International Journal of Hygiene and Environmental Health 232 (2021) 113689

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Urinary phthalates and body mass index in preschool children: The MIREC Child Development Plus study

Jillian Ashley-Martin [a], Linda Dodds [b], Tye E. Arbuckle [a], Bruce Lanphear [c], Gina Muckle [d], Warren G. Foster [e], Pierre Ayotte [f], Angelika Zidek [g], Elizabeth Asztalos [h], Maryse F. Bouchard [i], Stefan Kuhle [b,*]

[a] Environmental Health Science and Research Bureau, Health Canada, Ottawa, ON, K1A 0K9, Canada
[b] Perinatal Epidemiology Research Unit, Dalhousie University. 5850-5980 University Ave, Halifax, NS, B3K 6R8, Canada
[c] Faculty of Health Sciences. Simon Fraser University. 8888 University Drive Burnaby, B.C, V5A 1S6, Canada
[d] Laval University and CHU Research Centre, Quebec City, Quebec, Canada
[e] McMaster University Health Sciences Center, Hamilton, Ontario, Canada
[f] Institut National de Santé Publique du Québec (INSPQ), Quebec, Canada
[g] Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario, Canada
[h] University of Toronto, Toronto, ON, Canada
[i] Department of Environmental and Occupational Health, University of Montreal, Canada

ARTICLE INFO

Keywords:
Phthalates
Body mass index
Children
Biomonitoring
Skinfolds
Cohort study
Chemical mixtures

ABSTRACT

Childhood exposure to phthalates, a class of chemicals with known reproductive and developmental effects, has been hypothesized to increase the risk of obesity, but this association is not well understood in preschool children. We examined the association between urinary concentrations of phthalate metabolites and concurrently measured body mass index (BMI) and skinfolds among children between the ages of two and five years.

We collected anthropometric measures and biomonitoring data on approximately 200 children enrolled in the Maternal-Infant Research on Environmental Chemicals Child Development Plus study. We measured 22 phthalate metabolites in children's urine and used the 19 metabolites detected in at least 40% of samples. Our primary outcome was BMI z-scores calculated using the World Health Organization growth standards. Skinfold z-scores were secondary outcomes. We used multivariable linear regression to evaluate the association between tertiles of phthalate concentrations and each anthropometric measure. We also used weighted quantile sum regression to identify priority exposures of concern.

Our analytic sample included 189 singleton-born children with complete anthropometric data. Children with concentrations of the parent compound di-n-butyl phthalate (∑DnBP) in the third tertile had 0.475 (95% CI: 0.068, 0.883) higher BMI z-scores than those in the lower tertile. ∑DnBP was identified as a priority exposure in the weighted quantile sum regression BMI model.

In this population of Canadian preschool aged children, we identified DnBP as a potential chemical of concern in regard to childhood obesity. Future research with serial phthalate measurements and anthropometric measurements in young children will help confirm these findings.

1. Introduction[1]

The global prevalence of childhood obesity has increased dramatically in recent decades (GBD Obesity Collaboration, 2014). Obesity is a multifactorial condition rather than a simple caloric imbalance (Keith et al., 2006; Romano et al., 2014). Early life exposure to endocrine-disrupting chemicals, such as phthalates, is hypothesized to be one of the pathways leading to the development of childhood obesity (Grun and Blumberg, 2009; Heindel et al., 2015). Experimental data has shown that phthalate exposure may induce weight gain by stimulating

* Corresponding author. IWK Health Centre, PO Box 9700 5850-5980 University Ave, Halifax, NS B3K 6R8, Canada.
E-mail addresses: jillian.ashley-martin@canada.ca (J. Ashley-Martin), l.dodds@dal.ca (L. Dodds), Tye.arbuckle@canada.ca (T.E. Arbuckle), blanphear@sfu.ca (B. Lanphear), gina.muckle@psy.ulaval.ca (G. Muckle), fosterw@mcmaster.ca (W.G. Foster), pierre.ayotte@inspq.qc.ca (P. Ayotte), Angelika.zidek@canada.ca (A. Zidek), Elizabeth.asztalos@sunnybrook.ca (E. Asztalos), Maryse.bouchard@umontreal.ca (M.F. Bouchard), stefan.kuhle@dal.ca (S. Kuhle).

https://doi.org/10.1016/j.ijheh.2021.113689
Received 13 August 2020; Received in revised form 30 December 2020; Accepted 30 December 2020
Available online 11 January 2021

peroxisome proliferator-activated receptors (PPAR), decreasing androgenic activity, and increasing adipocyte number and size (Biemann et al., 2012; Casals-Casas and Desvergne, 2011; Desvergne et al., 2009; Hao et al., 2012; Hurst and Waxman, 2003). Findings from epidemiological studies in adults and children have been equivocal (Goodman et al., 2014), and hindered by the methodological challenges of multiple correlated exposures and inability to establish temporality. Furthermore, insufficient research has been dedicated to measuring the association between phthalate exposure and anthropometry during windows of developmental susceptibility such as early childhood.

Phthalates are synthetic organic chemicals that are commonly used as plasticizers, solvents and additives in numerous consumer products (Wang et al., 2019). Low molecular weight (LMW) phthalates, which contain between one to four carbon chains, are commonly found in personal care products and cosmetics, adhesives, enteric coated medications, and toys. High molecular weight (HMW) phthalates, which contain greater than five carbon chains, are commonly found in plastics, food packaging, and polyvinyl products (Wang et al., 2019; Wittassek et al., 2011). These chemicals readily leach out of these products and are absorbed by humans via ingestion or dermal absorption. Ingestion of both food and dust is one of the primary routes of exposure to many phthalates in young children (Weiss et al., 2018; Wormuth et al., 2006); the phthalates are then converted into monoesters or oxidative metabolites in the gastrointestinal tract, conjugated with glucuronide or sulfate and excreted via urine (Wang et al., 2019; Wittassek et al., 2011).

Young children tend to have higher phthalate exposure than adults due to their specific behaviors (e.g., mouthing and crawling), higher food requirements per unit body mass and ventilation rate. Furthermore, their rapid growth and under-developed detoxification mechanisms put young children at increased risk to the potential adverse effects of phthalate exposures (Braun, 2017; Gow et al., 2001; Wormuth et al., 2006). Still, the evidence from human studies on the association between phthalate exposure and measures of body fat in early childhood is scant and conflicting (Boas et al., 2010; Deierlein et al., 2016; Goodman et al., 2014; Maresca et al., 2016; Shoaff et al., 2017; Vafeiadi et al., 2018; Zhang et al., 2014).

In a 2014 systematic review of seven studies, including six cross-sectional studies, Goodman et al. concluded that the evidence was insufficient to determine whether childhood phthalate exposure is associated with childhood obesity (Goodman et al., 2014). More recently, longitudinal studies remain inconsistent with positive (Deierlein et al., 2016; Shoaff et al., 2017; Vafeiadi et al., 2018), null (Maresca et al., 2016; Shoaff et al., 2017), and inverse (Vafeiadi et al., 2018; Zhang et al., 2014) associations being reported. Of note, only three identified studies have examined the potential obesity-related effects of phthalate exposure in children younger than five years of age (Boas et al., 2010; Shoaff et al., 2017; Vafeiadi et al., 2018). Statistically significant positive associations in these studies were restricted to a specific subgroup defined by the child's age (Shoaff et al., 2017) and sex (Vafeiadi et al., 2018).

The objective of the present study was to determine the association between childhood urinary phthalate concentrations and concurrently measured body mass index (BMI) and skinfold measurements in a population of Canadian preschool children with extensive biomonitoring data. Given previous epidemiological (Vafeiadi et al., 2018; Yang et al., 2017; Zhang et al., 2014) and experimental (Grun and Blumberg, 2009; Hao et al., 2012) evidence of sex-specific obesity related effects of phthalate exposures, our secondary objective was to explore whether the association between phthalate exposure and anthropometric outcomes differed between boys and girls. To account for the challenges of analyzing multiple correlated exposures, we supplemented traditional regression models with weighted quantile sum regression (Carrico and Gennings, 2015; Tanner et al., 2019).

2. Methods

2.1. Study population

The Maternal-Infant Research on Environmental Chemicals (MIREC) study is a national-level pregnancy cohort of 2001 women from 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Toronto, Hamilton, Montreal, and Halifax) (Arbuckle et al., 2013). Briefly, women were recruited between 2008 and 2011 in their first trimester and followed through delivery. Compared to the national average, MIREC participants tended be of higher socioeconomic status, and have a lower rate of smoking, and lower BMI (Arbuckle et al., 2013). A follow-up study of child growth, neurodevelopment, and exposure biomonitoring was conducted in six study sites between 2013 and 2015 (Vancouver, Toronto, Hamilton, Kingston, Montreal, and Halifax) (MIREC-Child Development Plus (MIREC-CD Plus)). This follow-up group was similar to the overall MIREC population in terms of key characteristics such as pre-pregnancy BMI, smoking, and education. Biomonitoring of phthalate metabolites was conducted on 200 children between ages of 2 and 5 years of age. A spot random urine sample was collected from each participating child, during a home visit, unless the participant requested a clinic visit. Parents were reminded that urine would be collected at this visit and they might want to give their child a beverage before the appointment time. The study was reviewed by the Health Canada Research Ethics Board, CHU de Quebec Ethics Board, as well as by ethics committees at all recruitment sites, and parents signed an informed consent form for their child's participation.

2.2. Exposures: child urinary phthalate metabolite concentrations

We measured 22 phthalate metabolites in children's urine samples that were collected at the time of the biomonitoring visit (Suppl Table 1). To create summary indices of parent compounds, we calculated molar sums for metabolites of parent compounds by dividing metabolites by their molecular weight, summing all metabolites of a specific parent compound and multiplying the sum by 1000. We calculated molar sums for DEHP, DiNP, DnBP, DIDP, DiBP; the corresponding metabolites are shown in Suppl Table 1. Four metabolites (MMP, MEP, MBzP, MCPP) were distinct and treated as individual analytes (Table 1). We excluded three metabolites (MCHP, MOP, MNP) from the analyses that were detectable in less than 40% of samples (Suppl Table 1).

Analysis of the urine samples was performed at the Centre de toxicologie du Québec of the Institut National de Santé Publique du Québec. In brief, following an enzyme deconjugation step, the analytes were extracted using a robotized liquid-liquid extraction unit at pH 3 with a mixture of hexane:ethyl acetate. The extracts were brought to dryness, taken up in a solution of acetonitrile:water (25:75), and analyzed by an ultra-performance liquid chromatograph (Waters Acquity, Waters Limited Mississauga, ON, Canada) and tandem mass spectrometer (Waters Xevo TQ-S with MassLynx software version 4.1) in the multiple reaction monitoring mode with an "electrospray" source operated in the negative mode. Machine readings were used for all values below the limit of detection. To account for heterogeneity in urinary dilution, phthalate concentrations were standardized for urine specific gravity (SG), measured using a refractometer, according to the following formula: $P_c = P_i\ [(SG_m - 1)/(SG_i - 1)]$, where P_c = SG standardized metabolite concentration (μg/ml), P_i = observed metabolite concentration, SG_i = specific gravity of the urine sample, and SG_m = median SG for the cohort (Just et al., 2010). Descriptive statistics are presented for SG-standardized phthalate concentrations. To account for urinary dilution in multivariable models, SG was entered as a covariate with the raw phthalate concentrations. This approach facilitates comparison of crude and unadjusted models.

In the MIREC study, first trimester urinary concentrations of 11 phthalate metabolites were measured and descriptive statistics have been previously reported for the seven metabolites with a sufficient

Table 1
Urinary concentrations of specific gravity-standardized molar sum phthalate metabolite compounds (nmol/L) and individual metabolites (μg/L) among children enrolled in MIREC-CD Plus.

	N	Minimum	25th percentile	Median	75th percentile	Maximum
Parent Compounds						
∑DEHP	187	22.3	106	155	229	2023
∑DiNP	180	0.0030	10.1	19.1	33.6	707
∑DnBP	181	17.3	66.9	100	156	2595
∑DIDP	174	0.750	3.96	6.58	9.61	179
∑DiBP	187	11.1	62.1	89.8	141	1039
Metabolites						
MBzP	187	0.540	3.64	8.34	17.4	201
MEP	187	2.58	7.60	11.5	21.8	1020
MMP	186	0.729	2.68	3.74	5.89	72.5
MCPP	187	0.160	1.34	1.87	3.03	39.2
MCPP	187	0.160	1.34	1.87	3.03	39.2
2-OH-MiBP	187	1.13	5.72	8.58	12.7	76.8
MCMHP	187	0.009	2.77	4.01	6.39	44.3
MCiNP	179	0.251	0.999	1.43	2.16	885
MCiOP	184	0.001	1.02	1.69	3.26	92.3
MEHHP	187	1.79	8.02	12.5	17.8	200
MEHP	187	0.337	1.06	1.86	2.51	69.3
MEOHP	187	1.30	6.36	9.11	14.4	149
MHBP	181	0.493	2.28	3.53	5.27	99.6
MHiDP	182	0.001	0.102	0.290	0.491	39.6
MHiNP	183	0.002	1.13	2.15	3.72	83.1
MOiDP	186	0.001	0.129	0.262	0.576	59.6
MOiNP	187	0.002	0.714	1.39	2.31	34.7
MiBP	187	1.42	8.00	12.2	19.0	194
MiNP	187	0.001	0.137	0.362	0.865	13.4
MnOP	186	0.001	0.001	0.001	0.002	0.284

Abbreviations for all phthalate parent compounds and metabolites are provided in Supplemental table 1.

percentage of samples with detectable concentrations (Arbuckle et al., 2014). In the present study, we assess correlations between maternal and child phthalate concentrations, compare descriptive statistics with the child concentrations, and determine whether adjustment for these prenatal exposures influences results of the associations between child phthalate concentrations and anthropometric measures (Engle and Wolff, 2013). The maternal phthalate metabolites were standardized for SG in the same manner as described for the childhood phthalate metabolites.

2.3. Outcomes: child adiposity

Our primary outcome measure was the body mass index (BMI); secondary outcomes were triceps skinfold (TSF) and subscapular skinfold (SSF) z-scores. Trained research personnel followed a detailed protocol to measure child's weight with a calibrated scale (Seca model 874 [Seca Corporation, Hanover, MD, USA]), standing height using a calibrated stadiometer (Seca model 217 [Seca Corporation, Hanover, MD, USA]), and SSF and TSF using a Lange skinfold caliper (Cambridge Scientific Instruments, Cambridge, MA, USA). All measurements were completed during the home visit and the average of two measured values was used. If the two measures differed by a pre-determined value, research personnel performed a third measurement. A third measurement was performed in 24%, 10%, 3%, and less than 1% of individuals for weight, height, TSF, and SSF respectively. Research personnel were blinded to maternal and child phthalate exposure concentrations. Age- and sex-specific z-scores for BMI, TSF, and SSF were calculated for each child based on WHO Child Growth Standards (WHO, 2015).

2.4. Statistical analysis

We calculated counts and percentages for the study population. Pearson correlation coefficients were calculated for all log2-transformed summary indices and metabolites measured in children and in mothers. Due to the skewed distributions of all exposures, we log2-transformed all exposures prior to use in regression models or calculation of correlation coefficients.

We used three approaches to evaluate the association between phthalates and each anthropometric outcome. First, we developed individual linear regression models for tertiles of each phthalate parent compound (DEHP, DiNP, DnBP, DIDP, DiBP) and metabolite (MCPP, MBzP, MMP, MEP) measured at the time of the MIREC-CD Plus study visit. We categorized each exposure variable in this way to account for potential non-linear associations. Separate models were developed for each outcome. Confounders, identified via causal diagrams, included maternal postnatal weight status (Voerman et al., 2019), household income (Garí et al., 2019; Lewin et al., 2017), and maternal age (Lewin et al., 2017) (Suppl Figure 1). We entered these variables into all models, along with urine SG. We assessed effect modification by stratifying models by sex and calculating p values of products terms for sex and each exposure. To account for missingness in the covariates and the exposures, multiple imputation with chained equations was used (m = 30, 30 iterations) (van Buuren and Groothuis-Oudshoorn, 2011).

Second, we used weighted quantile sum (WQS) regression to evaluate the association of the phthalate mixture on each anthropometric outcome using the gWQS package in R (Renzetti et al., 2019). This approach estimates the body burden of the chemical mixture, identifies priority chemicals among a set of correlated exposures and has been shown to have superior performance to regularization techniques such as elastic net, particularly when dealing with multiple correlated exposures (Carrico and Gennings, 2015). A WQS index was created using our primary phthalate exposures (5 parent compounds, 4 metabolites) and each exposure was assigned a weight that reflects the strength of that phthalate-outcome association. Briefly, using 40% of the data designated as the training set, individual exposures were categorized into tertiles and assigned a weight between 0 and 1 using a non-linear modeling optimization algorithm. To increase the stability of the estimates, average weights were calculated based on analysis performed in 75 bootstrapped samples. Due to the unidirectional nature of WQS regression, we constrained the β coefficient of the index to be positive in the bootstrap samples based on our hypothesis that phthalates would be positively associated with childhood anthropometric measures;

however, the coefficients were not constrained to be positive when weights were estimated in the test data. We then modelled the association between the WQS index and each outcome using the data designated as the test set (60%). These models included specific gravity to account for urinary dilution. We report weights for chemicals determined to be of concern based on a weight greater than 11% (100/9 exposures) as this value is higher than expected by chance (Tanner et al, 2019, 2020). To further account for the relatively small sample size and potential instability of results due to division of data into training and test sets, we used repeated holdout validation to randomly partition the data and repeated the WQS estimation 100 times. Based on the distribution of results, we calculated mean weights for each exposure (Tanner et al, 2019, 2020). As the WQS index does not have an intuitive quantitative interpretation, the WQS model was primarily used to qualitatively identify phthalate metabolites that are associated with the child's BMI and skinfold thickness.

The third step of the analysis was to evaluate dose-response relationships using generalized additive models for parent phthalate compounds or phthalate metabolites that were identified as a key exposure in both the linear regression and WQS models. In these models, we trimmed the data to exclude the top and bottom 3% of observations to prevent the curves from being overly influenced by extreme observations.

To assess the potential confounding effect of prenatal phthalate exposure, we conducted a sensitivity analyses adjusting for maternal first trimester urine phthalate concentrations. This analyses was restricted to the phthalates where both maternal and child data was available (MCPP, MBzP, MEP, MBP, ∑DEHP) and was accomplished by adding the log2-transformed maternal phthalate exposure in each model for the corresponding child phthalate. For example, log2-transformed maternal MCPP was added to the model examining the association between childhood MCPP and BMI.

Analyses were conducted in SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 3.5.2 (R Core Team, 2019).

3. Results

Of the 200 children with phthalate biomonitoring data, 192 were singleton births, and 189 had complete height and weight data and were included in the analyses. Consistent with other reports from the MIREC Study, the study population is of moderate to high socioeconomic status and mothers tended to be, on average, of normal pre-pregnancy BMI, non-smokers, and born in Canada. Children were, on average, 32 months (standard deviation (SD): 2.7) at the time of the MIREC-CD Plus study visit (Table 2). Mean (SD) BMI, TSF, and SSF z-scores were 0.55 (0.87), 1.0 (1.4), and −0.15 (1.6), respectively.

Eighteen out of the twenty-two metabolites measured in MIREC-CD Plus participants were detectable in at least 80% of children (Suppl Figure 1). Descriptive statistics for the five summed parent compounds and four individual metabolites measured in children are reported in Table 1. Descriptive statistics for the metabolites measured in mothers are presented in Suppl Table 2. Median urinary MCPP and MBzP concentrations were nearly twice as high in children compared to their mothers (MCPP mother = 0.94 μg/L, child = 1.9 μg/L; MBzP mother = 4.5 μg/L, child = 8.3 μg/L) whereas the median urinary MBP concentrations in mothers was approximately two-thirds that of the childs (mother = 13 μg/L, child = 19 μg/L). In contrast, the median urinary MEP concentration in the mother was more than double that of the child (mother = 26 μg/L, child = 12 μg/L). Concentrations of certain DEHP metabolites were also higher in children than mothers (MEHHP mother = 9.4 μg/L, child = 12 μg/L; MEOHP mother = 6.5 μg/L, child = 9.4 μg/L) (Table 1 and Suppl 2).

We observed moderate to strong correlations among metabolites and parent compounds in children, with correlation coefficients ranging from 0.387 (MBzP and MEP) to 0.843 (MCPP and ∑DiNP). In contrast, we observed weak correlations between maternal and child phthalate concentrations; correlation coefficients ranged from −0.001 (child ∑DIDP and maternal MBzP) to 0.165 (child MEP and maternal MEP) (Suppl Table 6).

In the adjusted linear regression models (Fig. 1, Suppl Table 3), children with ∑DnBP concentrations in the highest tertile had 0.475 (95% CI: 0.068, 0.883) higher BMI z-scores than those in the lowest tertile; no other phthalate metabolite or parent compound was associated with BMI z-scores in the adjusted models. In the sex-specific models (Suppl Table 6), no statistically significant associations were found, and there was no evidence of effect modification based on a product term p-

Table 2
Characteristics of MIREC-CD Plus study participants (n = 189).

Parental	N	%
Maternal age at time of delivery (years)		
≤29	54	29
30–34	73	39
≥35	62	33
Household income (CAD)		
<50 000	32	17
50 000–100 000	80	43
>100 000	73	40
Maternal Education		
High school or less	4	2.1
College courses of diploma	48	26
University degree	135	72
Maternal Country of Birth		
Canada	152	80
Other	37	20
Maternal pre-pregnancy BMI (kg/m^2)		
<25	115	67
25–29	28	16
≥30	29	17
Maternal postnatal BMI (kg/m^2)		
<25	108	58
25–29	41	22
≥30	38	20
Maternal prenatal smoking		
Current	6	3.2
Former or quit when pregnant	51	27
Never	127	70
Paternal BMI (kg/m^2)		
<25	52	28
25–29	88	47
≥30	46	25
Child		
Age at follow-up (mths) (mean (SD))	32	2.7
Gestational age (wks) (mean (SD))	39	1.4
Sex		
Male	83	44
Female	106	56

Missing income (n = 4), education (n = 2), pre-pregnancy BMI (n = 17), postnatal BMI (n = 2).
Paternal BMI (n = 3).
Abbreviations: BMI body mass index, CAD Canadian dollar, mths months, SD standard deviation, wks weeks.

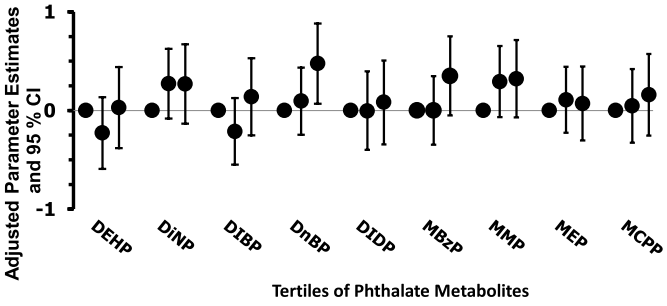

Fig. 1. Adjusted[1] parameter estimates and 95% confidence intervals (CI) for the association between tertiles of childhood urinary phthalate parent compounds (nmol/L), metabolites (μg/L), and BMI z-scores (n = 189).

value <0.05.

In the adjusted TSF z-score models, children with ∑DiNP, MMP, and MEP concentrations in the second tertile had higher TSF z-scores than children with levels in the referent category (∑DiNP 2nd tertile β = 0.623 (95% CI: 0.068, 1.178); MMP 2nd tertile β = 0.678 (95% CI: 0.113, 1.242); MEP 2nd tertile β = 0.551 (95% CI: 0.030, 1.071)) (Fig. 2, Suppl Table 4). We observed no statistically significant associations in the SSF z-score models (Fig. 3, Suppl Table 5). The magnitude and direction of effect in the sex-stratified models were largely consistent with the findings in the overall population (Suppl Tables 7–8). We observed no effect modification by sex in either the TSF or SSF models. We observed no material change in results for each of the outcomes when adjusted for prenatal phthalate exposures (data not shown).

According to the WQS regression models with repeated holdout validation (data not shown), ∑DnBP, ∑DIDP, and ∑DEHP accounted for 23%, 20%, and 13%, respectively, of the weight of the overall index of phthalate exposure in the BMI z-score models (range of weights 0.0–0.23). In the TSF models, MMP, ∑DnBP, and ∑DIDP accounted for 26%, 14%, and 14% of weights (range of weights: 0.03–0.26). The exposures of concern identified in the SSF z-score models were ∑DIDP (24%), ∑DnBP (20%), and MMP (12%) (range of weights: 0.04–0.24).

Based on the results of the categorical and the WQS models, we visualized the association between 1) log2-transformed ∑DnBP and BMI z-scores and 2) log2-transformed MMP and TSF z-scores using generalized additive models. Both models were characterized by a positive linear relationship with considerable imprecision (Fig. 4).

4. Discussion

In this population of Canadian preschool children, we evaluated concentrations of 22 phthalate metabolites in relation to concurrently measured BMI and skinfolds thickness. Compared to children with ∑DnBP concentrations in the referent tertile, those with concentrations in the highest tertile had moderately increased BMI z-scores. These results were consistent with the WQS analysis which identified ∑DnBP as a primary exposure of concern. In the TSF models, MMP was identified as a priority exposure in the WQS analysis and second tertile MMP concentrations were positively associated with TSF z-scores. We did not find effect modification by sex in any of the models. Our study provides a comprehensive assessment of phthalate metabolites in young children. Due to the number of measured metabolites, it is difficult to provide an overall summary comparison with other biomonitoring studies. Out of the LMW metabolites, median MEP and OH-MiBP concentrations in MIREC-CD Plus were lower than observed in children of similar ages enrolled in NHANES (CDC, 2009), GerES V (Schwedler et al., 2020), and a Polish study (Garí et al., 2019). With regard to HMW metabolites, median MEHHP, MEOHP, and MECPP concentrations were higher than observed in NHANES. A comparison table is provided in the supplemental material (Suppl Table 10). Moreover, the short half-life of these metabolites creates challenges to interpreting spot urine samples from different study populations and a more accurate comparison would include 24 h urine samples or repeated measurements over time.

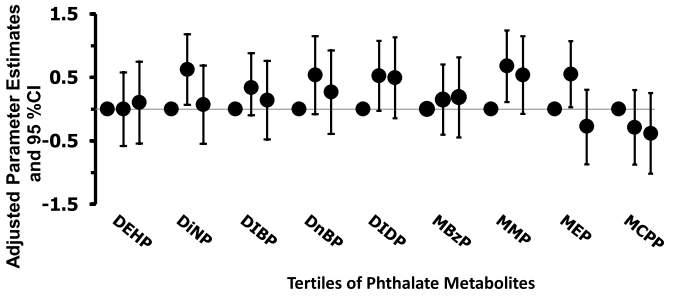

Fig. 2. Adjusted parameter estimates and 95% confidence intervals (CI) for the association between tertiles of childhood urinary phthalate parent compounds (nmol/L), metabolites (μg/L), and TSF z-scores (n = 186).

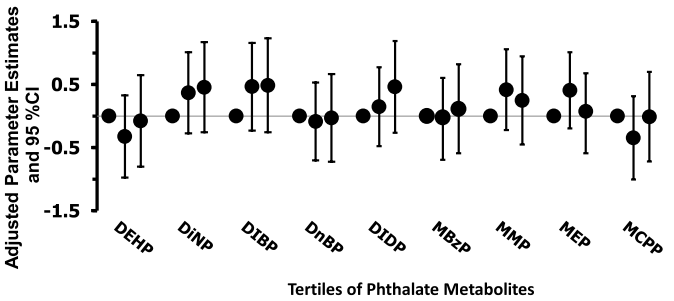

Fig. 3. Adjusted parameter estimates and 95% confidence intervals (CI) for the association between tertiles of childhood urinary phthalate parent compounds (nmol/L), metabolites (μg/L), and SSF z-scores (n = 184).

Children are most commonly exposed to DnBP via ingestion of food due to its ubiquitous presence in food packaging (Environment and Climate Change Canada, 2017; Fisher et al., 2019; Wormuth et al., 2006). In contrast, a primary route and source of exposure to DMP, the parent compound of MMP, is inhalation of indoor air resulting from the use of DMP as a solvent in building materials, such as paints and sealants (Wormuth et al., 2006). Children may also be exposed to DMP via dermal absorption of topical products (diaper creams, lotions), contact with toys (Environment and Climate Change Canada, 2015) (Fisher et al., 2019), and through diet (Correia-Sá et al., 2018). Previous epidemiological studies that examined the potential effects of these phthalates on childhood adiposity are equivocal due, in part, to heterogeneity in timing of measurement of both the exposures and the outcomes as well as different study designs and analytical methods. Despite this heterogeneity, our findings are consistent with one longitudinal and two cross-sectional studies of positive associations between concentrations of ∑DnBP, or its metabolite MBP, and measures of adiposity in children from Chinese and Mexican American populations between the ages 5 and 15 (Harley et al., 2017; Wang et al., 2013; Zhang et al., 2014). Exposure levels in MIREC-CD Plus participants (median MBP = 19 μg/L) tended to be lower than these studies (median MBP 30–43 μg/L in children ages 8–12 years of age) (Wang et al., 2013; Zhang et al., 2014). Our findings are also in line with reported positive associations between summary measures of LMW phthalates (which include both MBP and MMP) and childhood obesity (Buser et al., 2014; Deierlein et al., 2016; Teitelbaum et al., 2012). Some of these associations were only observed in subgroups of sex or weight status (Deierlein et al., 2016; Teitelbaum et al., 2012; Zhang et al., 2014). For example, authors of a study of children from New York City reported that the sum of LMW phthalates was associated with BMI and waist circumference only among children who were overweight (Teitelbaum et al., 2012). In a cross-sectional study of Chinese children, MBP and the sum of LMW phthalates were positively associated with BMI in boys, but not girls, older than 10 years of age (Zhang et al., 2014).

Our observed association between DnBP and increased BMI is further

[1] Abbreviations: BMI body mass index, DEHP di(2-ethylhexyl) phthalate, DiNP Di-iso-nonyl phthalate, DnBP Di-n-butyl phthalate, DIDP Diisodecyl phthalate, DiBP Diisobutyl phthalate, HMW high molecular weight, ICC intraclass correlation coefficient, LMW low molecular weight, MBzP monobenzyl phthalate, MEP monoethyl phthalate, MIREC Maternal-Infant Research on Environmental Chemicals, MMP mono-methyl phthalate, MCPP Mono-3-carboxypropyl phthalate, MOP Mono-n-octyl, MNP mono-isononyl phthalate, MCHP mono-cyclo-hexyl phthalate, PPAR peroxisome proliferator-activated receptors, SG specific gravity, SSF scapular skinfolds, TSF triceps skinfolds, WQS weighted quantile sum.

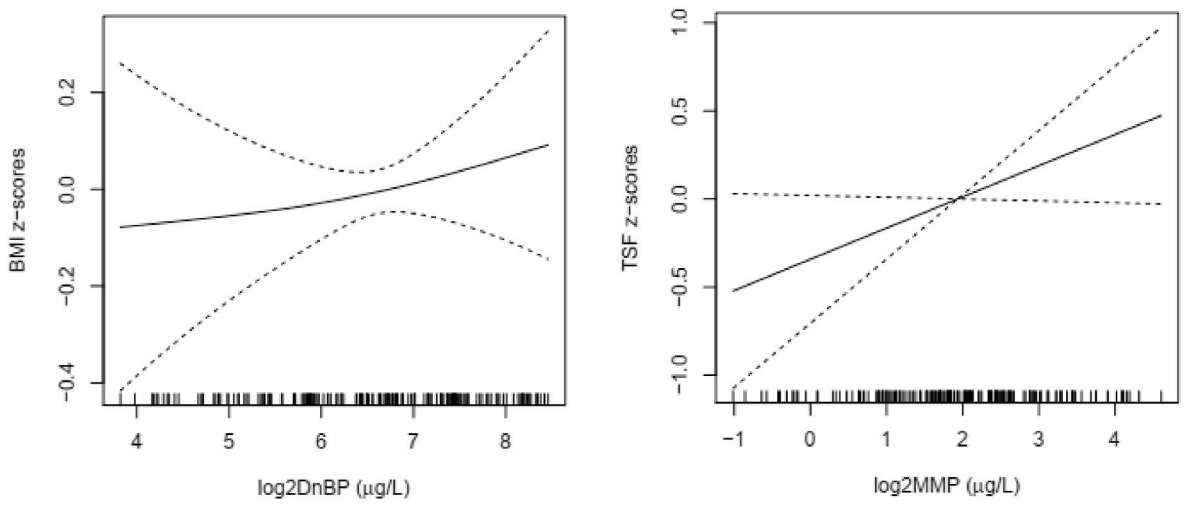

Fig. 4. Generalized additive models for associations and their 95% confidence intervals between log2DnBP and BMI z-scores and log2MMP and TSF z-scores.

supported by experimental data demonstrating that DnBP exhibits developmental and reproductive toxicity (Foster et al., 2000; Wittassek et al., 2011). Specifically, DnBP may promote adiposity via its anti-androgenic effects and subsequent influence on the estrogen/androgen ratio (Mylchreest et al., 1999). Other potential mechanisms underlying an association between DnBP and obesity are exposure-induced changes in testosterone, insulin, and thyroid levels (Mylchreest et al., 1999; Wittassek et al., 2011). Although other phthalates, such as DEHP, may disrupt lipid metabolism and promote adipogenesis via activation of PPAR-alpha and gamma receptors, DnBP may not operate through this pathway (Hurst and Waxman, 2003). MMP may modestly activate the PPAR-alpha pathway in a mouse model but not in humans (Hurst and Waxman, 2003). Further experimental research could help elucidate whether DnBP and DMP, the parent compound of MMP, have greater potential for inducing obesogenic effects than other phthalates.

Our study benefited from the extensive biomonitoring data and the use of two different analytical approaches that yielded consistent results. Although many studies have evaluated associations between phthalates and childhood growth, the present study is one of the first to examine these associations in children as young as 24 months. The extensive biomonitoring data available in the MIREC study also enabled us to evaluate more metabolites than any other identified biomonitoring study and to characterize how exposure patterns differed between pregnancy and early childhood. Consistent with previous data (CDC, 2009; Garí et al., 2019; Wormuth et al., 2006), we found that children had higher concentrations of certain metabolites than their mothers. These notable differences in exposure levels are compatible with the observed weak correlations between the maternal and child phthalate concentrations and confirm the variability in exposure patterns from the in utero to early life time periods. Additionally, this non-uniform pattern in exposure demonstrates the challenge in identifying a biomarker that is reflective of the critical window of exposure for childhood growth. Similar to our findings, authors of a Polish cohort study observed weak correlations between maternal third trimester and child phthalate concentrations (Garí et al., 2019). In contrast, a cross-sectional study of biospecimens from 17 European studies observed strong correlations between maternal and children's phthalate concentrations (Den Hond et al., 2015). The stronger correlation in this study could be due to the concurrent measurement of maternal and child phthalate concentrations and lack of influence from temporal trends in phthalate exposure concentrations (Zota et al., 2014).

The three primary limitations of our findings are the use of one spot urine sample, the small sample size, and the concurrent measurement of childhood phthalate metabolites and outcome measures. Phthalates are non-persistent chemicals and thus subject to intra-individual variability over time. However, MBP has been shown to be one of the most stable metabolites over time with an intraclass correlation coefficient for prenatal measurements ranging from 0.35 (Fisher et al., 2015) to 0.57 (Casas et al., 2018). A study of between-day and between-season phthalate variability in children reported intraclass correlation coefficients of 0.58 and 0.36 for MBP (Casas et al., 2018). Although the availability of only one childhood measurement precluded us from determining sensitive windows of exposure in the very early childhood years, we did consider the influence of in utero exposures by adjusting for certain prenatal phthalates. Future analyses of MIREC data will enable greater insight into this question by analyzing a wider range of prenatal phthalates and determining their association with children's anthropometric outcomes. At time of this writing, maternal urine samples are being re-analyzed to quantify levels of 22 phthalate metabolites. In addition to temporal variation, metabolite concentrations may be influenced by interindividual variations in urinary flow rate (Aylward et al., 2014; Hays et al., 2015). We addressed this potential source of misclassification bias by standardizing and adjusting metabolite concentrations for specific gravity.

It is also worth noting that our results are subject to type 1 error due to the multiple comparisons examined. It is possible, for example, that the statistically significantly results observed in middle tertiles of the TSF models are a result of chance rather than a true association. On the other hand, non-monotonic relationships have been observed between phthalate exposures and health outcomes (Hill et al., 2018). It is not possible within the present study to confirm or deny the presence of true non-linear relations; thus, we interpret our findings of positive associations between MMP and TSF z-scores with caution. The consistency in results between the traditional and weighted quantile sum regression models does, however, lessen concern that the observed positive associations between $\sum$DnBP and BMI and between MMP and TSF z-scorse are solely due to chance.

We cannot rule out the potential for reverse causality in the observed positive associations, given the cross-sectional data collection of child exposure and anthropometric data. Food packaging may serve as a source of exposure to DnBP as well as a surrogate of food consumption. A study of 112 children and adolescents aged 4–18 years found that adoption of a healthy diet low in packaged and processed food was associated with a reduction in phthalate metabolite levels (Correia-Sá et al., 2018). High consumption of processed food, which necessitates more packaging than fresh food, may contribute to risk of obesity (Poti et al., 2017), but we did not have the capacity to incorporate data on childhood dietary patterns or intake of sugar, fat, or processed foods in this study. It is, therefore, difficult to know whether associations

between ∑DnBP and BMI are real or an artifact of consumption and exposure patterns. It is also possible that we did not have the statistical power to detect associations and that the results are, therefore, subject to a type 2 error - particularly in the sex-specific analyses. Median ∑DnBP concentrations were higher in boys (110 nmol/L) than girls (89.9 nmol/L), whereas MMP concentrations were fairly similar (boys 4.48 nmol/L, girls 3.49 nmol/L). These modest differences may also reflect variation in the small sample and warrant further inquiry into potential sex-specific patterns of exposure and differences in potential exposure-related growth patterns.

MIREC study participants tend to have a healthier pregnancy profile (i.e. lower smoking rates and pre-pregnancy BMI) and are of higher socioeconomic status than the overall Canadian population (Arbuckle et al., 2013). Median concentrations of MnBP and MMP in MIREC-CD Plus participants were, however, comparable to concentrations in 3–6 year old children enrolled in the Canadian Health Measures Survey (Health Canada, 2019). Given the ubiquity of phthalate exposures across all socioeconomic groups, the sociodemographic composition of the MIREC cohort is not a notable barrier to the generalizability of our results. Our findings do, however, have limited generalizability to non-Caucasian racial and ethnic groups who may have heightened susceptibility to the growth-related effects of phthalate exposures (Trasande et al., 2013).

5. Conclusions

In this population of Canadian preschool-aged children, we identified DnBP as a potential chemical of concern with regard to childhood obesity. Future research with serial phthalate measurements and anthropometric measurements will help determine whether the observed findings are indicative of an obesogenic effect of these ubiquitous exposures. Follow-up studies being conducted in the MIREC study platform will facilitate this type of research.

Acknowledgements

We would like to acknowledge the MIREC Study Group as well as the MIREC study participants and staff for their dedication. We would also like to acknowledge Health Canada's Chemicals Management Plan for funding the MIREC-CD Plus study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113689.

Funding sources

The MIREC-CD Plus study was funded by Health Canada's Chemicals Management Plan.

Ethics review

The study was reviewed by the Research Ethics Board at Health Canada (Ottawa, ON), the Research Ethics committee at St Justine's Hospital (Montreal, QC, Canada), and ethics committees of each participating study site. Parents signed a written informed consent form prior to their and their child's participation in this study.

References

Arbuckle, T.E., Davis, K., Marro, L., Fisher, M., Legrand, M., Leblanc, A., Gaudreau, E., Foster, W.G., Choeurng, V., Fraser, W.D., 2014. Phthalate and bisphenol A exposure among pregnant women in Canada - results from the MIREC study. Environ. Int. 68, 55–65. https://doi.org/10.1016/j.envint.2014.02.010.

Arbuckle, T.E., Fraser, W.D., Fisher, M., Davis, K., Liang, C.L., Lupien, N., Bastien, S., Velez, M.P., von Dadelszen, P., Hemmings, D.G., Wang, J., Helewa, M., Taback, S., Sermer, M., Foster, W., Ross, G., Fredette, P., Smith, G., Walker, M., Shear, R., Dodds, L., Ettinger, A.S., Weber, J.-P., D'Amour, M., Legrand, M., Kumarathasan, P., Vincent, R., Luo, Z.-C., Platt, R.W., Mitchell, G., Hidiroglou, N., Cockell, K., Villeneuve, M., Rawn, D.F.K., Dabeka, R., Cao, X.-L., Becalski, A., Ratnayake, N., Bondy, G., Jin, X., Wang, Z., Tittlemier, S., Julien, P., Avard, D., Weiler, H., Leblanc, A., Muckle, G., Boivin, M., Dionne, G., Ayotte, P., Lanphear, B., Séguin, J.R., Saint-Amour, D., Dewailly, E., Monnier, P., Koren, G., Ouellet, E., 2013. Cohort profile: the maternal-infant research on environmental chemicals research platform. Paediatr. Perinat. Epidemiol. 27, 415–425. https://doi.org/10.1111/ppe.12061.

Aylward, L.L., Hays, S.M., Smolders, R., Koch, H.M., Cocker, J., Jones, K., Warren, N., Levy, L., Bevan, R., 2014. Sources of variability in biomarker concentrations. J. Toxicol. Environ. Health B Crit. Rev. 17, 45–61. https://doi.org/10.1080/10937404.2013.864250.

Biemann, R., Navarrete Santos, Anne, Navarrete Santos, Alexander, Riemann, D., Knelangen, J., Blüher, M., Koch, H., Fischer, B., 2012. Endocrine disrupting chemicals affect the adipogenic differentiation of mesenchymal stem cells in distinct ontogenetic windows. Biochem. Biophys. Res. Commun. 417, 747–752. https://doi.org/10.1016/j.bbrc.2011.12.028.

Boas, M., Frederiksen, H., Feldt-Rasmussen, U., Skakkebæk, N.E., Hegedüs, L., Hilsted, L., Juul, A., Main, K.M., 2010. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ. Health Perspect. 118, 1458–1464. https://doi.org/10.1289/ehp.0901331.

Braun, J.M., 2017. Early life exposure to endocrine disrupting chemicals and childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13, 161–173. https://doi.org/10.1038/nrendo.2016.186.

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. https://doi.org/10.1016/j.ijheh.2014.02.005.

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

Casals-Casas, C., Desvergne, B., 2011. Endocrine Disruptors: from endocrine to metabolic disruption. Annu. Rev. Physiol. 73, 135–162. https://doi.org/10.1146/annurev-physiol-012110-142200.

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

CDC, 2009. Fourth National Report on Human Exposure to Environmental Chemicals. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf. (Accessed 14 November 2020).

Correia-Sá, L., Kasper-Sonnenberg, M., Pälmke, C., Schütze, A., Norberto, S., Calhau, C., Domingues, V.F., Koch, H.M., 2018. Obesity or diet? Levels and determinants of phthalate body burden – a case study on Portuguese children. Int. J. Hyg Environ. Health 221, 519–530. https://doi.org/10.1016/j.ijheh.2018.02.001.

Deierlein, A., Wolff, M., Pajak, A., Pinney, S., Windham, G., Galvez, M.P., Silva, M.J., Calafat, A.M., Kushi, L.H., Biro, F.M., 2016. Longitudinal associations of phthalate exposures during childhood and body size measurements in young girls. Epidemiology 27, 492–499. https://doi.org/10.1097/EDE.0000000000000489.

Den Hond, E., Govarts, E., Willems, H., Smolders, R., Casteleyn, L., Kolossa-Gehring, M., Schwedler, G., Seiwert, M., Fiddicke, U., Castaño, A., Esteban, M., Angerer, J., Koch, H.M., Schindler, B.K., Sepai, O., Exley, K., Bloemen, L., Horvat, M., Knudsen, L.E., Joas, A., Joas, R., Biot, P., Aerts, D., Koppen, G., Katsonouri, A., Hadjipanayis, A., Krskova, A., Maly, M., Mørck, T.A., Rudnai, P., Kozepesy, S., Mulcahy, M., Mannion, R., Gutleb, A.C., Fischer, M.E., Ligocka, D., Jakubowski, M., Fátima Reis, M., Namorado, S., Gurzau, A.E., Lupsa, I.R., Halzlova, K., Jajcaj, M., Mazej, D., Tratnik, J.S., López, A., Lopez, E., Berglund, M., Larsson, K., Lehmann, A., Crettaz, P., Schoeters, G., 2015. First steps toward harmonized human biomonitoring in Europe: demonstration project to perform human biomonitoring on a European scale. Environ. Health Perspect. 123, 255–263. https://doi.org/10.1289/ehp.1408616.

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. https://doi.org/10.1016/j.mce.2009.02.017.

Engle, S., Wolff, M., 2013. Causal inference considerations for endocrine disruptor research in children's health. Annu. Rev. Publ. Health 34, 139–158. https://doi.org/10.1146/annurev-publhealth-031811-124556.

Environment and Climate Change Canada. Draft screening assessment phthalate substance grouping. http://ec.gc.ca/ese-ees/default.asp?lang=En&n=516A504A-1. (Accessed 14 November 2020).

Environment and Climate Change Canada, 2015. State of the science report phthalate substance grouping short-chain phthalate esters. http://ec.gc.ca/ese-ees/default.asp?lang=En&n=51624E94-1#Toc015. (Accessed 14 November 2020).

Fisher, M., Arbuckle, T.E., MacPherson, S., Braun, J.M., Feeley, M., Gaudreau, É., 2019. Phthalate and BPA exposure in women and newborns through personal care product use and food packaging. Environ. Sci. Technol. 53, 10813–10826. https://doi.org/10.1021/acs.est.9b02372.

Fisher, M., Arbuckle, T.E., Mallick, R., Leblanc, A., Hauser, R., Feeley, M., Koniecki, D., Ramsay, T., Provencher, G., Bérubé, R., Walker, M., 2015. Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability. J. Expo. Sci. Environ. Epidemiol. 25, 231–239. https://doi.org/10.1038/jes.2014.65.

Foster, P.M.D., Cattley, R.C., Mylchreest, E., 2000. Effects of Di-n-butyl phthalate (DBP) on male reproductive development in the rat: implications for human risk assessment. Food Chem. Toxicol. 38, S97–S99. https://doi.org/10.1016/s0278-6915(99)00128-3.

Garí, M., Koch, H.M., Pälmke, C., Jankowska, A., Wesołowska, E., Hanke, W., Nowak, D., Bose-O'Reilly, S., Polańska, K., 2019. Determinants of phthalate exposure and risk assessment in children from Poland. Environ. Int. 127, 742–753. https://doi.org/10.1016/j.envint.2019.04.011.

GBD 2013 Obesity Collaboration., 2014. Global, regional and national prevalence of overweight and obesity in children and adults 1980-2013: a systematic analysis. Lancet 384, 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8.

Goodman, M., Lakind, J.S., Mattison, D.R., 2014. Do phthalates act as obesogens in humans? A systematic review of the epidemiological literature. Crit. Rev. Toxicol. 44, 151–175. https://doi.org/10.3109/10408444.2013.860076.

Gow, P.J., Ghabrial, H., Smallwood, R.A., Morgan, D.J., Ching, M.S., 2001. Neonatal hepatic drug elimination. Pharmacol. Toxicol. 88, 3–15. https://doi.org/10.1034/j.1600-0773.2001.088001003.x.

Grun, F., Blumberg, B., 2009. Endocrine disrupters as obesogens. Mol. Cell. Endocrinol. 304, 19–29. https://doi.org/10.1016/j.mce.2009.02.018.

Hao, C., Cheng, X., Xia, H., Ma, X., 2012. The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice. Biosci. Rep. 32, 619–629. https://doi.org/10.1042/BSR20120042.

Harley, K., Berger, K., Rauch, S., Kogut, K., Henn, B., Calafat, A., Huen, K., Eskenazi, B., Holland, N., 2017. Association of prenatal urinary phthalate metabolite concentrations and childhood BMI and obesity. Pediatr. Res. 82, 405–415. https://doi.org/10.1038/pr.2017.112.

Hays, S.M., Aylward, L.L., Blount, B.C., 2015. Variation in urinary flow rates according to demographic characteristics and body mass index in NHANES: potential confounding of associations between health outcomes and urinary biomarker concentrations. Environ. Health Perspect. 123, 293–300. https://doi.org/10.1289/ehp.1408944.

Health Canada, 2019. Fifth report on human biomonitoring of environmental chemicals in Canada. Results of the Canadian Health Measures Survey Cycle 5.

Heindel, J.J., Newbold, R., Schug, T.T., 2015. Endocrine disruptors and obesity. Nat. Rev. Endocrinol. 11, 653–661. https://doi.org/10.1038/nrendo.2015.163.

Hill, C.E., Myers, J.P., Vandenberg, L.N., 2018. Nonmonotonic dose–response curves occur in dose ranges that are relevant to regulatory decision-making. Dose-Response 1–4. https://doi.org/10.1177/1559325818798282.

Hurst, C.H., Waxman, D.J., 2003. Activation of PPAR alpha and PPAR gamma by environmental phthalate monoesters. Toxicol. Sci. 308, 297–308. https://doi.org/10.1093/toxsci/kfg145.

Just, A.C., Adibi, J.J., Rundle, A.G., Calafat, A.M., Camann, D.E., Hauser, R., Silva, M.J., Whyatt, R.M., 2010. Urinary and air phthalate concentrations and self-reported use of personal care products among minority pregnant women in New York city. J. Expo. Sci. Environ. Epidemiol. 20, 625–633. https://doi.org/10.1038/jes.2010.13.

Keith, S.W., Redden, D.T., Katzmarzyk, P.T., Boggiano, M.M., Hanlon, E.C., Benca, R.M., Ruden, D., 2006. Putative Contributors to the Secular Increase in Obesity: Exploring the Roads Less Traveled, pp. 1585–1594. https://doi.org/10.1038/sj.ijo.0803326.

Lewin, A., Arbuckle, T.E., Fisher, M., Liang, C.L., Marro, L., Davis, K., Abdelouahab, N., Fraser, W.D., 2017. Univariate predictors of maternal concentrations of environmental chemicals: the MIREC study. Int. J. Hyg Environ. Health 220, 77–85. https://doi.org/10.1016/j.ijheh.2017.01.001.

Maresca, M.M., Hoepner, L.A., Hassoun, A., Oberfield, S.E., Mooney, S.J., Calafat, A.M., Ramirez, J., Freyer, G., Perera, F.P., Whyatt, R.M., Rundle, A.G., 2016. Prenatal exposure to phthalates and childhood body size in an urban cohort. Environ. Health Perspect. 124, 514–520. https://doi.org/10.1289/ehp.1408750.

Mylchreest, E., Sar, M., Cattley, R.C., Foster, P.M.D., 1999. Disruption of androgen-regulated male reproductive development by di(n- butyl) phthalate during late gestation in rats is different from flutamide. Toxicol. Appl. Pharmacol. 156, 81–95. https://doi.org/10.1006/taap.1999.8643.

Poti, J., Braga, B., Quin, B., 2017. Ultra-processed food intake and obesity: what really matters for health – processing or nutrient content ? Curr. Obes. Rep. 6, 420–431. https://doi.org/10.1007/s13679-017-0285-4.

R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://R-project.org.

Renzetti, S., Gennings, C., Curtin, P., 2019. gWQS: an R package for linear and generalized weighted quantile sum (WQS) regression. https://cran.asia/web/packages/gWQS/vignettes/gwqs-vignette.pdf. (Accessed 14 November 2020).

Romano, M.E., Savitz, D.A., Braun, J.M., 2014. Challenges and future directions to evaluating the association between prenatal exposure to endocrine-disrupting chemicals and childhood obesity. Curr. Epidemiol. Rep. 57–66. https://doi.org/10.1007/s40471-014-0007-3.

Schwedler, G., Rucic, E., Lange, R., Conrad, A., Koch, H.M., Pälmke, C., Brüning, T., Schulz, C., Schmied-Tobies, M.I.H., Daniels, A., Kolossa-Gehring, M., 2020. Phthalate metabolites in urine of children and adolescents in Germany. Human biomonitoring results of the German Environmental Survey GerES V, 2014–2017. Int. J. Hyg Environ. Health 225, 113444. https://doi.org/10.1016/j.ijheh.2019.113444.

Shoaff, J., Papandonatos, G.D., Calafat, A., Ye, Z., Chen, A., Lanphear, B.P., Yolton, K., Braun, J.M., 2017. Early-life phthalate exposure and adiposity at 8 years of age. Environ. Health Perspect. 125, 097008 https://doi.org/10.1289/ehp1022.

Tanner, E.M., Bornehag, C.G., Gennings, C., 2019. Repeated holdout validation for weighted quantile sum regression. Methods 6, 2855–2860. https://doi.org/10.1016/j.mex.2019.11.008.

Tanner, E.M., Hallerbäck, M.U., Wikström, S., Lindh, C., Kiviranta, H., Gennings, C., Bornehag, C.G., 2020. Early prenatal exposure to suspected endocrine disruptor mixtures is associated with lower IQ at age seven. Environ. Int. 134, 105185 https://doi.org/10.1016/j.envint.2019.105185.

Teitelbaum, S.L., Mervish, N., Moshier, E.L., Vangeepuram, N., Galvez, M.P., Calafat, A. M., Silva, M.J., Brenner, B.L., Wolff, M.S., 2012. Associations between phthalate metabolite urinary concentrations and body size measures in New York City children. Environ. Res. 112, 186–193. https://doi.org/10.1016/j.envres.2011.12.006.

Trasande, L., Attina, T.M., Sathyanarayana, S., Spanier, A.J., Blustein, J., 2013. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ. Health Perspect. 121, 501–506. https://doi.org/10.1289/ehp.1205526.

Vafeiadi, M., Myridakis, A., Roumeliotaki, T., Margetaki, K., Chalkiadaki, G., Dermitzaki, E., Venihaki, M., Sarri, K., Vassilaki, M., Leventakou, V., Stephanou, E. G., Kogevinas, M., Chatzi, L., 2018. Association of early life exposure to phthalates with obesity and cardiometabolic traits in childhood: sex specific associations. Front Public Health 6, 1–11. https://doi.org/10.3389/fpubh.2018.00327.

van Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: multivariate imputation by chained equations in R. J. Stat. Software 45, 1–67.

Voerman, E., Santos, S., Golab, B.P., Amiano, P., Ballester, F., Barros, H., Bergström, A., Charles, M.A., Chatzi, L., Chevrier, C., Chrousos, G.P., Corpeleijn, E., Costet, N., Crozier, S., Devereux, G., Eggesbø, M., Ekström, S., Fantini, M.P., Farchi, S., Forastiere, F., Georgiu, V., Godfrey, K.M., Gori, D., Grote, V., Hanke, W., Hertz-Picciotto, I., Heude, B., Hryhorczuk, D., Huang, R.C., Inskip, H., Iszatt, N., Karvonen, A.M., Kenny, L.C., Koletzko, B., Küpers, L.K., Lagström, H., Lehmann, I., Magnus, P., Majewska, R., Mäkelä, J., Manios, Y., McAuliffe, F.M., McDonald, S.W., Mehegan, J., Mommers, M., Morgen, C.S., Mori, T.A., Moschonis, G., Murray, D., Chaoimh, C.N., Nohr, E.A., Andersen, A.M.N., Oken, E., Oostvogels, A.J.J.M., Pac, A., Papadopoulou, E., Pekkanen, J., Pizzi, C., Polanska, K., Porta, D., Richiardi, L., Rifas-Shiman, S.L., Ronfani, L., Santos, A.C., Standl, M., Stoltenberg, C., Thiering, E., Thijs, C., Torrent, M., Tough, S.C., Trnovec, T., Turner, S., van Rossem, L., von Berg, A., Vrijheid, M., Vrijkotte, T.G.M., West, J., Wijga, A., Wright, J., Zvinchuk, O., Sørensen, T.I.A., Lawlor, D.A., Gaillard, R., Jaddoe, V.W.V., 2019. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: an individual participant data meta-analysis. PLoS Med. 16, 1–22. https://doi.org/10.1371/journal.pmed.1002744.

Wang, H., Zhou, Y., Tang, C., He, Y., Wu, J., Chen, Y., Jiang, Q., 2013. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PloS One 8, e56800. https://doi.org/10.1371/journal.pone.0056800.

Wang, Y., Zhu, H., Kannan, K., 2019. A review of biomonitoring of phthalate exposures. Toxics 7, 21. https://doi.org/10.3390/toxics7020021.

Weiss, J.M., Gustafsson, Å., Gerde, P., Bergman, Å., Lindh, C.H., Krais, A.M., 2018. Daily intake of phthalates, MEHP, and DINCH by ingestion and inhalation. Chemosphere 208, 40–49. https://doi.org/10.1016/j.chemosphere.2018.05.094.

WHO, 2015. The WHO Child Growth Standards. http://www.who.int/childgrowth/standards/en. (Accessed 14 November 2020).

Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. Assessing exposure to phthalates - the human biomonitoring approach. Mol. Nutr. Food Res. 55, 7–31. https://doi.org/10.1002/mnfr.201000121.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 26, 803–824. https://doi.org/10.1111/j.1539-6924.2006.00770.x.

Yang, T.C., Peterson, K.E., Meeker, J.D., Sánchez, B.N., Zhang, Z., Cantoral, A., Solano, M., Tellez-Rojo, M.M., 2017. Bisphenol A and phthalates in utero and in childhood: association with child BMI z-score and adiposity. Environ. Res. 156, 326–333. https://doi.org/10.1016/j.envres.2017.03.038.

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. https://doi.org/10.1371/journal.pone.0104852.

Zota, A.R., Calafat, A.M., Woodruff, T.J., 2014. Temporal trends in phthalate exposures: findings from the national health and nutrition examination survey, 2001-2010. Environ. Health Perspect. 122, 235–241. https://doi.org/10.1289/ehp.1306681.