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Prenatal exposure to phthalates and neuropsychological development during childhood

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ABSTRACT

There is inconsistent evidence regarding the effects of prenatal phthalate exposure on children's neuropsychological development. We evaluate the association between prenatal phthalate exposure and the cognitive, psychomotor and behavioral development of 367 children at repeated ages in a prospective birth cohort study. We measured phthalate metabolites (sum of four DEHP metabolites – Σ_4DEHP, MBzP, MEP, MiBP and MnBP) in urine samples collected during the 1st and 3rd trimesters of pregnancy in women participating in the INMA-Sabadell birth cohort study. We assessed cognitive and psychomotor development of their children at 1 and 4 years, and social competence, ADHD symptoms and other behavioral problems at 4 and 7 years. No associations were observed between prenatal phthalate exposure and cognitive and psychomotor scores at the age of 1 year and at the age of 4 years, except for an association between MBzP and lower psychomotor scores ($\beta = -1.49$ [95% confidence interval (CI) = −2.78, −0.21]). Σ_4DEHP concentrations were associated with increased social competence scores at 4 years and with reduced ADHD symptoms at age 4 and 7 years. Increasing MEP concentrations were associated with a reduced risk of inattention symptoms at 4 years. No associations were observed for MBzP, MiBP or MnBP in relation to behavioral problems. This study, with multiple phthalate exposure measurements and measures of neuropsychological domains at different ages, suggest that prenatal phthalate exposure does not adversely affect children's cognitive, psychomotor or behavioral development.

Abbreviations: ADHD, attention-deficit hyperactivity disorder; ADHD-DSM-IV, attention-deficit hyperactivity disorder – criteria of the diagnostic and statistical manual of mental disorders – 4th edition; BMI, body mass index; BSID, bayley scales of infant development; CPSCS, california preschool social competence scale; CSRS, conners' parent rating scales; DEHP, di-(2-ethylhexyl) phthalate; INMA, *infancia y medio ambiente*; LMWP, low molecular weight phthalates; LOD, limit of detection; MEHHP, mono-(2-ethyl-5-hydroxyl-hexyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxy-pentyl) phthalate; MBzP, mono-benzyl phthalate; MEP, mono-ethyl phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-*n*-butyl phthalate; MSCA, mccarthy scales of children's abilities; SDQ, strengths and difficulties questionnaire.

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Introduction

Phthalates are synthetic chemical compounds produced in large quantities and widely used in a range of consumer products including cosmetics, plastics, carpets, building materials, toys and medical and cleaning products (Bellinger, 2013; Braun et al., 2013; Miodovnik et al., 2014). The general population is exposed to phthalates mainly through diet, which is the most likely exposure route for the high molecular weight phthalates, and personal care products, which are the most likely exposure route for the low molecular weight phthalates (Hoppin et al., 2002; Braun et al., 2013; Miodovnik et al., 2014). Previous studies on animals have suggested that prenatal exposure to phthalates can increase the risk of impaired neurodevelopment (Bellinger, 2013; Martinez-Arguelles et al., 2013; Miodovnik et al., 2014). In humans, several prospective birth cohort studies, most of them including a relatively small study population, have reported adverse effects of prenatal phthalate exposure on the cognitive, psychomotor and behavioral development of children (Table 1). However, reported effects are not consistent across the different phthalate congeners and outcomes assessed and, additionally, there is not a clear gender specificity of the effects observed (Bellinger, 2013; Miodovnik et al., 2014). One of the main limitations of previous studies is that prenatal phthalate exposure was determined in a single spot urine measurement, which can lead to exposure misclassification, as phthalates have a short half-life (of hours) and are rapidly excreted from the body (Hoppin et al., 2002; Braun et al., 2013). Further, the only study with more than one phthalate measurement during pregnancy performed neurological examinations at the age of one month but did not follow-up children at later ages (Yolton et al., 2011). In the present study we collected urine samples in the 1st and the 3rd trimester of pregnancy in order to improve the assessment of phthalate exposure throughout pregnancy, and we conducted neuropsychological tests covering a wide range of cognitive, psychomotor and behavioral domains from the first year of life up to school ages. The aim of the present study, which includes more than 350 participants, is to evaluate whether urine biomarker measurements of phthalates during pregnancy are associated with impaired cognitive, psychomotor and behavioral development of children aged 1, 4 and 7 years in a longitudinal birth cohort study.

Methods

Study population

Pregnant women from the general population were recruited into the INMA-*INfancia y Medio Ambiente* (Environment and Childhood) birth cohort set up in Sabadell (Catalonia, Spain) between 2004 and 2006 ($N = 657$). Protocol details are described elsewhere (Guxens et al., 2012). Briefly, women were recruited during the 1st trimester routine antenatal care visit in the main public hospital or health centre of reference if they fulfilled the inclusion criteria: age ≥ 16 years, intention to deliver in the reference hospital, singleton pregnancy, no assisted conception, and no problems of communication. The study was conducted with the approval of the hospital ethics committee and written informed consent was obtained from the parents of all children.

Cognitive and psychomotor development assessment

At the age of 1 year (mean 14 months, range 12–18 months), we assessed children's cognitive and psychomotor development using the Bayley Scales of Infant Development (BSID) (Bayley, 1977), which includes the mental scale and the psychomotor scale. All testing was done in the health care centre in the presence of the mother by two specially trained psychologists, who followed a strict protocol to limit inter-observer variability (for further information see Forns et al., 2012). Scores were standardized for child's age in days at test administration using a parametric method for the estimation of age-specific reference intervals. The parameters of the distribution were modeled as a fractional polynomial function of age and estimated by maximum likelihood. Residuals were then normalized to a mean of 100 points with a standard deviation of 15 points to homogenize the scales and to be able to compare our results with other studies. At the age of 4 years, children completed a standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy, 1972). The global cognitive scale and five subscales (Verbal, Perceptive-Performance, Memory, Quantitative and Motor) were examined. The testing was done by one psychologist. The continuous MSCA scales were standardized to a mean score of 100 with a standard deviation of 15 to homogenize all the scales.

Behavioral development assessment

At the age of 4 years, teachers of children completed an adapted bilingual version (Spanish/Catalan) of the California Preschool Social Competence Scale (CPSCS) to evaluate children's social competence (Julvez et al., 2008). This test consists of 30 items and a single general score is obtained. Teachers also completed a form list of Attention-Deficit Hyperactivity Disorder (ADHD) Criteria of the Diagnostic and Statistical Manual of Mental Disorders – 4th edition (ADHD-DSM-IV), which is used to assess attention-deficit, hyperactivity and impulsivity. A global score and two subscores (Inattention and Hyperactivity/Impulsivity) were created. At the age of 7 years, parents completed the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) and the short form of the Conners' Parent Rating Scales (CSRS) (Gianarris et al., 2001). The SDQ, which covers common areas of emotional and behavioral difficulties, consists of 25 items that allow obtaining a global score and individual scores for five subscales (Emotional symptoms, Conduct problems, Hyperactivity/Inattention, Peer relationship problems and Prosocial behaviour). The short form of the CSRS intends to assess problematic behavior in children and consists of 27 items that result into scores for three subscales (Oppositional, Cognitive Problems/Inattention and Hyperactivity) and an ADHD Index.

In this study, all outcomes were assessed as continuous (score) variables rather than dichotomized according to a clinically relevant cut-off, because our aim was not to detect an effect of exposure on clinically diagnosed cases, but rather on the distribution of symptoms in the general population.

Exposure assessment

We collected spot urine samples of mothers at 12 and 32 weeks of gestation and stored in 10 mL polyethylene tubes at −20 °C. Echevarne laboratory of Barcelona (Spain) determined creatinine by the Jaffé method (kinetic with target measurement, compensated method) with Beckman Coulter© reactive in AU5400 (IZASA®), and the Bioanalysis Research Group at Hospital del Mar Medical Research Institute (IMIM, Barcelona, Spain) quantified urine concentrations of a total of eight phthalate metabolites: mono-(2-ethyl-5-hydroxyl-hexyl) phthalate (MEHHP), mono-(2-ethyl-hexyl) phthalate (MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxy-pentyl) phthalate (MECPP), MBzP, mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP) and mono-*n*-butyl phthalate (MnBP). The LOD for the different congeners ranged from 0.5 to 1 μg/L (further details at Valvi et al., 2015). We adjusted phthalates concentrations for creatinine (μg/g creatinine) to control for urine dilution. Because of the low correlation observed between phthalates concentrations

Table 1
Summary of the results of previous birth cohort studies (ordered by type of outcome and by age of study participants).

Study	N	Age(s)	Sample	Exposure	Test (items)	Results		
						Total study population	Females	Males
Neurological examinations								
Engel et al. (2009)	295	5d	MU (1st trim)	10 phthalates metabolites (6 HMWP & 4 LMWP)	BNBAS (46 items)	No associations	HMWP: ↓ orientation and quality of alertness	LMWP: ↑ motor performance
Yolton et al. (2011)	350	1 m	MU (1st & 3rd trim)	DEHP, DBP	NNNS (11 items)	DBP (3rd trim): ↓handling & ↑ regulation and movement quality DEHP (3rd trim): ↑non-optimal reflexes		DEHP (3rd trim): ↑non-optimal reflexes
Cognitive & psychomotor development								
Kim et al. (2011)	417	6 m	MU (3rd trim)	DEHP metabolites (MEHHP & MEOHP), MBP	BSID II (MDI & PDI)	DEHPs & MBP: ↓MDI and PDI		Stronger associations (no significant interaction)
Polanska et al. (2014)	150	24 m	MU (3rd trim)	11 phthalates metabolites (6 HMWP & 5 LMWP)	BSID III (Cognitive, language & motor)	HMWP, 3OH-MnBP & DnBP: ↓ motor		
Téllez-Rojo et al. (2013)	135	24, 30, 36 m	MU (3rd trim)	9 phthalates metabolites (4 DEHPs, MEP, MiBP, MnBP, MBzP, MCPP)	BSID II (MDI & PDI)	No associations	DEHPs: ↓ MDI	MBzP & MCPP: ↑ PDI
Whyatt et al. (2012)	297	36 m	MU (3rd trim)	4 DEHPs, MBzP, MnBP, MiBP	BSID II (MDI & PDI)	MnBP & MiBP: ↓ PDI	MnBP: ↓ MDI	
Factor-Litvak et al. (2014)	328	7y	MU (3rd trim)	2 DEHPs, MEP,MBzP, MnBP, MiBP	WISC-IV - (full IQ scale and 4 subscales)	MnBP: ↓ IQ & 3 subscales MiBP: ↓ IQ % 4 subscales MBzP: ↓ 1 subscale	MnBP: ↓ IQ & 2 subscales MiBP: ↓ IQ & 1 subscale	MnBP: ↓ 1 subscales MiBP: ↓ IQ & 1 subscale MBzP: ↓ 1 subscale
Engel et al. (2010)	177	5, 6.5, 7-9y	MU (3rd trim)	10 phthalates metabolites (6 HMWP & 4 LMWP)	BRIEF (8 scales)	LMWP: ↓ scores in the emotional scale and the Global Executive Composite index		
Behavioral development								
Whyatt et al. (2012)	277	36 m	MU (3rd trim)	4 DEHPs, MBzP, MnBP, MiBP	CBCL (5 subscales)	MnBP, MiBP, MBzP: ↑ behavioral problems		
Swan et al. (2010)	150	3-7y	MU (mid-pregnancy)	9 phthalates metabolites (DEHPs, DBPs & others)	PSAI (24 items; modified)	No associations		DEHPs: ↓ masculine playing behavior
Engel et al. (2010)	177	5, 6.5 and 7-9y	MU (3rd trim)	10 phthalates metabolites (6 HMWP & 4 LMWP)	BASC-PRS (9 scales)	LMWP: ↑ aggression, depression & attention, conduct and externalizing problems and behavioral symptom index		
Kobrosly et al. (2014)	153	6–10 y	MU (one sample along pregnancy)	9 phthalates metabolites (MEP, MiBP, MBzP, MBP, DEHPs)	CBCL	MiBP: ↑ attention problems, aggressive behavior	DEHPs, MBzP: ↓ anxiety/depression & internalizing behavior	MiBP: ↑ attention problems & rule-breaking, aggressive, internalizing and externalizing behavior
					CBCL DSM-oriented scores	DEHPs: ↑ somatic problems MEP: ↓ affective problems MBP: ↑ conduct problems MiBP: ↑conduct and oppositional problems	DEHPs, MBzP: ↓ anxiety problems	All associations for the total population found in boys & MBzP: ↑conduct and oppositional problems
Miodovnik et al. (2011)	137	7-9y	MU (3rd trim)	10 phthalates metabolites (6 HMWP & 4 LMWP)	SRS (5 subscales)	LMWP (MEP): ↓ social competences (3 subscales)		

Lien et al. (2015)	122	8-9y	MU (3rd trim)	7 phthalates metabolites (3 DEHPs, MBzP & 3 LMWP)	CBCL	MEOHP (DEHP): ↑ social problems, delinquent behavior, aggressive behavior, externalizing problemsMEHHP (DEHP): ↑ delinquent behaviorMEHP (DEHP): ↑ delinquent behavior, externalizing problemsMBP (LMWP): ↑ delinquent behavior, aggressive behavior, externalizing problems

ADD: Attention Deficit Disorder; ADHD: Attention-deficit/hyperactivity disorder; BASC-PRS: Behavior Assessment System for Children-Parent Rating Scale; BNBAS: Brazelton Neonatal Behavioral Assessment Scale; BSID II: Bayley Scales of Infant Development (MDI and PDI: Mental and Psychomotor Development Indexes, respectively); BRIEF: Behavior Rating Inventory of Executive Function; CBCL: Child Behavior Checklist; DSM-IV: Diagnostic and Statistical Manual of Mental Disorders; LD: Learning Disabilities; NNNS: NICU Netowrk Neurobehavioral Scale; IQ: intelligence quotient; PSAI: Pre-School Activities Inventory; SRS: Social Responsiveness Scale; WISC: Wechsler Intelligence Scale for Children.
HMWP: high-molecular weight phthalates; LMWP: low-molecular weight phthalates.
CU: child urine, MU: maternal urine, NA: not assessed.

in the 1st and the 3rd trimester of pregnancy (spearman values between 0.12 and 0.24) and the poor reproducibility between both trimesters (ICC < 0.25) for all metabolites (Valvi et al., 2015), we used the average of the 1st and 3rd trimester concentrations as our exposure variable in the main analyses in order to provide a better estimate of exposure throughout pregnancy, as recommended by previous studies for compounds with a short half-life (Hoppin et al., 2002; Braun et al., 2013). We performed our analysis grouping the DEHP metabolites [the sum of di-2-ethylhexyl phthalate (Σ_4DEHP) metabolites: MEHP, MEHHP, MEOHP, MECPP]. The other metabolites (MBzP, MEP, MiBP, MnBP) were analyzed separately.

Covariates

We obtained information on the following covariates through questionnaires answered by the mothers during the 1st and the 3rd trimesters of pregnancy and/or at age 1 year of the child: maternal age, social class, education and country of origin, maternal smoking during pregnancy, number of older siblings, day care attendance during the 1st year of life, duration of exclusive breastfeeding and pre-pregancy maternal body mass index (BMI). Information on gestational age, weight at birth, season of birth and child sex was collected from clinical records. The similarities subtest of the Wechsler Adult Intelligence Scales – 3rd edition (WAIS-III) was used as a proxy for maternal verbal IQ. Table 2 shows how each covariate has been treated (continuous or categorical).

Statistical methods

We imputed missing values in covariates (between 0 and 4.5%) using multiple imputation methods (Royston, 2005). We used the same method to impute phthalate concentrations below the LOD (between 0% and 0.9% of the samples) by defining the range of imputed values between 0 and the LOD value for each compound. Detailed information on the imputation process can be found in eTable 1 in the Supplemental material.

Out of the 657 pregnant women initially recruited in the INMA-Sabadell cohort, 391 had information on prenatal phthalate exposure at both trimesters of exposure assessment. Out of these, 347 children successfully completed the BSID, 367 the MSCA, 336 the CP-SCS, 352 the ADHD-DSM-IV, 362 the SDQ and 361 the CSRS. A different imputed dataset was generated for each of the outcomes.

As distributions of phthalate concentrations were right skewed we $\log_2$-transformed the exposure variables. To assess the association between phthalate exposure and BSID, MSCA and CPSCS scores we conducted linear regression analyses, as these are continuous outcomes. For outcomes with a negative binomial distribution (ADHD-DSM-IV, SDQ and CSRS scores) we conducted negative binomial generalized linear models. Linearity of the association between the different exposure variables and outcomes was assessed using Generalized Additive Models (GAM). Since there was no evidence of non-linearity, phthalate concentrations were treated as continuous variables. Multivariate models were directly adjusted by gender and age of the child at the time of the neuropsychological test (with the exception of the BSID scales, which were already standardized by age). To determine the rest of the covariates to be included in the final multivariate models we first analyzed which covariates were associated ($p < 0.05$) to at least one of the phthalates assessed and to at least one of the outcomes assessed. Covariates following this criterion were included in the initial multivariate models for all outcomes (maternal education, breastfeeding, season of birth, maternal country of origin, maternal smoking during pregnancy and siblings). Other variables that were related in the bivariate analyses only to at least one of the phthalates or to at least one of the outcomes were afterwards tested as potential confounders into the initial multivariate models (adding one

Table 2
Characteristics of the study population and maternal phthalate metabolite levels in urine.

Characteristics	$N = 367$[a]
Child	
Sex (%)	
Female	49
Male	52
Day-care attendance (%)	
No	65
Yes	35
Breastfeeding (%)	
No	7
≤2 m	11
>2 to <6 m	37
≥6 m	45
Siblings (%)	
No	58
1	36
≥2	6
Mother	
Country origin (%)	
Outside European Union	6
European Union	94
Age (median, 25th and 75th)	30 (28, 33)
Pre-pregnancy BMI (%)	
≤18.5	6
>18.5–25	68
>26–30	18
>30	9
Education (%)	
Primary	22
Secondary	42
University	36
Smoking during pregnancy (%)	
No	85
Yes	16
Maternal IQ (median, 25th and 75th)	101 (54, 136)
Urine phthalate metabolite levels (μg/g creatinine)[b] [median (25th, 75th)]	
High molecular weight phthalates	
DEHP	99.8 (68, 146)
MEHHP	27.1 (18, 41)
MEHP	10.7 (7, 17)
MEOHP	20.6 (14, 30)
MECPP	39.0 (27, 59)
MBzP	11.9 (7, 20)
Low molecular weight phthalates	
MEP	403.4 (199, 756)
MiBP	31.6 (22, 48)
MnBP	30.8 (20, 49)

DEHP: di-(2-ethylhexyl) phthalate; MEHHP: mono-(2-ethyl-5-hydroxyl-hexyl) phthalate; MEHP: mono-(2-ethylhexyl) phthalate; MEOHP: mono-(2-ethyl-5-oxo-hexyl) phthalate; MECPP: mono-(2-ethyl-5-carboxy-pentyl) phthalate; MBzP: mono-benzyl phthalate; MEP: mono-ethyl phthalate, MiBP: mono-iso-butyl phthalate, MnBP: mono-*n*-butyl phthalate.

[a] Based on the database of children with complete information on phthalates exposure and McCarthy Scales of Children's Abilities (MSCA), as this was the study population with more children included. Values obtained do not change in the other databases.

[b] Average of measurements at two time points in the 1st and 3rd trimester of pregnancy.

variable at the time); from this set of other variables, only maternal age changed the estimates by more than 10%, and therefore was also included in the final multivariate models. Although one single measurement might not be enough to properly assess exposure during a particular period, we conducted sensitivity analyses for the two trimesters of exposure separately. Finally, because previous animal and human studies have suggested that associations may differ by child sex, we tested potential effect modification by sex, including the product term between phthalates and sex, considering a $p < 0.10$ as the cut-off for interaction. Analyses were conducted using STATA software, version 12.0 (StataCorp, College Station, TX).

Results

Characteristics of the children included in the study are reported in Tables 2 and 3. Among the high molecular weight phthalates, Σ_4DEHP had median concentrations of 99.8 μg/g creatinine and MBzP of 11.9 μg/g creatinine. MEP was the low molecular weight phthalate metabolite present at highest concentrations in our study population (median = 403.4 μg/g creatinine), followed by MiBP (31.6 μg/g creatinine) and MnBP (30.8 μg/g creatinine). The distribution of the cognitive, psychomotor and behavioral scores obtained by the children was as expected in a population-based study, and no differences were observed with those children of the birth cohort excluded from the study (data not shown). Mothers of children not included in the present study were younger, more likely to come from non-European countries and less likely to breastfeed their children and to send them to a daycare service. These mothers also had lower education level (further details at Valvi et al., 2015).

Cognitive and psychomotor development

No associations were observed between prenatal phthalate exposure and cognitive and psychomotor development at the age of 1 or 4 years (Table 4). The only association observed was between prenatal MBzP and psychomotor development at the age of 4 years ($\beta = -1.49$ [95% confidence interval (CI) = −2.78, −0.21]) (Table 4). Overall, we did not observe consistent sex-specific associations between the different phthalates analyzed and cognitive or psychomotor development (data not shown). When we analyzed the effects of phthalate exposure by trimesters of pregnancy we observed that increasing concentrations of Σ_4DEHP in the 1st trimester were associated with better cognitive scores at the age of 4 years (eTable 2).

Behavioral development

At the age of 4 years increasing prenatal Σ_4DEHP concentrations were associated with better social competence ($\beta = 2.00$ [95% confidence interval (CI) = 0.22, 3.79]) (Table 5). Increasing prenatal Σ_4DEHP concentrations were also associated with a reduced risk of inattention symptoms at 4 years (IRR = 0.84 [95% confidence interval (CI) = 0.72, 0.98]) and 7 years (IRR = 0.83 [95% confidence interval (CI) = 0.71, 0.95]). At 7 years increasing Σ_4DEHP concentrations were also associated with a lower risk of ADHD symptoms (IRR = 0.88 [95% confidence interval (CI) = 0.77, 1.00]). The low molecular weight phthalate MEP was associated with a reduced risk of inattention symptoms at 4 years (IRR = 0.88 [95% confidence interval (CI) = 0.80, 0.97]) (Table 5). No associations were observed for MBzP, MiBP or MnBP (Table 5). Overall, we did not observe consistent sex-specific associations between the different phthalates analyzed and any of the behavioral outcomes assessed (data not shown). When we analyzed the effects of phthalate exposure by trimesters of pregnancy we did not observe different results between the two periods analyzed (eTable 3).

Discussion

Results of the present study suggest that prenatal phthalate exposure does not adversely affect children's cognitive, psychomotor or behavioral in children up to 7 years of age. The risk of certain ADHD symptoms and behavioral problems was reduced with increasing Σ_4DEHP and MEP concentrations.

In the present study we did not observe associations between prenatal phthalate exposures and the cognitive or the psychomotor development of children at the age of 1 year. Furthermore, we

Table 3
Description of the cognitive, psychomotor and behavioral scores of the children.

Age & outcome (*N*)	Test	Scores [median, (25th and 75th)]
Cognitive and psychomotor scores		
1 year (*N* = 347)	BSID	
	Mental scale	100.0 (91.0, 108.9)
	Psychomotor scale	99.9 (93.7, 112.2)
4 years (*N* = 367)	MCSA	
	Global score	101.2 (92.0, 110.3)
	Verbal	101.9 (89.9, 110.3)
	Performance	100.5 (88.8, 112.1)
	Quantitative	99.8 (90.1, 111.8)
	Memory	100.7 (89.7, 109.9)
	Motor	99.4 (90.3, 110.7)
Behavioral scores		
4 years		
Social competence (*N* = 336)	CP-SCS	4.4 (4.3, 4.5)
ADHD symptoms (*N* = 352)	ADHD-DSM-IV	
	Global score	4 (1, 10)
	Inattention	2 (0, 5)
	Hyperactivity/Impulsivity	2 (0, 5)
7 years		
Behavioral problems (*N* = 362)	SDQ	
	Global score	8 (5, 11)
	Emotional symptoms	2 (1, 3)
	Conduct problems	1 (0, 3)
	Hyperactivity/Inattention	3 (1, 5)
	Peer relationship problems	1 (0, 2)
	Poor prosocial behavior	1 (0, 3)
ADHD symptoms (*N* = 361)	CSRS	
	ADHD Index	6 (2, 12)
	Hyperactivity	2 (1, 6)
	Cognitive Problems/Inattention	2 (1, 5)
	Oppositional	2 (1, 4)

BSID: Bayley Scales of Infant Development, MCSA: McCarthy Scales of Children's Abilities; CP-SCS: California Preschool Social Competence Scale; ADHD-DSM-IV: Attention-Deficit Hyperactivity Disorder Criteria of the Diagnostic and Statistical Manual of Mental Disorders - 4th edition; SDQ: Strengths and Difficulties Questionnaire; CSRS: Conners' Parent Rating Scales.

showed that the null findings observed in the first year of life consistently persisted at the age of 4 years. Previous studies, which included less participants than the present study except Kim et al.'s (2011) study, observed reduced mental and psychomotor scores in relation to prenatal phthalates exposures. One study observed reduced psychomotor development at the age of 36 months and reduced IQ (intelligence quotient) at the age of 7 years in relation to prenatal MnBP and MiBP exposure (Factor-Litvak et al., 2014). The results obtained by the other studies were not as consistent, with associations observed not always for the same compounds or categories of compounds (low and high molecular weight phthalates) and/or for the same parameters (cognitive or psychomotor) of children's development (Engel et al., 2010; Kim et al., 2011; Whyatt et al., 2012; Téllez-Rojo et al., 2013; Polanska et al., 2014). Indeed, after stratifying by sex, results between studies were also inconsistent (Table 1).

The results obtained in some of the previous studies evaluating the effects of phthalates on behavioral outcomes suggest that low molecular weight phthalates might increase behavioral problems. However, there are inconsistencies across the different phthalate congeners and behavioral outcomes evaluated (Engel et al., 2010; Swan et al., 2010; Miodovnik et al., 2011; Whyatt et al., 2012;

Table 4
Associations between maternal urinary phthalate metabolite levels and cognitive and psychomotor outcomes at age 1 and 4 years.

Age (*N*)	Test	Σ_4DEHPs[a] β (95%CI)[b]	MBzP β (95%CI)[b]	MEP β (95%CI)[b]	MiBP β (95%CI)[b]	MnBP β (95%CI)[b]
1 year (*N* = 347)	BSID[c]					
	Mental scale	0.45 (−1.26, 2.15)	0.17 (−1.14, 1.48)	−0.49 (−1.57, 0.59)	0.62 (−0.84, 2.08)	0.85 (−0.59, 2.29)
	Psychomotor scale	0.37 (−1.52, 2.22)	−0.53 (−1.97, 0.91)	−0.80 (−1.97, 0.38)	−0.68 (−2.28, 0.92)	0.51 (−1.07, 2.09)
4 years (*N* = 367)	MCSA[d]					
	Global score	1.40 (−0.13, 2.93)	0.31 (−0.88, 1.50)	0.70 (−0.28, 1.68)	0.76 (−0.55, 2.08)	0.53 (−0.74, 1.81)
	Verbal	1.56 (−0.08, 3.19)	0.70 (−0.57, 1.97)	0.78 (−0.27, 1.84)	1.22 (−0.18, 2.63)	1.03 (−0.33, 2.40)
	Performance	0.98 (−0.57, 2.54)	0.27 (−0.94, 1.48)	0.32 (−0.68, 1.33)	0.19 (−1.15, 1.53)	−0.12 (−1.42, 1.18)
	Quantitative	0.63 (−1.02, 2.29)	−0.65 (−1.94, 0.62)	0.54 (−0.52, 1.60)	0.08 (−1.33, 1.50)	0.07 (−1.31, 1.45)
	Memory	1.41 (−0.27, 3.08)	0.37 (−0.93, 1.67)	0.40 (−0.68, 1.48)	0.77 (−0.67, 2.21)	0.73 (−0.66, 2.12)
	Motor	0.25 (-1.42, 1.93)	−1.49 (−2.78, −0.21)	0.41 (−0.67, 1.48)	−1.39 (−2.82, 0.04)	−0.28 (−1.67, 1.11)

BSID: Bayley Scales of Infant Development, MCSA: McCarthy Scales of Children's Abilities.
Σ_4DEHPs: sum of the four di-(2-ethylhexyl) phthalate metabolites; MBzP: Mono-benzyl phthalate, MEP: Mono-ethyl phthalate, MiBP: Mono-iso-butyl phthalate, MnBP: Mono-*n*-butyl phthalate.
[a] The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, MECPP.
[b] A unit increase per doubling concentration (levels were $\log_2$-transformed).
[c] Adjusted for: sex, maternal education, maternal smoking during pregnancy, birth season, breastfeeding, country origin of the mother, number of siblings, maternal age.
[d] Adjusted for: age of the child at the time of outcome assessment, sex, maternal education, maternal smoking during pregnancy, birth season, breastfeeding, country origin of the mother, number of siblings, maternal age.

Table 5
Associations between maternal urinary phthalate metabolite levels and behavioral development outcomes at ages 4 and 7 years.

Age & outcome (*N*)	Test	Σ_4DEHPs[a]	MBzP	MEP	MiBP	MnBP
		β (95%CI)[b]	β (95%CI)[b]	β (95%CI)[b]	β (95%CI)[b]	β (95%CI)[b]
4 years						
Social competence	CP-SCS[d]	2.00 (0.22, 3.79)	0.50 (−0.94, 1.95)	1.06 (−0.09, 2.21)	0.46 (−1.07, 1.98)	0.57 (−0.98, 2.13)
(*N* = 336)		IRR (95%CI)[c]	IRR (95%CI)[c]	IRR (95%CI)[c]	IRR (95%CI)[c]	IRR (95%CI)[c]
ADHD symptoms	ADHD-DSM-IV[d]					
(*N* = 352)	*Global score*	0.94 (0.82, 1.08)	1.02 (0.92, 1.12)	0.92 (0.85, 1.00)	0.94 (0.84, 1.06)	1.03 (0.92, 1.15)
	Inattention	0.84 (0.72, 0.98)	0.96 (0.86, 1.07)	0.88 (0.80, 0.97)	0.95 (0.84, 1.07)	0.98 (0.87, 1.11)
	Hyperactivity/Impulsivity	1.05 (0.91, 1.22)	1.07 (0.96, 1.18)	0.96 (0.88, 1.05)	0.94 (0.83, 1.06)	1.06 (0.94, 1.20)
7 years						
Behavioral problems	SDQ[d]					
(*N* = 362)	*Global score*	0.95 (0.84, 1.08)	1.03 (0.93, 1.13)	0.98 (0.90, 1.06)	1.00 (0.90, 1.12)	0.95 (0.85, 1.06)
	Emotional symptoms	0.96 (0.83, 1.11)	1.07 (0.96, 1.18)	0.96 (0.88, 1.06)	1.03 (0.91, 1.17)	0.92 (0.81, 1.04)
	Conduct problems	0.97 (0.84, 1.13)	1.04 (0.93, 1.16)	0.99 (0.90, 1.09)	1.06 (0.93, 1.20)	0.95 (0.84, 1.09)
	Hyperactivity/Inattention	0.95 (0.83, 1.08)	1.01 (0.90, 1.12)	1.00 (0.92, 1.09)	0.97 (0.86, 1.09)	0.98 (0.88, 1.11)
	Peer relationship problems	0.94 (0.79, 1.12)	0.99 (0.87, 1.12)	0.93 (0.83, 1.04)	0.94 (0.82, 1.09)	0.91 (0.79, 1.06)
	Poor prosocial behavior	0.93 (0.79, 1.08)	0.94 (0.84, 1.06)	0.95 (0.86, 1.06)	0.97 (0.85, 1.10)	0.94 (0.82, 1.08)
ADHD symptoms	CSRS[d]					
(*N* = 361)	ADHD index	0.88 (0.77, 1.00)	1.02 (0.93, 1.13)	0.95 (0.87, 1.03)	0.98 (0.89, 1.09)	0.98 (0.87, 1.09)
	Hyperactivity	0.90 (0.78, 1.03)	0.99 (0.89, 1.11)	0.94 (0.86, 1.03)	1.02 (0.91, 1.15)	0.98 (0.87, 1.11)
	Cognitive problems/inattention	0.83 (0.71, 0.95)	1.03 (0.93, 1.15)	0.95 (0.87, 1.04)	0.96 (0.86, 1.08)	0.94 (0.84, 1.06)
	Oppositional	0.90 (0.78, 1.04)	1.01 (0.91, 1.12)	0.96 (0.88, 1.05)	0.98 (0.87, 1.10)	0.92 (0.81, 1.04)

CP-SCS: California Preschool Social Competence Scale; ADHD-DSM-IV: Attention-Deficit Hyperactivity Disorder Criteria of the Diagnostic and Statistical Manual of Mental Disorders - 4th edition; SDQ: Strengths and Difficulties Questionnaire; CSRS: Conners' Parent Rating Scales. Σ_4DEHPs: sum of the four di-(2-ethylhexyl) phthalate metabolites; MBzP: Mono-benzyl phthalate, MEP: Mono-ethyl phthalate, MiBP: Mono-iso-butyl phthalate, MnBP: Mono-*n*-butyl phthalate.
[a] The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, MECPP.
[b] A unit increase per doubling concentration (levels were $\log_2$-transformed).
[c] A % increase per doubling concentration (levels were $\log_2$-transformed).
[d] Adjusted for: age of the child at the time of outcome assessment, sex, maternal education, maternal smoking during pregnancy, birth season, breastfeeding, country origin of the mother, number of siblings, maternal age.

Kobrosly et al., 2014; Lien et al., 2015) (Table 1). In the present study the results obtained at the age of 4 and 7 years did not show evidence of an adverse effect of prenatal exposure to phthalates for any of the different outcomes assessed. Indeed, we observed some associations that suggest reduced risks of some behavioral outcomes in relation to Σ_4DEHP and MEP. We evaluated many potential confounding variables related to maternal and child characteristics and none of them seemed to indicate negative residual confounding once included or excluded from the models. In the light of multiple testing issues and in the absence of consistency of results across ages and within phthalate metabolites, we consider it likely that the few statistically significant associations observed may be due to chance and they should therefore be interpreted with caution.

Despite inconsistencies between studies and the few associations obtained in the present work, several mechanisms have been described to explain the potential health effects of phthalates on the developing brain, including hormonal disruption (thyroid hormones), alteration of the calcium signaling and the lipid metabolism and the activation of peroxisome proliferator-activated receptors (PPAR) (Boas et al., 2012; Miodovnik et al., 2014). Several factors could explain our findings and those of previous studies. First, the in vivo biological activity of phthalates is quite weak, thus, larger study populations might be needed to detect weak effects (Miodovnik et al., 2014). Second, most of the studies based their prenatal exposure assessment in one single measurement, mostly in the 3rd trimester of pregnancy. For compounds with a short half-life, such phthalates, the use of one single measurement leads to the risk of miss-classification of the study subjects (Hoppin et al., 2002; Braun et al., 2013). If this misclassification is non-differential, as it is more likely in prospective birth cohort studies, this could lead to a dilution of the effect estimates (Pollack et al., 2013), and therefore to null findings. In this sense, in the present study we averaged the concentrations determined in two pregnancy periods to obtain a better estimate of prenatal phthalate exposure during the entire pregnancy, although the use of two measurements may still not be enough to avoid exposure misclassification (Hoppin et al., 2002; Braun et al., 2013; Frederiksen et al., 2013a). Furthermore, the critical period of exposure might have not been well captured by existing studies. In this sense, in the current study we also analyzed the association between neuropsychological developmental outcomes and the two trimesters (1st and 3rd) of prenatal exposure and no consistent results were obtained for any of the two periods. However, in this case we only had one measurement of phthalate exposure per period. There is controversy about whether phthalate concentrations measured in urine should be adjusted by creatinine concentrations to control for the dilution effect or else include creatinine values in the model as an independent variable (Hays et al., 2015). We conducted sensitivity analyses including creatitine as an independent variable and results were similar to those initially obtained (data shown). This may be because the correlation between creatitine and non-creatinine adjusted phthalates concentrations was high ($r > 0.73$).

As a further limitation, we did not measure postnatal phthalate exposure. Previous cross-sectional studies (Kim et al., 2009; Cho et al., 2010; Chopra et al., 2014) and one birth cohort (Factor-Litvak et al., 2014) have reported associations between postnatal phthalate exposures and neurodevelopmental outcomes, except for one (Lien et al., 2015). However, we do not think that the null results obtained in the present study are explained by a confounding effect of postnatal phthalates exposures. First, given the low correlation between pre- and postnatal phthalate exposure (Frederiksen et al., 2013b; Lewis et al., 2013), the results obtained for the first are not expected to be confounded by the later. And second, one birth cohort study observed that the associations between prenatal phthalate exposure and IQ of the children were not modified once postnatal measurements obtained at three different ages were included in the analysis model (Factor-Litvak et al., 2014).

Apart from using two measurements to assess prenatal exposure, an additional strength of the present study is that we assessed children's neuropsychological development at several ages using different validated neuropsychological tools and the results were

consistent between the different tests of cognitive and psychomotor development on one hand, and of behavioral development on the other hand. The fact that the study population included in the present study differed somewhat from the excluded birth cohort participants, with younger mothers and of lower education, results in an under-representation of these groups in our sample. It is unlikely that this has led to spurious associations between pollutant concentrations and health effects. However, our results may not be representative for other more disadvantaged populations with different (probably higher) levels of phthalate exposure (Valvi et al., 2015).

Conclusions

This study, with an improved exposure assessment and covering many different neuropsychological domains at different ages, does not show adverse effects of prenatal phthalate exposure on children's cognitive, psychomotor or behavioral development.

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The authors have no financial relationships relevant to this article to disclose.

Conflict of interest statement

No conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2015.05.006

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