Science of the Total Environment 461–462 (2013) 386–390

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

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

Short Communication

Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age

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HIGHLIGHTS

- In a cohort study, a differential effect of prenatal phthalate exposure by gender was found.
- A negative effect among girls between MDI and HMWP was estimated.
- There was no effect between MDI and phthalates among boys.
- A positive effect between prenatal LMWP exposure and PDI was estimated for boys.

ARTICLE INFO

Article history:
Received 9 January 2013
Received in revised form 8 May 2013
Accepted 9 May 2013
Available online 5 June 2013

Editor: Adrian Covaci

Keywords:
Prenatal exposure
Biomarkers
Phthalates
Child development
Longitudinal study

ABSTRACT

Background: Previous studies suggest that prenatal phthalate exposure affects neurodevelopment and behavior during the first years of life.
Objectives: To evaluate the effect of maternal urinary concentrations of phthalate metabolites during pregnancy on mental and psychomotor development in children 24–36 months of age.
Methods: This analysis was conducted on the first three years of life among a subsample of 136 mother–child pairs from the ELEMENT cohort studies conducted in Mexico City. Maternal urine samples collected during the third trimester of pregnancy were analyzed for 9 phthalate metabolites: Mono-ethyl phthalate (MEP), Mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP), Mono-3-carboxypropyl phthalate (MCPP), and four di-2-ethylhexyl phthalate (DEHP) metabolites [mono-2-ethylhexyl-phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)]. Among the 136 children, 135 (99.3%) completed the study period. Child neurodevelopment was assessed using mental and psychomotor development indexes (MDI and PDI) from a Bayley (BSID II) test at 24, 30, and 36 months of age. The effect of prenatal phthalate exposure on neurodevelopment was estimated using linear regression models for longitudinal data clustered at the individual level.
Results: No significant associations were observed among all children combined, but differential effects by gender were found. Among girls, there was a negative association between MDI and DEHP metabolites MEHP ($\beta = -2.11$ [95% CI: −3.73, −0.49]), MEHHP ($\beta = -1.89$ [95% CI: −3.64, −0.15]), MEOHP ($\beta = -1.80$ [95% CI: −3.58, −0.03]) MECPP ($\beta = -2.52$ [95% CI: −4.44, −0.61]), and ΣDEHP ($\beta = -3.41$ [95% CI: −5.26, −1.55]); there was no significant effect among boys. Male PDI was positively related to MBzP ($\beta = 1.79$ [95% CI: 0.14, 3.45]) and MCPP ($\beta = 1.64$ [95% CI: 0.15, 3.12]); there was no significant effect on PDI among girls.

Abbreviations: BSID II, the Bayley Scales of Infant Development II; CDC, Centers for Disease Control and Prevention; CI, confidence interval; DEHP, di-2-ethylhexyl phthalate; HMWP, high molecular weight phthalates; LMWP, low molecular weight phthalates; LOD, limit of detection; MDI, Mental Development Index; PDI, Psychomotor Development Index; MBzP, mono-benzyl phthalate; MEP, Mono-ethyl phthalate; MCPP, Mono-(3-carboxypropyl) phthalate; MnBP, Mono-n-butyl phthalate; MiBP, mono-isobutyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; ΣDEHP, sum of di-2-ethylhexyl phthalate metabolites; SG, specific gravity.

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0048-9697/$ – see front matter
http://dx.doi.org/10.1016/j.scitotenv.2013.05.021

Conclusion: This study demonstrates that sex plays a role of an effect modifier in the association between prenatal phthalate exposure and neurodevelopment.

1. Introduction

Phthalates are a group of chemicals used in a wide range of industrial applications and consumer products. Higher molecular-weight phthalates, such as di(2-ethylhexyl) phthalate (DEHP), are used in flexible vinyl plastic and other consumer products, while low-molecular-weight phthalates, such as di-n-butyl phthalate (DnBP), diethyl phthalate (DEP), and butyl benzyl phthalate (BBzP) are used in pharmaceuticals and personal care products (Afshari et al., 2004; Schettler, 2006). Due to their widespread and diverse usage, detectable levels of phthalates have been found in a variety of populations worldwide including Mexico (Casas et al., 2011; Romero-Franco et al., 2011; Svensson et al., 2011). During the last decade, there has been growing concern for health risks associated with exposure to phthalates, especially in susceptible populations, such as pregnant women and children (Jurewicz and Hanke, 2011). Phthalates have been shown to cross the fetoplacental barrier with detectable levels found in human amniotic fluid and fetal circulation (Huang et al., 2009; Swan, 2008; Wittassek et al., 2009).

Phthalate metabolites measured in maternal urine during pregnancy have been associated with adverse effects on neurodevelopment and behavior of their offspring (Boas et al., 2006; Jurewicz and Hanke, 2011) and some reports suggest a sex–phthalate interaction affecting neurodevelopment (Cho et al., 2010; Engel et al., 2009; Kim et al., 2009, 2011; Whyatt et al., 2013). Potential mechanisms for these associations remain unclear.

Studies in animals and in vitro have shown that certain phthalates may act as endocrine-disrupting compounds (EDCs) that could lead to adverse reproductive and developmental effects through a number of biological pathways, ranging from effects on hormone receptors to effects on hormone synthesis, secretion, or metabolism (Boas et al., 2006; NRC, 1999). Other mechanisms, such as oxidative stress, may also play a role (Ferguson et al., 2012).

Thus, the aim of our study is to estimate the association between maternal urinary concentrations of phthalate metabolites during the third trimester of pregnancy and child mental and psychomotor development in children 24 to 36 months of age in a subsample of the *Early Life Exposure in Mexico to ENvironmental Toxicants* (ELEMENT) project in Mexico City.

2. Methods

The present study was conducted in a subsample of the ELEMENT birth cohort studies developed in Mexico City. The study comprises 1710 mother and child pair from which 646 women were recruited during prenatal visits in the first trimester of pregnancy and with the same inclusion criteria at the Mexican Institute of Social Security (1997–2003) and have been describe elsewhere (Ettinger et al., 2009). Archived third trimester urine sample was available for 136 women and their children were followed at 24, 30 and 36 months of age. One girl was excluded due to a diagnosis of Down's syndrome, resulting in a final sample of 135 children (64 boys and 71 girls). Questionnaires were administered to collect sociodemographic information such as mother's age, maternal education and marital status. Study personnel were trained to measure children's weight and height during all visits.

The research protocol was approved by the Ethics and Research Committees of all participating institutions. The study was described in detail to all participating mothers, and all study participants signed an informed consent.

Measurement of Child Development: All children were assessed for the infant's mental and psychomotor development indexes (MDI and PDI) using the Bayley Scales of Infant Development II (BSID II) at 24, 30, and 36 months of age (Bayley, 1993): from the 135 children participating in the study, we had 127, 120 and 113 kids with MDI and PDI assessments at 24, 30 and 36 months that entered in the analyses, respectively. The instructions and prompts were translated into Spanish by L.S.A., the psychologist personnel coordinator, who administered the BSID II and was also responsible for personnel standardization, training and supervision. Quality control checks were conducted by reviewing videotaped evaluations.

2.1. Measure of phthalate in urine

A spot (second morning void) urine sample was collected from each woman during her third-trimester visit to the project's research center and frozen at −80 °C. Samples were shipped on dry ice overnight to either the U.S. Centers for Disease Control and Prevention (CDC; n = 36) or the University of Michigan (n = 99), where samples were analyzed for nine phthalate metabolites: Mono-ethyl phthalate (MEP), Mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP), Mono-3-carboxypropyl phthalate (MCPP), and four di-2-ethylhexyl phthalate (DEHP) metabolites [mono-2-ethylhexyl-phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)]. Both laboratories utilized previously published methods involving enzymatic deconjugation of the metabolites from their glucuronidated form, solid-phase extraction, separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry using methods developed at CDC (Silva et al., 2007). The limits of detection (LODs) were similar for both laboratories and in the low nanogram per milliliter range for each phthalate metabolite. There was also good agreement in phthalate concentrations between laboratories among a small subset of samples that were analyzed by both labs. Isotopically labeled internal standards and conjugated internal standards were used to increase precision of measurements. Along with the unknown samples, each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentration to monitor for accuracy and precision. Analysts were blind to all information concerning subjects.

Urinary phthalate metabolite concentrations were corrected for urine dilution by specific gravity (SG) using the formula $Pc = P[(1.014 - 1)/(SG - 1)]$, where Pc is the SG-corrected phthalate metabolite concentration (micrograms per liter), P is the observed phthalate metabolite concentration, 1.014 is the median SG value among the present study population, and SG is the specific gravity of the individual urine sample. SG was measured using a handheld digital refractometer (ATAGO Company Ltd., Tokyo, Japan). Finally, we calculated (in nanomoles per milliliter) the sum of concentrations of DEHP metabolites that were measured (i.e., MEHP, MEHHP, MEOHP, and MECPP).

2.2. Statistical analysis

Since the distributions of phthalate metabolite concentrations were right-skewed, these variables were transformed by the natural logarithm (ln) to improve their linear relation with the outcome variables (MDI and PDI scores). Phthalate metabolite values below their respective LODs were assigned the value of one-half of the LOD. Descriptive statistics from mothers and kids were compared by sex of