Prenatal and early postnatal phthalate exposure and child neurodevelopment at age of 7 years – Polish Mother and Child Cohort

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Abstract

Phthalates are among of the most frequently investigated environmental chemicals influencing children's health and particularly their neuropsychological development. However, the reported effects of these compounds on child behavior, cognitive and psychomotor outcome are not fully consistent. The aim of this study is to evaluate the associations between prenatal and early postnatal phthalate exposures and child neurodevelopment at age of 7 years. A total of 134 mother-child pairs from Polish Mother and Child cohort (REPRO_PL) constitute the basis for current analysis. Eleven phthalate metabolites were measured in urine samples collected from mothers in the 3rd trimester of pregnancy and from children at the age of 2 years. Child neuropsychological development at early school age (7 years) was assessed by both the Strengths and Difficulties Questionnaire (SDQ) filled by mothers and the Polish adaptation of the Intelligence and Development Scales (IDS) performed by psychologists. MEP concentration during pregnancy was significantly associated with increased risk of peer relationship problems in SDQ (OR=2.7, p=0.03). The concentration of two phthalate metabolites (oxo-MEHP and MiBP) in the urine collected from mothers during pregnancy, as well as MnBP concentration at age of 2 years were of borderline significance on prosocial behaviour and hyperactivity/inattention problems, respectively (OR=2.6, p=0.08; OR=2.2, p=0.07; OR=2.6, p=0.08). The results of the IDS analyses focused on child's cognitive and psychomotor development are not fully conclusive. Negative associations were evident between some phthalates in early childhood period and fluid intelligence and cognition (MEP: β=-5.2; p=0.006; β=-4.2; p=0.006; MnBP: β=-4.9; p=0.03; β=-4.0; p=0.03; respectively) and psychomotor skills (OH-MiNP: β=-1.2; p=0.07), while positive associations have been found in the prenatal period (MEP: β=2.9; p=0.07 for cognition; β=1.4; p=0.06 for language skills; oxo-MEHP: β=3.6; p=0.03 for fluid intelligence; β=2.9; p=0.03 for cognition; β=1.2; p=0.08 for psychomotor development). Further studies are required in order to elucidate which are the most critical periods of phthalate exposure on children's neurodevelopmental outcomes.

Keywords: Behaviour; Neurodevelopment; Children; Phthalates; Prenatal and early postnatal exposure.

Highlights:

- Prenatal and early postnatal phthalate exposure is associated with neurodevelopment.
- Prenatal MEP concentrations are associated with peer relationship problems.
- Postnatal MEP and MnBP concentrations are inversely associated with cognition.
- Further studies are needed to understand the most critical periods of exposure.

1. **Introduction**

Child neurodevelopment is a multi-causal phenomenon, influenced by both genetic and environmental exposures as well as interactions between them. Among the variety of environmental factors to which children are exposed, phthalates are the most frequently investigated ones. They are widely used as plasticizers and additives in a variety of products, including personal care products, plastic containers, toys, building materials, medical devices and food packaging (NRC, 2008). These chemicals are rapidly metabolized in the human body and eliminated in urine within hours after exposure (Heudorf et al., 2007; Wittassek and Angerer, 2008). Thus urinary concentrations of phthalate metabolites are a good indicator of the internal exposure (Calafat et al., 2015).

The brain is particularly vulnerable *in utero* as well as during infancy and early childhood, and insults occurring during these critical periods have the potential to cause long-term damage. A growing body of literature is evaluating the associations between prenatal and postnatal phthalate exposures and a wide spectrum of adverse neurodevelopmental outcomes. However, reported effects are not fully consistent across the different phthalate compounds, the neuropsychological domains implicated and the sex-specificity of the effects (Bellinger, 2013; Miodovnik et al., 2014; Ejaredar et al., 2015; Katsikantami et al., 2016; Vrijheid et al., 2016; Braun, 2017**;** Lee et al., 2018; Bornehag et al., 2018; Zhang et al. 2019). Among the large amount of the existing studies in this field only few are based on prospective study design that take into account more than a single time point for exposure assessments as well as multiple phthalate metabolites and neuropsychological outcomes.

Neuropsychological assessment around school age revealed a wide range of adverse effects of the different phthalate classes on social competencies, peer relationships, perceptual reasoning, working memory, activity levels and attention (Zhang et al. 2019).

According to epidemiological studies, experimental studies on rodents showed that prenatal exposure to phthalates induce neurobehavioural abnormalities including anxiety, impairment in social behaviour and cognition (Barakat et al., 2018; Kougias et al., 2018; Wang et al., 2016). In parallel to the behavioural changes, several mechanisms of toxicity have been described in offspring exposed prenatally to a single parent compound (mainly DEHP, di(2-ethylhexyl) phthalate; DBP, dibutyl phthalate) including endocrine effects, disruption of

metabolic pathways and interference with neuronal maturation and neurotransmission in specific brain areas (Miodovnik et al 2014).

A previously published analysis based on data from the Polish Mother and Child cohort (REPRO_PL) indicated a rather low phthalate exposure level in prenatal (3rd trimester of pregnancy) and early postnatal (at age of two years) periods, in comparison with later stages (Polańska et al., 2014; Garí et al., 2019). Despite low exposures, child motor development at age of two years was inversely associated with urinary concentrations of several phthalate metabolites measured in their mothers during pregnancy (Polańska et al., 2014). By contrast, postnatal children's exposure to phthalates was not associated with any of the measured scores of child psychomotor development. This is of particular interest, since the consequences of prenatal or early postnatal exposures may not be observed or reliably diagnosed within the first two years of life but can appear in later age. It is also of interest if the consequences observed within the first two years of life still persist until school age or they are compensated by other factors.

Thus the aim of the study was to evaluate the associations between prenatal and early childhood phthalate exposure and child neurodevelopment at the age of 7 years.

2. Materials and methods

2.1 Study population

The current study is based on data from Polish Mother and Child Cohort (REPRO_PL) which has been described in details elsewhere (Polańska et al., 2009; 2011; 2016a). Briefly, this prospective birth cohort comprises the following phases (from which data relevant for current analyses have been selected): pregnancy period (prenatal phthalate exposure and relevant covariates), early childhood period (phthalate exposure at age of 2 years and relevant covariates), and early school age period (child neurodevelopmental assessment at age of 7 years and relevant covariates). The study was approved by the Ethical Committee of the Nofer Institute of Occupational Medicine, Lodz, Poland (Decision No. 7/2007; No. 3/2008 and No. 22/2014). Written informed consent was obtained from the pregnant women and the children's parents for the procedures within each phase of the study.

Data regarding phthalate exposure were available for 150 samples collected in prenatal period and 148 samples collected from children at 2 years of age (Polańska et al., 2014). A total of 134 children (89%) evaluated at the age of 7 years for neurodevelopmental outcomes constitute the sample included in the current analysis.

2.2 Prenatal and early childhood phthalate exposure assessment

Prenatal and early childhood phthalate exposures were determined by measuring 11 phthalate metabolites in urine samples: mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), 3OH-mono-n-butyl phthalate (OH-MnBP), mono-benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (OH-MEHP), mono-2-ethyl-5-oxo-hexyl phthalate (oxo-MEHP), 7-OH-mono-methyloctyl phthalate (OH-MiNP), 7-oxo-mono-methyloctyl phthalate (oxo-MiNP), mono-n-octyl phthalate (MnOP).

The samples were collected from mothers during the third trimester of pregnancy and from children at the age of 2 years, and analyzed by high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). The detailed description of the methodology, including limits of detection (LOD) for each phthalate metabolite, has been published elsewhere (Polańska et al., 2014; 2016b). Creatinine concentrations in urine collected from pregnant women were measured using the Jaffe static method with a working range of 0.05–5.00 g creatinine/l.

2.3 Neurodevelopmental assessments

2.3.1 Child behavior based on Strengths and Difficulties Questionnaire (SDQ)

Health and neurodevelopmental assessment has been scheduled when the child achieved 7 years of age. During the visit at pediatric clinic mothers filled in the Strengths and Difficulties Questionnaire (SDQ), which is a widely used tool for evaluation of the child behaviour (www. sdqinfo.com, parent reported) (Goodman and Scott, 1997). SDQ is a 25-item questionnaire that consists of four subscales measuring mental health problems (conduct problems, emotional symptoms, peer relationship problems, hyperactivity-inattention problems) and one subscale measuring strengths (prosocial behaviour) (Duinhof et al., 2019).

For each of the statements describing child behavior three response categories are possible: “not true”, “somewhat true”, and “certainly true” (with the scoring 0-2). That results in the final scoring for subscales that range from 0 to 10. All four subscales measuring mental health problems were summed as total difficulties score (ranging from 0 to 40). Higher total, emotional, conduct, hyperactivity and peer SDQ scores indicate higher difficulties, whereas for prosocial scores the opposite interpretation needs to be applied. For each scales the following cut-offs were used: total difficulties: 0–13 were defined as normal, 14–16 as borderline, and 17–40 as clinical; conduct as well as peer relationship problems: 0–2 = normal, 3 = borderline, 4–10 = clinical; emotional problems: 0–3 = normal, 4 = borderline, 5–10 = clinical; hyperactivity/inattention: 0–5 = normal, 6 = borderline, 7–10 = clinical; prosocial behaviour: 6–10 = normal, 5 = borderline, 0–4 = clinical (www. sdqinfo.com, parent reported).

2.3.2 Child cognition and psychomotor development based on Intelligence and Development Scales (IDS)

Child cognitive and psychomotor development at the age of 7 years was assessed by trained psychologists using a Polish adaptation of the Intelligence and Development Scales (IDS) (Grob et al., 2009). The IDS allows for assessment of general intellectual ability (fluid and crystallized intelligence) and six developmental domains. In the current study the following domains were evaluated: cognition, mathematical skills, language skills and psychomotor skills. Reliability for fluid and crystallized intelligence equals 0.94, and for the full scale 0.96. The correlations with analogous Wechsler Intelligence Scale for Children (WISC-R) are about 0.80. (Jaworowska et al., 2012).

2.4 Confounding factors included in the models

The following potential confounding factors were evaluated: child’s sex and age at examination, parental age (at child birth) and educational levels (years of completed education at child evaluation at age of 7 years: ≤12, >12), socio-economic status (SES) of the family (poor/very poor, good, very good), household status (parents living together, single parent household), place of residence (urban, rural), number of siblings (0, ≥1), child age when he/she have started school education (at age 6 or 7 years), traumatic events including death of family member (yes, no), maternal pre-pregnancy body mass index (BMI), prenatal (with 10

ng/ml as cut-off point for cotinine level in maternal saliva) and postnatal (with 2.1 ng/ml as cut-off point for cotinine level in child urine collected at examination) tobacco smoke exposure (as described by Polańska et al., 2017; Lupsa et al., 2016), child BMI (based on height and weight measured by trained staff at child examination), gestational age and birth weight (from medical records collected after birth), breastfeeding duration (0-2 months, 2-6 months, >6 months). As child cognitive and psychomotor development was assessed by 7 psychologists, the evaluator was also included in the final model. Details regarding the covariates are presented in tables S1 and S2.

2.5 Statistical analysis

Data analyses were performed using the Statistical Package for Social Science (SPSS) version 25.

The following descriptive statistics have been calculated: medians, arithmetic means, standard deviations and 95% confidence intervals (CI) for continuous variables; absolute frequencies and percentages for categorical variables. Statistical differences among subgroups of children based on socio-demographic variables were tested for significance using the Chi-square test for categorical outcomes, t student's test and one-way analysis of variance for quantitative outcomes.

Multivariate regression analyses were used to assess the association of sociodemographic/lifestyle related covariates and phthalate concentrations with the scores of the SDQ (logistic regression) and IDS (linear regression) scales. In the logistic regression for the SDQ scales, the subjects were dichotomized in normal vs borderline/clinical (http://www.sdqinfo.com/).

The metabolites that were found in above the LOD in less than 70% of the samples (e.g. MEHP, oxo-MiNP and MnOP in mothers and children, and MBzP in mothers) were not included in multivariate regression models. Before inclusion in the models, phthalate concentrations were log10 transformed. The models were adjusted by both prenatal and postnatal phthalate concentrations.

The other covariates to be included in the regression models were selected based on their significant association to at least one outcome, or considered relevant in the statistical analyses of psychosocial epidemiological studies. For SDQ logistic regression covariates were as follow: child's sex, child's age at examination, birth weight, SES, maternal educational level, prenatal and childhood tobacco smoke exposure, breastfeeding duration and maternal BMI. For IDS linear regressions, the final model included the following covariates: child's sex, child's age at examination, maternal educational level, place of residence, birth weight, prenatal and childhood tobacco smoke exposure and psychologist who have performed child examination.

For SDQ logistic regression, Odds Ratio (OR) are shown, while for IDS linear regression, beta coefficients (β) are presented in the Tables.

3. Results

3.1 Characteristics of the study population

Most of the study population was from urban areas (89%) (Tables S1 and S2). 55% of the children were girls and similar percentages of children (56%) have started school attendance at age of 7 years. A high proportion of the children had mothers with university degree (60%) and lived in families declaring good or very good SES (92%). Based on cotinine level in biological samples, about 11% of the children were classified as exposed to tobacco smoke in the prenatal period, and 30% at the age of 7 years.

Details regarding phthalate exposure in the prenatal period (3rd trimester of pregnancy) and at the age of 2 years has been previously published (Polańska et al., 2014). Five out of 11 phthalate metabolites were quantifiable in more than 80% of the samples collected from mothers and their children. In addition, 90% and 95% detection rate was found for OH-MEHP in child urine and for oxoMEHP in maternal urine, respectively. For both prenatal and early childhood, MEP was the phthalate metabolite with the highest median concentration (Table 1).

The description of child behavioural problems (based on SDQ scales) is presented in Table 2. About 18% of the studied children were in borderline or clinical range for total difficulties scale. Similar percentages were observed for peer relationship problem. High proportion of

the children were identified as having conduct (32%), hyperactivity/inattention (27%) and emotional problems (25%). For the scores on prosocial behaviour, 9% of the children were classified in the borderline or clinical range.

Intellectual efficiency and psychomotor skills (IDS) of the studied children are presented in Table 3. Although a considerable variability in individual test scores were observed, mean values indicate that the children are within the normal range.

3.2 Phthalate exposure and behavioral development (based on SDQ)

The results of multivariate logistic regression models for the association between phthalate metabolites concentrations in prenatal and early childhood period and child behavioural development at the age of 7 years are presented in Table 4. Prenatal MEP concentration was significantly associated with increased risk of peer relationship problems (OR=2.7, p=0.03). oxo-MEHP and MiBP concentrations in pregnant mothers (prenatal exposure) was associated with prosocial behaviour, while early childhood MnBP concentration (postnatal exposure) was related to hyperactivity/inattention problems, all the associations with borderline significance (OR=2.6, p=0.08; OR=2.2, p=0.07; OR=2.6, p=0.08; respectively).

3.3 Phthalate exposure and cognitive and psychomotor development (based on IDS)

The analysis focusing on the impact of prenatal and early postnatal phthalate exposure on child cognitive and psychomotor development indicates conflicting results (Table 5). While the exposure in prenatal period was positively associated with children's intellectual abilities, early childhood exposure was negatively associated with intellectual development at the age of 7. In details, concentrations of oxo-MEHP in maternal urine was positively associated with fluid intelligence (β=3.6; p=0.03) and cognition (β=2.9; p=0.03). The following other associations were of borderline significance: oxo-MEHP with psychomotor development (β=1.2; p=0.08), MEP with cognition (β=2.9; p=0.07) and language skills (β=1.4; p=0.06). Increased urinary concentrations of MEP and MnBP in children at age of 2 years were associated with lower scores in fluid intelligence and cognition (MEP: β=-5.2; p=0.006; β=-4.2; p=0.006; MnBP: β=-4.9; p=0.03; β=-4.0; p=0.03; respectively). In addition, early postnatal exposure to OH-MiNP is identified with lower scores on psychomotor skills (β=-

1.2; p=0.07). There were no impact of any analyzed phthalate metabolite on child crystallized intelligence and mathematical skills.

4. Discussion

The current analysis, based on a prospective mother-child cohort study, showed that MEP concentration during the 3rd trimester of pregnancy was associated with increased risk of peer relationship problems at early school age, with statistically significant results. Furthermore negative associations were found between urinary MEP and MnBP concentrations in early childhood period (children at 2 years of age) and fluid intelligence and cognition. On the other hand, the results showed a positive effect of prenatal oxo-MEHP concentrations on child intelligence and cognition.

The concentrations of phthalate metabolites' in prenatal and early postnatal periods have been thoroughly discussed and compared in our previous publications (Polańska et al., 2014; Garí et al., 2019). In this regard, it should be pointed out that the Polish children population is characterized by rather low exposure levels in both prenatal and early postnatal periods (3rd trimester of pregnancy and at the age of two years, respectively) with MEP representing the phthalate metabolite with a higher median concentration. The low level of exposure found in this population could be explained by sociodemographic and lifestyle related characteristics, as well as other external factors, such as legislative measures against the use of certain phthalates in selected products.

Prenatal MEP concentrations found in the studied population were associated with increased risk of peer relationship problems. The existing studies, although not fully conclusive, report that prenatal exposures to phthalates might be associated with Attention Deficit and Hyperactivity Disorder (ADHD), autism spectrum disorder (ASD) and other specific behavioural problems (Bellinger, 2013; Miodovnik et al., 2014; Ejaredar et al., 2015; Katsikantami et al., 2016; Vrijheid et al., 2016; Braun, 2017**;** Lee et al., 2018; Bornehag et al., 2018,). Our findings are in agreement with the Mount Sinai Children's Environmental Health study in the US where prenatal phthalate exposure was associated with childhood social impairment at age of 7-9 years (Miodovnik et al., 2011**)**. Another study reported an association between prenatal

exposure to low-molecular weight phthalates (LMWP) and poorer parent-related behavioural and executive functioning profiles in children between 4 and 9 years of age (Engel et al., 2010). Data from the EDEN cohort performed in France (in this selected analysis limited to boys only) also confirmed that some phthalates (in fact, the same as in our analysis: MnBP, MBzP, MEP and DEHP) were positively associated with SDQ scores or increased the risk of specific behavioural problems at age of 3 and/or 5 years, including internalizing behaviour, peer relationship problems, and emotional symptoms (Philippat et al., 2017). In another study conducted in the US, exposure to certain phthalates (namely MiBP, MBzP and DEHP) in late pregnancy was associated to behavioural problems in boys, including inattention, rule-breaking behavior, aggression, conduct problems and oppositional behavior, while among girls, exposure to MBzP was related to reduced anxiety scores (Kobrosly et al., 2014). A very recently published longitudinal study (with the assessment of urinary concentrations of 7 phthalate metabolites in women at the 3rd trimester of pregnancy and in children between 2 and 8 years of age) has shown that prenatal exposures were associated with higher internalizing and externalizing problems, as well as delinquent behaviour scores at pre-adolescence and adolescence (8-14 years of age). Furthermore, children's urinary MBzP levels were associated with higher scores of social problems (Huang et al., 2019). In accordance, the analysis performed in Taiwant indicated that externalizing problems, delinquent behavior and aggressive behaviour scores were directly related to increased concentrations of several phthalates in maternal urine (Lien et al., 2015). Conversely, the Spanish INMA-Sabadell birth cohort, which assessed several phthalate metabolites in the 1st and 3rd trimesters of pregnancy, reported that phthalate exposures do not adversely affect behavioural development in children up to 7 years of age. The risk of certain ADHD symptoms and behavioural problems was even reduced with increasing DEHP and MEP concentrations in the prenatal period (Gascón et al., 2015).

Our results focusing on child's cognitive and psychomotor development by the IDS scale are not fully conclusive. We found negative effects of some phthalate metabolites (MEP, MnBP) in early childhood period and positive effects of prenatal oxo-MEHP on fluid intelligence and cognition. In agreement with our observations, the results of other studies in this field are also not consistent.

In a recent study, evaluating phthalate exposure during pregnancy and several times in childhood period, higher prenatal exposure to specific phthalates was associated with lower motor function among 11- year old girls while higher postnatal exposure to DEHP metabolites was

associated with lower scores among boys (Balalian et al., 2019). The negative impact of prenatal exposure to several phthalates on child motor functions at age of 2 years was observed in our previous analysis (based on a subsample of the REPRO_PL cohort) (Polańska et al., 2014). However, these associations were not confirmed at the age of 7 years.

In another study performed on the US, maternal prenatal urinary metabolite concentrations of DnBP and DiBP measured in late pregnancy were associated with deficits in children's intellectual development at age 7 years (Factor-Litvak et al., 2014). Conversely, results from the above-mentioned Spanish INMA-Sabadell birth cohort suggest that prenatal phthalate exposure does not adversely affect children's cognitive and psychomotor development (Gascón at al., 2015). In a study performed in Taiwan, with phthalate metabolites assessed in pregnancy period and in children at several follow-up visits, and children's neurocognitive functions and intelligence evaluated at four different time points, no significant associations between maternal phthalate exposure and the children's IQ scores were found. The authors concluded that exposure to phthalates during early childhood but not during the prenatal period is associated with decreased cognitive development in young children (Huang et al., 2015). Similar results were obtained by Kim et al. (2017), who pointed out that children's phthalate exposure, but not maternal exposure, has an adverse effect on IQ and attentional performance. These associations were found to be independent from each other (Kim et al., 2017).

A systematic review of the literature including studies until year 2014 suggests that elevated prenatal and childhood concentrations of phthalate metabolites are associated with cognitive and behavioural outcomes in children below 12 years of age (Ejaredar et al., 2014). The neurodevelopmental outcomes related to phthalate exposures mentioned in this review included lower IQ, problems with attention and hyperactivity, as well as poorer social communication. In addition, sex-specific differences in cognitive and behavioural functions were revealed, with a higher amount of studies pointing out adverse outcomes among boys than girls. A more recent systematic review and meta-analysis pointed out significant associations between the concentrations of DEHP metabolites and the neurodevelopmental outcomes in children among cross-sectional results, and significant association between DEHP exposure in the prenatal period and the psychomotor outcomes measured later in childhood (Lee et al., 2018). The aforementioned reviews highlighted a number of inconsistencies in the literature, which might be explained by several reasons, such as different levels of exposure among the studies, the potential exposure misclassification, varying mixtures of phthalates, the timing of the exposure measurement, the diverse behavioural functions assessed as well as the use of different

tools to measure these functions, and the genetic background of the populations and other unknown factors.

Some positive associations observed for prenatal oxo-MEHP and child cognitive development may result from not controlled confining factors (although we evaluated many potential covariates related to maternal and child characteristics) or due to chance and they should therefore be interpreted with caution.

The biological mechanisms underlying the adverse effects of prenatal phthalate exposure on neurobehavioural development are still unclear. Phthalates may perturb multiple downstream physiologic processes including thyroid hormones homeostasis, calcium signaling, lipid metabolism, and steroid receptor activity (Miodovnik et al., 2014). Phthalates easily cross the placenta (Mose et al., 2007), and thus the gestational period is a critical window for their endocrine- and metabolic-disrupting effects. Additionally, considering the important role of hormones (e.g. both steroid and thyroid hormones) in brain and synaptic development, it is not surprising that levels of urinary phthalate metabolites during pregnancy have an impact on children's social abilities (Miodovnik et al., 2011; Ejaredar et al., 2015; Huang et al. 2017) and executive function (Engel et al., 2010; Lien et al., 2015; Kobrosly et al., 2014). Moreover, a large body of animal data indicate that different phthalates induce hyperactivity in rats, likely through their well described effects on dopaminergic neurotransmission (Wang et al., 2016; Tanida et al. 2009). Moreover, the association between phthalate exposure, neurobehavioural development and cognitive functions may be also ascribed to interference the homeostasis of thyroid hormones which are important modulators of brain development (Ghisari and Bonefeld-Jorgensen 2009; Boas et al., 2010; Johns et al., 2016). Altogether the wide range of behavioural perturbations described in animal and human studies are likely the result of interference with multiple endocrine and neurochemical pathways implicated in brain development. In such complex framework, there is still a considerable degree of uncertainty concerning the vulnerable time windows to phthalate toxicity in neurodevelopment. Conflicting results when comparing the consequences of prenatal vs postnatal exposure on later neurobehavioural outcome should be seen in this perspective.

The prospective study design of the current analysis constitutes the main strength of the study. Despite the significant scientific interest in the impact of phthalates on child neurodevelopment, only few studies are in fact based on several time points of exposure, with

assessments in both prenatal and early postnatal periods. In this regard, our analyses have considered the two periods, which are described as the most critical ones for early and long-term neuropsychological consequences. It needs to be pointed out that the exposure assessment to phthalates was also performed at age of 7 years in a sub-sample of the children from the REPRO_PL cohort (Garí et al., 2019). Unfortunately, mother-child pairs data on phthalate metabolites available for each three time points (prenatal, at the age of 2 and at the age of 7 years) were not fully comparable and thus, it was not possible to include such data in the current analysis. The second strength of the study is related to the extensive set of phthalate metabolites assessed (11 phthalate metabolites indicating exposure to 7 phthalates) comparing to few (most frequently DEHP metabolites) evaluated in other epidemiological studies (Vrijheid et al., 2016; Braun, 2017; Lee et al., 2018). Furthermore, our analysis has considered a broad spectrum of child neurodevelopment, including behavioural, cognitive and psychomotor outcomes, performed by SDQ (one of the most used test for child behavioural assessments) and IDS (that has high correlation with the analogous WISC-R scales, Jaworowska et al., 2012). Finally, a variety of confounding factors, evaluated prospectively and also by biomarkers measurements, have been considered and included in the statistical analyses.

The main limitation of this study is related to the small sample size (n=134), which limits the power of the resulted associations. In addition, and as above-mentioned, the urinary phthalate metabolites concentrations in children at the age of 7, although available for a sub-sample, were not included in the current analysis. On the other hand, and despite the variety of covariates considered in the analyses, there might be some other covariates, such as the quality of home environment, or parental IQ, that could be responsible for the observed associations. Thus residual confounding cannot be excluded. Finally, due to the small sample size, a sex-depended impact of phthalate exposures on child neuropsychological functions has not been evaluated.

5. Conclusions

The present study confirms the influence of phthalate exposure on children's neuropsychological potential, especially in the early postnatal period on cognitive and social development of children. However, for the prenatal period, results are not fully conclusive. Its consequences depend on the characteristics of the environmental exposure itself, as well as on

social, economic and demographic factors, which determine individual susceptibility to their effects. Due to complexity and heterogeneity of the mechanisms involved in the influence of the analyzed chemicals on the children's development, further prospective studies with larger sample size and multiple time points of assessment are required, in order to elucidate which are the most critical periods of phthalate exposure on children's neurodevelopmental outcomes.

Acknowledgements

This work has received financial support from the European Union's Seventh Framework Programme under grant agreement No. 603946 (Health and Environment-wide Associations based on Large population Surveys, HEALS) and the National Science Centre, Poland, under grant No. DEC-2014/15/B/NZ7/00998 and DEC-2011/01/B/NZ7/06462. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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