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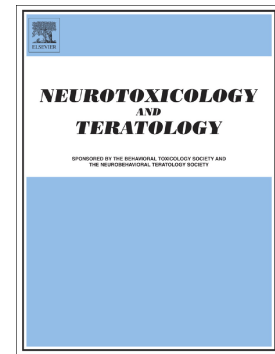
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Prenatal Phthalate Exposure and Language Development in Toddlers from the Odense Child Cohort

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Abstract:

Background: Phthalates are a group of chemicals found in a variety of consumer products. They have anti-androgenic properties and human studies have reported associations between prenatal phthalate exposure and neuropsychological development in the offspring despite different cognitive tests, different ages and varying timing of exposure.

Objectives: To investigate the association between prenatal phthalate exposure and language development in children aged 20-36 months.

Methods: In the Odense Child Cohort, we analyzed 3rd trimester urine samples of 518 pregnant women for content of metabolites of diethyl, di-n-butyl, diisobutyl, butylbenzyl, di(2-ethylhexyl), and diisononyl phthalate, adjusted for osmolality. Language development was addressed using the Danish version of the MacArthur-Bates Communicative Development Inventories “Words and Sentences”. Associations were assessed using logistic regression models comparing children below and above the 15th percentile while stratifying by sex and adjusting for maternal age and educational level.

Results: Phthalate metabolites were detectable in all samples although in lower levels than previous studies. Among boys, increased prenatal phthalate exposure was associated with lower scores in language development; odds ratios for vocabulary score below the 15th percentile with doubling in monoethyl phthalate, and summed di-(2-ethylhexyl) phthalate metabolites were respectively 1.24 (95% confidence interval: 1.05,1.46), and 1.33 (1.01,1.75). Similar associations were found for language complexity. No associations were found for girls.

Conclusions: Our findings are notable, as adverse associations were suggested even in this low-level exposed population, with only one spot urine sample for exposure assessment and control for confounders. Lower scores in early language development are of relevance to health as this test predicts later educational success.

Introduction:

Phthalates are a group of synthetic industrially produced chemicals used as plasticizers in polyvinyl chloride (PVC), as well as solvents and fixatives (Katsikantami et al., 2016). Phthalates are used in a large proportion of everyday consumer products, including building materials (Bornehag et al., 2005), personal care products, cosmetics (Braun et al., 2014) and food packaging (Rudel et al., 2011). As a result of their chemical properties, phthalates can migrate into food (Serrano et al., 2014), drinking water (Shi et al., 2012), dust (Bergh et al., 2012; Langer et al., 2014) and air (Adibi et al., 2008). Therefore, they can be ingested, inhaled and dermally absorbed (Wormuth et al., 2006). Internal exposure is therefore strongly affected by personal habits and lifestyle (Den Hond et al., 2015; Schettler, 2006).

The European population is widely exposed to these substances (Den Hond et al., 2015; Katsikantami et al., 2016), posing a potential risk, especially for pregnant women and their offspring, as phthalates can pass the placental barrier and occur in amniotic fluid (Jensen et al., 2012). Hence, they can potentially affect the vulnerable, developing fetus (Bergman et al., 2013). Previous research has found phthalate exposure to be inversely associated with adverse birth and health outcomes in both sexes, including neurodevelopment (Braun et al., 2013; Katsikantami et al., 2016). Eleven studies (summarized in (Ejaredar et al., 2015; Miodovnik et al., 2014; Ponsonby et al., 2016)) have examined the relationship between phthalate exposures and child neurodevelopment. Interestingly, all but two previous studies (Gascon et al., 2015 and Polanska et al., 2014) have found association between prenatal or childhood phthalate exposure and neurodevelopment, despite differences between the cognitive domains, child age at testing and time of exposure measurement across studies (Ejaredar et al., 2015; Miodovnik et al., 2014; Ponsonby et al., 2016). The two studies which did not find associations were smaller ($n = 367$ (Gascon et al., 2015) and $n = 165$ (Polanska et al., 2014) mother-child pairs) and none of them stratified by sex, which may explain the lack of association.

Few studies have examined whether the effects are sex specific, none has studied detailed language development and none has been conducted among Danish children. Early language development has been found to be associated with IQ scores (Liao et al., 2015) and predicts school performance (Bleses et al., 2016) and subsequent professional achievement (Duncan et al., 2007; Elbro et al., 2011; Hawa and Spanoudis, 2014) which makes it a sensible marker for neurodevelopment.

We therefore investigated the association between maternal phthalate exposure during pregnancy and language development scores in 518 offspring aged 20-36 months in the Odense Child Cohort.

Materials and methods:***Study settings and design***

From January 1st 2010 to December 31st 2012, all newly pregnant women living in the Municipality of Odense (Denmark) were invited to participate in The Odense Child Cohort (OCC). The women were contacted at a voluntary information meeting introducing the routine ultrasound examinations at Odense University Hospital (OUH) or at their first antenatal visit at gestational weeks 8 to 16

(Kyhl et al., 2015). Of the 6,707 pregnant women registered in Odense during that period, 4,017 were informed of the study, of which 3,605 (54%) accepted enrolment. The inclusion criteria were met by 2,874 (43% of all pregnant women), of which 374 (13%) dropped out for various reasons (Kyhl et al., 2015). By November 2014, the cohort included 2,500 active families with 2,549 children (Kyhl et al., 2015) (Figure 1). At gestational week 28 i.e., the formal time of inclusion, women were asked to deliver a serum sample and a urine sample, and both were stored in freezers at the Odense Patient data Explorative Network (OPEN). Information regarding maternal mental, physical and social health was obtained through questionnaires filled in during pregnancy. Self-reported education was obtained from questionnaires, but was missing for 131 women, and therefore instead coded from the occupational status listed in the birth records. Maternal ethnicity was obtained from the municipality data.

Data on birth characteristics was extracted from obstetric and pediatric hospital records. Information regarding the child's health, including duration of breastfeeding, was obtained from questionnaires answered by the parents and supplemented with data from the clinical examination performed by trained technicians. Information from clinical examination included anthropometric measurements (length (cm), weight (g), head circumference (cm)), at approximately 18 months of age (median = 19.2, range = 17.5 - 23.5 months).

Phthalate measurement

Concentrations of phthalate metabolites in maternal pregnancy spot urine samples (collected before 9:30 AM after overnight fasting) were measured in a subset of OCC-participants ($n = 870$). Samples were stored at -80°C until chemical analysis and analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) with preceding enzymatic deconjugation followed by solid phase extraction for the concentration of 12 metabolites of six different phthalates, free as well as conjugated (table S1). Details regarding preparation and chemical analysis have been described previously (Frederiksen et al., 2010).

The analyzed phthalate metabolites were monoethyl phthalate (MEP) of diethyl phthalate (DEP); mono-*n*-butyl phthalate (MnBP) and mono-*iso*-butyl phthalate (MiBP) of the isomers di-*iso*-butyl phthalate (DiBP) and di-*n*-butyl phthalate (DnBP), respectively; monobenzyl phthalate (MBzP) of butylbenzyl phthalate (BBzP); mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) all metabolites of di-(2-ethylhexyl) phthalate (DEHP) and metabolites mono-*iso*-nonyl phthalate (MiNP), mono-hydroxy-*iso*-nonyl phthalate (MHiNP), mono-oxo-*iso*-nonyl phthalate (MHiOP) and mono-carboxy-*iso*-octyl phthalate (MCiOP) all metabolites of di-*iso*-nonyl phthalate (DiNP).

The first subset of 196 urine samples were selected at random (analyzed from September 2011 to January 2012), whereas the subsequent set of samples ($n = 674$) (analyzed from December 2012 to January 2013) “were selected based on the availability of information from questionnaires, birth records and clinical examination of the child at 3 months.” (Jensen et al., 2016). A total of 565 of the 870 participants were included in previous studies (Frederiksen et al., 2014; Jensen et al., 2016; Tefre de Renzy-Martin et al., 2014). The concentrations of some phthalate metabolites were slightly higher in the first subset than the second, although not significantly so. Nevertheless, 20 samples from the two subsets were reanalyzed in the same batch, yielding no differences from the first analysis (Jensen et al., 2016).

In order to adjust measured urinary concentrations for the urinary dilution (Frederiksen et al., 2013; Johns et al., 2015), the osmolality (Osm/kg) of each individual urine sample (Osm/kg) was measured by the freezing point depression method using automatic cryoscopic osmometer (Osmomat® 030 from Gonotec, Berlin, Germany). To assess the precision of the osmolality measurements, a control standard urine pool was also measured. The mean urinary osmolality in this pool ($n = 77$) was 0.825 Osm/kg, with a relative standard deviation of 1.85%. The median (range) osmolality of all urine samples included in this study was 0.639 (0.094-1.117) Osm/kg ($n = 518$).

Language development measurement

Language skills were assessed using the validated Danish adaptation (Bleses and Syddansk, 2007; Bleses et al., 2008a, b) of the MacArthur-Bates Communicative Development Inventories (MB-CDI) (Fenson et al., 1994). The MB-CDI is a checklist-based parent report instrument that measures understanding and production of common words and expressions in young children. The test is built on a rating-scale based on a particular country’s linguistics, and cultural idiosyncrasies, while also taking into account the differences in language development between the sexes.

The parents answered an electronic version of the MB-CDI questionnaire (MB-CDI “Words and Sentences”) regarding their child’s current language development, every third month from the age of 16 to 36 months. The questionnaires contained seven categories, two of which were chosen, as they focus on different important aspects of language development at that age span and have previously been shown to have good correlations (Fenson et al., 1994): (a) Vocabulary (725 items - 22 semantic categories) which focuses on the individual child’s use of commonly used words for toddlers (lexicon), and (b) Complexity (33 items) which focuses on the child’s use of correct grammar and syntactic complex sentences (morphosyntax). The parents were instructed to check all words and sentences their child produced on the questionnaire including previously learned words and sentences.

The first questionnaire completed for each child was used to generate a productive vocabulary summary score (number of produced words). Complexity data was used to generate a complexity summary score (number of produced complexity items), starting from the age of 30 months for

boys, and 26 months for girls, since more than 15 % of the children in the reference study scored zero before that age (Bleses 2007). We then assigned each child a percentile language score, which is sex and age specific, according to the Danish MB-CDI reference study (Bleses and Syddansk, 2007), conducted among 3,714 Danish children aged 16-36 months. Using the reference study as basis for language scores eliminates the need to condition on sex and age at testing. Vocabulary and complexity yields two separate language scores for each child. A percentile-score of ≤ 15 was considered as impaired language development (Bleses and Syddansk, 2007).

In the Danish MB-CDI reference study (Bleses and Syddansk, 2007) children whose fathers had different ethnicity (bilingual homes), whose parents were living apart, or who suffered from any chronic diseases or speech/speech-hearing problems were excluded. We did not obtain information about these factors and were therefore not able to exclude children like in the reference population.

The study was performed in accordance with the second Helsinki Declaration, with written, informed consent, and approved by the regional Ethical Committee (Project ID S-20090130).

Data analysis

Phthalate metabolite concentrations above LOD (see table S1) were adjusted for urinary dilution by dividing each raw individual phthalate metabolite concentration by its appertaining urine sample's osmolality and subsequently multiplying it with the median osmolality of all urine samples. If the phthalate metabolite concentration was below LOD the value was replaced by the appropriate $\text{LOD}/\sqrt{2}$. To quantify the significance of exposure to all DEHP and DiNP metabolites the individual molar concentration of phthalate metabolites, adjusted for urinary osmolality, were summed for DEHP (MEHP + MEHHP + MEOHP + MECPP) and DiNP (MHiNP + MCiOP + MOiNP + MiNP) and multiplied by the molecular weight of the parent compound in order to have homogenous exposure measurements as previously done (Jensen et al., 2016), henceforth termed as $\sum\text{DEHP}_m$ and $\sum\text{DiNP}_m$. Additionally, as the two isomers of DiBP and DnBP have been shown to be highly correlated, their metabolites (ng/mL) were summed ($\text{MiBP} + \text{MnBP} = \sum\text{MBP}_{(i+n)}$) (Frederiksen et al., 2010).

The distribution of the osmolality-adjusted phthalate metabolites were all skewed toward the right and therefore transformed by the use of the natural logarithm or divided into quartiles. However, MBzP was divided into levels below and above the median as 30.7% of the measurements were $<\text{LOD}$. Analysis of data on MiNP was omitted, due to the high proportion of samples with values $<\text{LOD}$ (90.2%). The language estimates were left untransformed, except for use in logistic regression where they were converted into a binary variable with scores above or below the 15th percentile.

Correlations between phthalate metabolites and vocabulary and complexity scores were assessed using Spearman Correlation Coefficients.

Possible confounders of the association between phthalate metabolites and language scores were found through a careful review of the literature and evaluated using a directed acyclic graph (DAG) (Hernán and Robins, 2018). Those factors that *a priori* were found to influence both exposure and outcome were considered possible confounders, and included in our logistic regression model. These factors were maternal age and maternal education (supplementary figure 1). Therefore, we finally adjusted for maternal age and maternal education.

The differences in the distribution of phthalates in relation to vocabulary and complexity scores were assessed by using non-parametric Mann-Whitney/Kruskal-Wallis tests (table S4). As language scores tended to be lower among the highest exposed, we used multiple logistic regression to analyze the association between the prenatal phthalate exposure (continuous osmolality adjusted ln-transformed) and MB-CDI (0 = >15, 1 = ≤15) vocabulary and complexity score, adjusting for potential confounders. Estimates of association were converted by multiplying the estimate with ln(2) to express the change in odds of being in ≤15 percentile score with a doubling of the exposure (table 1, figure 2a and figure 2b). As the phthalate concentrations were low and because the results from the descriptive analyses indicated that phthalate exposures in the upper quartiles tended to be associated with lower language scores, we repeated all logistic regression analyses with phthalate levels divided into exposures below or above the 75th percentile (0 = < 75 percentile, 1 = ≥75 percentile). We did not attempt adjustment for confounders in the latter logistic analyses (table 2) due to the small number of highly exposed women with children in the 15th percentile lowest language score bracket (*n* between 7 and 32, table 2).

All analyses were performed separately for boys and girls. Results with *p*-values <0.05 were considered statistically significant.

Results:

Of the 2,549 participants in the OCC the following were excluded; twins (*n* = 101), non-ethnic Danish mothers (*n* = 230), insufficient phthalate measurements (*n* = 18) and incomplete/erroneously answered language questionnaires (*n* = 3) (figure 1). A total of 870 mothers (39.2%) had phthalates measured, and 24 urine samples (2.8%) were unusable due to missing osmolality values. The women with and without phthalate measurements did not differ according to age, parity or gestational age at birth. However, the women with usable phthalate data had higher BMI and less often smoked (data not shown). A total of 1,363 of the 2,217 Danish singletons' parents (61.5%) completed the Danish MB-CDI. Three questionnaires were excluded because the children were older than 36 months. Responders of the MB-CDI did not differ from non-responders according to BMI and parity. However, the women who responded to the MB-CDI questionnaires were older, had fewer preterm births and fewer of them were smokers (data not shown). Ultimately, 518 had data on maternal phthalate exposure and MB-CDI questionnaire "Vocabulary", with 384 providing data on complexity as well.

The 12 phthalate metabolites were detectable in almost all urine samples, except for MBzP and MiNP, where only 69.3% and 9.8% respectively of the samples had concentrations above LOD (see table S1). The highest median concentrations were seen for MiBP, MEP and MnBP. The $\sum\text{MBP}_{(i+n)}$ metabolites were correlated ($r = 0.77$ $p < 0.0001$), $\sum\text{DEHP}_m$ metabolites were correlated ($r \geq 0.64$ $p < 0.0001$) and $\sum\text{DiNP}_m$ metabolites were correlated ($r \geq 0.82$ $p < 0.0001$ (MiNP excluded in correlation analysis)). The urinary concentrations of $\sum\text{DEHP}_m$ and $\sum\text{DiNP}_m$ were significantly ($p < 0.2$) higher for mothers with a pre-pregnancy BMI > 35 kg/m². For $\sum\text{MBP}_{(i+n)}$ and $\sum\text{DEHP}_m$ there was a significantly higher exposure in women with a parity ≥ 3 . Women with low education (high school or less) had significantly higher concentrations of $\sum\text{DEHP}_m$ and $\sum\text{DiNP}_m$, while higher educational level was associated with a higher concentrations of $\sum\text{MBP}_{(i+n)}$ (data not shown).

The initial MB-CDI questionnaires on vocabulary were completed for 271 boys and 247 girls, at a median age (range) of 21 months (boys range = 20-34, girls range = 20-35). The median age (range) of the boys when parents completed the complexity subscale score was 31 months (30-36), and for the girls 27 months (26-35). For vocabulary, the boys had a median (25-75 percentile) percentile score of 50 (25-75), while girls had a median score of 50 (25-80). For complexity, the boys had a percentile score median of 40 (15-75), while girls had a median score of 45 (15-70). The correlation between vocabulary and complexity was $r = 0.67$ ($p < 0.0001$) for boys and 0.62 ($p < 0.0001$) for girls. The percentile scores were not significantly associated ($p > 0.2$) with any of the baseline characteristics (e.g. maternal age, parity, education, BMI, smoking) (data not shown).

Lower vocabulary and complexity percentile scores were in general found among boys in the highest quartile of prenatal phthalate exposure compared to the three other quartiles (table S2). No dose-response relationships were apparent, as scores were similar in the three lowest quartiles and lower in the highest quartile. When comparing the vocabulary and complexity scores of the 75% lowest exposed and the 25% highest exposed individuals, elevated MEP ($p = 0.011$) and $\sum\text{DEHP}_m$ ($p = 0.012$) were associated with significantly lower vocabulary scores in the boys. Complexity scores were significantly lower in boys highly exposed to MEP ($p = 0.020$), MBzP ($p = 0.032$), $\sum\text{DEHP}_m$ ($p = 0.006$), MEHHP ($p = 0.018$), MEOHP ($p = 0.010$) and MECPP ($p = 0.006$) as compared to the lowest quartiles (data not shown).

The adjusted logistic regression yielded statistically significant higher odds for a vocabulary score below the 15th percentile for a doubling in MEP and $\sum\text{DEHP}_m$ (as well as its' metabolites) with the following ORs (boys only): 1.24 (95% CI: 1.05,1.46) and 1.33 (1.01,1.75) (table 1, figure 2a). Similar associations were found for complexity scores among boys, but with a significant OR for MBzP (1.28 (95% CI: 1.02,1.59) rather than MEP (table 1, figure 2b). No association between prenatal phthalate exposure and vocabulary nor complexity score was found among girls. The unadjusted logistic regression analyses were repeated with maternal phthalate exposure as a binary variable below or above the 75th percentile, which strengthened the associations between phthalate exposure and language scores in boys (table 2).

As both MEP and Σ DEHP_m were seen to be significantly associated with lower language scores and as they were correlated (Spearman Correlation Coefficient = 0.28, $p < 0.0001$), they were both included in the adjusted multiple logistic regression. This attenuated the association between MEP and Σ DEHP_m and lower language scores (both vocabulary and complexity) among boys although still in the same direction (data not shown). The same was done for girls, with no change to initial findings.

Discussion:

In this prospective cohort study of 518 mother-child pairs we found an association between higher prenatal exposure to most of the detectable phthalates and increased odds of lower language scores in boys aged 20-36 months, whereas no such association was apparent among the girls. In particular, a statistically significant association was found between higher maternal concentrations of DEP, BBzP and DEHP metabolites and lower vocabulary and complexity language scores in the male offspring. This association was mainly driven by mothers within the highest quartile of phthalate exposure, perhaps due to the fact that this population in general has a fairly low exposure, as compared to other populations studied so far (table S3). However, even within this low-level exposed population an association between maternal phthalate exposure and lower language scores in the male offspring was evident.

Many studies have addressed the adverse effects of phthalate exposure on behavior (Ejaredar et al., 2015; Miodovnik et al., 2014; Ponsonby et al., 2016), but our report appears to represent the largest cohort study addressing the adverse effects of prenatal phthalate exposure on childhood language development.

Eight studies reported on cognitive evaluation as a summed score of several subcategories of which language was one in relation to phthalate exposure (table S4) (Cho et al., 2010; Doherty et al., 2016; Factor-Litvak et al., 2014; Huang et al., 2015; Kim et al., 2011; Polanska et al., 2014; Tellez-Rojo et al., 2013; Whyatt et al., 2012). However, only three of these studies reported on the language subcategory (Cho et al., 2010; Factor-Litvak et al., 2014; Polanska et al., 2014), and of these only one stratified by sex (Factor-Litvak et al., 2014). An inverse association between third trimester MiBP exposure and verbal concept formation, in boys aged 7-years was reported in a U. S. study of 328 mother-child pairs using the Weschler Intelligence Scale (WISC) (Factor-Litvak et al., 2014). Similar to our findings, no inverse association was found for girls. In addition, Factor-Litvak et al. also observed an inverse association between MBP metabolites and full-scale IQ for both boys and girls. Their exposure levels were higher than in our study with the exception of MiBP. In the Polish study by Polanska et al. 2014 of 150 children aged 23-28 months using the Bayley Scales of Infant Development (BSID) they did not find any association between 3rd trimester prenatal exposure to MEP, MBP or DEHP metabolites and language abilities, nor the mental development index (MDI). They did not, however, stratify by sex, and exposure levels were lower than in our study, which

may explain the lack of association. An inverse association between postnatal MEHP and MEOHP exposure and vocabulary score in boys aged 8 to 11 years was found in 618 children from South Korea, whereas no association was found for girls (Cho et al., 2010). However, in this study only postnatal phthalate exposure was reported with no data on prenatal exposures.

Of the five studies that reported on the association between 3rd trimester phthalate exposure and cognitive evaluation by the use of BSID, but did not report on the language estimates, one study (Kim et al., 2011) found an inverse association between DEHP metabolite exposure and cognitive development in boys, whereas the other four did not (Doherty et al., 2016; Huang et al., 2015; Tellez-Rojo et al., 2013; Whyatt et al., 2012); three found inverse associations between prenatal MBP and DEHP exposure and MDI-scores in girls (Doherty et al., 2016; Tellez-Rojo et al., 2013; Whyatt et al., 2012).

Our findings of sex-specific sensitivity to phthalates are in accordance with several animal studies (Dai et al., 2015; Holahan and Smith, 2015), however, the mechanism is not fully determined. Prenatal DEHP exposure has been shown to reduce the lipid content in fetal rat brains (Xu et al., 2007, 2008), reduce the density of axonal innervation and decrease the density of neuronal cells in the CA3 region of the hippocampus in male rats (Miodovnik et al., 2014). Additionally, a recent study (Engel et al., 2017) has shown that several phthalates interfere with estrogen receptors ER α , ER β as well as androgen receptor. The developing brain has high levels of estrogen receptors (McCarthy 2008) and as one of the factors influencing sex differences in brain development is estrogen stimulation (McCarthy 2016), this may explain the observed sex differences. Furthermore, several studies have reported lower testosterone levels in male rats exposed to DEHP both pre- and postnatally (Holahan and Smith, 2015) and testosterone is known to be important for male brain development (Schore, 2017). Additionally, DBP and DEHP exposure in rats has been found to be associated with lower total and free T4 and in some human studies, with an increase in TSH postnatally (Gore et al., 2015). Subclinical hypothyroidism of the mother is known to affect the fetal brain development (Henrichs et al., 2013).

The longitudinal design, the high number of participants, and the amount of available information from the multiple questionnaires and medical journals are major strengths of this study. In addition, we had access to a large Danish MB-CDI reference population (Bleses and Syddansk, 2007). There are, however, some limitations as only 42% of the invited women participated in the OCC. These participants were older, more often nulliparous and more often non-smokers than non-participants (Kyhl et al., 2015). Moreover, mothers with available phthalate measurements and available MB-CDI questionnaires had higher BMI, were older, smoked less and had fewer preterm births than mothers with unavailable information. However, the parents were unaware of their phthalate exposure and their children's language development at time of inclusion making selection bias less

likely. Also, non-participation was probably in part due to the method of recruitment, and given the weak effects of the covariates, it seems unlikely that serious selection bias would occur and affect the study findings.

The use of the validated MB-CDI has several strengths (Bleses et al., 2008a; Fenson et al., 2000). First, the MB-CDI language score is based on the parents' knowledge of their child's language and is therefore neither dependent on the child's current state, nor on the test setting or the examiner. Secondly, there are differences in the development of early language skills between the sexes (Eriksson et al., 2012) and taking this into account leads to a more precise estimate of language scores. Thirdly, the language estimates are comparable between countries of different languages and cultures (Bleses et al., 2008b). Finally, a strong correlation was seen between the two language estimates in this study, as described in the literature (Fenson et al., 1994). However, a possible inclusion of children whose fathers had different ethnicity (bilingual homes), whose parents were living apart, or who suffered from any chronic diseases or speech/speech-hearing problems may have occurred. Furthermore, parental reporting may also lead to misclassification of the child's current language developmental score, however, this is likely non-differential, as the parents were not aware of their phthalate exposure level. While some imprecision may have occurred, we also recognize that the long-term validity of these measures in early childhood may be limited.

All urine samples were collected as fasting morning spot urine and adjusted for osmolality, which likely provided a more precise estimate of the exposures (Frederiksen et al., 2007; Johns et al., 2015). The time of the sampling and fasting of the participants may have contributed to lower concentrations of phthalates compared to other studies but may not have affected the ratios that were already standardized throughout all samples. Use of more than a single sample would have been preferable and would have made the exposure estimates more precise as phthalates have a substantial temporal intra-individual variability due to their rapid breakdown and excretion within hours to days, especially DiNP and DEHP (Johns et al., 2015). However, this imprecision is probably random and therefore most likely biasing the estimates towards the null-hypothesis. Analysis of phthalates was performed during two different periods, but no differences were seen in the measurements when reanalyzed.

Sociodemographic characteristics may indeed confound results, and we adjusted for maternal age and maternal education. Unfortunately, we did not obtain information on maternal IQ or home conditions. Most women in our cohort were, however, relatively well educated (Kyhl et al., 2015), and 63.8% had at least a high school education whereas 22.5% had an education of four or more after completed high school. Residual confounding from other dietary, lifestyle or behavioral factors cannot be excluded. Co-exposure to other endocrine disrupting chemicals and adjustments for maternal IQ deserve to be addressed in future studies.

Early language development predicts later reading skills, one of the most important predictors of educational success (Bleses et al., 2016; Duncan et al., 2007; Elbro et al., 2011; Hawa and Spanoudis, 2014). Interestingly, most previous studies are in agreement with our findings of a potential link between prenatal phthalate exposure and language and/or neurodevelopment despite testing of different cognitive domains at different ages and varied timing of phthalate exposure measures across studies. Few studies have examined whether the effects are sex-specific and none have been conducted among Danish children.

Conclusions:

In this study of 518 mother-child pairs, we showed an inverse association between 3rd trimester prenatal phthalate exposure and language scores in boys that was statistically significant for DEP, BBzP and DEHP metabolites. No association between maternal phthalate exposure and language scores of girls was apparent. Our findings are notable as associations were suggested even in this low-level exposed population with only a single spot urine sample for exposure measure and control for confounders. Early language development is of relevance as it is thought to be an important predictor of later reading skills, which again is linked to educational achievement. Follow-up of these children should include assessment of their own postnatal phthalate exposure.

Figure Legends

Figure 1. Flowchart describing selection of study sample of 518 mother-child pairs derived from the Odense Child Cohort (2010-2012).

MB-CDI = MacArthur-Bates Communicative Development Inventories "Words and Sentences" (Danish version).

Figure 2a. Graphical representation of data from back-transformation of logistic regression with ln-transformed continuous osmolality adjusted maternal urinary phthalate concentrations and dichotomized MB-CDI vocabulary score (0 = >15 percentile, 1 = ≤15percentile), adjusted for maternal education level and maternal age.

Blue squares = Boys

Red circles = Girls

Figure 2b. Graphical representation of data from back-transformation of logistic regression with ln-transformed continuous osmolality adjusted maternal urinary phthalate concentrations and dichotomized MB-CDI complexity score (0 = >15 percentile, 1 = ≤15percentile), adjusted for maternal education level and maternal age.

Blue squares = Boys

Red circles = Girls

Supplementary figure 1. Directed acyclic graph depicting selection of confounders

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Table 1. Odds ratios (95% CI) of having a vocabulary and complexity score below 15th percentile with a doubling of prenatal phthalate exposure. Adjusted for maternal age and maternal education

	Vocabulary Scores		Complexity Scores	
	Boys (n = 271) OR (95%CI)	Girls (n = 247) OR (95%CI)	Boys (n = 179) OR (95%CI)	Girls (n = 205) OR (95%CI)
MEP	1.24 (1.05,1.46)*	1.08 (0.92,1.27)	1.16 (0.97,1.39)	1.10 (0.94,1.30)
MiBP	0.99 (0.76,1.30)	0.86 (0.65,1.13)	1.03 (0.77,1.39)	1.01 (0.77,1.32)
MnBP	1.11 (0.86,1.42)	0.81 (0.63,1.05)	1.31 (0.97,1.77)	0.89 (0.69,1.16)
ΣMBP (i+n)	1.01 (0.77,1.32)	0.81 (0.61,1.08)	1.12 (0.81,1.54)	0.97 (0.74,1.27)
MBzP	1.08 (0.89,1.32)	0.93 (0.74,1.18)	1.28 (1.02,1.59)*	0.96 (0.76,1.21)
MEHP	1.02 (0.85,1.23)	0.88 (0.71,1.09)	1.14 (0.92,1.42)	1.08 (0.86,1.35)
MEHHP	1.32 (1.03,1.70)*	0.87 (0.67,1.13)	1.38 (1.04,1.83)*	0.86 (0.66,1.11)
MEOHP	1.35 (1.04,1.73)*	0.81 (0.63,1.05)	1.41 (1.06,1.87)*	0.89 (0.68,1.16)
MECPP	1.37 (1.02,1.85)*	0.83 (0.62,1.11)	1.48 (1.04,2.09)*	0.90 (0.67,1.21)
ΣDEHP_m	1.33 (1.01,1.75)*	0.82 (0.61,1.11)	1.43 (1.03,1.97)*	0.88 (0.65,1.18)
MHiNP	1.16 (0.96,1.41)	0.96 (0.79,1.15)	0.97 (0.80,1.18)	0.93 (0.76,1.14)
MOiNP	1.19 (0.99,1.43)	1.00 (0.83,1.20)	1.03 (0.85,1.24)	0.90 (0.74,1.11)
MCiOP	1.17 (0.95,1.45)	0.98 (0.78,1.23)	0.96 (0.76,1.21)	0.88 (0.66,1.16)
ΣDiNP_m	1.18 (0.95,1.48)	0.98 (0.78,1.23)	0.97 (0.76,1.23)	0.88 (0.67,1.15)

ΣMBP(i + n), sum of isomers MiBP and MnBP. ΣDEHP_m, molar sum of DEHP metabolites expressed as excreted DEHP (ng/mL). ΣDiNP_m, molar sum of DiNP metabolites expressed as excreted DiNP (ng/mL).

Back-transformation of multiple logistic regression with ln-transformed continuous osmolality adjusted maternal urinary phthalate concentrations and dichotomized MB-CDI vocabulary and complexity score (0 = >15 percentile, 1 = ≤15 percentile), adjusted for maternal education and maternal age.

* $p < 0.05$

Table 2. Odds ratios of having a vocabulary or complexity score ≤ 15 th percentile and an osmolality adjusted prenatal phthalate exposure ≥ 75 th percentile.

	Vocabulary Scores				Complexity Scores			
	Boys		Girls		Boys		Girls	
	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)	N	OR (95%CI)
MEP	26/271	3.50* (1.88,6.54)	14/247	1.31 (0.65,2.65)	18/179	1.52 (0.76,3.06)	15/205	1.42 (0.70,2.90)
MiBP	13/271	1.03 (0.51,2.06)	9/247	0.54 (0.25,1.18)	18/179	1.83 (0.90,3.73)	14/205	1.08 (0.53,2.20)
MnBP	15/271	1.21 (0.62,2.37)	7/247	0.41 (0.17,0.97)	17/179	1.61 (0.79,3.28)	10/205	0.61 (0.28,1.32)
ΣMBP(i+n)	14/271	1.08 (0.54,2.13)	9/247	0.58 (0.26,1.28)	19/179	1.99 (0.98,4.02)	10/205	0.63 (0.29,1.37)
MBzP	32/271	1.37 (0.75,2.48)	24/247	0.86 (0.46,1.60)	36/179	2.09* (1.08,4.03)	20/205	0.59 (0.31,1.12)
MEHP	12/271	0.84 (0.41,1.70)	13/247	1.03 (0.51,2.10)	14/179	1.07 (0.51,2.23)	16/205	1.39 (0.70,2.79)
MEHHP	20/271	1.76 (0.94,3.30)	11/247	0.95 (0.45,2.01)	22/179	2.18* (1.11,4.32)	10/205	0.78 (0.35,1.70)
MEOHP	20/271	1.80 (0.96,3.39)	10/247	0.80 (0.37,1.72)	22/179	2.29* (1.15,4.53)	11/205	0.88 (0.41,1.88)
MECPP	22/271	1.95* (1.05,3.63)	7/247	0.55 (0.23,1.31)	23/179	2.16* (1.10,4.22)	9/205	0.74 (0.33,1.66)
ΣDEHPm	23/271	2.39* (1.28,4.45)	10/247	0.82 (0.38,1.77)	23/179	2.25* (1.15,4.42)	11/205	0.98 (0.45,2.12)
MHiNP	17/271	1.25 (0.65,2.38)	14/247	1.48 (0.73,3.00)	16/179	1.00 (0.50,2.02)	11/205	0.94 (0.44,2.04)
MOiNP	21/271	1.68 (0.90,3.13)	15/247	1.98 (0.98,4.02)	18/179	1.14 (0.58,2.26)	11/205	1.07 (0.49,2.31)
MCiOP	18/271	1.18 (0.63,2.23)	12/247	1.41 (0.67,2.97)	19/179	1.19 (0.61,2.34)	11/205	1.16 (0.53,2.53)
ΣDiNPm	20/271	1.56 (0.83,2.91)	13/247	1.47 (0.71,3.03)	20/179	1.45 (0.74,2.85)	10/205	0.91 (0.41,2.01)

Univariate logistic regression of dichotomized osmolality adjusted prenatal phthalate exposure (0 = < 75 percentile, 1 = ≥ 75 percentile) and unadjusted language score (0 = > 15 percentile, 1 = ≤ 15 percentile) for vocabulary and complexity.

* $p < 0.05$

Figure 1

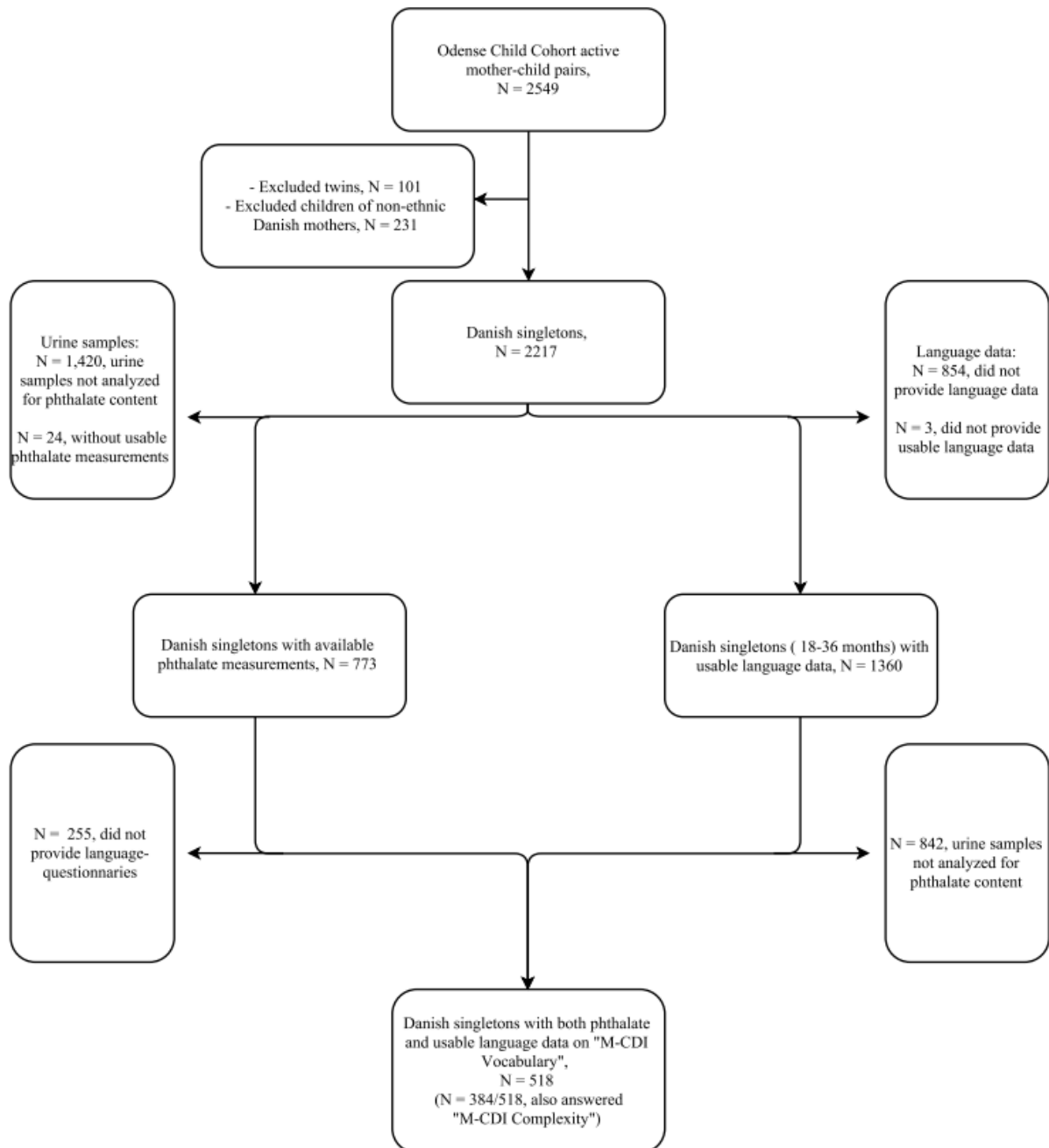


Figure 2a: Odds-ratio (95% CI) of having a vocabulary score ≤ 15 th percentile with a doubling of prenatal phthalate exposure.

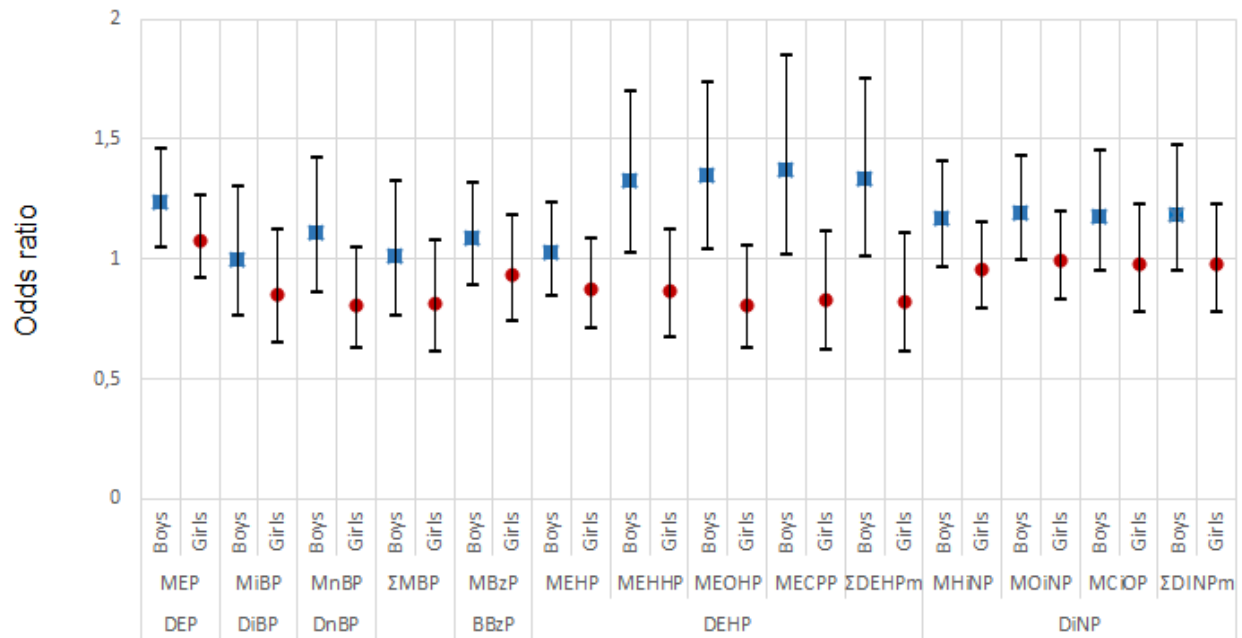
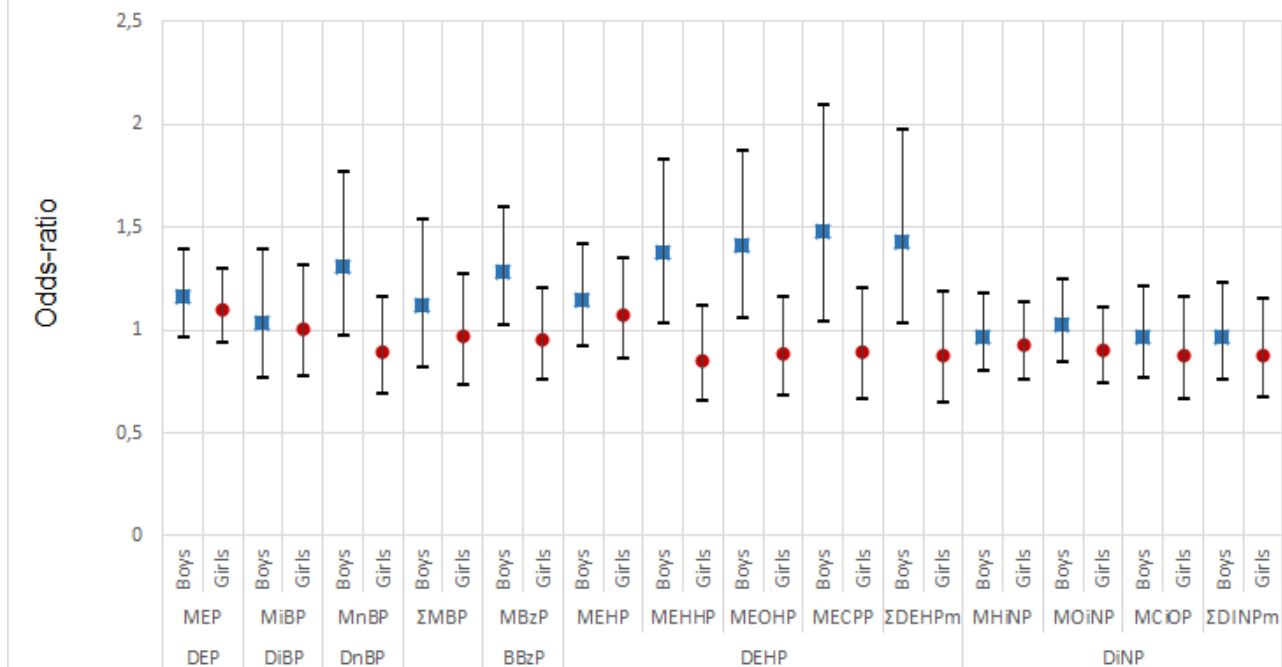


Figure 2b: Odds-ratio of having a complexity score ≤ 15 th percentile with a doubling of prenatal phthalate exposure.



Highlights

- There may not be a dose-response relation between prenatal phthalate and language
- High phthalate exposure in utero may have a negative effect on early language
- Prenatal phthalate exposure may have an adverse effect on male early language
- Prenatal phthalate exposure may not have adverse effect on female early language

ACCEPTED MANUSCRIPT