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Effects of early exposure to phthalates on cognitive development and visual behavior at 24 months

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A B S T R A C T

Background and objectives: Studies focusing on the neurodevelopmental effects of phthalates seldom consider exposure during infancy, a critical period for brain development. Most rely on parent-completed questionnaires to assess child neurodevelopment, which may be subject to reporting error. We studied the associations between prenatal and infancy exposure to phthalates and objective measures of neurodevelopment at the age of two.

Methods: We relied on 151 mother-child pairs from the SEPAGES mother-child cohort. Women were asked to collect three spot urine samples per day over seven consecutive days during the second (median: 18.0 gestational weeks) and third (median: 34.2 gestational weeks) trimesters of pregnancy. They then collected one urine sample per day over seven consecutive days from their infants around the age of 12 months. Metabolites of phthalates and non-phthalate plasticizers were measured in within-subject and within-period pools of repeated urine samples. Eye tracking tasks were performed at two years allowing to compute four indicators linked with cognitive development and visual behavior: mean fixation duration, novelty preference, percent time spent looking at the eyes and mean reaction time.

Results: Pre-natal exposure to monobenzyl phthalate at the second and third trimesters was associated with shorter fixation durations. In models allowing for interaction with child sex, these associations were only observed among girls. Exposure to di(2-ethylhexyl) phthalate at the third but not the second trimester was associated with increased time spent looking at a novel face and eyes. We observed faster reaction times and decreased time spent looking at the eyes in a face recognition task, with increased post-natal exposure to monoethyl, mono-iso-butyl and mono-n-butyl phthalates.

Discussion: Relying on improved exposure assessment, we highlighted associations of pre- and post-natal exposure to phthalates with indicators derived from eye tracking tasks, mainly in girls. Some of these indicators have been affected in individuals with neurodevelopmental disorders.

1. Introduction

Phthalates are widely used as plasticizers (Dodson et al., 2012; Hauser and Calafat, 2005). In the European Union, some are banned or regulated in specific products. Use of diethylhexyl phthalate (DEHP), dibutyl phthalate (DBP) and benzyl butyl phthalate (BBzP) is regulated in material intended to come into contact with food while BBzP, DEHP, dibutyl phthalate (DBP) and bis(2-methoxyethyl) phthalate (DMEP) are banned from use in cosmetics. Despite this restriction recent cohort studies still reported widespread exposure to these chemicals in the general population (Philippat et al., 2021; Warembourg et al., 2019). This is of concern as phthalates have endocrine disrupting properties and early life exposure is suspected to affect child health including child neurodevelopment (Miodovnik et al., 2014; Braun, 2017). Several epidemiological studies have reported detrimental associations between pregnancy exposure to phthalates and child behavior (Lien et al., 2015; Kobrosly et al., 2014; Whyatt et al., 2012; Kamai et al., 2021) or cognition (Whyatt et al., 2012; Kim et al., 2011; Téllez-Rojo et al., 2013; Polanska et al., 2014). Effect estimates (Radke et al., 2020) of a recent meta-analysis were also suggestive of deleterious effects, however, confidence intervals often included zero and the evidence was considered as limited by the authors for most phthalate – neurodevelopmental outcome pairs. The authors mentioned that reliance on spot urine samples and the resulting exposure measurement error may partly explain this null finding. Additionally, studies might also be affected by

Abbreviations: DMEP, bis(2-methoxyethyl) phthalate; T3, Third trimester; T2, Second trimester; M12, 12 month infancy; HAD, Hospital Anxiety and Depression scale score; DPHP, bis(2-propylheptyl) phthalate.

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Abbreviations

cx-MiNP	mono-4-methyl-7-carboxyoctyl phthalate
DEHP	di(2-ethylhexyl) phthalate
DEP	diethyl phthalate
DiNP	diisononyl phthalate
DINCH	di(isononyl)cyclohexane-1,2-dicarboxylate
LOD	limit of detection
LOQ	limit of quantification
MBzP	monobenzyl phthalate
MECPP	mono-2-ethyl-5-carboxypentyl phthalate
MEHP	mono-2-ethylhexyl phthalate
MEHHP	mono-2-ethyl-5-hydroxyhexyl phthalate
MEOHP	mono-2-ethyl-5-oxohexyl phthalate
MEP	monoethyl phthalate
MiBP	mono-iso-butyl phthalate
MMCHP	mono-2-carboxymethyl hexyl phthalate
MnBP	mono-n-butyl phthalate
Oh-MiNP	mono-4-methyl-7-hydroxyoctyl phthalate
Oh-MINCH	2-(((hydroxy-4-methyloctyl) oxy) carbonyl) cyclohexanecarboxylic acid
Oh-MPHP	mono-6-hydroxy-propylheptyl phthalate
oxo-MINCH	2-(((4-Methyl-7-oxooctyl) oxy) carbonyl) cyclohexanecarboxylic acid
oxo-MiNP	mono-4-methyl-7-oxooctyl phthalate
ΣDEHP	molar sum of di(2-ethylhexyl) phthalate metabolites
ΣDiNP	molar sum of diisononyl phthalate metabolites
ΣDINCH	molar sum of di(isononyl)cyclohexane-1,2-dicarboxylate metabolites
WAIS-IV	Wechsler Adult Intelligence Scale – Fourth Edition

measurement error in the outcome, especially in the case of parent-completed questionnaires, which are subjective and may be prone to bias (Wilson et al., 2000; Ross et al., 1995; Rosenman et al., 2011). Another limitation of the current literature is the lack of exposure assessment during infancy, a period of rapid brain growth, and a potentially sensitive window (Dekaban and Sadowsky, 1978; Rice and Barone, 2000).

To overcome these issues, we assessed 15 biomarkers of phthalate exposure in repeated urine samples collected during pregnancy and the first year of life and studied their associations with indicators derived from eye-tracking tasks performed at 24-months. Eye tracking is non-invasive and provides an objective measurement of several aspects of the cognitive function and visual behavior at an early age. Its use is steadily growing in neurodevelopment research as a number of eye tracker measures have been shown to be affected in children later diagnosed with atypical neurodevelopment disorders like Autism Spectrum Disorders (ASD) (Yurkovic et al., 2021; Sasson and Elison, 2012; Wan et al., 2019; Falck-Ytter et al., 2013; Jones and Klin, 2013; Constantino et al., 2017)¯(Yurkovic et al., 2021; Sasson and Elison, 2012; Wan et al., 2019; Falck-Ytter et al., 2013; Jones and Klin, 2013; Constantino et al., 2017), Attention Deficit/Hyperactivity Disorder (ADHD) or Williams syndrome (Riby and Hancock, 2008).

2. Methods

2.1. Study population

Our study population was a sub-sample of the French SEPAGES mother-child cohort that recruited 484 pregnant women from the Grenoble area (France) between July 2014 and July 2017 (Lyon-Caen et al., 2019). The eligibility criteria were: 1) being pregnant by less than 19 gestational weeks at inclusion, 2) being older than 18 years old, 3) reading and speaking French fluently, 4) being affiliated to the French national security system, and 5) planning to deliver in one of the four maternity clinics of the area. Multiple pregnancies were excluded from the study.

The study was approved by the relevant ethical committees. Both parents of the expected child signed an informed consent form for themselves and their infants prior to inclusion.

2.2. Urine sample collection and exposure assessment

Women were asked to collect three spot urine samples (morning, midday, evening) per day over seven consecutive days during the second (median of 18.0 gestational weeks, IQR: 2.3, T2) and third (median of 34.2 gestational weeks, IQR: 3.1, T3) trimesters of pregnancy. Postnatally they were asked to collect one urine sample per day over seven consecutive days from their infant around the age of 12 months (median of 12.1 months, IQR: 0.7, M12). Samples were stored in the participant's freezer until a field worker came to pick them up at the end of each urine collection week. For each individual and for each collection week, we pooled an equal volume of each of the urine samples that were collected (Vernet et al., 2019; Philippat and Calafat, 2021).

For each mother-child pair, an aliquot of each weekly pool was sent on dry ice to the Norwegian Institute of Public Health (Oslo, Norway) to assess the urinary concentrations of 12 phthalate metabolites and two 1,2-Cyclohexane dicarboxylic acid, diisononyl ester (DINCH) metabolites (listed in Supplemental Material, Table S1) using high performance liquid chromatography coupled with mass spectrometry (HPLC-MS-MS) (Sabaredzovic et al., 2015). An in-depth descriptive analysis of these concentrations has been published (Philippat et al., 2021).

2.3. Eye tracking assessment

During the SEPAGES follow-up, among the 479 families whose phthalate urinary concentrations were measured at least once, 151 participated in an ancillary study that consisted of an eye tracking experiment around the child's 24 months (Fig. 1). The relatively low participation rate can in part be explained by the fact that there was a delay in the set-up of the sub-study (it started in September 2017 while SEPAGES children started turning 2 years old in January 2017) and the constraints in the eye tracking lab's schedule.

Each child performed a battery of well-known tasks in the following order: 1) scene exploration task, 2) visual paired recognition task with faces, 3) saccade to target task and 4) smooth-pursuit task. Extensive details regarding these tasks can be found in the Supplemental Material.

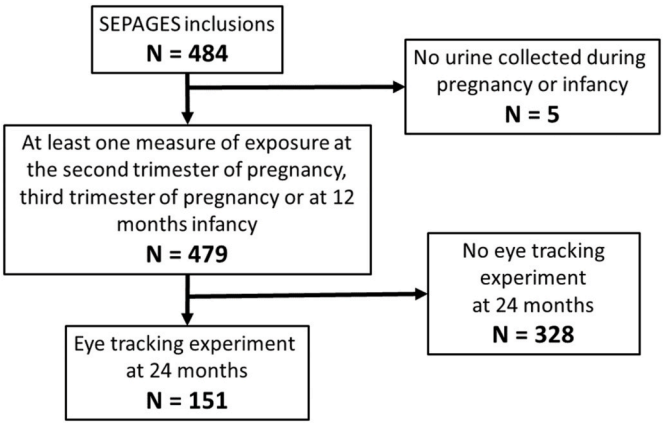

Fig. 1. Study population flowchart.

Below, we describe only the indicators that were derived and used as outcomes for this analysis.

1) Mean fixation durations (in milliseconds (ms)) is a marker of attentional control that was derived from two tasks: the scene exploration task and the visual paired recognition task with faces. Shorter mean fixation durations in infancy have been associated with higher hyperactivity-inattention scores (Papageorgiou et al., 2014) and observed in children with ASD (Wass et al., 2015; Al-Haddad et al., 2020).
2) Novelty preference (i.e, percentage of time spent looking at a novel face) was derived from the visual paired recognition task with faces. It is a measure of recognition/visual memory (Dzwilewski et al., 2020) rather than a measure of processing speed. It predicts mental development index scores of the Bayley Scale of Early Learning (BSID) at 3 years (Rose et al., 2009) as well as intellectual quotient scores at 6 (Rose et al., 1992) and 11years (Rose and Feldman, 1995).
3) Percent time spent looking at the eyes was derived from the visual paired recognition task with faces. It has been used in autism research (Yurkovic et al., 2021; Sasson and Elison, 2012; Wan et al., 2019; Falck-Ytter et al., 2013), with autistic individuals showing reduced time spent looking at eyes as early as 6 months (Jones and Klin, 2013).
4) Mean reaction time (in ms) was computed from two tasks: the saccade to target task and the smooth-pursuit task. It reflects the capacity of the child to disengage from a previous location before attending to a new stimulus. It is considered a marker of processing speed. A shorter visual reaction time during infancy has been associated with higher IQ scores at later ages (Dougherty and Haith, 1997). Interestingly, infants with ASD have been found to excel in visual search and have shown shorter reaction times than non ASD children (Joseph et al., 2009).

The sample size slightly varied across scores as a few children did not perform all four tasks (Table 1).

2.4. Statistical analysis

Our statistical analysis plan was published prior to analysis on osf. io (https://osf.io/7xpwt).

Biomarker concentrations below the limit of detection and quantification were singly imputed using the compound's probability distribution (Helsel, 1990). Standardization for sampling (sample transportation time between the participant's home to the biobank and sample thawing time during the pooling procedure) and analytical conditions (analytical batch) was then performed (Mortamais et al., 2012; Guilbert et al., 2021). We additionally computed the molar sum of metabolites of a same parent compound, di-isononyl phthalate (DiNP), DINCH and DEHP (Supplemental Material, Table S1). The concentrations were ln transformed prior to modelling.

Mean fixation duration showed a log-linear distribution as in (Papageorgiou et al., 2014) and was also ln-transformed prior to modelling.

Candidate model covariates were selected through a literature search and included factors likely to be associated with both phthalate exposure and eye tracking scores without being a consequence of them, and eye tracking score predictors (see detailed list in Supplemental Material). Regression models with all candidate covariates and no exposure were run for each eye tracking score. When the p value (F-test) for a given covariate was smaller than 0.2 in at least one of the models, it was retained as a covariate in all final models. This selection was motivated by the relatively small N. The retained covariates were: child sex (male/female), maternal age (continuous, linear), breastfeeding duration (continuous, linear), maternal tobacco consumption after conception (Yes/No), maternal cognitive function assessed with the verbal component of the Wechsler Adult Intelligence Scale – Fourth Edition

Table 1
Study population descriptive table, with comparison between the SEPAGES families whose child passed the eye-tracker test at 24 months and those that did not.

	Included (N = 151)		Not included (N = 333)		p[a]
	N	%	N	%	
Maternal education					
High school + 2 years or less	21	14%	62	19%	0.13
High school + 3 years or more	130	86%	271	81%	
Ethnicity					
European	139	92%	262	79%	<0.001
Other[b]	3	2%	7	2%	
Missing	9	6%	64	19%	
Child sex					
Boy	85	56%	171	51%	0.30
Girl	66	44%	157	47%	
Parity					
First child	76	50%	146	44%	0.10
Second child	65	43%	149	45%	
Third child or more	10	7%	38	11%	
Maternal depression during pregnancy					
HAD score ≤11	103	68%	180	54%	0.08
HAD score >11	46	30%	109	33%	
Missing	2	1%	44	13%	
Maternal tobacco consumption during pregnancy[c]					
No	128	85%	252	76%	0.62
Yes	17	11%	38	11%	
Missing	6	4%	43	13%	
Professional status					
Working	127	84%	257	77%	0.87
Not working, not looking for work	13	9%	26	8%	
Not working, looking for work	6	4%	15	5%	
Missing	5	3%	35	11%	
Eye tracking experiment hour					
8AM - 12PM	122	81%			
12PM–14PM	7	5%			
14PM–16PM	22	14%			
	N	**Median (IQR)**	**N**	**Median (IQR)**	
Maternal age at delivery	151	32.1 (4.8)	333	32.2 (5.5)	0.55
Breastfeeding duration (months)	150	7.1 (7.1)	295	7.0 (6.9)	0.84
Gest. age at the second trim. sample	151	18.0 (2.3)	326	17.6 (2.6)	0.07
Gest. age at the third trim. sample	150	34.2 (3.1)	306	34.0 (3.0)	0.91
Infant age at sampling (years)	145	1.01 (0.06)	241	1.02 (0.07)	0.04
Number of samples in the second trim. pool	151	21 (1)	326	21 (1)	0.29
Number of samples in third trim. pool	150	21 (1)	305	21 (1)	0.10
Number of samples in the infant pool	145	7 (1)	242	6 (2)	0.19
Age at eye tracking experiment in months	151	24.8 (0.7)			
Fixation duration (ms)	150	352 (65)			
Reaction time (ms)	145	282 (64)			
Time spent looking at the eyes (%)	150	73 (23)			
Time spent looking at the novel face (%)	150	55 (9)			

Abbreviations: HAD, Hospital Anxiety and Depression scale score.
[a] Kolmogorov-Smirnov test.
[b] Includes Africa, America, Oriental Mediterranean countries, South East Asia and Other.

(WAIS-IV, continuous, linear), parity (1st child/2nd child/3rd child or more), maternal professional status at sampling time (working, not working and not looking for work, not working and looking for work) and experiment time of the day (continuous, 4-degree natural spline).

The adjusted associations between phthalate concentration, fixation duration, and reaction time were modelled using adjusted linear regressions. Percentages (percent time spent looking at the eyes and at the novel face) were modelled with beta regression (Ferrari and Cribari-Neto, 2004) (R package *betareg* (Cribari-Neto and Zeileis, 2010)). Regarding model interpretation, betas are provided for an increase by one in the ln-transformed concentrations, except for fixation duration for which betas represent the percent change in fixation duration for doubling in biomarker concentration.

Since several studies (Radke et al., 2020), including one conducted within the SEPAGES cohort (Guilbert et al., 2021), reported effect modification by child sex for various neurodevelopmental outcomes, in an additional analysis, we added an interaction term between sex and phthalate metabolite concentration. If the p-value for this interaction term was <0.2, sex-specific exposure effects were computed.

To test the robustness of our results, analyses were run with phthalate metabolite concentrations that were not standardized for sampling and analytical conditions. Additional adjustment for specific gravity (assessed in each pool using a handheld Atago PAL 10-S refractometer (Atago)) was also performed. Finally, models were re-run without outlier observations identified using Cook's distance above the threshold of 0.026 (4/sample size) (Bollen and Jackman, 1985).

All tasks and eye tracking data postprocessing were performed using MatLab (R2007b) and the Eyelink® toolbox (Cornelissen et al., 2002) while statistical analyses were performed using R version 4.2.0 (R Core Team, 2021). The R codes are available at https://gricad-gitlab.univ-grenoble-alpes.fr/iab-env-epi.

3. Results

3.1. Study population

A relatively low number of women reported smoking during pregnancy (N = 17, 11%). Their median age at enrollment was 32.1 years (IQR: 4.8). More than half of the children were boys (N = 85, 56%), and children were breastfed for a median of 7.1 months (IQR: 7.1).

3.2. Eye tracking scores

The median age at the eye tracking test was 24.8 months (IQR: 0.7, Table 1). Most assessments (81%) were performed between 8AM and 12PM (Table 1). Absolute Spearman correlation coefficients between eye tracking scores were all below 0.2 (Supplemental Material, Table S2).

3.3. Exposure

Detection frequencies were above 99% for all phthalate metabolites at all sampling time points (Table 2).

Overall, the characteristics and urinary concentrations of our study population did not differ significantly from those of the rest of the SEPAGES cohort (Tables 1 and 2).

3.4. Adjusted associations between phthalate exposure and eye tracking scores

Fixation duration decreased by 2.5% (95% confidence interval (CI): −5.4; 0.44) and 3.0% (95%CI: −5.5; −0.43) for each doubling in MBzP concentration assessed at the second and third trimesters of pregnancy respectively. The p-values of interaction with child sex were below 0.2 for both periods and in the models with interaction these associations were only observed among girls (β = −4.4% (95%CI: −8.4; −0.4) and −4.7% (95%CI: −8.3; −1.1) in girls at the second and third trimesters, respectively, Fig. 3, Supplemental Material Table S4). Infant MBzP levels were not associated with any of the eye tracking scores (Fig. 2, Supplemental Material Table S3).

ΣDEHP at the third trimester, but not at the other time points, was

Table 2
Phthalate urinary concentrations at the three collection time points (second and third trimesters of pregnancy and 12 month of infancy) with comparison between families whose child passed the eye-tracker test at 24 months and those that did not.

	Included			Not included			
	N	**% > LOD**	**Median (IQR)**	**N**	**% > LOD**	**Median (IQR)**	**p[b]**
Concentrations[a] at the second trimester of pregnancy (µg/l)							
MEP	151	100	24.558 (33.29)	326	100	23.669 (35.75)	0.43
MnBP	151	100	10.828 (8.95)	326	100	10.477 (7.58)	0.74
MiBP	151	100	15.050 (11.82)	326	100	15.033 (14.23)	0.61
MBzP	151	100	4.440 (3.86)	326	100	4.435 (3.98)	0.97
Oh-MPHP	151	100	0.818 (0.36)	326	100	0.869 (0.5)	0.26
∑DEHP[c]	151	/	0.105 (0.07)	326	/	0.108 (0.07)	0.61
∑DiNP[c]	151	/	0.039 (0.05)	326	/	0.041 (0.05)	0.94
∑DINCHH[c]	151	/	0.010 (0.01)	326	/	0.010 (0.01)	0.76
Concentrations[a] at the third trimester of pregnancy (µg/l)							
MEP	150	100	19.790 (32.02)	306	100	20.980 (33.5)	0.90
MnBP	150	100	10.877 (9.59)	306	100	11.729 (8.94)	0.81
MiBP	150	100	13.754 (11.26)	306	100	15.221 (14.91)	0.29
MBzP	150	100	3.750 (4.32)	306	100	4.307 (3.88)	0.08
Oh-MPHP	150	100	0.770 (0.32)	306	100	0.833 (0.45)	0.07
∑DEHP[c]	150	/	0.036 (0.04)	306	/	0.037 (0.04)	0.18
∑DiNP[c]	150	/	0.009 (0.01)	306	/	0.010 (0.01)	0.42
∑DINCH[c]	150	/	0.103 (0.08)	306	/	0.111 (0.08)	0.52
Concentrations[a] at 12 months of infancy (µg/l)							
MEP	145	100	11.352 (14.47)	242	100	12.494 (13.55)	0.51
MiBP	145	100	14.418 (14.19)	242	100	14.219 (15.28)	0.98
MnBP	145	100	11.216 (11.66)	242	100	11.407 (10.73)	0.86
MBzP	145	100	3.461 (5.9)	242	100	3.154 (5.92)	0.54
Oh-MPHP	145	99	0.679 (0.49)	242	100	0.752 (0.53)	0.22
∑DEHP[c]	145	/	0.024 (0.03)	242	/	0.026 (0.02)	0.83
∑DiNP[c]	145	/	0.012 (0.01)	242	/	0.011 (0.01)	0.77
∑DINCH[c]	145	/	0.080 (0.09)	242	/	0.079 (0.08)	0.46

Abbreviations: MEP, Monoethyl phthalate; MnBP, Mono-n-butyl phthalate; MiBP, Mono-iso-butyl phthalate; MBzP, Monobenzyl phthalate; oh-MPHP, Mono-6-hydroxy-propylheptyl phthalate; DEHP, Di (2-ethylhexyl) phthalate; DiNP, Di-isononyl phthalate; DINCH, 1,2-Cyclohexane dicarboxylic acid, diisononyl ester, ∑ molar sum.

[a] Concentrations standardized on the following variables when needed: analytical batch, sample transportation time between the participant's home to the biobank and sample thawing time during the pooling procedure.

[b] Kolmogorov-Smirnov test.

[c] Molar sum of metabolites of a same parent compound, measured in µmol/l.

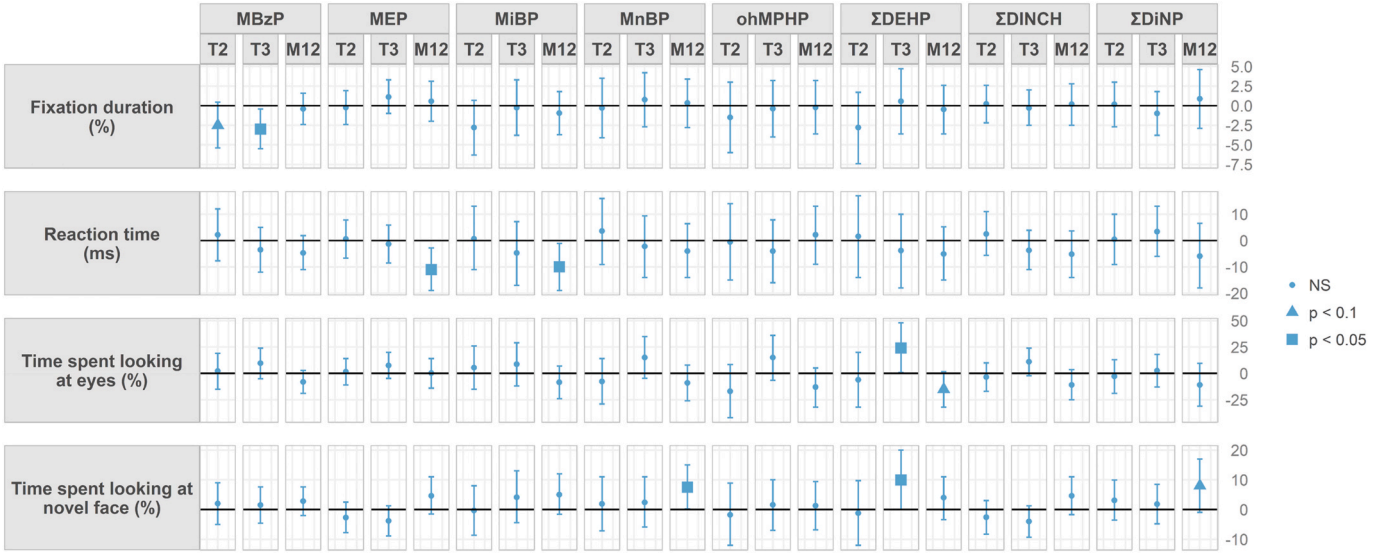

Fig. 2. Adjusted associations between urinary concentrations of phthalate metabolites and eye tracking scores for each exposure period in the SEPAGES mother-child cohort

Note: Beta and 95% confidence intervals are expressed in standardized units (divided by standard deviation) for each indicator to preserve a comparable scale. Values can be found in Supplemental Material, Table S3.

Abbreviations: MEP, Monoethyl phthalate; MnBP, Mono-n-butyl phthalate; MiBP, Mono-iso-butyl phthalate; MBzP, Monobenzyl phthalate; ohMPHP, Mono-6-hydroxy-propylheptyl phthalate; DEHP, Di (2-ethylhexyl) phthalate; DiNP, Di-isononyl phthalate; DINCH, 1,2-Cyclohexane dicarboxylic acid, diisononyl ester; T2, Second trimester of pregnancy; T3, Third trimester of pregnancy; M12, 12 month infancy.

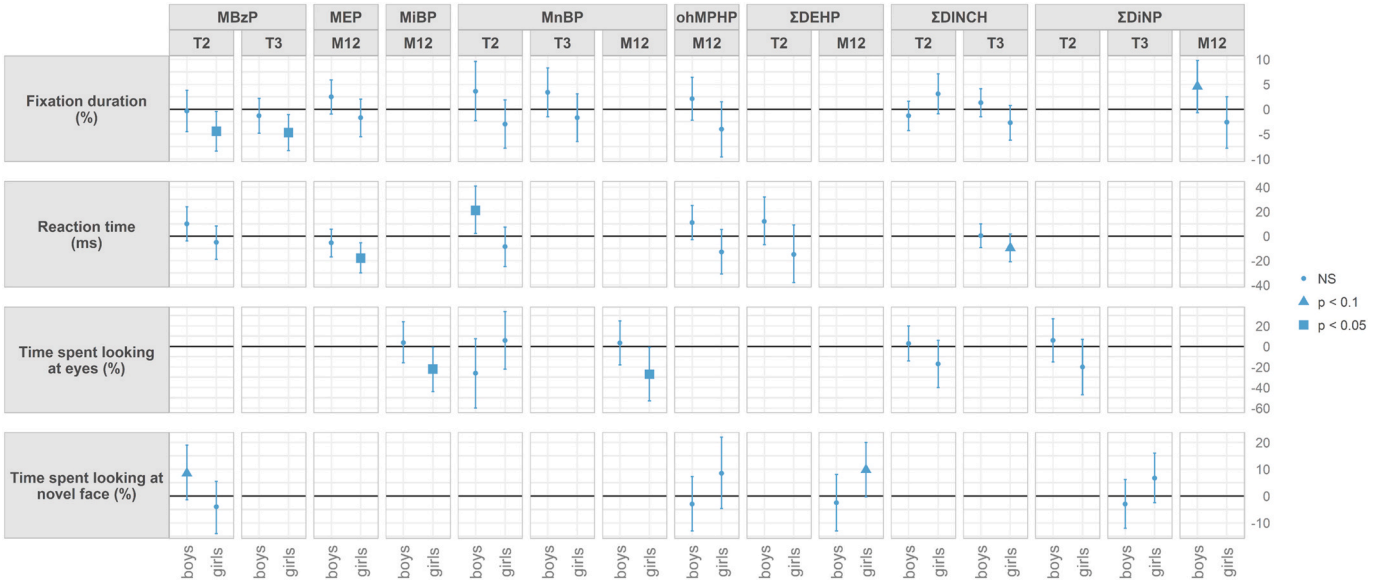

Fig. 3. Adjusted associations between urinary concentrations of phthalate metabolites and eye tracking scores for each exposure period in the SEPAGES mother-child cohort according to child sex (we only reported associations for which there was signs of an effect modification (p-value for interaction< 0.2) between biomarker urinary concentration and child sex).

Note: Beta and 95% confidence intervals are expressed in standardized units (divided by standard deviation) for each indicator to preserve a comparable scale. Values can be found in Supplemental Material, Table S4.

Abbreviations: MEP, Monoethyl phthalate; MnBP, Mono-n-butyl phthalate; MiBP, Mono-iso-butyl phthalate; MBzP, Monobenzyl phthalate; ohMPHP, Mono-6-hydroxy-propylheptyl phthalate; DEHP, Di (2-ethylhexyl) phthalate; DiNP, Di-isononyl phthalate; DINCH, 1,2-Cyclohexane dicarboxylic acid, diisononyl ester; T2, Second trimester of pregnancy; T3, Third trimester of pregnancy; M12, 12 month infancy.

positively associated with the percent time spent looking at a novel face (β = 9.9% (95%CI: 0.05; 20)) and the percent time spent looking at the eyes (β = 24% (95%CI: 0.75; 48)).

The only other metabolite for which we observed an association with pregnancy exposure was mono-n-butyl phthalate (MnBP). MnBP concentration during the second trimester was associated with longer reaction times in boys (β = 21 ms (95%CI: 2.1; 41) but not in girls (β = −8.6 ms (95%CI: −25; 7.5). Infancy exposure to MnBP, was also positively associated with the time spent looking at a novel face (β = 7.5% (95%CI: 0.23; 15)) when boys and girls were studied together, and negatively with the time spent looking at the eyes in girls (β = −27% (95%CI: −53; −0.73)) but not in boys (β = 3.4% (95%CI: −18; 25)).

Infancy urinary concentration of both MiBP and MEP were negatively associated with reaction time which decreased by −10 ms (95%CI:

−19; −1.1) and −11 ms (95%CI: −19; −2.8) for each one unit increase in ln-transformed concentrations of MiBP and MEP, respectively. For MEP, this association seemed to be driven by girls (p-value for interaction = 0.15; β = −18 ms (95%CI: −30; −5.5) compared to −5.5 (95%CI: −17; 5.7) for boys). Finally, we observed a negative association between infant exposure to MiBP and the time spent looking at the eyes for girls (β = −22% (95%CI: −44; −0.80)), but not for boys (β = 3.7% (95%CI: −16; 24), p-value for interaction = 0.08).

No clear association was observed with ΣDiNP and mono-6-hydroxy-propylheptyl phthalate (oh-MPHP), a metabolite of Bis(2-propylheptyl) phthalate (DPHP, Figs. 2 and 3, Supplemental Material Tables S3 and S4).

3.5. Sensitivity and robustness analyses

Associations for unstandardized exposure, excluding outliers (N ranged from 0 to 13 depending on the outcome and the exposure) and additionally adjusted for specific gravity were overall similar to those from our main models (Supplemental Material, Tables S5, S6, S7).

4. Discussion

Relying on repeated urine samples to assess phthalate exposure during pregnancy and infancy we analyzed associations with eye tracking scores at 24 months. These scores are markers of cognitive development and visual behavior and have been shown to predict several aspects of child neurodevelopment later in life (Dzwilewski et al., 2020).

Given the high number of tests performed, we cannot exclude the possibility that some of the associations we observed resulted from chance findings. Therefore, we focused on associations for which 1) consistency was observed across time points (e.g., the association between MBzP and fixation duration observed both at the second and third trimesters of pregnancy) or within time points (e.g., associations between several low molecular weight phthalates at 12 months and eye tracking scores).

4.1. Infancy exposure to MEP, MiBP and MnBP and eye tracking scores

At 12 months, metabolites of low molecular weight phthalates, namely MEP, MiBP and MnBP were associated with several eye tracking scores usually affected in individuals with ASD (shorter reaction times (Joseph et al., 2009) and less time looking at eyes (Jones and Klin, 2013; Al-Haddad et al., 2020; Klin et al., 2002; Sterling et al., 2008)). When an effect modification by child sex was detected, these associations were mainly seen in girls. The fact that we did not observe any of these associations with pregnancy exposure may suggest that infancy is a more sensitive period for the effects of these chemicals. None of the previous studies looking at visual behavior (either using eye trackers (Merced--Nieves et al., 2021; Dzwilewski et al., 2021) or the Fagan Test of Infant Intelligence (Ipapo et al., 2017)), computed reaction time or the time spent looking at eyes, limiting possible comparisons with our results. However, several studies have examined the associations between prenatal exposure to phthalates and childhood autistic traits or ASD diagnosis, and among them there have been reports of increased social problems associated with prenatal urinary concentrations of low molecular weight phthalates (molar sum (Miodovnik et al., 2011), MEP (Patti et al., 2021; Haggerty et al., 2021; Day et al., 2021; Ponsonby et al., 2020), MiBP (Patti et al., 2021) and MnBP (Patti et al., 2021; Oulhote et al., 2020)). Sex specific associations have also been reported, in girls (Kim et al., 2018) as in our study, but also in boys (Haggerty et al., 2021). Overall, these results suggest that early exposure to low molecular weight phthalates may lead to alterations in visual behavior, similar to those observed in ASD. To fully explore sex-specific effects, further studies with a stronger focus on infancy exposure and a larger sample size are needed.

Finally, we observed a positive association between MnBP urinary concentrations at 12 months and novel face preference. Such a protective association was not observed in previous eye tracking studies (Dzwilewski et al., 2021; Ipapo et al., 2017).

4.2. Prenatal exposure to phthalates and eye tracking scores

In our study, second trimester MnBP concentration was associated with longer reaction times in boys, a marker of lower processing speed. In a previous study relying on eye tracking scores, the molar sum of all phthalates was associated with increased run duration, suggesting slower information processing speed. As in our study, this association was seen in boys (Dzwilewski et al., 2021). While the sum included MnBP concentration, the authors did not provide effect estimates for this specific metabolite and further investigations are needed.

We observed a decrease in mean fixation duration with increased second and third pregnancy trimester MBzP concentrations. These associations were only present for girls when we included an interaction with child sex. Shorter fixation durations have been observed in children with ASD (Wass et al., 2015; Al-Haddad et al., 2020) and have been associated with higher scores of hyperactivity-inattention (Papageorgiou et al., 2014). Among the three studies assessing visual behavior in association with prenatal exposure to phthalates (either using eye trackers or the Fagan Test of Infant Intelligence) only one studied MBzP individually (Ipapo et al., 2017). In this study among younger children (6–7 months), the authors reported detrimental associations in girls, however this association was observed with novelty preference, a marker not associated with MBzP in our study. Among the cohorts that investigated associations between MBzP and autistic traits, only two reported increased social problems (Patti et al., 2021; Day et al., 2021), while those relying on ASD diagnosis did not report associations for this phthalate (Philippat et al., 2015; Shin et al., 2018). However they had a limited sample size and one assessed BBzP in dust instead of relying on exposure biomarkers (Philippat et al., 2015). Finally, in our cohort, third trimester ΣDEHP levels were positively associated with novelty preference and time spent looking at eyes. These associations were unexpected since these two markers have been associated with improved neurodevelopmental outcomes later in life (Dzwilewski et al., 2020; Rose et al., 2009; Rose et al., 1992; Rose and Feldman, 1995).

4.3. Strengths and limitations

We relied on pools of many urine samples to assess exposure during both pregnancy and infancy reducing the risk of exposure misclassification compared to previous studies relying on a smaller number of urine samples (Perrier et al., 2016). The collection of urine samples at 12 months is a major strength because very few studies have assessed exposure during this period even though it is a critical period for brain development (Dekaban and Sadowsky, 1978; Villagomez et al., 2019).

The use of eye trackers and eye tracking data is relatively new in the field of environmental health, and holds many promises. They provide objective and quantitative measurements of visual behavior that have been shown to predict components of neurodevelopment at a later age (mainly cognitive function, risk of developing ASD (Wass et al., 2015; Al-Haddad et al., 2020; Joseph et al., 2009), ADHD (Papageorgiou et al., 2014) or Williams syndrome (Riby and Hancock, 2008)). Eye trackers can be used with infants a few months old (Aslin, 2012; Aslin and McMurray, 2004; Richmond and Nelson, 2009; Turati et al., 2010) which is of high interest. Current neurodevelopmental measurement scales are indeed limited for children before the ages of 3 to 4 (Fernandes et al., 2014), which is problematic as poor performances at a young age can predict neurodevelopmental deficits at a later age (Rose et al., 1992; Dietrich et al., 2005) and early diagnosis increases the chance of successful intervention. Unfortunately, there is still a lack of standardized eye tracking procedures, which leads to a large variability in stimuli and experimental settings that limits between study comparison

(Mastergeorge et al., 2021). Additionally, although some of the eye tracking scores used in this study have been shown to be affected in individuals with neurodevelopmental disorders they are not used for diagnosis, calling for cautious interpretation of our results.

5. Conclusion

Exposure to low molecular weight phthalates at 12 months and exposure to MBzP during pregnancy were associated with eye tracking scores measured at 24 months. These early markers of visual behavior may predict several aspects of child neurodevelopment, including the risk of developing autism like behaviors later in life. Despite our relatively small sample size, effect modification by child sex was detected and suggested that effects were overall stronger in girls. Further studies should assess exposure during both pregnancy and infancy.

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Role of funder/sponsor (if any)

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Registration (if any)

Our statistical analysis plan was published prior to analysis on osf. io (https://osf.io/7xpwt). Data used in this study are confidential and can only be provided upon a reasonable request to the corresponding authors, after approval by the SEPAGES steering comity.

Contributors statement page

Matthieu Rolland: formal analysis, visualization, writing – original draft, Remy Slama: review and editing, resources, cohort design, Sarah Lyon-Caen: review and editing, resources, data collection, Cathrine Thomsen: review and editing, chemical assessments, Amrit K. Sakhi: review and editing, chemical assessments, Azemira Sabaredzovic: review and editing, chemical assessments, Sam Bayat: review and editing, cohort design, David Meary: review and editing, eye tracking assessments, Claire Philippat: writing – original draft, funding acquisition, scientific supervision, cohort design.

All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.115068.

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