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Urinary concentrations of phthalate/DINCH metabolites and body mass index among European children and adolescents in the HBM4EU Aligned Studies: A cross-sectional multi-country study

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A B S T R A C T

Background: Phthalates are ubiquitous in the environment. Despite short half-lives, chronic exposure can lead to endocrine disruption. The safety of phthalate substitute DINCH is unclear.
Objective: To evaluate associations between urinary concentrations of phthalate/DINCH metabolites and body mass index (BMI) z-score among children and adolescents.
Method: We used Human Biomonitoring for Europe Aligned Studies data from 2876 children (12 studies, 6–12 years, 2014–2021) and 2499 adolescents (10 studies, 12–18 years, 2014–2021) with up to 14 phthalate/DINCH urinary metabolites. We used multilevel linear regression to assess associations between phthalate/DINCH concentrations and BMI z-scores, testing effect modification by sex. In a subset, Bayesian kernel machine regression (BKMR) and quantile-based g-computation assessed important predictors and mixture effects.
Results: In children, we found few associations in single pollutant models and no interactions by sex (p-interaction > 0.1). BKMR detected no relevant exposures (posterior inclusion probabilities, PIPs < 0.25), nor joint mixture effect. In adolescent single pollutant analysis, mono-ethyl phthalate (MEP) concentrations were associated with higher BMI z-score in males ($\beta = 0.08$, 95 % CI: 0.001,0.15, per interquartile range increase in ln-transformed concentrations, p-interaction $= 0.06$). Conversely, mono-isobutyl phthalate (MiBP) was associated with a lower BMI z-score in both sexes ($\beta =$ -0.13, 95 % CI: −0.19, −0.07, p-interaction $= 0.74$), as was sum of di(2-ethylhexyl) phthalate ($\sum$DEHP) metabolites in females only ($\beta =$ -0.08, 95 % CI: −0.14, −0.02, p-interaction $= 0.01$). In BKMR, higher BMI z-scores were predicted by MEP (PIP=0.90) and MBzP (PIP=0.84) in males. Lower BMI z-scores were predicted by MiBP (PIP=0.999), OH-MIDP (PIP=0.88) and OH-MINCH (PIP=0.72) in both sexes, less robustly by DEHP (PIP=0.61) in females. In quantile g-computation, the overall mixture effect was null for males, and trended negative for females ($\beta =$ -0.11, 95 % CI: −0.25, 0.03, per joint exposure quantile).
Conclusion: In this large Europe-wide study, we found age/sex-specific differences between phthalate metabolites and BMI z-score, stronger in adolescents. Longitudinal studies with repeated phthalate measurements are needed.

1. Introduction

Phthalates are a group of synthetic chemicals with a wide range of applications in the manufacturing of plastics as softeners to enhance flexibility and durability. Moreover, they are used in numerous personal care and household products, including nail polish, soaps, shampoos, body lotions, hair sprays, toys, food packaging, vinyl flooring, shoes, medication and dietary supplements (Braun, 2017, Bilal and Iqbal, 2019, Kelley et al., 2012, Serrano et al., 2014). Despite the short half-lives of phthalates, chronic exposure to some phthalates has been shown to disrupt the endocrine system, which has led to the regulation of certain phthalates in Europe and globally (European Commission, 2021). The phthalate substitute, DINCH, was introduced in 2002 to replace many of the higher molecular weight phthalate esters in food packaging materials, medical devices, and children's toys (Testai et al., 2016).

Due to widespread use, phthalates are ubiquitous in the environment. Across Europe, there exists substantial regional variation in phthalate exposure. Among children and adolescents, the exposure is most pronounced for di-(2-ethyl-hexyl) phthalate (DEHP), di-*iso*-butyl phthalate (DiBP), and di-ethyl phthalate (DEP), while it is lower for di-*iso*-decyl phthalate (DiDP) and di-*iso*-nonyl-cyclohexane-1,2-dicarboxylate (DINCH) (Vogel et al., 2023, Lange et al., 2022).

Concentrations of phthalate metabolites in the human body have decreased in recent years. Analysis of 24-hour urine samples collected from 1988 to 2015 by the German Environmental Specimen Bank (ESB) revealed a ten-fold reduction in levels of DEHP, DnBP, and BBzP following the implementation of EU regulations in 1999. Despite these improvements, some samples continue to exceed established health-based guidance values (Koch et al., 2017). Conversely, there has been an increasing trend for DINCH metabolites (Gyllenhammar et al., 2017).

Overweight/obesity among children and adolescents is a strong predictor of cardiovascular disease later in life. The global increase in the prevalence of overweight and obesity ranges from 4 % in 1975 to over 18 % in 2016 among 5–19 year olds and represents a major public health challenge (WHO, 2021). While poor nutrition and lack of physical activity are the primary drivers of the obesity epidemic, evidence indicates that endocrine-disrupting chemicals can increase the risk of obesity (Heindel et al., 2015).

The evidence linking phthalate exposure and body mass index (BMI), adiposity and cardiometabolic status in children and adolescents is inconsistent, with both positive (Trasande et al., 2013, Deierlein et al., 2016) and negative associations (Hatch et al., 2008, Boas et al., 2010). Findings from systematic reviews are inconsistent overall (Lee et al., 2022). Some associations vary by race/ethnicity (Trasande et al., 2013), specific phthalate types, particularly low molecular weight phthalates (Ribeiro et al., 2019; Zhang et al., 2014), and sex (Vafeiadi et al., 2018, Zhang et al., 2014, Deierlein et al., 2016, Perng et al., 2017). Furthermore, there is limited evidence about the safety of co-exposure to phthalate substitutes such as DINCH (Rodríguez-Carmona et al., 2020). Our study aims to address these gaps by using multi-pollutant analysis to examine a wide range of phthalates, including newer substitutes, across diverse populations in different regions of Europe and considering differences by age and sex.

In this study, we evaluated the associations between 14 phthalate/DINCH metabolites and BMI z-score in children (12 studies, 6–12 years) and adolescents (10 studies, 12–18 years) in a large European population. We used data from 14 countries in Europe, where metabolites of phthalates and DINCH were measured in urine under strict quality control methods (Esteban López et al., 2021). This study was conducted in the framework of Human Biomonitoring for Europe (HBM4EU), an innovative large-scale European project operating at the science-policy interface aimed at the generation of EU-wide harmonised and accessible data on internal human exposure (Ganzleben et al 2017; Kolossa-Gehring et al 2023; https://www.hbm4eu.eu) to support chemicals policy and the improvement of health and wellbeing in Europe.

2. Methods

2.1. Study population

The study population was selected from HBM4EU-Aligned studies (2014–2021), in which biomonitoring of several prioritized chemicals was performed. The HBM4EU-Aligned Studies are a survey aimed at collecting HBM samples and data that are as harmonized as possible from (national) studies to derive current internal exposure data for the

European population/citizens across a geographic spread. These studies are described in detail elsewhere (Gilles et al., 2021, Govarts et al., 2023, Gilles et al., 2022). In brief, 23 countries covering all geographical areas of Europe participated across selected age groups (children aged 6–11 years, adolescents aged 12–18 years, and young adults aged 20–39 years). Even though national representativeness was preferred for each country, regional level representativeness was also accepted. The number of participants within each country was limited to a maximum of 300 participants per age group with an approximate 1:1 male to female ratio.

The eligibility criteria for being included in the HBM4EU-Aligned studies included availability of biobanked urine samples, new studies adopting HBM4EU standardised protocols for producing harmonized data (Pack et al., 2023), studies that target the general population, availability of a basic set of variables, ethical approval, compliance with the HBM4EU data management plan and data policy, and analysis of HBM4EU priority chemicals in a laboratory that passed the HBM4EU quality assurance quality control and follows the HBM4EU quality assurance protocol (Govarts et al., 2022, Gilles et al., 2022, Esteban López et al., 2021, Gilles et al., 2021). The 12 studies targeting children (6–11 years) included NEBII (Norwegian Environmental Biobank II – a sub-cohort of the Norwegian Mother, Father and Child Study, MoBa (Magnus et al., 2016); Norway), OCC (Odense Child Cohort; Denmark), InAirQ (Transnational Adaption Actions for Integrated Indoor Air Quality Management; Hungary), PCB cohort (Endocrine disruptors and health in children and adolescents in Slovakia; Slovakia), POLAES (Polish Aligned Environmental Study; Poland), SLO CRP (Exposure of children and adolescents to selected chemicals through their habitat environment; Slovenia), CROME (Cross-Mediterranean Environment and Health Network; Greece), NACII (Northern Adriatic cohort II; Italy), ESTEBAN (Étude de santé sur l'environnement, la biosurveillance, l'activité physique et la nutrition; France), GerES V-sub unweighted (German Environmental Survey subsample; Germany), 3xG (Gezondheid, Gemeenten, Geboorte studie; Belgium) and SPECIMEn-NL (Survey on PEstiCide Mixtures in Europe, The Netherlands) resulting in a total study population of 2876 children from across Europe.

For the adolescents, 10 studies targeting teenagers between 12 and 18 years of age were included: NEBII (Norway), Riksmaten Adolescents (Sweden), POLAES (Poland), PCB cohort follow-up (Slovakia), SLO CRP (Slovenia), CROME (Greece), BEA (Biomonitorización en Adolescentes; Spain), ESTEBAN (France), GerES V-sub unweighted (Germany), and FLEHS IV (Flemish Environment and Health Study IV; Belgium), resulting in a total study population of 2499 adolescents from across Europe.

2.2. Exposure

Chemical analyses for phthalates/DINCH were performed on urine (first morning void or spot urine) samples per study within HBM4EU. As the measured concentration in urine is influenced by dilution level, creatinine was used for standardizing phthalate/DINCH metabolite levels in urine in both children and adolescents, i.e., metabolite concentrations divided by the concentration of creatinine (μg/g creatinine). Urine samples with a creatinine concentration of $\geq$ 5 mg/dL were defined as acceptable samples for screening of phthalates/DINCH based on the U.S. Department of Transportation definitions which is less stringent than the World Health Organisation (WHO) definitions (Barbanel et al., 2002).

The concentrations (μg/L) of 14 phthalate/DINCH metabolites quantified in urine samples included: mono-ethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-*iso*-butyl phthalate (MiBP), mono-benzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxy-hexyl) phthalate (5OH-MEHP), mono(2-ethyl-5-oxo-hexyl) phthalate (5oxo-MEHP), mono (2-ethyl-5-carboxy- pentyl) phthalate (5cx-MEPP), mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MiNP), mono(4-methyl-7-carboxyheptyl) phthalate (cx-MiNP), mono-hydroxy-isodecyl phthalate (OH-MiDP), mono(2,7-methyl-7carboxy-heptyl) phthalate (cx-MiDP), cyclohexane-1,2- dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH), and cyclohexane-1,2-dicarboxylate-mono-(7- carboxylate-4- methyl)heptyl ester (cx-MINCH). A detailed description of parent compounds and metabolites in this study can be found in Supplementary Table S1. The number of metabolites measured ranged from 10 to 14 for children and 12–14 for adolescents across countries (Supplementary Table S2 and S3, respectively). The European HBM dashboard (https://hbm.vito.be/eu-hbm-dashboard) makes the summary statistics for the exposure data publicly available.

Table 1 is a summary of exposure and percent of samples above the limit of detection (LOD) for the total population of children and adolescents. Of the compounds, ΣDiDP had the fewest measures above the limit of detection: 89.7 % of child and 82.7 % of adolescent concentrations were above the LOD (Table 1, Supplementary Table S2 and S3). Values below LOD were imputed for each biomarker in each data collection by single random imputation from a truncated lognormal distribution. The molar sum of the metabolites was calculated by dividing each metabolite concentration by its molecular weight and then summing them. We used the molar sums to represent exposure to the parent compound in single and multi-pollutant models since it is the parent compound (pollutant) that can be regulated and not the individual metabolites. Since not all studies had all metabolites measured, multi-pollutant analyses were performed in a subset of studies/individuals with all exposures available (n = 9 studies, 1873 children; n = 7 studies, 1583 adolescents, see Supplementary Table S2 and S2). To retain as many studies as possible, and because cx-MiDP and cx-MINCH were not available in some cohorts (two in the children's study: NEB II and POLAES, and three in the adolescents' study: ESTEBAN, Riksmaten, and POLAES), OH-MiDP and OH-MINCH were used as biomarkers for DIDP and for DINCH, respectively, instead of the molar sum of ΣDiDP and ΣDINCH metabolites.

Table 1
Phthalates (DINCH) concentrations (μg/L) in urine from children in 12 HBM4EU Aligned Studies (N=2876, 6–11 years, 2014–2021) and adolescents in 10 HBM4EU-Aligned Studies (N=2499, 12–18 years, 2014–2021).

Exposure	Total children (N=2876)			Total adolescents (N=2499)		
	N	>LOD (%)	Median (IQR)	N	>LOD (%)	Median (IQR)
MEP	2876	99.7	23.8 (10.8–55.8)	2499	100.0	37.5 (18.3–88.4)
MiBP	2575	100.0	28.8 (15.7–53.1)	1918	99.8	25.3 (15.3–46.0)
MnBP	2875	99.9	23.7 (13.5–43.7)	2199	99.9	23.5 (13.2–44.1)
MBzP	2875	97.5	3.4 (1.4–7.6)	2499	97.1	2.9 (1.3–6.8)
ΣDEHP	2574	91.1	37.6 (21.1–67.0)	2498	93.3	30.5 (18.3–50.7)
ΣDiNP	2280	97.6	9.6 (5.0–17.9)	2499	99.8	11.0 (6.0–20.5)
ΣDiDP	1819	89.7	1.8 (1.0–3.2)	1881	82.7	1.9 (1.2–3.2)
ΣDINCH	2575	96.2	3.4 (1.9–6.7)	2317	97.8	2.4 (1.2–4.7)

Abbreviations: HBM4EU Human Biomonitoring for Europe, LOD limit of detection, IQR interquartile range, MEP mono-ethyl phthalate, MiBP mono-*iso*-butyl phthalate, MnBP mono-n-butyl phthalate, MBzP mono-benzyl phthalate, ΣDEHP sum of di(2-ethylhexyl) phthalate metabolites, ΣDiNP sum of diisononyl phthalate metabolites, ΣDiDP sum of diisodecyl phthalate, ΣDINCH sum of di (isononyl) cyclohexane-1,2-dicarboxylate metabolites. Full description of parent compounds and metabolites in this study can be found in Supplementary Table S1. Exposure concentrations per Aligned Study can be found in Supplementary Tables S2 (children) and S3 (adolescents).

2.3. Outcome

The outcome BMI (kg/m^2) derived from weight and height was available as a continuous variable for all the studies included while waist circumference (used in a sensitivity analysis) was available only for three children's and three adolescents' studies (children: 3xG, NEBII, PCB Cohort; adolescent: FLEHS IV, NEBII, PCB Cohort). Anthropometric measures were self-reported (four children's studies: CROME, POLAES, NEB II, and SPECIMEn-NL; four adolescents' studies: CROME, POLAES, BEA and NEBII) or measured by a study nurse or physician (seven children's studies: ESTEBAN, NAC II, InAirQ, OCC, PCB cohort, GerES V-sub and 3xG; six adolescents' studies: ESTEBAN, Riksmaten, CRP, PCB cohort, GerES V-sub, FLEHS IV). Age- and sex-specific z-scores for BMI and waist circumference were calculated using the WHO (World Health Organization, 2007) using the 'zanthro' package in STATA (Vidmar et al., 2004). Overweight and obesity were defined based on International Obesity Task Force (IOTF) age and sex specific cut off values for BMI (Cole et al., 2000).

2.4. Covariates

The covariates for this study were collected using self-reported questionnaires from the Aligned Studies, and post-harmonised according to the codebook for the HBM4EU-Aligned Studies that can be found on Zenodo (Govarts, 2022a, Govarts, 2022b). The harmonized data of each study were uploaded to the Personal Exposure and Health Data Platform (PEH) (https://hbm.vito.be/peh-data-platform). Directed acyclic graphs (DAGs) were used prior to analysis to select a minimal sufficient adjustment set of confounders to allow unbiased effect estimations for the associations between the exposure biomarker phthalates/DINCH and the outcome BMI z-score (Figure S1) (Textor et al., 2011). The covariates identified for this study were age (years), sex (male/female), highest education level of the household (ISCED scale: low/medium, 0–4 vs medium/high education, ≥5), breastfeeding (weeks), birthweight (grams), frequency of food eaten from plastic packaging (≤1/week, > 1/week), maternal smoking during pregnancy (yes/no) (Supplementary Figure S1). Only the first three covariates (age, sex, highest education level) were available for all studies and thus the main analyses, while the others were included in sensitivity analyses described below.

2.5. Statistics

The correlations between the 14 phthalate/DINCH metabolites were tested using Spearman's rank coefficient. There were missing in the following potential covariates across all studies: education (0.3–11.2 %), maternal smoking (0.3–14.4 %), breastfeeding (0.7–56.5 %), and food from plastic packaging (0.3–2.7 %). Studies that did not include a particular covariate were excluded from the calculation of missing data percentages, as well as from imputation and analyses involving that covariate. We used multiple imputation with predictive mean matching (White et al., 2011) to impute these missing covariate data for each Aligned Study separately creating 20 multiply imputed datasets.

To study the cross-sectional associations between creatinine-standardized urinary concentrations of phthalate/DINCH metabolites and BMI z-score, we used multilevel generalized linear models adjusting for age, sex, and household education status, with random intercept for Aligned Study. Potential effect modification for sex was assessed in single pollutant models including main effects and cross-product terms, with a Wald test p-value of < 0.1 suggestive of an interaction. In the studies with available data, we investigated the sensitivity of our results by individually testing the effect of further adjusting for the specific covariates that were not provided by all Aligned Studies (birthweight, breastfeeding, maternal smoking during pregnancy, and consumption of food from plastic packaging). We also tested sensitivity to using adjustment for specific gravity instead of creatinine in the adolescent population who had these measurements. Random-effects *meta*-analysis was used to test for heterogeneity between the Aligned Studies for selected phthalate/DINCH metabolites.

For our multi-pollutant analyses, we used Bayesian kernel machine regression (BKMR) to assess the joint effect of the exposures on BMI z-score. In BKMR, the health outcome is regressed on a flexible kernel function of the mixture components, allowing for non-linearity and interactions (Bobb et al., 2014). We assessed (i) the posterior inclusion probability (PIP) (from 0 to 1) that a chemical is included in the model with PIP>0.60–0.80 considered possible predictors and PIP>0.80 the most robust predictors, (ii) the shape and direction of the exposure–response association of each exposure in relation to BMI z-score when holding the other exposures at their median concentrations, (iii) two-way exposure interactions while holding the other exposures at their median values, and (iv) overall mixture effects at different exposure percentiles. BKMR was fitted with the R package "bkmr" using the default settings and Markov chain Monte Carlo algorithm with 50,000 iterations (Bobb et al., 2014). We also estimated the joint effect of phthalate/DINCH metabolite concentrations on BMI z-score using quantile-based g-computation (hereafter, quantile g-computation) using qgcomp package in R (Keil et al., 2020).

For all analyses, exposures were natural logarithm (ln) transformed and scaled to their interquartile range (IQR). The multiply imputed dataset was used for the single pollutant analyses, while only the first imputed dataset was used in BKMR and g-computation models. Stata (version17; Stata Corp LP, College Station, Texas) was used for all statistical analyses except for BKMR and quantile g-computation where we used R version 4.2.2 (R Core Team, 2022).

3. Results

Table 2.1 and 2.2 describe the study participants for the children's (n = 12 studies, 6–11 years) and adolescents' (n = 10, 12–18 years) studies across Europe, respectively. The overall prevalence of overweight/obesity was 18.7 % (n = 539) for children and 20.8 % (n = 520) for adolescents. The highest prevalence of overweight/obesity was reported in Slovakia for both children (35.1 %) and adolescents (26.8 %), while the lowest was reported in the Netherlands (4.5 %) for children, and Norway (8.8 %) for adolescents. The highest and lowest proportions of households with high educational levels were reported in Norway and Slovakia, respectively, for both children and adolescents. The number of study participants ranged from 89 to 304 in the different aligned studies and the percentage of females ranged from 44.8-57.5 %.

3.1. Concentrations/correlation

Although the distribution of the metabolites varied across studies, the highest median (IQR) raw concentration (μg/L) of phthalate metabolites in the children's study was observed for MiBP [28.8 (15.7–53.1)] followed by MEP [23.8 (10.8–55.8)], and vice versa for adolescents (MEP, 37.5 (18.3–88.4); MiBP, 25.3 (15.3–46.0). ΣDiDP [children: 1.8 (1.0–3.2); adolescents: 1.9 (1.2–3.2)] and DINCH [children: 3.4 (1.9–6.7); adolescents: 2.4 (1.2–4.7)] had the lowest median concentrations (Supplementary Table S2 and S3). In general, there were low ($0.25 \leq rp < 0.50$) to moderate ($0.5 \leq rp < 0.75$) correlations among phthalate metabolites, and weak or no ($-0.25 \leq rp < 0.25$) correlations between other phthalate and DINCH metabolites (Figure S2).

3.2. Associations between urinary concentrations of phthalates/DINCH and BMI

3.2.1. Single pollutant models

In single pollutant models, there were few consistent associations between urinary concentrations of phthalates/DINCH and BMI z-score in children, although there was a tendency for the molar sum of DINCH

Table 2.1
Child characteristics (n (%) or median (IQR) in 12 HBM4EU Aligned Studies (N=2876, 6–12 years, 2014–2021).

Region	**Europe**	**North**		**South**			**West**				**East**		
Country	12 studies	NO (Norway)	DK (Denmark)	IT (ITALY)	SI (Slovenia)	GR (Greece)	FR (France)	DE (Germany)	BE (Belgium)	NL Netherland	PL (Poland)	HU (Hungary)	SK (Slovakia)
Study (Year)	HBM4EU, 2014–2021	NEB II, 2016–2017	OCC, 2018–2019	NAC II, 2014–2016	SLO CRP, 2018	CROME, 2020–2021	ESTEBAN, 2014–2016	GerES V-sub, 2015–2017	3xG, 2019–2020	SPECIMEn, 2020	POLAES, 2017	InAirQ, 2017–2018	PCB cohort, 2014–2017
N	N=2,876	N=300	N=300	N=300	N=149	N=161	N=286	N=300	N=133	N=89	N=300	N=262	N=296
Age (years)[1]	9 (7–10)	10 (9–11)	7 (7–7)	7 (7–7)	9 (8–10)	8 (7–10)	9 (7–10)	9 (7–10)	7 (7–7)	9 (7–10)	9 (7.5–10)	9 (9–10)	11 (11–11)
Sex													
Male	1,435 (49.9 %)	160 (53.3 %)	165 (55.0 %)	150 (50.0 %)	67 (45.0 %)	78 (48.4 %)	145 (50.7 %)	150 (50.0 %)	66 (49.6 %)	41 (46.1 %)	150 (50.0 %)	131 (50.0 %)	132 (44.6 %)
Female	1,438 (50.0 %)	140 (46.7 %)	135 (45.0 %)	150 (50.0 %)	82 (55.0 %)	83 (51.6 %)	141 (49.3 %)	150 (50.0 %)	67 (50.4 %)	45 (50.6 %)	150 (50.0 %)	131 (50.0 %)	164 (55.4 %)
Missing	3 (0.1 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	−0 (0 %)	0 (0 %)	0 (0 %)	3 (3.4 %)	0 (0 %)	0 (0 %)	0 (0 %)
ISCED HH													
Low/medium	1,127 (39.2 %)	14 (4.7 %)	195 (65.0 %)	165 (55.0 %)	49 (32.9 %)	22 (13.7 %)	121 (42.3 %)	131 (43.7 %)	20 (15.0 %)	0 (0.0 %)	79 (26.3 %)	95 (36.3 %)	236 (79.7 %)
High	1,678 (58.3 %)	273 (91.0 %)	105 (35.0 %)	132 (44.0 %)	100 (67.1 %)	135 (83.9 %)	164 (57.3 %)	169 (56.3 %)	111 (83.5 %)	79 (88.8 %)	221 (73.7 %)	145 (55.3 %)	44 (14.9 %)
Missing	71 (2.5 %)	13 (4.3 %)	0 (0 %)	3 (1.0 %)	0 (0 %)	4 (2.5 %)	1 (0.3 %)	0 (0 %)	2 (1.5 %)	10 (11.2 %)	0 (0 %)	22 (8.4 %)	16 (5.4 %)
Birthweight (gram)[1]	3440 (3130–3790)	3708 (3365–4040)	–	3380 (3100–3715)	–	–	–	3375 (3020–3750)	3420 (3170–3730)	–	–	3400 (3100–3720)	3350 (3010–3670)
BMI z-sscore[1]	0.24 (−0.53–1.10)	0.26 (−0.49–0.86)	−0.14 (−0.69–0.47)	0.60 (−0.06–1.41)	0.22 (−0.57–1.16)	0.58 (−0.33–1.51)	0.02 (−0.72–0.80)	0.12 (−0.60–0.95)	0.09 (−0.58–0.64)	−0.07 (−1.21–0.50)	0.33 (−0.44–1.41)	0.24 (−0.68–1.28)	0.81 (−0.09–1.89)
WSA z-score[1]	1.12 (0.45–2.01)	0.89 (0.25–1.53)	–	–	–	–	–	–	0.76 (0.31–1.53)	–	–	–	1.65 (0.82–2.58)
BMI categorized													
Underweight	297 (10.3 %)	12 (4.0 %)	41 (13.7 %)	12 (4.0 %)	21 (14.1 %)	16 (9.9 %)	40 (14.0 %)	33 (11.0 %)	13 (9.8 %)	20 (22.5 %)	34 (11.3 %)	39 (14.9 %)	16 (5.4 %)
Normal weight	1,916 (66.6 %)	195 (65.0 %)	224 (74.7 %)	188 (62.7 %)	97 (65.1 %)	98 (60.9 %)	211 (73.8 %)	219 (73.0 %)	108 (81.2 %)	47 (52.8 %)	194 (64.7 %)	161 (61.5 %)	174 (58.8 %)
Overweight/ Obese	539 (18.7 %)	25 (8.3 %)	29 (9.7 %)	75 (25.0 %)	31 (20.8 %)	47 (29.2 %)	35 (12.2 %)	48 (16.0 %)	12 (9.0 %)	4 (4.5 %)	72 (24.0 %)	57 (21.8 %)	104 (35.1 %)
Missing	124 (4.3 %)	68 (22.7 %)	6 (2.0 %)	25 (8.3 %)	–	–	–	–	–	18 (20.2 %)	–	5 (1.9 %)	2 (0.7 %)
Breastfeeding[1] **(weeks)**	32 (14–52)	44 (32–56)	34 (16–47)	49 (27–72)	–	33 (15–52)	–	24 (12–40)	15 (4–24)	–	–	25.7 (17.1–42.9)	20 (8–52)
Maternal Smoking													
No	1,447 (90.9 %)	285 (95.0 %)	–	268 (89.3 %)	–	–	–	272 (90.7 %)	127 (95.5 %)	–	–	232 (88.5 %)	263 (88.9 %)
Yes	111 (6.9 %)	7 (2.3 %)	–	32 (10.7 %)	–	–	–	23 (7.7 %)	6 (4.5 %)	–	–	10 (3.8 %)	33 (11.1 %)
Missing	33 (2.1 %)	8 (2.7 %)	–	0 (0 %)	–	–	–	5 (1.7 %)	0 (0 %)	–	–	20 (7.6 %)	0 (0 %)

[1]Median (Interquartile range) is presented for continuous variables (), and percentage n (%) for the categorical measures. ISCED: International Standard Classification of Education, BMI: Body mass index, and it is categorized based on International Obesity Task Force (IOTF) body mass index cut-offs.

Table 2.2
Adolescents' characteristics (n (%) or median (IQR) in 10 HBM4EU Aligned Studies (N=2499, 12–18 years, 2014–2021).

Region	**Europe**	**North**		**South**			**West**			**East**	
Country	**10 studies**	SE (Sweden)	NO (Norway)	GR (Greece)	ES (Spain)	Sl (Slovenia)	DE (Germany)	BE (Belgium)	FR (France)	PL (Poland)	SK (Slovakia)
Study (Year)	*HBM4EU, 2014–2021*	*Riksmaten Ung, 2016–2017*	*NEB II, 2016–2017*	*CROME, 2020–2021*	*BEA, 2017–2018*	*SLO CRP, 2018*	*GerES V-sub, 2015–2017*	*FLEHS IV, 2017–2018*	*ESTEBAN, 2014–2016*	*POLAES, 2017*	*PCB cohort follow-up, 2019–2020*
N	N=2,499	N=300	N=181	N=150	N=300	N=96	N=300	N=300	N=304	N=281	N=287
Age (years)[1]	14 (13–15)	14 (14–17)	12 (12–13)	14 (12–15)	15 (14–15)	14 (13–14)	14 (13–16)	14 (14–15)	14 (13–16)	13 (12–14)	16 (15–16)
Sex											
Male	1,219 (48.8 %)	150 (50.0 %)	77 (42.5 %)	75 (50.0 %)	144 (48.0 %)	53 (55.2 %)	150 (50.0 %)	150 (50.0 %)	144 (47.4 %)	151 (53.7 %)	125 (43.6 %)
Female	1,280 (51.2 %)	150 (50.0 %)	104 (57.5 %)	75 (50.0 %)	156 (52.0 %)	43 (44.8 %)	150 (50.0 %)	150 (50.0 %)	160 (52.6 %)	130 (46.3 %)	162 (56.4 %)
Missing	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
ISCED HH											
Low/medium	1,059 (42.4 %)	126 (42.0 %)	11 (6.1 %)	35 (23.3 %)	135 (45.0 %)	55 (57.3 %)	130 (43.3 %)	116 (38.7 %)	150 (49.3 %)	69 (24.6 %)	232 (80.8 %)
High	1,395 (55.8 %)	174 (58.0 %)	155 (85.6 %)	110 (73.3 %)	155 (51.7 %)	41 (42.7 %)	170 (56.7 %)	184 (61.3 %)	154 (50.7 %)	212 (75.4 %)	40 (13.9 %)
Missing	45 (1.8 %)	0 (0 %)	15 (8.3 %)	5 (3.3 %)	10 (3.3 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	15 (5.2 %)
Birthweight[1] (gram)	3460 (3100–3780)	–	3626 (3315–3939)	–	–	–	3430 (3100–3750)	3410 (3030–3780)	–	–	3380 (3000–3700)
BMI z-sscore[1]	0.26 (−0.46–1.04)	0.40 (−0.26–1.01)	0.08 (−0.48–0.64)	0.42 (−0.09–1.06)	0.32 (−0.38–1.03)	0.19 (−0.50–1.17)	0.12 (−0.52–0.94)	0.24 (−0.51–1.05)	−0.03 (−0.76–0.86)	0.45 (−0.46–1.28)	0.33 (−0.41–1.14)
WSA z-score[1]	1.05 (0.41–1.94)	–	0.90 (0.22–1.55)	–	–	–	–	1.04 (0.51–1.94)	–	–	1.21 (0.34–2.14)
BMI catagories											
Underweight	198 (7.9 %)	17 (5.7 %)	14 (7.7 %)	12 (8.0 %)	21 (7.0 %)	3 (3.1 %)	24 (8.0 %)	18 (6.0 %)	38 (12.5 %)	24 (8.5 %)	27 (9.4 %)
Normal weight	1,730 (69.2 %)	219 (73.0 %)	119 (65.7 %)	106 (70.7 %)	199 (66.3 %)	69 (71.9 %)	222 (74.0 %)	217 (72.3 %)	210 (69.1 %)	187 (66.5 %)	182 (63.4 %)
Overweight/Obese	520 (20.8 %)	64 (21.3 %)	16 (8.8 %)	32 (21.3 %)	63 (21.0 %)	24 (25.0 %)	54 (18.0 %)	65 (21.7 %)	55 (18.1 %)	70 (24.9 %)	77 (26.8 %)
Missing	51 (2.0 %)	0 (0 %)	32 (17.7 %)	0 (0 %)	17 (5.7 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (0.3 %)	0 (0 %)	1 (0.3 %)
Breastfeeding (weeks)	20 (8–38)	20 (20–38)	44 (32–56)	24 (8–36)	–	–	32 (12–44)	6 (0–20)	–	–	16 (8–40)
Maternal Smoking											
No	915 (85.1 %)	–	144 (79.6 %)	–	–	–	270 (90.0 %)	256 (85.3 %)	–	–	245 (85.4 %)
Yes	120 (11.2 %)	–	11 (6.1 %)	–	–	–	29 (9.7 %)	41 (13.7 %)	–	–	39 (13.6 %)
Missing	33 (3.1 %)	–	26 (14.4 %)	–	–	–	1 (0.3 %)	3 (1.0 %)	–	–	3 (1.0 %)
Plastic food consumption											
<= 1 time/week	766 (67.3 %)	–	–	137 (91.3 %)	174 (58.0 %)	25 (26.0 %)	–	–	144 (47.4 %)	–	286 (99.7 %)
> 1 time/week	353 (31.0 %)	–	–	13 (8.7 %)	118 (39.3 %)	71 (74.0 %)	–	–	151 (49.7 %)	–	0 (0.0 %)
Missing	18 (1.6 %)	–	–	0 (0 %)	8 (2.7 %)	0 (0 %)	–	–	9 (3.0 %)	–	1 (0.3 %)

Median (Interquartile range) is presented for continuous variables (), and percentage n (%) for the categorical measures. ISCED: International Standard Classification of Education, BMI: Body mass index, and it is categorized based on International Obesity Task Force (IOTF) body mass index cut-offs.

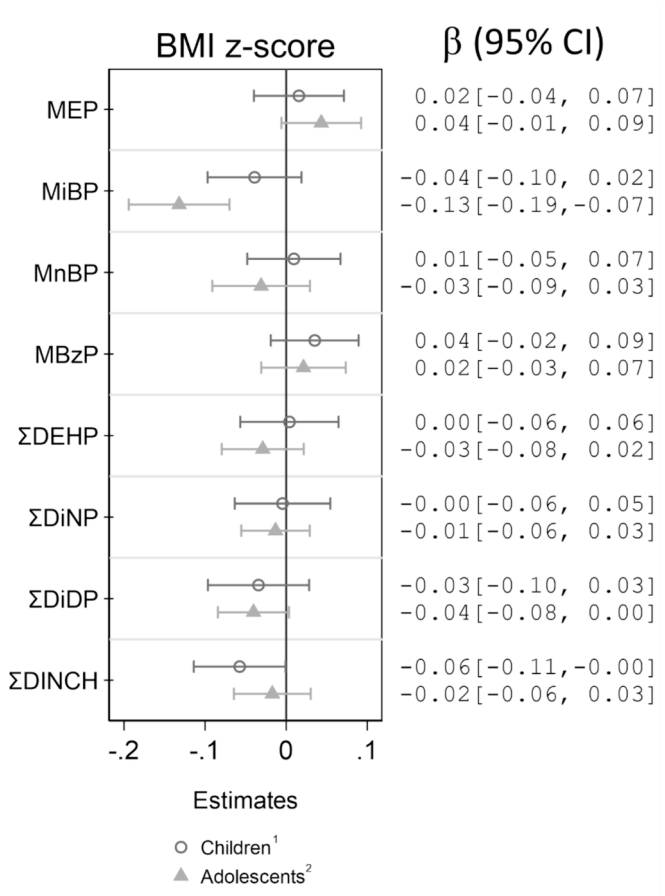

Fig. 1. Association between creatinine-adjusted urinary concentrations of phthalate and DINCH metabolites and body mass index (BMI) z-score in 22 HBM4EU Aligned Studies (12 studies n = 2,876 children, 6–11 years, 10 studies n = 2,499 adolescents, 12–18 years, 2014–2021). For the children's study, 12 studies had measured MEP (n = 2876), MiBP (n = 2575, except POLAES-PL), MnBP (n = 2875), MBzp (n = 2875), ΣDEHP (n = 2574, except NAC II, Italy), ΣDiNP (n = 2280, except NAC II & PCB cohort), ΣDiDP (n = 1819, except NAC II, NEB II, PCB cohort, CROME), and ΣDINCH (n = 2575, except NEB II).[2]For the adolescents' study, 10 studies had measured MEP (n = 2499), MBzP (n = 2499), MnBP (n = 2199, except Riksmaten-SE), MiBP (n = 1918, except POLAES-PL, PCB cohort-SK, Riksmaten-SE), ΣDiDP (n = 1881, NEBII-NO, PCB cohort-SK, CROME-EL), and ΣDINCH (n = 2317, except GerSV-DE). The Σ refers to the molar sum of the metabolites as shown in Supplemental Table S1. The total number of metabolites and the percentage above the limit of detection/quantification for each chemical across all cohorts are provided in the Supplementary Table S2 (Children study) and Table S3(Adolescent study). Models adjusted for age (years), gender, and household educational status, and fitted with a random intercept for aligned study. Exposures were natural log (ln)-transformed and scaled to their interquartile range. For each exposure, the first estimate (circle) is for children while the second estimate (triangle) is for adolescents. Abbreviations: HBM4EU Human Biomonitoring for Europe, BMI body mass index, CI confidence interval, MEP mono-ethyl phthalate, MiBP mono-*iso*-butyl phthalate, MnBP mono-n-butyl phthalate, MBzP mono-benzyl phthalate, ΣDEHP molar sum of di(2-ethylhexyl) phthalate metabolites (MEHP+5OH-MEHP+5oxo-MEHP+5cx-MEPP), ΣDiNP molar sum of diisononyl phthalate metabolites (OH-MiNP+cx-MiNP), ΣDiDP molar sum of diisodecyl phthalate (OH-MiDP+cx-MiDP), ΣDINCH molar sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites (OH-MINCH+cx-MINCH) (SupplementaryTable S1).

Table 3
Sex-stratified associations between creatinine-adjusted urinary concentrations of phthalate and DINCH metabolites and body mass index (BMI) z-score in 22 HBM4EU Aligned Studies (12 studies n = 2,876 children, 6–11 years, 10 studies: n = 2,499 adolescents, 12–18 years, 2014–2021).

	Children's studies BMI z-score								p-int sex	Adolescents' studies BMI z-score							p-int sex
	Total			Males		Females				Total			Males		Females		
	N	β	(95 % CI)	β	(95 % CI)	β	(95 % CI)			N	β	(95 % CI)	β	(95 % CI)	β	(95 % CI)	
MEP	2876	0.02	(−0.04, 0.07)	0.01	(−0.07, 0.08)	0.03	(−0.04, 0.1)	0.74		2 499	0.04	(−0.001, 0.09)	**0.08**	**(0.001, 0.15)**	0.01	(−0.05, 0.07)	**0.06**
MiBP	2575	−0.04	(−0.10, 0.02)	0.003	(−0.08, 0.08)	−0.06	(−0.14, 0.02)	0.13		1 918	**−0.13**	**(−0.19, −0.07)**	**−0.12**	**(−0.21, −0.02)**	**−0.14**	**(−0.21, −0.07)**	0.76
MnBP	2875	−0.001	(−0.05, 0.07)	0.05	(−0.03, 0.13)	−0.06	(−0.13, 0.02)	0.11		2 199	−0.03	(−0.09, 0.03)	−0.04	(−0.13, 0.05)	−0.03	(−0.11–0.05)	0.8
MBzP	2875	0.04	(−0.02, 0.09)	0.06	(−0.01, 0.14)	−0.02	(−0.09, 0.05)	0.60		2 499	0.02	(−0.03, 0.07)	0.004	(−0.07, 0.08)	0.03	(−0.04, 0.09)	**0.09**
ΣDEHP	2574	0.001	(−0.06, 0.06)	0.0004	(−0.07, 0.08)	0.01	(−0.06, 0.09)	0.45		2 498	−0.03	(−0.08, 0.02)	0.01	(−0.07, 0.08)	**−0.08**	**(−0.14, −0.02)**	**0.01**
ΣDiNP	2280	−0.01	(−0.06, 0.05)	−0.01	(−0.09, 0.07)	−0.01	(−0.08, 0.06)	0.35		2 499	−0.01	(−0.06, 0.03)	0.01	(−0.06, 0.07)	−0.03	(−0.08, 0.02)	0.28
ΣDiDP	1819	−0.03	(−0.10, 0.03)	−0.04	(−0.13, 0.05)	−0.03	(−0.10, 0.05)	0.72		1 881	−0.04	(−0.08, 0.002)	−0.02	(−0.09, 0.04)	**−0.07**	**(−0.12, −0.02)**	0.16
ΣDINCH	2575	−0.06	(−0.11, 0.001)	−0.04	(−0.12, 0.04)	−0.05	(−0.12, 0.03)	0.19		2 317	−0.02	(−0.06, 0.02)	−0.01	(−0.09, 0.06)	−0.03	(−0.09, 0.02)	0.59

Models adjusted for age (years), gender, and household educational status, and fitted with a random intercept for aligned study. Exposures were natural log (ln)-transformed and scaled to their interquartile range. Details of the chemical measurement for each study/cohort is provided in the supplementary Table S2 and Table S3.

For the children's study, 12 studies had measured MEP, MiBP (except POLAES-PL), MnBP, MBzp, ΣDEHP (except NAC II, Italy), ΣDiNP (except NAC II & PCB cohort), ΣDiDP (except NAC II, NEB II, PCB cohort, CROME), and ΣDINCH (except NEB II).[2]For the adolescents' study, 10 studies had measured MEP, MBzP, MnBP (except Riksmaten-SE), MiBP (except POLAES-PL, PCB cohort-SK, Riksmaten-SE), ΣDiDP (NEBII-NO, PCB cohort-SK, CROME-EL), and ΣDINCH (except GerSV-DE). The Σ refers to the molar sum of the metabolites as shown in Supplemental Table S1.

The total number of metabolites and the percentage above the limit of detection/quantification for each chemical across all cohorts are provided in the Supplementary Table S2 (Children study) and Table S3 (Adolescent study).

Abbreviations: BMI body mass index, HBM4EU Human Biomonitoring for Europe, MEP mono-ethyl phthalate, MiBP mono-*iso*-butyl phthalate, MnBP mono-n-butyl phthalate, MBzP mono-benzyl phthalate, ΣDEHP sum of di(2-ethylhexyl) phthalate metabolites, ΣDiNP sum of diisononyl phthalate metabolites, OH-MiDP 6-OH-mono-propyl-heptyl phthalate, cx-MiDP mono (2,7-methyl-7- carboxy-heptyl) phthalate, ΣDiDP sum of diisodecyl phthalate, OH-MINCH cyclohexane-1,2- dicarboxylate-mono-(7- hydroxy-4-methyl) octyl ester, cx-MINCH cyclohexane-1,2- dicarboxylate-mono-(7- carboxylate-4- methyl) heptyl ester, ΣDINCH sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites, p-int sex p-value for interaction by sex. (Table 1.).

metabolites to be associated with lower BMI z-score (β = -0.06, 95 %CI: −0.11, −0.001 per IQR increase in ln concentrations) (Fig. 1). There was no evidence of effect modification by sex (*p*-interaction > 0.1 for all metabolites, Table 3). However, in adolescents, creatinine-standardized urinary concentrations of low molecular weight phthalate metabolites were associated with BMI z-scores: MEP concentrations were associated with higher BMI z-score (β = 0.04, 95 % CI: −0.01, 0.09), while MiBP was associated with lower BMI z-score (β = -0.13, 95 % CI: −0.19, −0.07) per IQR increase in ln concentration, as was the molar sum of DiDP metabolites (β = -0.04, 95 % CI: −0.08, 0.001) (Fig. 1). In this age group, there was evidence of effect modification by sex: for MEP, the association with higher BMI z-score was present in adolescent males (β = 0.08, 95 % CI: 0.001, 0.15, *p*-interaction = 0.06), while in females the molar sum of metabolites of the high molecular weight DEHP were negatively associated with BMI z-score (β = -0.08, 95 % CI: −0.14, −0.02, *p*-interaction = 0.01) (Table 3).

In the children's study heterogeneity was no/low (I^2 < 25 %) for MEP, MBzP, ΣDiNP and DINCH, moderate (I^2 ≥ 25 to < 50 %) for MiBP, MnBP and ΣDiDP and high (I^2 ≥ 50 %) for ΣDEHP. In the adolescents' study heterogeneity was no/low for MEP, MnBP, ΣDEHP, moderate for MBzP and DINCH, and high for ΣDiNP and ΣDiDP (Figure S3).

Sensitivity analysis by further adjustment for breastfeeding (Figure S4), birth weight and maternal smoking during pregnancy (Figure S5), and consumption of food from plastic packaging (Figure S6) did not materially alter the effect estimates. In analyses of studies where both BMI z-score and waist circumference z-scores outcomes were available for analysis, the results for BMI and waist circumference z-scores were similar in the adolescents' population; however, waist circumference had stronger negative associations for MnBP and ∑DEHP in the children's study for the three aligned studies with available data (Figure S7). In the adolescents' study, there was no material difference between results using specific gravity-adjusted and creatine-adjusted phthalate measurements (Figure S8).

3.2.2. *Multi-pollutant models*

3.2.2.1. Children. For the children's study, BKMR had low posterior inclusion probabilities for all metabolites (PIPs < 0.26) and there was no overall mixture effect (Figure S9). There was also no joint mixture effect using quantile g-computation (β = -0003, 95 % CI: −0.10, 0.09, per joint exposure quantile).

3.2.2.2. Adolescents. In the adolescents' study, BKMR predicted higher BMI z-scores in males with increasing MEP (PIP=0.90) and MBzP (PIP=0.84) when other metabolites were fixed at their median (Fig. 2A and B, Males). For both sexes combined, lower BMI z-scores were predicted for MiBP (PIP=0.999 total, PIP=0.91 males, PIP=84 females), OH-MiDP (PIP=0.88 total, PIP=0.71, males, PIP=0.74 females), and to a lesser extent OH-MINCH (PIP=0.72 total, PIP=0.63 males, PIP=0.55 females) (Fig. 2A and B, Total, Males and Females). While there was evidence of lower BMI z-score in females with DEHP in the single pollutant model, this was not robustly selected in the multipollutant BKMR model (PIP=0.61). In the BKMR analyses, there was evidence of non-linearity only in the males for MBzP (Fig. 2A, Males). There was no overall mixture effect for adolescent males and suggestion of an overall negative association for females, although the credible intervals crossed 0 (Fig. 2C, Males and Females). There were limited interaction effects only for the males between MiBP and OH-MiDP, and DiNP and MBzP (Figure S10). In the quantile g-computation analysis the joint effect was null for adolescent males (β −0.002, 95 % CI: −0.17, 0.16 per joint exposure quantile), and suggested negative for females (β = -0.11, 95 % CI −0.25, 0.03, per joint exposure quantile) (Figure S11).

4. Discussion

In this large cross-sectional study of children and adolescents from all regions in Europe, some age- and sex-specific associations between phthalate exposure and BMI z-score were observed. We found little evidence of clear associations between urinary concentrations of phthalate/DINCH metabolites exposure and BMI z-score in children. However, in adolescents, the most robust associations were between MEP and MBzP and higher BMI z-score in males, and between MiBP and lower BMI z-score in both sexes. In this age group there was less robust support for negative associations with DEHP in females and DINCH metabolite OH-MINCH and DiDP metabolite OH-MiDP in both sexes. There appeared to be a negative overall mixture effect from phthalate/DINCH metabolites in females only.

Urinary concentrations of MEP were associated with higher BMI z-score in adolescent males. This was evident in the single pollutant model and the association remained in the multipollutant BKMR analysis when other metabolites were fixed at their median (i.e., adjusting for them). By contrast, there was no clear association in the children in our study. Some previous studies have found a positive association between MEP and BMI in childhood. Among participants aged 6–19 years in the National Health and Nutrition Examination Survey (NHANES, 2005–2010), MEP was cross-sectionally positively associated with BMI z-score applying three different statistical models, but they did not test for sex differences (Wu et al., 2020). A *meta*-analysis of the cross-sectional association between phthalates exposure and adiposity (BMI and waist circumference) reported a positive but non-significant association for MEP in both children and adolescents, also not stratified by sex (Ribeiro et al., 2019). However, some cross-sectional studies have investigated the sex-specific effects of MEP on BMI or overweight/obesity with similar findings to this study. In China, the sum of low molecular-weight phthalate metabolites (MBP, MMP and MEP) was positively associated with boys' obesity (Zhang et al., 2014). Analysis from NHANES (2007–2010) reported higher odds of obesity in male children and adolescents (Buser et al., 2014). In contrast, metabolites of low molecular weight phthalates (MEP, MBP, and MiBP) were positively associated with BMI in a study of girls aged 6 to 8 years enrolled in the U. S. Breast Cancer and Environment Research (Deierlein et al., 2016), as was MEP in adolescent girls and not in adolescent boys in an earlier analysis from NHANES (1999–2002) (Hatch et al., 2008). The concentrations in the U.S. studies are substantially higher than in our study limiting direct comparison. However, also contrary to our findings are multipollutant studies with phthalate concentrations similar to ours, such as the European study, HELIX, which did not find any associations between prenatal or childhood MEP or other phthalate exposures and obesity using deletion-substitution-addition (DSA) variable selection to assess 77 different exposures, nor evidence of interactions by sex (*p*-interaction > 0.10) (Vrijheid et al., 2020). A recent Spanish longitudinal cohort also reported no association between prenatal MEP and BMI z-score at 11 years of age nor sex-differences using BKMR (Güil-Oumrait et al., 2022).

High molecular weight metabolite MBzP had a positive but non-linear association with BMI z-scores in adolescents, more robust in males. This was clearer in the BKMR model because non-linearity was not accounted for in the single pollutant linear regression model. There also appeared to be an interaction with DiNP, highlighting the need to consider the co-occurring chemicals. Cross-sectional analyses from NHANES (1999–2002) reported that the most consistent findings were for males 20–59 years with higher BMI and waist circumference with increasing quartiles of MBzP (Hatch et al., 2008.). A small cross-sectional study from Iran with children aged 6–18 years, also reported a positive association with BMI z-score and waist circumference, however, this was not stratified by sex (Amin et al., 2018). A large cross-sectional study of children 3–17 years from the Korean National Environmental Health Survey (KoNEHS, 2015–2017), with concentrations similar to our study, reported a non-significant increased odds of obesity

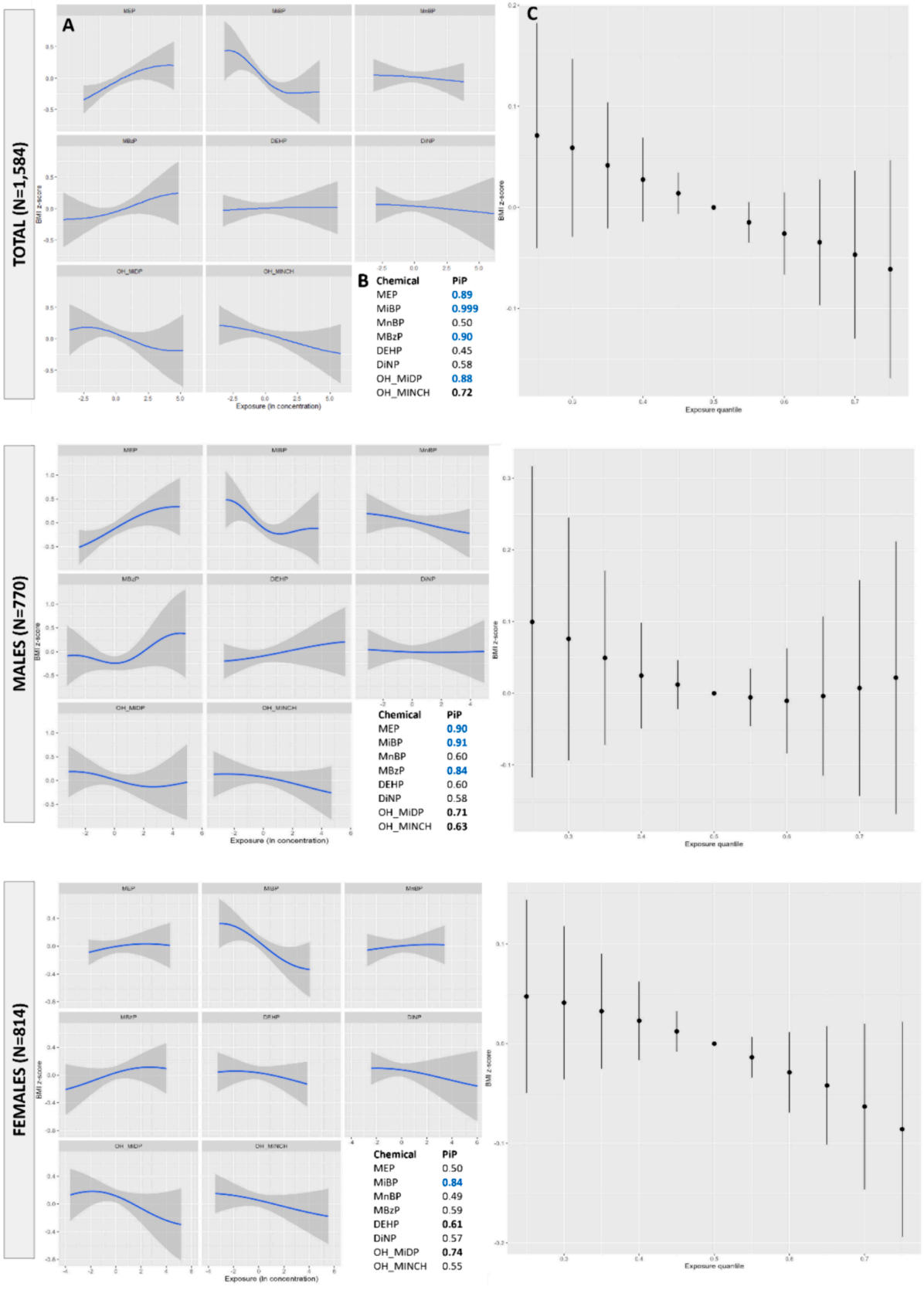

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Fig. 2. Summary estimates from BKMR on the association between mixtures of natural logarithm (ln) transformed creatinine-adjusted urinary concentrations of phthalate and DINCH metabolites and BMI z-score in adolescents (12–18 years) by Total population (n = 1584), Males (n = 770) and Females (n = 814) in 7 HBM4EU aligned studies (2014–2021). Models adjusted for household income, gender and child's age at outcome assessment, and fitted with a random intercept for aligned study. The results are shown in panels for Total, Males and Females. (A) Exposure-response associations for each chemical when the others are fixed at their median. (B) Posterior inclusion probabilities (PiP) of each chemical in the mixture-response function for BMI z-score. Highlighted black, PIP>0.60–0.80 possible predictors, highlighted blue PIP>0.80 robust predictors. (C) Joint effect of phthalates/DINCH mixture on BMI z-score at adolescence. Aligned Studies included: Esteban (France, n = 303), Chrome (Greece, n = 150), BEA (Spain, n = 287), SLO CRP (Slovenia, n = 96), NEB II (Norway, n = 149), GerEs V-sub (Germany, n = 299), FLEHS IV (Belgium, n = 300). The total number of metabolites and the percentage above the limit of detection/quantification for each chemical across all cohorts are provided in the Supplementary Table S2 (Children study) and Table S3 (Adolescent study). Abbreviations: BMI body mass index, HBM4EU Human Biomonitoring for Europe, MEP mono-ethyl phthalate, MiBP mono-*iso*-butyl phthalate, MnBP mono-n-butyl phthalate, MBzP mono-benzyl phthalate, DEHP sum of di (2-ethylhexyl) phthalate metabolites, DiNP sum of diisononyl phthalate metabolites, OH-MiDP 6-OH-mono-propyl-heptyl phthalate, OH-MINCH cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester (Supplementary Table S1). Note: In the figure, an underscore (_), is read as a hyphen (e.g., OH_MINP is OH-MINP).

at the highest quartile of exposure only(Seo et al., 2022). The potential non-linear exposure–response relation should be explored in future studies.

MiBP was associated with lower BMI z-score in adolescents of both sexes, in single and multipollutant analysis. There was also a suggestion of a negative association for children in the single pollutant model, but this was non-significant. A previous *meta*-analysis based on longitudinal data also reported negative associations between MiBP and BMI z-score in children aged 8–15 years (Ribeiro et al., 2019). Contrary to our finding, MiBP was found to be positively associated with obesity among participants aged 6–19 in the National Health and Nutrition Examination Survey (NHANES) 2005–2010 (Wu et al., 2020). As noted already, the high concentrations from the earlier U.S. studies make direct comparison difficult.

∑DiDP and ∑DINCH also had a tendency for a negative association with BMI z-score in the children's and adolescents' single-pollutant models. However, this was only significant for ∑DiDP in adolescent females. Their metabolites OH-MiDP and OH-MINCH were identified by BKMR as possible predictors of lower BMI z-score only in the adolescent population. The urinary metabolite concentrations of both DiDP and DINCH were comparatively the lowest among the compounds measured in both children and adolescent populations, and DINCH metabolites were weakly (or not at all) correlated with the other phthalate metabolites. Human studies assessing DINCH metabolites and adiposity are still scarce, and there is a lack of *in vivo* studies assessing the obesogenic properties of DINCH (Langsch et al., 2018). However, given indications that DINCH metabolites can dysregulate metabolism to a greater extent than those from DEHP (Crobeddu et al., 2022), and the prevalence of DINCH as a replacement plasticizer (Vogel et al., 2023), our findings need corroboration in other studies.

∑DEHP metabolites were associated with lower BMI z-score in girls in the single pollutant model. However, this was not robustly supported in the multipollutant BKMR model. Previous studies have also found similar negative associations in girls in single pollutant models. For example, in China, the sum of DEHP metabolites was negatively associated with girls' obesity (Zhang et al., 2014). A U.S. study using cross-sectional data from the National Health and Nutrition Examination Study (NHANES, 1999–2002) reported that MEHP was inversely associated with BMI in adolescent girls (Hatch et al., 2008), while a pooled analysis of cohort studies in the U.S. reported a negative association between prenatal DEHP (and MEP) and BMI z-scores only in girls and of similar magnitude to our study (Buckley et al., 2016). However, in KoNEHS (2015–2017), DEHP metabolite MECPP, was the only phthalate metabolite significantly associated with increased odds of obesity in children of both sexes (Seo et al., 2022). While many of the phthalate concentrations in KoNEHS were similar to our study, DEHP metabolites were substantially higher, which may contribute to the difference in our results.

4.1. Joint mixture effect of phthalates/DINCH

We found a suggested negative joint mixture effect in adolescent females, although this was non-significant. A recent study investigating prenatal chemical mixtures and risk of metabolic syndrome (which includes BMI z-score in the calculation) found that high-molecular weight phthalate mixtures (DEHP and DiNP metabolites and MBzP) were associated with lower MetS score in girls, while and low molecular weight phthalate mixtures (MEP, MiBP, MnBP) were associated with a lower MetS score in both girls and boys (Güil-Oumrait et al., 2024). By contrast, Spanish study found a trend for a positive joint effect of prenatal exposure to eight phthalate metabolites and six phenols on BMI z-scores in girls (Güil-Oumrait et al., 2022). For the adolescent males in our study, the combination of positive association from MEP and negative association from MiBP cancelled each other out resulting in a null association. This is contrary to previous cross-sectional studies, for example in NHANES where using Weighted Quantile Sum (WQS) Regression of seven xenobiotics (including MEP and bisphenols A and S), the WQS index was significantly associated with obesity in adults of both sexes (Zhang et al., 2019). There are few mixture studies of phthalates and BMI, and it is difficult to compare results from joint mixture analysis, due to differing exposure profiles (i.e., other populations may have different proportions of the phthalate/DINCH metabolites or may include metabolites or compounds from a different class), or methods that do not highlight the specific mixture effect of just phthalates.

4.2. Age- and sex-specific associations

MEP and ∑DEHP metabolites showed age and sex-specific association in this study, with MEP associated with higher BMI z-score in adolescent males, and DEHP lower z-score in adolescent females. Age and sex-specific differences in associations may be explained by non-biological factors such as varying levels of exposure sources (e.g., personal care products, diet) and behavioural factors (e.g., dietary habits, lifestyle differences). These factors can influence phthalate exposure profiles as well as anthropometry differently between sexes and across different age groups.

Some biologically plausible mechanisms might also explain the sex-specific associations observed between phthalates and BMI. One of the potential mechanisms is the sex-specific difference in hepatic peroxisome proliferator-activated receptors (PPARs) expression (Jalouli et al., 2003). PPARs have important roles in carbohydrate and lipid metabolism, and have been found to be affected by phthalate diesters or some of their metabolites such as MEHP (Desvergne et al., 2009). Another potential mechanism that could explain the sex differences is the anti-androgenic effects of phthalates. Exposure to DEHP metabolites was found to inhibit human testis steroidogenesis (testosterone production) (Desdoits-Lethimonier et al., 2012), and urinary levels associated with low testosterone levels in adult men (Meeker et al., 2009). Low testosterone levels in men, but higher androgen levels in females are associated with higher BMI or greater risk for overweight/obesity and metabolic syndrome (Wang et al., 2011, Barber et al., 2006). This may explain the inverse association between ∑DEHP and BMI z-score among female adolescents in our study.

We found no clear evidence for an association between phthalate metabolites and BMI z-score in the children in this study, and NHANES

(1999–2002) study data revealed a notable difference by age and gender with most of the associations among adult males (≥18 years) and no important associations among children for associations of urinary phthalate metabolite (MEP, MBzP, MEHHP, MEOHP) concentrations with BMI or waist circumference (Hatch et al., 2008). These age-specific findings may be due to an interaction with hormones as the adolescents approach or enter puberty, but we could not include information on puberty status in our study.

4.3. Strengths and limitations

The primary strength of our study is a large sample size and the pooling of several studies (n = 22), representing the general population across various regions of Europe. The study covers both children (n = 12 studies, 6–11 years of age) and adolescents (n = 10, studies 12–18 years of age) with an approximate 1:1 ratio of sexes allowing us to study both age and sex-specific associations of phthalates metabolites. The HBM4EU harmonised data set increased the power and pooling the data in this way controls better for unmeasured confounding since the underlying confounder structure varies across studies and reduces the risk of publication bias. In addition, the exposure assessment included urinary concentrations of many phthalate metabolites, including two alternative phthalate metabolites (DINCH). Moreover, we undertook multi-pollutant analysis to address potential multicollinearity and assess mixtures of phthalates/DINCH using two methods (BKMR and quantile g-computation), which gave consistent results.

There are some limitations to this study. First, the study design is cross-sectional which precludes making causal inference about the direction of detected associations. Thus, we cannot exclude reverse causality as individuals with higher BMI may use a greater quantity of phthalate-containing personal care products and consume higher energy/food contaminated with DEHP than those with lower BMI, potentially leading to higher absorption and subsequently increased excretion of phthalate metabolites in urine (Braun et al., 2013, Campbell et al., 2018). BMI was measured by study nurse in most of the studies; however, in four adolescents' and four children's studies, the outcome, BMI, was based on self-reported data, potentially leading to outcome misclassification and a negative bias if those with higher BMI (and corresponding higher phthalate concentrations) reported a lower BMI. Furthermore, the BMI measure has low sensitivity to distinguish between fat mass and lean mass or to accurately reflect variations in body fat distribution in children and adolescents, leading to misclassification in younger populations (Freedman et al., 2005) (Javed et al., 2015). To address these limitations, we conducted sensitivity analyses using waist circumference measurements, finding stronger associations in the children's study, suggesting this is a more sensitive outcome (Janssen et al., 2004). However, few studies had data on waist circumference precluding its use in the large, pooled analysis. Also, exposure misclassification cannot be ruled out because single-spot urine measurements only reflect recent exposure due to the relatively short half-life of phthalates, and not necessarily long-term exposure, which may explain the uncertainty in some of the results. However, the potential source of exposure for some phthalates such as personal care products may occur regularly, and therefore the concentration of these phthalate metabolites at one point in time is likely to be representative of regular exposure (Hoppin et al., 2002, Hauser et al., 2004, Teitelbaum et al., 2008). Furthermore, although outcome and exposure assessment were obtained during fieldwork by a study nurse in seven children's and six adolescents' studies, for the self-reported outcome assessment we cannot be certain of the timing. Despite the limitations of creatinine adjustment due to BMI and body composition influences (Bulka et al., 2017), our sensitivity analysis using specific gravity adjustments in the adolescent study revealed no material differences between creatinine-adjusted and specific gravity-adjusted phthalate measurements, confirming the robustness of our findings. Few confounders were measured across all studies, thus the estimates may have been confounded, although sensitivity analysis with the subset of the studies with additional variables available did not suggest confounding. There are also some differences in the age range included across individual studies, although this was accounted for in the analyses. We also cannot rule out potential confounding from unmeasured or unknown confounders affecting the association between phthalates exposure and BMI. Lastly, even though we used two multi-pollutant models, the potential for spurious associations due to multiple comparisons cannot be discounted.

5. Conclusion

We found age- and sex-specific differences of the association between urinary concentrations of phthalate/DINCH metabolites and BMI z-score. Phthalates/DINCH were associated both positively (MEP, MBzP) and negatively (MiBP) with BMI z-score in adolescents. Longitudinal studies that measure phthalate levels at various points throughout the day, across a diverse range of age groups, and in both sexes are warranted to make causal inference.

CRediT authorship contribution statement

Anteneh Desalegn: Writing – original draft, Writing review & editing, Formal analysis, Visualization. **Tessa Schillemans:** Methodology, Writing – review & editing. **Eleni Papadopoulou:** Writing – review & editing, Methodology, Resources. **Amrit K. Sakhi:** Writing – review & editing, Resources. **Line S. Haug:** Writing – review & editing, Resources. **Ida Henriette Caspersen:** Writing – review & editing, Methodology, Resources . **Andrea Rodriguez-Carrillo:** Writing – review & editing, Methodology. **Sylvie Remy:** Writing – review & editing, Resources, Methodology, Project administration, Conceptualization. **Greet Schoeters:** Writing – review & editing, Resources, Methodology. **Adrian Covaci:** Writing – review & editing, Resources. **Michelle Laeremans:** Writing – review & editing, Resources. **Mariana F Fernández:** Writing – review & editing, Resources. **Susana Pedraza-Diaz:** Writing – review & editing, Resources. **Tina Kold Jensen:** Writing – review & editing, Resources. **Hanne Frederiksen:** Writing – review & editing, Resources. **Agneta Åkesson:** Writing – review & editing, Resources, Methodology. **Bianca Cox:** Writing – review & editing, Methodology. **Shereen Cynthia D'Cruz:** Writing – review & editing, Resources. **Loïc Rambaud:** Writing – review & editing, Resources. **Margaux Riou:** Writing – review & editing, Resources. **Marike Kolossa-Gehring:** Writing – review & editing, Resources, Funding acquisition. **Antje Gerofke:** Writing – review & editing, Resources. **Aline Murawski:** Writing – review & editing, Resources. **Nina Vogel:** Writing – review & editing, Resources. **Catherine Gabriel:** Writing – review & editing, Resources. **Spyros Karakitsios:** Writing – review & editing, Resources. **Nafsika Papaioannou:** Writing – review & editing, Resources. **Dimosthenis Sarigiannis:** Writing – review & editing, Resources. **Fabio Barbone:** Writing – review & editing, Resources. **Valentina Rosolen:** Writing – review & editing, Resources. **Sanna Lignell:** Writing – review & editing, Resources. **Anna Karin Lindroos:** Writing – review & editing, Resources. **Janja Snoj Tratnik:** Writing – review & editing, Resources. **Anja Stajnko:** Writing – review & editing, Resources. **Tina Kosjek:** Writing – review & editing, Resources. **Žiga Tkalec:** Writing – review & editing, Resources. **Lucia Fabelova:** Writing – review & editing, Resources.**Lubica Palkovicova Murinova:** Writing – review & editing, Resources. **Branislav Kolena:** Writing – review & editing, Resources. **Sona Wimmerova:** Writing – review & editing, Resources. **Tamás Szigeti:** Writing – review & editing, Resources. **Szilvia Középesy:** Writing – review & editing, Resources. **Annick van den Brand:** Writing – review & editing, Resources. **Jan-Paul Zock:** Writing – review & editing, Resources. **Beata Janasik:** Writing – review & editing, Resources. **Wojciech Wasowicz:** Writing – review & editing, Resources. **Annelies De Decker:** Writing – review & editing, Resources. **Stefaan De Henauw:** Writing – review & editing, Resources. **Eva Govarts:** Writing – review & editing, Resources, Conceptualization, Data curation, Project

administration. **Nina Iszatt:** Writing – review & editing, Methodology, Formal analysis, Visualization, Supervision.

Declaration of competing interest

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

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

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References

Amin, M.M., Parastar, S., Ebrahimpour, K., Shoshtari-Yeganeh, B., Hashemi, M., Mansourian, M., Kelishadi, R., 2018. Association of urinary phthalate metabolites concentrations with body mass index and waist circumference. Environ. Sci. Pollut. Res. 25, 11143–11151.

Barbanel, C.S., Winkelman, J.W., Fischer, G.A., King, A.J., 2002. Confirmation of the Department of Transportation criteria for a substituted urine specimen. J. Occup. Environ. Med. 44, 407–416.

Barber, T.M., McCarthy, M.I., Wass, J.A.H., Franks, S., 2006. Obesity and polycystic ovary syndrome. Clin. Endocrinol. 65, 137–145.

Bilal, M., Iqbal, H.M.N., 2019. An insight into toxicity and human-health-related adverse consequences of cosmeceuticals - A review. Sci. Total Environ. 670, 555–568.

Bobb, J.F., Valeri, L., Claus Henn, B., Christiani, D.C., Wright, R.O., Mazumdar, M., Godleski, J.J., Coull, B.A., 2014. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16, 493–508.

Braun, J.M., 2017. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13, 161–173.

Braun, J.M., Sathyanarayana, S., Hauser, R., 2013. Phthalate exposure and children's health. Curr. Opin. Pediatr. 25, 247–254.

Buckley, J.P., Engel, S.M., Braun, J.M., Whyatt, R.M., Daniels, J.L., Mendez, M.A., Richardson, D.B., Xu, Y., Calafat, A.M., Wolff, M.S., Lanphear, B.P., Herring, A.H., Rundle, A.G., 2016. Prenatal phthalate exposures and body mass index among 4- to 7-year-old children: a pooled analysis. Epidemiology 27, 449–458.

Bulka, C.M., Mabila, S.L., Lash, J.P., Turyk, M.E., Argos, M., 2017. Arsenic and obesity: a comparison of urine dilution adjustment methods. Environ. Health Perspect. 125, 087020.

Buser, M.C., Murray, H.E., Scinicariello, F., 2014. Age and sex differences in childhood and adulthood obesity association with phthalates: Analyses of NHANES 2007–2010. Int. J. Hyg. Environ. Health 217, 687–694.

Campbell Jr., J.L., Yoon, M., Ward, P.L., Fromme, H., Kessler, W., Phillips, M.B., Anderson, W.A., Clewell 3rd, H.J., Longnecker, M.P., 2018. Excretion of Di-2-ethylhexyl phthalate (DEHP) metabolites in urine is related to body mass index because of higher energy intake in the overweight and obese. Environ. Int. 113, 91–99.

Cole, T.J., Bellizzi, M.C., Flegal, K.M., Dietz, W.H., 2000. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ (clinical Research Ed.) 320, 1240–1243.

Core, R., Team,, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Crobeddu, B., Jutras-Carignan, A., Kolasa, É., Mounier, C., Robaire, B., Plante, I., 2022. Gestational and Lactational Exposure to the Emergent Alternative Plasticizer 1,2-Cyclohexane Dicarboxylic Acid Diisononyl Ester (DINCH) Impairs Lipid Metabolism to a Greater Extent Than the Commonly Used Di(2-Ethylhexyl) Phthalate (DEHP) in the Adult Rat Mammary Gland. Toxicol. Sci. 189, 268–286.

Deierlein, A.L., Wolff, M.S., Pajak, A., Pinney, S.M., Windham, G.C., Galvez, M.P., Silva, M.J., Calafat, A.M., Kushi, L.H., Biro, F.M., Teitelbaum, S.L., 2016. Longitudinal Associations of Phthalate Exposures During Childhood and Body Size Measurements in Young Girls. Epidemiology 27, 492–499.

Desdoits-Lethimonier, C., Albert, O., le Bizec, B., Perdu, E., Zalko, D., Courant, F., Lesné, L., Guillé, F., Dejucq-Rainsford, N., Jégou, B., 2012. Human testis steroidogenesis is inhibited by phthalates. Hum. Reprod. 27, 1451–1459.

Desvergne, B., Feige, J.N., Casals-Casas, C., 2009. PPAR-mediated activity of phthalates: A link to the obesity epidemic? Mol. Cell. Endocrinol. 304, 43–48.

Esteban López, M., Göen, T., Mol, H., Nübler, S., Haji-abbas-Zarrabi, K., Koch, H.M., Kasper-sonnenberg, M., Dvorakova, D., Hajslova, J., Antignac, J.-P., Vaccher, V., Elbers, I., Thomsen, C., Vorkamp, K., Pedraza – Díaz, S., Kolossa-Gehring, M., Castaño, A., 2021. The European human biomonitoring platform - Design and implementation of a laboratory quality assurance/quality control (QA/QC) programme for selected priority chemicals. International Journal of Hygiene and Environmental Health 234, 113740.

EUROPEAN COMMISSION 2021. amending Annex XIV to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Brussels,: Official Journal of the European Union.

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castaño, A., Esteban López, M., Rodriguez Martin, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L.E., Kolossa-gehring, M., Schoeters, G., 2021. HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity - The HBM4EU survey. Int. J. Hyg. Environ. Health 237, 113809.

Gilles, L., Govarts, E., Rodriguez Martin, I, Andersson, A.M., Appenzeller, B.M.R., Barbone, F., Castaño, A., Coertjens, D., Den Hond, E., Dzhedzheia, V., Eržen, I., López, M.E., Fábelová, L., Fillol, C., Franken, C., Frederiksen, H., Gabriel, C., Haug, L.S., Horvat, M., Halldórsson, T.I., Janasik, B., Holcer, N.J., Kakucs, R., Karakitsios, S., Katsonouri, A., Klánová, J., Kold-jensen, T., Kolossa-gehring, M., Konstantinou, C., Koponen, J., Lignell, S., Lindroos, A.K., Makris, K.C., Mazej, D., Morrens, B., Murínová, I., P., Namorado, S., Pedraza-diaz, S., Peisker, J., Probst-hensch, N., Rambaud, L., Rosolen, V., Rucic, E., Rüther, M., Sarigiannis, D., Tratnik, J.S., Standaert, A., Stewart, L., Szigeti, T., Thomsen, C., Tolonen, H., Eiríksdóttir, Á., Van Nieuwenhuyse, A., Verheyen, V.J., Vlaanderen, J., Vogel, N., Wasowicz, W., Weber, T., Zock, J.P., Sepai, O., Schoeters, G., 2022. Harmonization of Human Biomonitoring Studies in Europe: Characteristics of the HBM4EU-Aligned Studies Participants. Int. J. Environ. Res. Public Health 19.

Govarts, E., Gilles, L., Bopp, S., Holub, P., Matalonga, L., Vermeulen, R., Vrijheid, M., Beltran, S., Hartlev, M., Jones, S., Rodriguez Martin, L., Standaert, A., Swertz Morris, A., Theunis, J., Trier, X., Vogel, N., Van Espen, K., Sylvie, R., Schoeters, G., 2022. Position paper on management of personal data in environment and health research in Europe. Environ. Int. 165, 107334.

Govarts, E., Rodriguez Martin, L., Gilles, L., Remy, S., 2022a. HBM4EU Harmonized Codebook Children - Aligned studies. Zenodo.

Govarts, E., Rodriguez Martin, L., Gilles, L., Remy, S., 2022b. HBM4EU Harmonized Codebook Teenagers - Aligned studies. Zenodo.

Govarts, E., Gilles, L., Rodriguez Martin, L., Santonen, T., Apel, P., Alvito, P., Anastasi, E., Andersen, H.R., Andersson, A.M., Andryskova, L., Antignac, J.P., Appenzeller, B., Barbone, F., Barnett-itzhaki, Z., Barouki, R., Berman, T., Bil, W., Borges, T., Buekers, J., Cañas-portilla, A., Covaci, A., Csako, Z., Den Hond, E., Dvorakova, D., Fabelova, L., Fletcher, T., Frederiksen, H., Gabriel, C., Ganzleben, C., Göen, T., Halldorsson, T.I., Haug, L.S., Horvat, M., Huuskonen, P., Imboden, M., Jagodic Hudobivnik, M., Janasik, B., Janev Holcer, N., Karakitsios, S., Katsonouri, A., Klanova, J., Kokaraki, V., Kold Jensen, T., Koponen, J., Laeremans, M., Laguzzi, F., Lange, R., Lemke, N., Lignell, S., Lindroos, A.K., Lobo, Vicente, J., Luijten, M., Makris, K.C., Mazej, D., Melymuk, L., Meslin, M., Mol, H., Montazeri, P., Murawski, A., Namorado, S., Niemann, L., Nübler, S., Nunes, B., Olafsdottir, K., Palkovicova Murinova, L., Papaioannou, N., Pedraza-diaz, S., Piler, P., Plichta, V., Poteser, M., Probst-hensch, N., Rambaud, L., Rauscher-gabernig, E., Rausova, K., Remy, S., Riou, M., Rosolen, V., Rousselle, C., Rüther, M., Sarigiannis, D., Silva, M.J., Šlejkovec, Z., Snoj Tratnik, J., Stajnko, A., Szigeti, T., Tarazona, J.V., Thomsen, C., Tkalec, Ž., Tolonen, H., Trnovec, T., Uhl, M., Van Nieuwenhuyse, A., Vasco, E., Verheyen, V.J., Viegas, S., Vinggaard, A.M., Vogel, N., Vorkamp, K., Wasowicz, W., Weber, T., et al., 2023. Harmonized human biomonitoring in European children, teenagers and adults: EU-wide exposure data of 11 chemical substance groups from the HBM4EU Aligned Studies (2014–2021). Int. J. Hyg. Environ. Health 249, 114119.

Güil-Oumrait, N., Cano-Sancho, G., Montazeri, P., Stratakis, N., Warembourg, C., Lopez-Espinosa, M.J., Vioque, J., Santa-Marina, L., Jimeno-Romero, A., Ventura, R., Monfort, N., Vrijheid, M., Casas, M., 2022. Prenatal exposure to mixtures of phthalates and phenols and body mass index and blood pressure in Spanish preadolescents. Environ. Int. 169, 107527.

Güil-Oumrait, N., Stratakis, N., Maitre, L., Anguita-Ruiz, A., Urquiza, J., Fabbri, L., Basagaña, X., Heude, B., Haug, L.S., Sakhi, A.K., Iszatt, N., Keun, H.C., Wright, J., Chatzi, L., Vafeiadi, M., Bustamante, M., Grazuleviciene, R., Andrušaityte, S., Slama, R., McEachan, R., Casas, M., Vrijheid, M., 2024. Prenatal Exposure to Chemical Mixtures and Metabolic Syndrome Risk in Children. JAMA Netw. Open 7, e2412040.

Hatch, E.E., Nelson, J.W., Qureshi, M.M., Weinberg, J., Moore, L.L., Singer, M., Webster, T.F., 2008. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ. Health 7, 27.

Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal Variability of Urinary Phthalate Metabolite Levels in Men of Reproductive Age. Environ. Health Perspect. 112, 1734–1740.

Heindel, J.J., Newbold, R., Schug, T.T., 2015. Endocrine disruptors and obesity. Nat. Rev. Endocrinol. 11, 653–661.

Hoppin, J.A., Brock, J.W., Davis, B.J., Baird, D.D., 2002. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ. Health Perspect. 110, 515–518.

Jalouli, M., Carlsson, L., Améen, C., Lindén, D., Ljungberg, A., Michalik, L., Edén, S., Wahli, W., Oscarsson, J., 2003. Sex difference in hepatic peroxisome proliferator-activated receptor alpha expression: influence of pituitary and gonadal hormones. Endocrinology 144, 101–109.

Janssen, I., Katzmarzyk, P.T., Ross, R., 2004. Waist circumference and not body mass index explains obesity-related health risk. Am. J. Clin. Nutr. 79, 379–384.

Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A Quantile-Based g-Computation Approach to Addressing the Effects of Exposure Mixtures. Environ. Health Perspect. 128, 047004.

Kelley, K.E., Hernández-Díaz, S., Chaplin, E.L., Hauser, R., Mitchell, A.A., 2012. Identification of phthalates in medications and dietary supplement formulations in the United States and Canada. Environ. Health Perspect. 120, 379–384.

Lange, R., Vogel, N., Schmidt, P., Gerofke, A., Luijten, M., Bil, W., Santonen, T., Schoeters, G., Gilles, L., Sakhi, A.K., Haug, L.S., Jensen, T.K., Frederiksen, H., Koch, H.M., Szigeti, T., Szabados, M., Tratnik, J.S., Mazej, D., Gabriel, C., Sarigiannis, D., Dzhedzheia, V., Karakitsios, S., Rambaud, L., Riou, M., Koppen, G., Covaci, A., Zvonař, M., Piler, P., Klánová, J., Fábelová, L., Richterová, D., Kosjek, T., Runkel, A., Pedraza-Díaz, S., Verheyen, V., Bastiaensen, M., Esteban-López, M., Castaño, A., Kolossa-Gehring, M., 2022. Cumulative risk assessment of five phthalates in European children and adolescents. International Journal of Hygiene and Environmental Health 246, 114052.

Langsch, A., David, R.M., Schneider, S., Sperber, S., Haake, V., Kamp, H., Leibold, E., Ravenzwaay, B.V., Otter, R., 2018. Hexamoll(®) DINCH: Lack of in vivo evidence for obesogenic properties. Toxicol. Lett. 288, 99–110.

Magnus, P., Birke, C., Vejrup, K., Haugan, A., Alsaker, E., Daltveit, A.K., Handal, M., Haugen, M., Høiseth, G., Knudsen, G.P., Paltiel, L., Schreuder, P., Tambs, K., Vold, L., Stoltenberg, C., 2016. Cohort Profile Update: The Norwegian Mother and Child Cohort Study (MoBa). Int. J. Epidemiol. 45, 382–388.

Meeker, J.D., Calafat, A.M., Hauser, R., 2009. Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. J. Androl. 30, 287–297.

Pack, L.K., Gilles, L., Cops, J., Tolonen, H., van Kamp, I., Esteban-López, M., Pedraza-Díaz, S., Lacasaña, M., Alzaga, B.G., Lermen, D., Bartel-Steinbach, M., Katsonouri, A., Fiddicke, U., Castaño, A., Kolossa-Gehring, M., 2023. A step towards harmonising human biomonitoring study setup on European level: Materials provided and lessons learnt in HBM4EU. Int. J. Hyg. Environ. Health 249, 114118.

Ribeiro, C., Mendes, V., Peleteiro, B., Delgado, I., Araújo, J., Aggerbeck, M., Annesi-Maesano, I., Sarigiannis, D., Ramos, E., 2019. Association between the exposure to phthalates and adiposity: A meta-analysis in children and adults. Environ. Res. 179, 108780.

Rodríguez-Carmona, Y., Ashrap, P., Calafat, A.M., Ye, X., Rosario, Z., Bedrosian, L.D., Huerta-Montanez, G., Vélez-Vega, C.M., Alshawabkeh, A., Cordero, J.F., Meeker, J. D., Watkins, D., 2020. Determinants and characterization of exposure to phthalates, DEHTP and DINCH among pregnant women in the PROTECT birth cohort in Puerto Rico. J. Eposure Sci. Environ. Epidemiol. 30, 56–69.

Seo, M.Y., Moon, S., Kim, S.H., Park, M.J., 2022. Associations of Phthalate Metabolites and Bisphenol A Levels with Obesity in Children: The Korean National Environmental Health Survey (KoNEHS) 2015 to 2017. Endocrinol Metab (seoul) 37, 249–260.

Serrano, S.E., Braun, J., Trasande, L., Dills, R., Sathyanarayana, S., 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ. Health 13, 43.

Teitelbaum, S.L., Britton, J.A., Calafat, A.M., Ye, X., Silva, M.J., Reidy, J.A., Galvez, M.P., Brenner, B.L., Wolff, M.S., 2008. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. Environ. Res. 106, 257–269.

Testai, E., Hartemann, P., Rastogi, S.C., Bernauer, U., Piersma, A., de Jong, W., Gulliksson, H., Sharpe, R., Schubert, D., Rodríguez-Farre, E., 2016. The safety of medical devices containing DEHP plasticized PVC or other plasticizers on neonates and other groups possibly at risk (2015 update). Regulatory Toxicology and Pharmacology: RTP 76, 209–210.

Textor, J., Hardt, J., Knuppel, S., 2011. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 22, 745.

Vidmar, S., Carlin, J., Hesketh, K., Cole, T., 2004. Standardizing anthropometric measures in children and adolescents with new functions for egen. Stata J. 4, 50–55.

Vogel, N., Schmidt, P., Lange, R., Gerofke, A., Sakhi, A.K., Haug, L.S., Jensen, T.K., Frederiksen, H., Szigeti, T., Csákó, Z., Murinova, L.P., Sidlovska, M., Janasik, B., Wasowicz, W., Tratnik, J.S., Mazej, D., Gabriel, C., Karakitsios, S., Barbone, F., Rosolen, V., Rambaud, L., Riou, M., Murawski, A., Leseman, D., Koppen, G., Covaci, A., Lignell, S., Lindroos, A.K., Zvonar, M., Andryskova, L., Fabelova, L., Richterova, D., Horvat, M., Kosjek, T., Sarigiannis, D., Maroulis, M., Pedraza-Diaz, S., Cañas, A., Verheyen, V.J., Bastiaensen, M., Gilles, L., Schoeters, G., Esteban-López, M., Castaño, A., Govarts, E., Koch, H.M., Kolossa-Gehring, M., 2023. Current exposure to phthalates and DINCH in European children and adolescents – Results from the HBM4EU Aligned Studies 2014 to 2021. International Journal of Hygiene and Environmental Health 249, 114101.

Vrijheid, M., Fossati, S., Maitre, L., Márquez, S., Roumeliotaki, T., Agier, L., Andrusaityte, S., Cadiou, S., Casas, M., de Castro, M., Dedele, A., Donaire-Gonzalez, D., Grazuleviciene, R., Haug, L.S., McEachan, R., Meltzer, H.M., Papadopouplou, E., Robinson, O., Sakhi, A.K., Siroux, V., Sunyer, J., Schwarze, P.E., Tamayo-Uria, I., Urquiza, J., Vafeiadi, M., Valentin, A., Warembourg, C., Wright, J., Nieuwenhuijsen, M.J., Thomsen, C., Basagaña, X., Slama, R., Chatzi, L., 2020. Early-Life Environmental Exposures and Childhood Obesity: An Exposome-Wide Approach. Environ. Health Perspect. 128, 67009.

Wang, C., Jackson, G., Jones, T.H., Matsumoto, A.M., Nehra, A., Perelman, M.A., Swerdloff, R.S., Traish, A., Zitzmann, M., Cunningham, G., 2011. Low Testosterone Associated With Obesity and the Metabolic Syndrome Contributes to Sexual Dysfunction and Cardiovascular Disease Risk in Men With Type 2 Diabetes. Diabetes Care 34, 1669–1675.

White, I.R., Royston, P., Wood, A.M., 2011. Multiple imputation using chained equations: Issues and guidance for practice. Stat. Med. 30, 377–399.

WHO. 2021. *Obesity and overweight* [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [Accessed].

World health organization, 2007. WHO child growth standards : head circumference-for-age, arm circumference-for-age, triceps skinfold-for-age and subscapular skinfold-for-age : methods and development. World Health Organization, Geneva.

Wu, B., Jiang, Y., Jin, X., He, L., 2020. Using three statistical methods to analyze the association between exposure to 9 compounds and obesity in children and adolescents: NHANES 2005–2010. Environ. Health 19, 94.

Zhang, Y., Meng, X., Chen, L., Li, D., Zhao, L., Zhao, Y., Li, L., Shi, H., 2014. Age and sex-specific relationships between phthalate exposures and obesity in Chinese children at puberty. PLoS One 9, e104852.

Zhang, Y., Dong, T., Hu, W., Wang, X., Xu, B., Lin, Z., Hofer, T., Stefanoff, P., Chen, Y., Wang, X., Xia, Y., 2019. Association between exposure to a mixture of phenols, pesticides, and phthalates and obesity: Comparison of three statistical models. Environ. Int. 123, 325–336.