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Phthalate exposure and child development: The Polish Mother and Child Cohort Study

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ABSTRACT

Background: Widespread phthalate exposure has prompted investigations concerning their potential adverse health effects.
Aim: The objective of this study was to evaluate the impact of pre and early postnatal phthalate exposure on child psychomotor development based on the data from the prospective Polish Mother and Child Cohort Study (REPRO PL).
Study design, subjects and outcome measures: Phthalate exposure was determined by measuring 11 phthalate metabolites (MEP, MiBP, MnBP, 3OH-MnBP, MBzP, MEHP, 5OH-MEHP, 5oxo-MEHP, OH-MiNP, oxo-MiNP, and MnOP) in the urine collected from mothers during the third trimester of pregnancy (prenatal exposure) and from their children at the 24th month of age (postnatal exposure). The analysis was performed by the HPLC–MS/MS method. Psychomotor development was assessed in children at the age of 2 years by the Bayley Scales of Infant and Toddler Development.
Results: Child motor development was inversely associated with natural log concentrations (μg/g creatinine) of 3OH-MnBP ($\beta = -2.3$; 95% CI −4.0 to −0.6), 5OH-MEHP ($\beta = -1.2$; 95% CI −2.2 to −0.3), 5oxo-MEHP ($\beta = -1.8$; 95% CI −3.3 to −0.2) and sum of DEHP metabolites ($\beta = -2.2$; 95% CI −3.6 to −0.8), DnBP metabolites ($\beta = -1.9$; 95% CI −3.4 to −0.4), and high molecular weight phthalates ($\beta = -2.5$; 95% CI −4.1 to −0.9) in the urine collected from mothers during pregnancy after adjustment for a variety of potential confounders. Additional adjustment for postnatal phthalate exposure did not change the results. Postnatal child exposure to phthalates was not associated with any of the measured scores of child psychomotor development.
Conclusions: The study findings add further support to the possibility that prenatal phthalate exposure may be detrimental to child neurodevelopment and underscore the importance of policies and public health interventions aiming at reduction of such exposure.

1. Introduction

Phthalates are a widely used class of chemicals that are dialkyl- or alkylarylesters of 1,2-benzenedicarboxylic acid [1]. They are divided into two distinct groups, with different applications, toxicological properties and classifications. High molecular weight phthalates (High-MWP) such as di(2-ethylhexyl) phthalate (DEHP) or di-iso-nonyl phthalate (DiNP) are added to plastics to enhance their flexibility and durability. Typical products containing these phthalates include floorings, roofings, wall coverings and cables, clothing, packaging materials, toys, furniture and car upholstery. Low molecular weight phthalates (Low-MWP) including diethyl phthalate (DEP) and di-n-butyl phthalate (DnBP) are used in adhesives, detergents, and solvents and are present in some medicines (tablet coating, capsules), personal-care products, cosmetics, fragrances and nail polish [1,2]. Humans are exposed to these compounds via multiple pathways — through food and water (oral), through air (inhalation) and through care products (dermal) [1]. Phthalates are rapidly metabolized to monoesters and can be further oxidized to oxidative metabolites. Urinary phthalate metabolites have been used extensively as biomarkers of human exposure [1,3].

Widespread exposure to phthalates has prompted investigations concerning their potential adverse health effects. Fetal exposure is of particular concern because phthalate metabolites cross the placenta and have been found in amniotic fluid, placental tissue, cord blood and neonatal meconium [1,4–7]. Experimental animal studies have indicated their adverse reproductive and developmental effects and suggest that phthalates may have endocrine-disrupting properties [8]. In humans, prenatal exposure to phthalates has been associated with poor birth outcomes, reduced anogenital distance in boys, neurological

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and behavioral problems, reduced masculine play in boys and social impairment in childhood [9–20]. In addition, reproductive, respiratory, metabolic and thyroid effects in children as well as in adults have been reported [20].

The aim of this study was to evaluate the impact of pre and early postnatal phthalate exposure on child psychomotor development.

2. Materials and methods

2.1. Study design and population

The present study was a part of the Polish Mother and Child Cohort (REPRO_PL, www.repropl.com), a multicenter prospective cohort study of environmental factors contributing to pregnancy outcomes, children's health and neurodevelopment, established in 2007. The mothers' recruitment and mothers–children follow-up procedures have been previously published [21,22]. Briefly, women were recruited during the first trimester of pregnancy at maternity units or clinics in selected regions of Poland if they fulfilled the following inclusion criteria: single pregnancy up to 12 weeks of gestation, no assisted conception, no pregnancy complications and no chronic diseases as specified in the study protocol. The women were interviewed 3 times during the pregnancy (once in each trimester) in order to collect and update demographic and socio-economic data, medical and reproductive history, and information about occupational exposure and lifestyle factors. After delivery, detailed information regarding pregnancy outcomes and children's health was obtained. Two years after birth, assessment of exposure, health status as well as neurodevelopment of each child was performed by a pediatrician as well as a psychologist/child development specialist. At that time also information concerning socio-demographic, environmental and lifestyle factors has been updated by conducting interviews with mothers.

Current analysis was restricted to 165 children from Lodz (central Poland) district.

The study was approved by Ethical Committee of the Nofer Institute of Occupational Medicine, Łódź, Poland (Decision Nos. 7/2007 and 3/2008) and a written consent was obtained from all the subjects.

2.2. Assessment of children's prenatal and postnatal exposure to phthalates and tobacco constituents

Prenatal phthalate exposure was determined by measuring phthalate metabolites in the urine collected from the mothers during the third trimester of pregnancy (range 30–34 weeks) while postnatal exposure was determined by measuring phthalate metabolites in the urine from children at around the 24th month of age (range 23–28 months). Spot urine samples were collected into polypropylene cups and were stored at −20 °C until the analysis, which was performed at the Nofer Institute of Occupational Medicine (NIOM), Lodz, Poland. A portion of each sample (1 ml) was analyzed. The following 11 phthalate metabolites were measured: a) five Low-MWP: monoethyl phthalate (MEP) (metabolite of diethyl phthalate; DEP), mono-iso-butyl phthalate (MiBP) (metabolite of di-iso-butyl phthalate; DiBP), mono-n-butyl phthalate (MnBP) (metabolite of di-n-butyl phthalate; DnBP), 3OH-mono-n-butyl phthalate (3OH-MnBP) (secondary metabolite of DnBP), and monobenzyl phthalate (MBzP) (metabolite of butyl-benzyl phthalate; BBzP), and b) six High-MWP: mono(2-ethylhexyl) phthalate (MEHP) (metabolite of di(2-ethylhexyl) phthalate; DEHP), 5OH-mono(2-ethylhexyl) phthalate (5OH-MEHP) and 5oxo-mono(2-ethylhexyl) phthalate (5oxo-MEHP) (secondary metabolites of DEHP), 7OH-mono-methyloctyl phthalate (OH-MiNP) and 7oxo-mono-methyloctyl phthalate (oxo-MiNP) (secondary metabolites of di-iso-nonyl phthalate; DiNP), and mono-n-octyl phthalate (MnOP) (metabolite of di-n-octyl phthalate; DnOP).

Molecular concentrations of MnBP and 3OH-MnBP were summarized as total DnBP; MEHP, 5OH-MEHP and 5oxo-MEHP as total DEHP and OH-MiNP and oxo-MiNP as DiNP.

For quality control, the ICI "P" and ICI "S" control urine samples (obtained from COPHES WP3) were used.

MEP, $^{13}C_4$-MEHP, 5OH-MEHP, 5oxo-MEHP, MnBP, MBzP, MnOP, MiBP and internal standards labeled with $^{13}C_4$: $^{13}C_4$-MEP, $^{13}C_4$-MEHP, $^{13}C_4$-5OH-MEHP, $^{13}C_4$-5oxo-MEHP, $^{13}C_4$-MnBP, $^{13}C_4$-MBzP and $^{13}C_4$-MOnP, were purchased from Cambridge Isotopes Laboratory — CIL.

Additional standards: OH-MiNP, oxo-MiNP, 3OH-MnBP and deuterium labeled internal standards D_4-OH-MiNP, D_4-oxo-MiNP, D_4-MiBP and D_4-3OH-MnBP were the kind gift of dr Holger Koch from the Institut für Prävention und Arbeitsmedizin der Deutschen Gesetzlichen Unfallversicherung, Institut der Ruhr-Universität-Bochum (IPA).

Acetonitrile of LC–MS purity, water — Milli-Q purity, acetic acid and ammonium acetate (99%) were purchased from J.T. Baker. Whereas, β-Glucuronidase – *Escherichia coli* K12 – EC 3.2.1.31 was purchased from ROCHE and an ISOLUTE C18 96-well Plate was purchased from Biotage.

Calibration — 6 calibration points, concentration range — from 0.4 to 500 μg/l, correlation coefficients — between 0.987 and 0.9985 for OH-MiNP and 3OH-MnBP respectively.

Limit of quantification (LOQ) was 0.1 μg/l for the following metabolites: MEHP, MBzP, oxo-MiNP, MiBP, and MnOP and 0.4 μg/l for: MEP, 5OH-MEHP, 5oxo-MEHP, OH-MiNP, MnBP, and 3OH-MnBP. Limit of detection, (LOD) for each of the metabolites is presented in Table 2. Concentrations of phthalate metabolites below LOD were imputed with a value equal to ½ LOD.

Stock solutions of each single native and labeled standards were prepared by diluting with 50% acetonitrile to a concentration of 10 μg/ml. Mixtures of all the native standards as well as mixtures of both $^{13}C_4$ and D_4-labeled standards were prepared in 10% acetonitrile. Calibration samples were prepared in 10% acetonitrile by diluting working solutions of the native standards to final concentrations of 0.4, 2, 10, 50, 250 and 500 μg/l.

Before chemical analysis all the samples were deconjugated using enzymatic hydrolysis with β-Glucuronidase — *E. coli* K12 (pH 6.5) and purified by SPE on an ISOLUTE C18 96-well Plate (Biotage).

Phthalate metabolites in the urine were determined by the use of a HPLC–MS/MS (Waters Alliance 2695 HPLC-Waters QuattroMicro API tandem mass spectrometer) with negative electrospray ionization.

Chromatographic separation was performed on a 2.5 μm 3.0 × 100 mm XSelect CSH Ph-Hexyl (Waters) column with a flow rate of 0.25 ml/min and gradient mode of A — acetonitrile, B — water: 1–8 min 10% A; 8–15 min 92% A; and 18 min 10% A.

Cone and collision energy were optimized for all the analytes – native and labeled ones – for two parent–daughter transmissions (quantitative and confirmation of identity of compounds).

For prenatal exposure the urinary phthalate metabolite concentrations were corrected for dilution by the urinary creatinine concentration. Creatinine concentration was measured using the Jaffe static method with a working range of 0.05–5.00 g creatinine/l. Only urine samples with creatinine values between 0.2 and 3.0 g/l were considered. As creatinine in children is more of a function of the stage of physical development rather than urine dilution, creatinine correction was not performed for phthalate assessment in the child population [23–25].

Out of 165 eligible urine samples from the mothers the creatinine assessment was not performed for 5 samples, and for 10 samples creatinine level was below the selected value. The urine samples for phthalate metabolites measurement were not available in the case of 17 children.

Prenatal exposure to tobacco constituents was assessed based on the cotinine level in saliva collected from the women during the third trimester of pregnancy. Child exposure to environmental tobacco smoke (ETS) was assessed based on the cotinine level in the urine collected during the visit scheduled for assessment of child health and neurodevelopment. The cotinine level in saliva and urine was analyzed