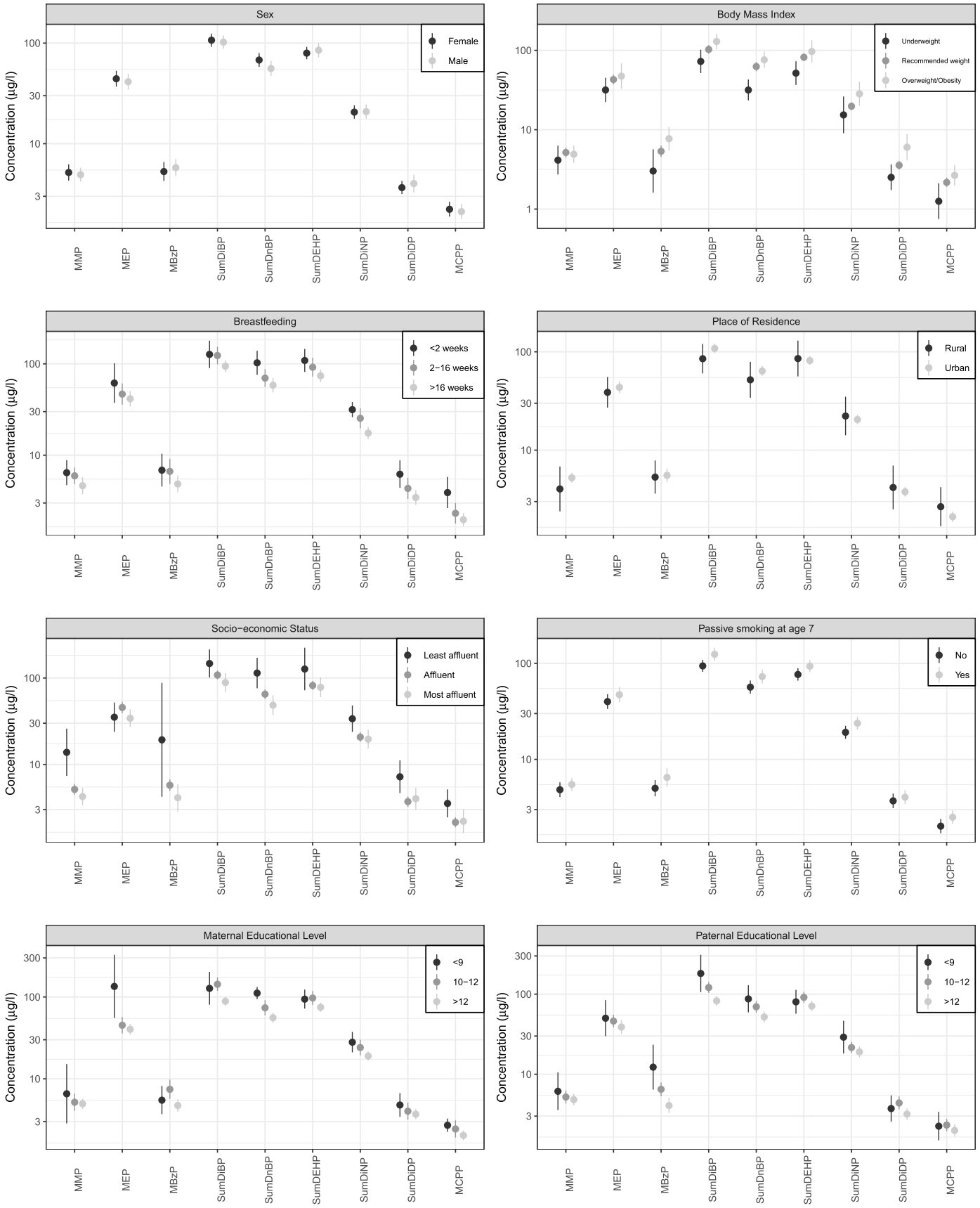


Fig. 4. Geometric means and 95% confidence intervals (µg/l) of the phthalate metabolite concentrations in 7-year old children for several socio-demographic characteristics: children's sex and BMI, breastfeeding duration, place of residence, socio-economic status, passive smoking at age 7 and maternal and paternal educational level. The y-axis is shown in logarithmic scale.

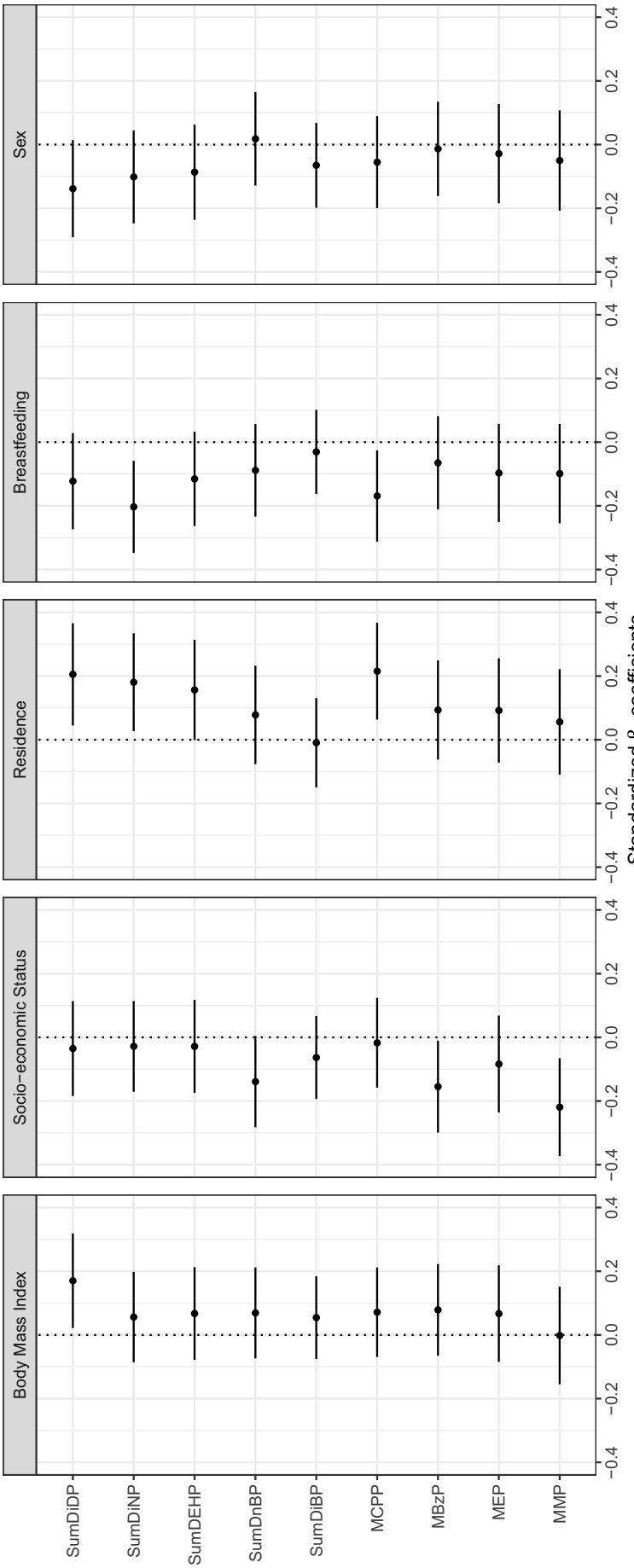


Fig. 5. Standardized beta-coefficients from multivariate regression models for several socio-demographic and individual characteristics. Models were adjusted by sex, body mass index, breastfeeding duration, place of residence, urinary cotinine levels, socio-economic status, parental occupational status and educational level. BMI was kept as continuous and, together with the rest of covariates, was standardized (centred at zero and scaled to two SD) for cross-comparison of the results within covariates and between compounds.

were not breastfed and those who breastfed > 6 months are statistically significant (Fig. 4). Other covariates such as children's sex, number of siblings, place of residence and maternal age did not show statistically significant concentration differences for any of the compounds analysed (Fig. 4 and Fig. S1).

Multivariate regression models confirmed the aforementioned tendencies on socio-demographic variables (Fig. 5). DiDP metabolites were significantly associated with increasing body mass index. HMW phthalate concentrations were systematically higher in rural areas, while LMW phthalates were found to be lower in the most affluent social class, both covariates showing statistically significant results ($p < 0.05$). Several phthalate metabolites (DiNP metabolites and MCPP) were associated with breastfeeding duration, with lower concentrations among children who were breastfed during longer periods (> 6 months). Girls showed lower phthalate concentrations than boys, although only two phthalates showed significant difference at a 90% confidence. Finally, paternal educational level was also found to be associated with phthalate concentrations (MBzP, ΣDiBP), while urinary cotinine levels and parental occupational status did not involve any significant result (data not shown).

Differences in environment and lifestyle (different habits, different product uses, or different market uses) influenced individual phthalate metabolite values and country-specific averages (Den Hond et al., 2015). In DEMOCOPHES assessments a significant influence of SES (represented by the highest educational level within the family) on phthalate metabolite levels was observed (Den Hond et al., 2015). Specifically, phthalate metabolite concentrations were lower with increasing education, which is in line with the present results (Fig. 4). However, after multivariate analyses, a statistically significant negative association between SES and phthalate metabolite concentrations was found only for certain LMW phthalates (*e.g.* DMP, BBzP and DnBP metabolites, Fig. 5 and Fig. S2). Therefore, underlying lifestyle factors (*e.g.* personal-care products, home equipment, car upholstery or food consumption, among others) that vary with SES may account for these findings, although they were not considered in detail in the study questionnaires. Concerning sex differences, the present results concur with other studies which found higher concentrations of most phthalate metabolites in boys than in girls (Liao et al., 2018). Maybe this relates to increases physical activity, mouthing behavior or other sex specific differences in child behavior. Several studies have examined associations of childhood phthalate exposure and adiposity/obesity (Teitelbaum et al., 2012; Deierlein et al., 2016; Hatch et al., 2008; Trasande et al., 2013; Braun, 2017; Correia-Sá et al., 2018). A recent review concluded that the associations between early-life phthalate exposure and child adiposity or obesity risk have been inconsistent (showing positive, negative or no association) (Braun, 2017). It is pointed out that inconsistent findings of the previous studies can be explained by the fact that obese and regular weight children differ strongly in many physical characteristics (*e.g.* urine flow rate, urinary volume, surface area leading to the application of amounts of phthalate-containing personal care products to their skin, among others) which can directly influence all measures ranging from urinary metabolite concentration to body weight related calculated (daily) intakes. The study performed on Portuguese children indicates that obese children following a healthy diet composed of fresh and less packaged/processed food can considerably reduce their intake for most phthalates and can have lower phthalate intakes than regular weight/regular diet children (Correia-Sá et al., 2018). Given these caveats, in our study we found that increases of BMI were associated with certain phthalate metabolites, namely MEHP and OH-MiDP (Fig. S2).

In the present study, children living in rural areas had higher levels of HMW phthalate metabolites compared to those from urban areas. This finding, however, is not consistent with the literature. For instance, some reports found higher concentrations of MBzP, MnBP and MiBP (which belong to LMW phthalates) in children from urban areas when compared to residents from the rural area (Larsson et al., 2014; Schwedler et al., 2017). On the other hand, place of residence was not a significant determinant of internal phthalate exposure in DEMOCOPHES assessment (Den Hond et al., 2015; Černá et al., 2015). Also Liao et al. (2018) pointed out that residential characteristics were less associated with urinary phthalate metabolites compared with lifestyles and dietary habits. Those results indicate that the levels and sources of exposure to phthalates can be regional or country specific. It could be suspected that higher exposures to HMW phthalates among children living in rural areas from Poland are related to home equipment, since such phthalates are primarily used in polyvinyl chloride (PVC) polymer and can be found in building and construction materials, including floorings and furnishings (Katsikantami et al., 2016; Benjamin et al., 2017).

Phthalates are present in breast milk, as already shown in previous studies (Fromme et al., 2011; Katsikantami et al., 2016). Lactating women are exposed to them through food consumption or use of cosmetics (Katsikantami et al., 2016). However, infants nourished with formula are also exposed to phthalates, and their daily intakes might be similar or even higher than for exclusively breast-fed infants (Fromme et al., 2011). The present study found an unexpected association, with higher phthalate levels among children who had longer breastfeeding (> 6 months) (Fig. 5). However, this finding seems to be rather related to a residual confounding than to a direct impact of breastfeeding at age 7. In this regard, family lifestyle, home environment and other socio-economic characteristics not controlled in the current study could be the main factors for the observed associations.

3.5. Calculated daily intakes and cumulative risk assessment

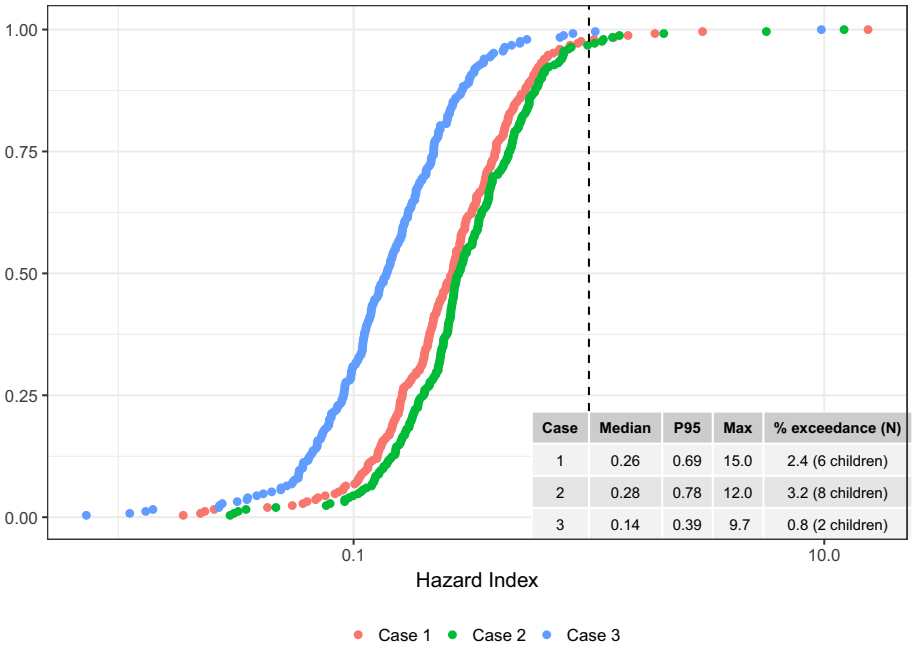
Daily intakes (DI) for the parent phthalates investigated based on their urinary metabolites were calculated following Eq. (1). Results are shown in Table 4. DEHP was the phthalate with the highest median DI (6.1 μg/kg bw/day), followed by DiBP, DiNP, DnBP and DEP, with median daily intakes of 3.7, 2.3, 2.2 and 1.8 μg/kg bw/day, respectively (Table 4).

For DEHP and DiNP, one child (0.4%, the same for both compounds) exceeded the TDI established by EFSA, whereas for DnBP, six children (2.4%) exceeded the EFSA TDI (Table 4). For DiBP, assuming the same TDI as for DnBP, a total of 25 children (10% of the children) would exceed the TDI. However, no exceedances on TDIs were found for BBzP and DiDP. The percentages of children exceeding the U.S. EPA Reference Dose differ: 4% for DEHP (corresponding to 10 children) and 0.4% for DnBP (corresponding to 1 child). Similar numbers were

Table 4
Daily intakes (μg/kg bw/day) of selected phthalates for the studied population (n = 249).

	Median	P95	Max	Values set (% exceedance)		
				TDI (EFSA)[a]	RfD (U.S. EPA)[b]	RfD AA[c]
DMP	0.19	1.1	321.6	–	–	–
DEP	1.8	8.8	85.5	–	800 (0%)	–
BBzP	0.22	1.3	22.1	500 (0%)	200 (0%)	330 (0%)
DiBP	3.7	15.0	48.8	10[d] (10%)	100[d] (0%)	200 (0%)
DnBP	2.2	8.5	252.4	10 (2.4%)	100 (0.4%)	100 (0.4%)
DEHP	6.1	17.3	451.9	50 (0.4%)	20 (4%)	30 (1.6%)
DiNP	2.3	8.1	334.4	150 (0.4%)	–	–
DiDP	0.38	1.6	96.5	150 (0%)	–	–

[a] Tolerable daily intakes set by the European Food and Safety Agency (EFSA, 2005a, 2005b, 2005c, 2005d, 2005e; EC, 2013).
[b] Reference dose set by the U.S. Environmental Protection Agency (U.S. EPA, 2007).
[c] Reference dose for anti-androgenicity (Kortenkamp and Faust, 2010; Soeborg et al., 2012).
[d] For DiBP, no information is available, hence values set are derived for DnBP, based on a conservative approach.



Case	Median	P95	Max	% exceedance (N)
1	0.26	0.69	15.0	2.4 (6 children)
2	0.28	0.78	12.0	3.2 (8 children)
3	0.14	0.39	9.7	0.8 (2 children)

Fig. 6. Relative cumulative frequencies for the estimated hazard indices in children from Poland (n = 249), for each CHAP case. Vertical dashed line marks the value in which exceedance may indicate a health risk (HI > 1).

applied to the anti-androgenicity approach proposed by Kortenkamp and Faust (2010) and applied by Soeborg et al. (2012) in an study performed in Denmark, as shown in Table 4 (1 child exceeding the RfD AA for DnBP, and 4 children for DEHP).

In agreement with the rather high urinary metabolite levels found in this study, daily intakes in the present study are higher (by a factor between 2 and 8) than those reported in other studies performed in children from Portugal, Denmark and Austria, except in the case of DEP in the Portuguese report and BBzP and DiBP in both the Austrian and Danish studies, in which DIs were similar (Correia-Sá et al., 2018; Hartmann et al., 2015; Frederiksen et al., 2014). In general, the daily intakes of our study population (sampled in 2014–15) for most phthalates are quite similar to daily intakes of other studies performed in the early 2000s, when regulatory measures on phthalates were not set yet.

Fig. 6 shows the Hazard Indices calculated for each of the three cases of CHAP (Lioy et al., 2015). Median HI values ranged between 0.14 and 0.28, and percentile 95 was lower than 1 in all the cases (between 0.39 and 0.78). However, between 2 and 8 children (0.8–3.2% of the study population) exceeded the HI of 1, indicating that the cumulative phthalate exposure level cannot be regarded as safe anymore. Median hazard indices for the three cases found in the present study (0.14–0.28) were higher than those reported for Infants in the CHAP report (0.12–0.22) or in the aforementioned Portuguese report (0.03–0.10) (CHAP, 2014; Correia-Sá et al., 2018), but lower than in studies from Canada (0.37–0.82) or Austria and Denmark (0.18–0.41) (Environment and Climate Change Canada and Health Canada, 2017; Hartmann et al., 2015; Frederiksen et al., 2013). In any case, the latter studies are not directly comparable, since HIs were calculated based on EFSA's TDI, while the rest of the reports uses the derived PEAA, as already explained above. Nevertheless, irrespective of the calculation model and points of departures used, all studies have in common that median phthalate exposures seem to be below any recognizable risk level, but exposures in the upper percentiles exceed levels that can be regarded as safe.

3.6. Study strengths and limitations

The strength of the current study is related to the prospective study design (comparison of the exposure level in the same children during prenatal period, at age of 2 and 7 years) and assessment of 21 phthalate metabolites which represents the most extensive set of phthalate metabolites ever determined for Poland and rarely performed in other studies. The main limitation of this study needs to be underlined. Although the present study is focused on socio-demographic factors related to the exposure level, some lifestyle-related determinants, including diet, were not evaluated. For instance, as reported in a recent study performed in Shanghai (China) on children aged 5–10 years, non-usage household air cleaner, changing the child's pillowcase less than one time a week, dusting furniture in the child's bedroom less than three times a week, using higher proportion of plastic toys, as well as drinking soft drinks or eating candies were significantly associated with higher phthalate levels (Liao et al., 2018). In general, food consumption, as well as the use of plastic containers, such as those used for ready-to-eat food, have been found to be associated to the estimated dietary exposure to HMW phthalates (*e.g.* DEHP and DiNP) (Larsson et al., 2014; Martínez et al., 2017, 2018). The present prospective REPRO_PL cohort was designed to evaluate the impact of a variety of environmental exposures on children's health and neurodevelopment, hence such detailed assessment was not possible. On the other hand, and as already known and discussed thoroughly in many previous studies, single urine measurements of non-persistent chemicals with short half-lives, such as phthalates, is a general limitation in HBM studies (Aylward et al., 2014, 2017). In any case, the purpose of the study, aimed to show an overview of the general exposure to phthalates in the Polish children population, is accomplished.

4. Conclusions

This study represents the most extensive set of phthalate metabolites ever determined for Poland. The presented data confirms the ubiquitous phthalate exposure of children and, specially, that the levels in Polish children (sampled in 2014–15) are higher than levels found in other European populations sampled in recent years. Thus we confirm some of the heterogeneity in phthalate exposures over Europe already observed in the EU DEMOCOPHES approach. Several socio-demographic and lifestyle characteristics were associated with phthalate body burdens, including the BMI, breastfeeding duration, place of residence, parental educational level and socio-economic status, mostly in

agreement with previous reports.

The individual and cumulative risk assessment of exposure to the studied phthalates confirmed that children in Poland are still broadly exposed to several anti-androgenic phthalates, and exposures in the upper percentiles cannot be regarded as safe. Substantial declines in exposure levels to critical phthalates, as observed in other European or North American populations, are yet to find their reflection in the Polish population. Taking into account the level of exposure and existing knowledge on the potential impact of phthalates on children's health, exposure reduction measures need to be intensified in Poland, including policies and public health interventions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.04.011.

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Declaration of interest

The authors declare there is no conflict of interest.

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