# Phthalate exposure and neurodevelopmental outcomes in early school age children from Poland

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**Abstract**

Some phthalates are known endocrine disrupting chemicals (EDC). They are widely present in the environment thus their impact on children's health is of particular scientific interest. The aim of the study was to evaluate the association between phthalate exposure and neurodevelopmental outcomes, in particular behavioral, cognitive and psychomotor development, in 250 early school age children from the Polish Mother and Child Cohort (REPRO_PL). Urine samples were collected at the time of children's neurodevelopmental assessment and were analyzed for 21 metabolites of 11 parent phthalates. Behavioral and emotional problems were assessed by the Strengths and Difficulties Questionnaire (SDQ) filled in by the mothers. To assess children's cognitive and psychomotor development, Polish adaptation of the Intelligence and Development Scales (IDS) was administered. The examination was performed by trained psychologists. Dimethyl phthalate (DMP) and Di-n-butyl phthalate (DnBP) were the two phthalates showing the highest statistically significant associations, with higher total difficulties scores (β=1.5, 95% CI 0.17; 2.7; β=1.5, 95% CI 0.25; 2.8, respectively) as well as emotional symptoms and hyperactivity/inattention problems for DnBP (β=0.46, 95% CI -0.024; 0.94; β=0.72, 95% CI 0.065; 1.4, respectively), and peer relationships problems for DMP (β=0.37, 95% CI -0.013; 0.76). In addition, DnBP and DMP have been found to be negatively associated with fluid IQ (β=-0.14, 95% CI -0.29; 0.0041) and crystallized IQ (β = -0.16, 95% CI -0.29; -0.025), respectively. In the case of mathematical skills, three phthalates, namely DMP (β= -0.17, 95% CI -0.31; -0.033), DEP (β=-0.16, 95% CI -0.29; -0.018) and DnBP (β=-0.14, 95% CI -0.28; 0.0012), have also shown statistically significant associations. This study indicates that exposure to some phthalates seems to be associated with adverse effects on behavioral and cognitive development of early school age children. Further action including legislation, educational and interventional activities to protect this vulnerable population is still needed.

*Keywords: Behavior; Children; Cognition; Neurodevelopment; Phthalates.*

## 1. Introduction

Some phthalates are endocrine disrupting chemicals (EDC). They are used in a variety of products including personal care products, flooring, medical supplies, food packaging, toys and other plastics, and they are widely present in the environment (NRC, 2008). Humans are exposed to phthalates through ingestion, inhalation or dermal absorption, but usually as a combination of various exposure sources and routes (Koch *et al.*, 2013; Lioy *et al.*, 2015; Salthammer *et al.*, 2018; Giovanoulis *et al.*, 2018). Phthalates can be measured in a variety of biological matrices, though urine is the most common, reliable and non-invasive matrix for measuring their metabolites in human population studies (Koch and Calafat, 2009; Wittassek *et al.*, 2011; Calafat *et al.*, 2015).

Previous studies underlined that the concentrations of phthalate metabolites in urine were higher in children than in adults, which can be associated to age-specific behaviors, diet and metabolism (Frederiksen *et al.*, 2014; Hartmann *et al.*, 2015; Kasper-Sonnenberg *et al.*, 2012; Katsikantami *et al.*, 2016; Larsson *et al.*, 2014; Martínez *et al.*, 2018; Correia-Sá *et al.*, 2018). A recent European human biomonitoring (HBM) study reported phthalate exposure levels for children and adults in several countries, with children from Poland experiencing one of the highest levels among the European populations investigated (Den Hond *et al.*, 2015). This was recently confirmed in a previous analysis performed on 7-year old children from the Polish Mother and Child Cohort, REPRO_PL (the same population as included in the current study), indicating that 18 out of 21 phthalate metabolites were quantifiable in more than 90% of the samples and pointing to high exposure level for most of the analyzed phthalates (Garí *et al.*, 2019).

There is a public health concern on the effects of phthalate exposures, including their impact on child neurodevelopment. The existing studies to date suggest that both prenatal and postnatal phthalate exposures might be associated with behavioral problems and cognitive decrement in children (Ejaredar *et al.*, 2015; Katsikantami *et al.*, 2016; Vrijheid *et al.*, 2016; Braun, 2017**;** Lee *et al.*, 2018; Bornehag *et al.*, 2018). However, findings are not fully conclusive; inconsistencies across studies may be due to differences in analysed phthalates and their metabolites (usually few phthalates have been measured), the time of exposure

assessment (prenatal and/or postnatal periods), misclassification of exposure, differences in neurodevelopmental domains assessed or child age at evaluation, to mention but a few. The sex-specificity of the neurodevelopmental effects is also underlined in some publications (Ejaredar *et al.*, 2015; Vrijheid *et al.*, 2016; Bornehag *et al.*, 2018). As far as mechanisms of developmental neurotoxicity, experimental studies have indicated that several pathways relevant to brain development are targeted by phthalates, including dopaminergic neurotransmission (Chen *et al.*, 2011; Dhanya *et al.*, 2003; Tully *et al.*, 2000; Wang *et al.*, 2016), thyroid hormone homeostasis (Liu *et al.*, 2015), and steroids' action through their receptors (Dombret *et al.*, 2017). Very recent data obtained in rats also showed decreased number of neurons and synapses in the medial prefrontal cortex following developmental exposure to an environmentally relevant mixture of major phthalates *(*Kougias *et al.*, *2018)*.

Considering the high phthalate exposure levels among Polish children there is concern on the impact of these chemicals on child neuropsychological development. Thus, the aim of the study was to evaluate the associations between phthalate exposures at age 7 years and their neurodevelopmental outcomes. Children's behavior, as well as cognitive and psychomotor development were included in the assessment.

## 2. Materials and methods

### 2.1 Study population

The prospective Polish Mother and Child Cohort study (REPRO_PL) was established in 2007 with the aim to evaluate the impact of environmental and life-style related factors, taking into account individual susceptibility, on pregnancy outcomes, children's health and neurodevelopment (Polańska *et al.*, 2009; 2011; 2016). The women were recruited in maternity units during a 4-year period and under the following inclusion criteria: first trimester of healthy singleton pregnancy not assisted with reproductive technology. The study comprised the following phases: Phase I: pregnancy period; phase II: child exposure, health status and neurodevelopment evaluated at age of 1 and 2 years; and phase III: follow-up examination at age of 7 years. The current analysis is focused on phthalate exposure and neuropsychological outcomes in early school age children (based on phase III of the study).

However, relevant covariates collected during pregnancy and/or postnatal periods were also taken into account. Each phase of 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 scheduled phases of the cohort.

Taking into account organizational purposes, financial resources and the availability of exposure measures (phthalate), outcome data (child behavior, cognitive and psychomotor development) and the covariates of interest, the current assessment has been focused on 250 out of 407 children (61%).

**2.2 Phthalate exposure assessment**

The details regarding the analysis of phthalate metabolites has been published elsewhere (Garí *et al*., 2019). Briefly, a spot urine sample was collected at the time of child examination at age of 7 years. 21 metabolites corresponding to 11 phthalate compounds (including both low-molecular weight (LMW) and high-molecular weight (HMW) phthalates) were analyzed using on-line high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) with isotope dilution for quantification, as described by Koch *et al*. (2003, 2017). Limits of quantification ranged between 0.2 to 1 µg/l, depending on the metabolite (Garí *et al*., 2019). The present study focuses on 18 metabolites, which are those found above LQ in more than 90% of the analysed children. Therefore, a total of 8 phthalates were considered: five LMW phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), butyl-benzyl phthalate (BBzP), di-iso-butyl phthalate (DiBP) and di-n-butyl phthalate (DnBP), represented by 7 metabolites) and three HMW phthalates (di(2-ethylhexyl) phthalate (DEHP), di-iso-nonyl phthalate (DiNP) and di-iso-decyl phthalate (DiDP), represented by 10 metabolites). In addition, mono-3-carboxypropyl phthalate (MCPP), a common metabolite of various LMW and HMW phthalates, was also included in the analyses.

**2.3 Neurodevelopmental assessments**

***2.3.1 Child behavior***

Child behavioral and emotional problems at age of 7 years were assessed by the Strengths and Difficulties Questionnaire (SDQ) (www. sdqinfo.com, parent reported) (Goodman and Scott, 1997). The questionnaire was filled in by the mothers at the period as the child examination was performed. The 25 items in the SDQ constitute of five scales (conduct problems, hyperactivity/inattention problems, emotional symptoms, peer relationship problems and prosocial behavior) of five items each. Each item has three response categories: “not true”, “somewhat true”, and “certainly true” (with the scoring 0-2). For each of the five scales the score can range from 0 to 10. All sub-scale scores excluding prosocial behavior were summed as total difficulties score (ranged from 0 to 40) to assess the behavioral problems. Higher total, emotional, conduct, hyperactivity/inattention and peer SDQ scores indicate higher difficulties, whereas higher prosocial scores indicate better functioning in a social group. In this study, the outcomes were assessed both as continuous (score) variables and as dichotomized, according to a clinically relevant cut-off (normal *vs.* clinical) (http://www.sdqinfo.com/).

### *2.3.2 Child cognition and psychomotor development*

To assess children’s cognitive and psychomotor development, a Polish adaptation of the Intelligence and Development Scales (IDS) was administered (Grob *et al.*, 2009). The IDS allows assessing general intellectual ability (Fluid and Crystallized intelligence) and six developmental domains. In the current study, in addition to general intellectual ability, the following scales were evaluated:

(i) Cognition (based on the following domains: Visual perception, Selective attention, Phonological span, Visual-spatial working memory, Figural reasoning, Conceptual reasoning and Verbal long-term memory);

(ii) Mathematical skills;

(iii) Language skills (covering Expressive and Receptive language);

(iv) Psychomotor skills (including Gross motor, Fine motor and Visual-motor skills).

Total score (IQ Total) can be calculated if difference between IQ Fluid and IQ Crystallized is equal or below 10. In our population, 35% of the children did not meet this condition, hence IQ Total was not calculated.

Reliability and validity values of the IDS for Polish population are satisfactory. Reliability for fluid and crystallized intelligence equals 0.94, and for the full scale, 0.96. The correlations with analogous scales from Wechsler Intelligence Scale for Children (WISC-R) are about 0.80 (Jaworowska *et al.*, 2012; Grob *et al.*, 2009). Trained psychologists carried out the individual examinations and further evaluations, according to the standard procedure.

### 2.4 Covariates included in the assessment

Potential confounding factors were defined a priori based on previous literature. The following variables related to child characteristics were considered: child's sex and age at the neurodevelopmental assessment, prenatal exposure to tobacco smoke (with 10 ng/ml as cut off point for cotinine level in maternal saliva) and postnatal tobacco smoke exposure (with 2,1 ng/ml as cut off point for cotinine level in child urine collected at examination) (Polańska *et al.*, 2016; Lupsa *et al.*, 2015), traumatic events (including death of close family member or parental divorce) experienced by the child (yes/no), child age when he/she has started school education (at age of 6 years/at age of 7 years), child body mass index (BMI) based on height and weight measured by trained staff at child examination, breastfeeding duration (0-2 months/2-6 months/>6 months) and number of siblings (0/1/≥2). The parental factors included were maternal age at childbirth, parental educational level at child examination (years of completed education: ≤9/10-12/>12), socio-economic status (SES) of the family (very poor and poor/good/very good), household status (parents living together/single parent household) and place of residence (urban/rural).

### 2.5 Data analysis

Data analysis and graphics were performed using the statistical software R (R Development Core Team, 2018). Statistical analyses were focused on 9 compounds: mono-methyl phthalate, MMP (metabolite of DMP); mono-ethyl phthalate, MEP (metabolite of DEP); mono-benzyl phthalate, MBzP (metabolite of BBzP); ΣDiBP (sum of mono-isobutyl phthalate

(MiBP) and 2OH-mono-iso-butyl phthalate (OH-MiBP)); ΣDnBP (sum of mono-n-butyl phthalate (MnBP) and 3OH-mono-n-butyl phthalate (OH-MnBP)); ΣDEHP (sum of mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (5OH-MEHP), mono-2-ethyl-5-oxo-hexyl phthalate (5oxo-MEHP) and mono-2-ethyl-5-carboxypentyl phthalate (5cx-MEPP)); ΣDiNP (sum of 7-OH-mono-methyloctyl phthalate (OH-MiNP), 7-oxo-mono-methyloctyl phthalate (oxo-MiNP) and 7-carboxy-mono-methylheptyl phthalate (cx-MiNP)); ΣDiDP (sum of 6-OH-mono-propylheptyl phthalate (OH-MiDP), 6-oxo-mono-propylheptyl phthalate (oxo-MiDP), mono-2-7-methyl-7-carboxyheptyl phthalate (cx-MiDP)); and MCPP (a common metabolite of several phthalates).

Bivariate correlations between outcome variables and phthalate concentrations were used for descriptive analysis. A linear trend was added to all the figures.

Multivariate linear regression models were used to assess the neurodevelopmental outcomes (both SDQ and IDS) and phthalate concentrations, controlling for the above-mentioned covariates. Phthalate metabolite concentrations (ng/ml) were transformed into the natural logarithm for normalization. In addition, all continuous variables were scaled and centered for cross-comparison of the models.

As an alternative model specification for SDQ, multivariate logistic regressions were performed, categorizing the outcome variables into two groups: normal *vs.* clinical. In addition, SDQ sub-scales were calculated using confirmatory factor analysis (CFA), and the resulting scores underwent multivariate linear regression models. For CFA analysis and subsequent regression models, prosocial behavior was transformed to asocial behavior, hence the resulting associations are the same for all the SDQ sub-scales. All these alternative approaches are presented as supporting information.

All the regression models were performed using a Bayesian approach, through the arm package (Gelman and Su, 2018). The default package priors were employed (mean 0, scale 2.5 for logit model). In addition, the models were tested for goodness of fit, through the Jarque Bera test (for linear regression) and the Hosmer-Lemeshow test (for logistic regression). In general, no evidence of lack of fit was found.

## 3. Results

### 3.1 Characteristics of the study population

Socio-demographic characteristics of the cohort have been described in a previously published paper (Garí *et al.*, 2019). Briefly, there were no statistically significant differences between the subset of children included and not included in current analyses except for age at examination (7.2 ± 0.23 years vs. 7.5 ± 1.1 years; $p < 0.05$). Most of the children were living in urban areas (86%). Girls represented 54% of the study population. A high proportion of the parents declared university degree (65% of the mothers and 42% of the fathers) and affluent (78%) or most affluent (20%) SES. Almost 70% of the children had siblings and 85% were living with both parents. There was almost equal distribution of the children regarding the age of the school attendance for the first time (at age of 6 or at age of 7 years). About 10% of the children were exposed to tobacco smoke during the whole prenatal period, and 40% at age of 7 years.

The previous study focused on a detailed description and interpretation of the levels and determinants of phthalate exposure in the Polish children's population (Garí *et al.*, 2019). Briefly, the highest concentrations were found for ΣDiBP, ΣDEHP and ΣDnBP metabolites (median values of 97.3 µg/l, 89.3 µg/l and 62.6 µg/l, respectively), followed by the DEP metabolite MEP (42.0 µg/l) and ΣDiNP metabolites (21.9 µg/l).

The description of child behavioral problems (SDQ) as well as cognitive and psychomotor development (IDS) are presented in Tables 1 and 2, respectively. Based on SDQ scores, the prevalence for conduct problems, emotional symptoms and hyperactivity/inattention was 28%, 25% and 24%, respectively (Table 1). Peer relationship problems were noted among 18% and total difficulties among 19% of the studied population. For the 7% of the children the scores for prosocial behavior scales were within borderline and clinical range.

Intellectual efficiency and psychomotor skills (IDS) of the analysed samples are within the normal range (Table 2). However considerable variability in individual test scores were observed (*e.g.* 104 (±14) for fluid IQ mean score, range 56-136).

### 3.2 Phthalate exposure and behavioral development (SDQ)

As for univariate analyses, SDQ scales are associated with certain phthalate metabolites in children at age 7 (Figure 1). Except for prosocial behavior scale, in which the scores are inversely related, higher phthalate concentrations have shown a positive association with poorer SDQ performance. Negative associations have been found only for MCPP in hyperactivity/inattention problems, and for both ΣDiNP and ΣDEHP in peer-relationships problems. In multivariate regression models, MMP and ΣDnBP were the two phthalate metabolites showing the highest statistically significant associations, namely with higher total difficulties scores for both compounds (β = 1.5, 95% CI 0.17; 2.7; β = 1.5, 95% CI 0.25; 2.8, p-values<0.05, respectively), as well as with emotional symptoms and hyperactivity/inattention problems for ΣDnBP (β = 0.50, 95% CI -0.024; 0.94; β =0.72, 95% CI 0.065; 1.4, p-values<0.05, respectively), and peer relationships problems for MMP (β = 0.37, 95% CI -0.013; 0.76, p-value<0.1) (Figure 2). The results of confirmatory factor analysis and subsequent multivariate linear regression models, as well as multivariate logistic regression models using a binomial distribution (normal *vs.* clinical) indicate comparable results, even with stronger associations in the former (Supplementary Materials, Figures S1-S3).

### 3.3 Phthalate exposure and cognitive and psychomotor development (IDS)

Considering univariate analyses, lower IDS scores have been generally found to be positively associated with higher phthalate concentrations, with some exceptions (Figure 3). For instance, higher language skills have been found to be correlated with higher levels of phthalates, except MMP and ΣDiDP. Other phthalate compounds have been found to have a positive correlation in other IDS scales: MBzP with psychomotor skills, MCPP in crystallized IQ, and ΣDEHP in total cognition (Figure 3). These tendencies have been confirmed in the multivariate regression models (Figure 4). ΣDnBP and MMP have been found to be negatively associated with fluid IQ (β = -0.14, 95% CI -0.29; 0.0041, p-value<0.1) and crystallized IQ (β = -0.16, 95% CI -0.29; -0.025, p-value<0.05), respectively, with statistically significant results. Specifically for the mathematical skills, three phthalates, namely MMP (β

= -0.17, 95% CI -0.31; -0.033, p-value<0.05), MEP (β = -0.16, 95% CI -0.29; -0.018, p-value<0.05) and ΣDnBP (β = -0.14, 95% CI -0.28; 0.0012, p-value<0.1), have also shown statistically significant negative associations.

## 4. Discussion

The current study indicated high phthalate exposure levels among early school age children in Poland. The exposure to two phthalates, namely DMP and DnBP was associated with SDQ total difficulties score, as well as with specific behavioral problems, including emotional, hyperactivity/inattention and peer relationships problems. Furthermore, exposure to phthalates has been found to be negatively associated with child's intelligence (ΣDnBP and MMP metabolites) and mathematical skills (ΣDnBP, MMP and MEP metabolites).

The current study represents the most extensive set of phthalate metabolites ever determined for Poland, as already discussed in the previous article (Garí *et al.*, 2019). This assessment is consistent with the results presented at the DEMOCOPHES study performed among children with a comparable age group (6–11 years), indicating that such a vulnerable population in Poland has a higher phthalate exposure level than other European populations (Den Hond *et al.*, 2015).

The impact of phthalate exposure on children's health and development is of growing scientific and public health concern. Our study indicated the impact of phthalate exposure on child behavioral performance. A recent analysis published by Huang *et al.* (2019) also pointed out that phthalate exposure during childhood is associated with increased scores of social problems in later life (even after adjustment for maternal phthalate exposure simultaneously) (Huang *et al.*, 2019). Other studies have reported that DEHP metabolites in particular may be associated with attention-deficit–hyperactivity disorder symptoms in school-age children (Hu *et al.*, 2017; Kim *et al.*, 2009; Won *et al.*, 2016).

Recently published review papers pointed out that exposure to some phthalates in childhood is associated with reduced cognitive abilities and behavioral problems (Ejaredar *et*

*al.*, 2015; Katsikantami *et al.*, 2016; Vrijheid *et al.*, 2016; Braun, 2017; Lee *et al.*, 2018). The meta-analysis of studies investigating the association between DEHP exposure and neurodevelopment in children indicated that two-fold increase in DEHP metabolites of childhood was associated with a 0.8 point reduction in IQ (Lee *et al.*, 2018). Although some of the longitudinal studies indicate that exposure in the prenatal period has a higher impact on child development than the postnatal period, the results of the above-mentioned meta-analysis did not provide any further insight on the specific susceptibility period of phthalate effects on neurodevelopmental disturbances (Lee *et al.*, 2018). Previous published results from REPRO_PL cohort indicated that child motor development at age of 2 years was inversely associated with natural log concentration of phthalates in the urine collected from mothers during pregnancy even after adjustment for the postnatal exposure. In addition, that study found that postnatal child exposure to phthalates was not associated with any of the measured scores of child psychomotor development (Polańska *et al.*, 2014). It needs to be pointed out that the exposure level for prenatal period and in early childhood was much lower than that recorded at the age of 7 years (Garí *et al.*, 2019). In addition, for the child neurodevelopment at the age of 2 years, exposures during the prenatal period can have a higher impact than the postnatal one, whereas at older ages, the current exposure level can be crucial (especially when it is of high level, such as that registered in Polish children).

The results of the present study confirm the reports of other authors focusing on child intellectual potential (although they are focusing on different phthalates and/or pointing out different cognitive/psychomotor outcomes). For instance, Cho *et al.* (2010) found a relationship between the levels of some urinary phthalate metabolites and the intellectual performance. In that study, and for the whole group of children analysed, a significant negative correlation between DEHP levels and vocabulary sub-scale scores was found. In addition, within the group of boys, a similar negative correlation was found for MEHP. Two studies performed on Asian children revealed an association between higher levels of phthalate metabolites and lower total IQ (Huang *et al.*, 2015) and learning disabilities (Arbuckle *et al.*, 2016). In comparison to the aforementioned studies, our analysis provides a wider range of chemicals included for the assessment of neurodevelopmental outcomes.

The biological mechanisms implicated in the adverse effects of phthalate exposure on neurobehavioral development are so far unclear. A significant effect of environmental relevant exposure to phthalates on the brain is consistently supported by data showing impairment of hippocampal structural and functional plasticity (Holahan and Smith, 2015) and by a very recent study that found decreased number of neurons and synapses in the medial prefrontal cortex following developmental exposure to a mixture of major phthalates (Kougias *et al.*, 2018*)*.

Whether these changes occur as a direct neurotoxic effect of phthalates or an indirect effect through disruption of other neurotransmitter or metabolic pathways is not fully understood. Experimental research indicated that phthalates could interfere with dopamine receptor D2, tyrosine hydroxylase, and homeostasis of calcium-dependent neurotransmitters, resulting in reduced dopamine release (Chen *et al.*, 2011; Dhanya *et al.*, 2003; Tully *et al.*, 2000; Wang *et al.*, 2016). In addition, phthalates could alter the lipid metabolism of the rat fetal brain mediated by the peroxisome proliferator-activated receptor, which may cause aberrant neurodevelopment (Xu *et al.*, 2007). Finally, phthalates interfere with endogenous endocrine functions relevant to neurodevelopment, namely thyroid hormones biosynthesis, biotransformation and metabolism (Liu *et al.*, 2015; Huang *et al.*, 2017; Johns *et al.*, 2016), and steroids' action through their receptors and metabolism.

The strength of the current study is related to the assessment of 18 phthalate metabolites which represent the most extensive set of phthalates evaluated for Poland and rarely performed in other studies (Garí *et al.*, 2019). Although HBM studies have pointed out that humans are exposed to a mixture of phthalates, most of the previously published epidemiological studies have examined the health impacts of one or few of them (Vrijheid *et al.*, 2016; Braun, 2017**;** Lee *et al.*, 2018). Although our report did not perform a global and comprehensive assessment, it included a large set of phthalates. Secondly, the current study has considered a broad spectrum of child neurodevelopment, including behavioral, cognitive and psychomotor domains. The child behavior has been assessed by SDQ, which is the tool widely used in other studies in this field (increasing comparability of the results obtained). Child cognitive and psychomotor assessments have been performed by psychologists trained

and certified in performing IDS. As it was mentioned previously, IDS has high reliability as well as high correlation with analogous WISC-R scales (Jaworowska *et al.*, 2012). Finally, a variety of confounding factors have been considered and included in the models; most of them were already evaluated prospectively, while others were based on biomarker measurements (*e.g.* saliva and urine cotinine levels for prenatal period and postnatal tobacco smoke exposures, respectively).

The limitations of the study also need to be mentioned. First of all, the current assessment has been focused on the postnatal exposure and its impact on child neurodevelopment. Although the prenatal and early life (at age of 2 years) exposure assessment to phthalates was performed in a sub-sample of the children from the REPRO_PL cohort, it was not possible to include such data in current analysis (Polańska *et al.*, 2014; Garí *et al.*, 2019) as the prenatal measurements have been performed in a lower number and not fully comparable mother-child pairs were available. Additionally, different phthalate metabolites were analysed within each period of the assessment (11 phthalate metabolites for prenatal and early postnatal assessment *vs.* 21 phthalates metabolites among early school age children). As it was mentioned above, the phthalate concentrations in 7-year old children were higher than those measured in the same children at age 2, and also in their mothers during pregnancy. Furthermore, except for MEP and MBzP (mother-child at age of 7 years) there were no significant correlations between phthalate metabolites measured in different periods. This could indicate a different profile of phthalate exposures. For these reasons, the effects of prenatal/early postnatal exposure formed the subject of a specific study (Polańska *et al.*, 2014). Despite the fact that the analysis covered a variety of covariates, we did not consider the quality of home environment or parental IQ. In that case, SES and parental education can be regarded as a reliable proxy. And finally, even that sex specificity of the effects has been underlined in existing studies, we were not able to perform such analysis in the current study. In this regard, additional urine samples from children of the REPRO_PL cohort will be available for phthalate analyses in the following months, and therefore, with a higher sample size, a new assessment on the sex-dependent neurodevelopmental outcomes will be performed.

## 5. Conclusions

This study indicates that phthalate exposures in children from Poland might have an adverse effect on behavioral and cognitive development at early school age. Specifically, MMP and DnBP have been found to be related to poorer behavior scores from the SDQ test, as well as lower intellectual abilities from the IDS test. Those results underline that further actions including legislation, educational and interventional activities to protect vulnerable population such as that in Poland are needed.

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**References**


1. Arbuckle, T.E., Davis, K., Boylan, K., Fisher, M., Fu, J., 2016. Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6–11 years of age: CHMS 2007–2009. Neurotoxicology 54: 89–98.
2. Bornehag CG, Lindh C, Reichenberg A, Wikström S, Unenge Hallerback M, Evans SF, Sathyanarayana S, Barrett ES, Nguyen RHN, Bush NR, Swan SH. 2018. Association of Prenatal Phthalate Exposure With Language Development in Early Childhood. JAMA Pediatr. 172(12): 1169-1176.
3. Braun, J.M., 2017. Early life exposure to Endocrine Disrupting Chemicals and childhood obesity and neurodevelopment. Nat Rev Endocrinol. 13(3): 161–173.
4. Calafat AM, Longnecker MP, Koch HM, Swan SH, Hauser R, Goldman LR, Lanphear BP, Rudel RA, Engel SM, Teitelbaum SL, Whyatt RM, Wolff MS. 2015. Optimal Exposure Biomarkers for Nonpersistent Chemicals in Environmental Epidemiology. Environ Health Perspect. 123(7): A166-8.
5. Chen, T., Yang, W., Li, Y., Chen, X., Xu, S., 2011. Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: inhibition of proliferation and promotion of differentiation in PC12 cells. Toxicol. Lett. 201. 34–41.
6. Cho, S.C., Bhang, S.Y., Hong, Y.C., Shin, M.S., Kim, B.N., Kim, J.W., Kim, H.W., 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ. Health Perspect. 118 (7): 1027–1032.
7. Correia-Sá L, Kasper-Sonnenberg M, Pälmke C, Schütze A, Norberto S, Calhau C, Domingues VF, Koch HM. 2018. Obesity or diet? Levels and determinants of phthalate body burden - A case study on Portuguese children. Int J Hyg Environ Health. 221(3): 519-530.
8. 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., Reis, M.F., 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 (3): 255–263.
9. Dhanya, C.R., Indu, A.R., Deepadevi, K.V., Kurup, P.A., 2003. Inhibition of membrane Na (+)-K+ Atpase of the brain, liver and RBC in rats administered di (2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. Indian J. Exp. Biol. 41: 814–820.
10. Dombret, C., Capetal, D., Poissenot, K., Parmentier, C., Bergsten, E., Pionneau, C., Chardonnet, S., Hardin-Pouzet, H., Grange-Messent, V., Keller, M., Franceschini, I., Mhaouty-kodja, S., 2017. Neural Mechanisms Underlying the Disruption of Male Courtship Behavior by Adult Exposure to Di(2-ethylhexyl) Phthalate in Mice. Environ Health Perspect. 125(9): 097001.
11. Ejaredar, M., Nyanza, E.C., Eycke, T.K., Dewey, D., 2015. Phthalate exposure and

childrens neurodevelopment: A systematic review. Environ Res. 142: 51-60.
12. Frederiksen, H., Jensen, T.K., Jorgensen, N., Kyhl, H.B., Husby, S., Skakkebaek, N.E., Main, K.M., Juul, A., Andersson, A.M., 2014. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. Reproduction 147(4): 555–565.
13. 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.
14. Gelman, A., Su, Y-S., 2018. Data Analysis Using Regression and Multilevel/Hierarchical Models. R package version 1.10-1. https://CRAN.R-project.org/package=arm.
15. Giovanoulis G, Bui T, Xu F, Papadopoulou E, Padilla-Sanchez JA, Covaci A, Haug LS, Cousins AP, Magnér J, Cousins IT, de Wit CA. 2018. Multi-pathway human exposure assessment of phthalate esters and DINCH. Environ Int. 112: 115-126.
16. Goodman, R., Scott, S. Child Psychiatry. Wiley, 1997.
17. Grob, A., Meyer,Ch.S., Hagmann-von Arx, P., 2009. IDS Intelligence and Development Scales - Children aged 5-10.
18. Hartmann, C., Uhl, M., Weiss, S., Koch, H.M., Scharf, S., König, J., 2015. Human bio-monitoring of phthalate exposure in Austrian children and adults and cumulative risk assessment. Int. J. Hyg. Environ. Health 218: 489–499.
19. Holahan, M.R., Smith, C.A., 2015. Phthalates and neurotoxic effects on hippocampal network plasticity. Neurotoxicology. 48: 21-34.
20. Hu, D., Wang, Y.X., Chen, W.J., Zhang, Y., Li, H.H., Xiong, L., Zhu, H.P., Chen, H.Y., Peng, S.X., Wan, Z.H., Zhang, Y., Du, Y.K., 2017. Associations of phthalates exposure with attention deficits hyperactivity disorder: a case-control study among Chinese children. Environ. Pollut. 229: 375–385.
21. Huang, H.B., Chen, H.Y., Su, P.H., Huang, P.C., Sun, C.W., Wang, C.J., Chen, H.Y., Hsiung, C.A., Wang, S.L., 2015. Fetal and childhood exposure to phthalate diesters and cognitive function in children up to 12 years of age: Taiwanese Maternal and Infant Cohort Study. PLoS One. 10(6): e0131910.
22. Huang, H.B., Chuang, C.J., Su, P.H., Sun, C.W., Wang, C.J., Wu, M.T., Wang, S.L., 2017. Prenatal and childhood exposure to phthalate diesters and thyroid function in a 9year follow-up birth cohort study: Taiwan maternal and infant cohort study. Epidemiology 28 (Suppl 1) (S10-s18).
23. Huang, H-B., Kuo, P-H., Su, P-H., Sun, Ch-W., Chen, W.J., Wang, S-L., 2019. Prenatal and childhood exposure to phthalate diesters and neurobehavioral development in a 15-year follow-up birth cohort study. Environ. Res. 172: 569–577.
24. Jaworowska, A., Matczak, A., Fecenec, D., 2012. Skale Inteligencje i Rozwoju - dzieci w wieku 5-10 lat. Podręcznik. Pracownia Testów Psychologicznych Polskiego Towarzystwa Psychologicznego. Warszawa. [Scales of Intelligence and Development - children aged 5-10. Manual. Laboratory of Psychological Tests of the Polish Psychological Association. Warsaw.]
25. Johns, L.E., Ferguson, K.K., McElrath, T.F., Mukherjee, B., Meeker, J.D., 2016. Associations between repeated measures of maternal urinary phthalate metabolites and thyroid hormone parameters during pregnancy. Environ. Health Perspect. 124: 1808–1815.
26. Kasper-Sonnenberg, M., Koch, H.M., Wittsiepe, J., Wilhelm, M., 2012. Levels of phthalate metabolites in urine among mother-child-pairs–results from the Duisburg

birth cohort study, Germany. Int. J. Hyg. Environ. Health 215: 373–382.
27. Katsikantami, I., Sifakis, S., Tzatzarakis, M.N., Vakonaki, E., Kalantzi, O.I., Tsatsakis, A.M., et al., 2016. A global assessment of phthalates burden and related links to health effects. Environ. Int. 97: 212–236.
28. Kim, B.N., Cho, S.C., Kim, Y., Shin, M.S., Yoo, H.J., Kim, J.W., Yang, Y.H., Kim, H.W., Bhang, S.Y., Hong, Y.C., 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol. Psychiatry 66: 958–963.
29. Koch, H.M., Gonzalez-Reche, L.M., Angerer, J., 2003. On-line clean-up by multi-dimensional liquid chromatography-electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine. J. Chromatogr. BAnalyt. Technol. Biomed. LifeSci. 784 (1): 169-182.
30. Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals use dinplastic manufacture. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 364 (1526): 2063–2078.
31. Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., Brüning, T., Kolossa-Gehring, M., 2017. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg. Environ. Health 220 (2PtA): 130–141.
32. Koch HM, Lorber M, Christensen KL, Pälmke C, Koslitz S, Brüning T. 2013. Identifying sources of phthalate exposure with human biomonitoring: results of a 48h fasting study with urine collection and personal activity patterns. Int J Hyg Environ Health 216(6): 672-681.
33. Kougias, D.G., Sellinger, E.P., Willing, J., Juraska, J.M., 2018. Perinatal Exposure to an Environmentally Relevant Mixture of Phthalates Results in a Lower Number of Neurons and Synapses in the Medial Prefrontal Cortex and Decreased Cognitive Flexibility in Adult Male and Female Rats. J Neurosci. 38(31): 6864-6872.
34. Larsson, K., Ljung Björklund, K., Palm, B., Wennberg, M., Kaj, L., Lindh, C.H., Jönsson, B.A., Berglund, M., 2014. Exposure determinants of phthalates, parabens, bisphenol A and triclosan in Swedish mothers and their children. Environ. Int. 73: 323–333.
35. Lee, D.W., Kim, M.S., Lim, Y.H., Lee, N., Hong, Y.C., 2018. Prenatal and postnatal exposure to di-(2-ethylhexyl) phthalate and neurodevelopmental outcomes: A systematic review and meta-analysis. Environ Res. 167: 558-566.
36. Lioy PJ, Hauser R, Gennings C, Koch HM, Mirkes PE, Schwetz BA, Kortenkamp A. 2015. Assessment of phthalates/phthalate alternatives in children's toys and childcare articles: Review of the report including conclusions and recommendation of the Chronic Hazard Advisory Panel of the Consumer Product Safety Commission. J Expo Sci Environ Epidemiol 25(4): 343-353.
37. Liu, C., Zhao, L., Wei, L., Li, L., 2015. DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ. Sci. Pollut. Res. Int. 22: 12711–12719.
38. Lupsa, I.R., Nunes, B., Ligocka, D., Gurzau, A.E., Jakubowski, M., Casteleyn, L., Aerts, D., Biot, P., DenHond, E., Castaño, A., Esteban, M., Kolossa-Gehring, M., Fiddicke, U., Knudsen, L.E., Schoeters, G., Reis, M.F., 2015. Urinary cotinine levels and environmental tobacco smoke in mothers and children of Romania, Portugal and Poland within the European human biomonitoring pilot study. Environ. Res. 141: 106–117.
39. Martínez, M.A., Rovira, J., Prasad Sharma, R., Nadal, M., Schuhmacher, M., Kumar, V.,

2018. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: a Catalonia(Spain) case study. Environ. Res. 166: 25–34.
40. National Research Council, 2008. Phthalates and Cumulative Risk Assessment: The Tasks Ahead. Committee on the Health Risks of Phthalates. National Academies Press (US), Washington DC.
41. Polańska, K., Hanke, W., Gromadzińska. J., Ligocka, D., Gulczyńska, E., Sobala, W., Wąsowicz, W., 2009. Polish mother and child cohort study —defining the problem, the aim of the study and methodological assumptions. Int. J. Occup. Med. Environ. Health 22 (4): 383–391.
42. Polańska, K., Hanke, W., Jurewicz, J.,Sobala, W., Madsen, C., Nafstad, P., et al., 2011. Polish mother and child cohort study (REPRO_PL)–methodology of follow-up of the children. Int. J. Occup. Med. Environ. Health 24: 391–398.
43. Polańska, K., Ligocka, D., Sobala, W., Hanke, W., 2014. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. Early Hum Dev. 90(9): 477-85.
44. Polańska, K., Hanke, W., Król, A., Potocka, A., Waszkowska, M., Jacukowicz, A., et al., 2016a. Polish Mother and Child Cohort Study (REPRO_PL) – methodology of the follow-up of the children at the age of 7. Int. J. Occup. Med. Environ. Health 29(6): 883–893.
45. Polańska,K., Ligocka,D., Sobala,W.,Hanke,W., 2016b. Effect of environmental phthalate exposure on pregnancy duration and birth outcomes. Int. J. Occup. Med. Environ. Health 29(4): 683–697.
46. R Core Team., 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
47. Salthammer T, Zhang Y, Mo J, Koch HM, Weschler CJ. 2018. Assessing Human Exposure to Organic Pollutants in the Indoor Environment. Angew Chem Int Ed Engl. 57(38): 12228-12263.
48. Tully, K., Kupfer, D., Dopico, A.M., Treistman, S.N., 2000. A plasticizer released from IV drip chambers elevates calcium levels in neurosecretory terminals. Toxicol. Appl. Pharmacol. 168: 183–188.
49. Vrijheid, M., Casas, M., Gascon, M., Valvi, D., Nieuwenhuijsen, M., 2016. Environmental pollutants and child health - A review of recent concerns. Int J Hyg Environ Health. 219(4-5): 331-342.
50. Wang, R., Xu, X., Zhu, Q., 2016. Pubertal exposure to di-(2-ethylhexyl) phthalate influences social behavior and dopamine receptor D2 of adult female mice. Chemosphere 144: 1771–1779.
51. Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. Assessing exposure to phthalates–the human biomonitoring approach. Mol. Nutr. Food Res. 55(1): 7–31.
52. Won, E.K., Kim, Y., Ha, M., Burm, E., Kim, Y.S., Lim, H., Jung, D.E., Lim, S., Kim, S.Y., Kim, Y.M., Kim, H.C., Lee, K.J., Cheong, H.K., Kang, H.T., Son, M., Sakong, J., Oh, G.J., Lee, C.G., Kim, S.Y., Ryu, J.M., Kim, S.J., 2016. Association of current phthalate exposure with neurobehavioral development in a national sample. Int. J. Hyg. Environ. Health 219: 364–371.
53. Xu,Y., Agrawal, S., Cook, T.J., Knipp,G.T.,2007. Di-(2-ethylhexyl)-phthalate affects lipid profiling in fetal rat brain upon maternal exposure. Arch. Toxicol. 81: 57–62.

**Figure captions**

**Figure 1**. Univariate associations between phthalate metabolite concentrations (µg/l) in 7-year old children for behavioral scales (SDQ): a) Associations for low-molecular weight phthalates; b) Associations for high-molecular weight phthalates. The x-axes are shown in logarithmic scale.

**Figure 2**. Standardized beta-coefficients from multivariate linear regression models for phthalate concentrations on the behavioral scales (SDQ) in children at 7 years of age. Models are adjusted by child's sex and age at examination, age at school attendance, household status, SES, parental educational level, maternal age at birth, traumatic events, children's BMI, place of residence, number of siblings, exposure to tobacco during pregnancy and in children's at 7 years of age.

**Figure 3**. Univariate associations between phthalate metabolite concentrations (µg/l) in 7-year old children for intelligence and development scales (IDS): a) Associations for low-molecular weight phthalates; b) Associations for high-molecular weight phthalates. The x-axes are shown in logarithmic scale.

**Figure 4**. Standardized beta-coefficients from multivariate linear regression models for phthalate concentrations on intelligence and development scales (IDS) in children at 7 years of age. Models are adjusted by child's sex and age at examination, age at school attendance, examiner, household status, SES, parental educational level, maternal age at birth, breastfeeding duration, place of residence, number of siblings, exposure to tobacco during pregnancy and in children's at 7 years of age.