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Cytokines as mediators of the associations of prenatal exposure to phenols, parabens, and phthalates with internalizing behaviours at age 3 in boys: a mixture exposure and mediation approach

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List of abbreviations

2,4-DC: 2,4-dichlorophenol

2,5-DC: 2,5-dichlorophenol

ASD: autism spectrum disorder

BKMR-CMA: Bayesian Kernel Machine Regression Causal Mediation Analysis

BP: butyl-paraben

BPA: bisphenol A

BzP-3: benzophenone-3

DEHP: di(2-ethylhexyl) phthalate

ED: endocrine disruptor

EP: ethyl-paraben

IL: interleukin

mBzP: monobenzyl phthalate

mCNP: monocarboxynonyl phthalate

mCOP: monocarboxyoctyl phthalate

mCPP: mono(3-carboxypropyl) phthalate

mECPP: mono(2-ethyl-5-carboxypentyl)

mEHHP: mono(2-ethyl-5-hydroxy-hexyl) phthalate

mEHP: mono(2-ethylhexyl) phthalate

mEOHP: mono(2-ethyl-5-oxohexyl) phthalate

mEP: monoethyl phthalate

miBP: mono-isobutyl phthalate

mnBP mono-n-butyl phthalate

MP: methyl-paraben

PP: propyl-paraben

Tc: triclosan

TNF: Tumor Necrosis Factor

PIP: Posterior Inclusion Probability

ABSTRACT

Childhood internalizing disorders refer to inwardly focused negative behaviours such as anxiety, depression, and somatic complaints. Interactions between psychosocial, genetic, and environmental risk factors adversely impact neurodevelopment and can contribute to internalizing disorders. While prenatal exposure to single endocrine disruptors (EDs) is associated with internalizing behaviours in infants, the associations with prenatal exposure to ED in mixture remain poorly addressed. In addition, the biological mediators of EDs in mixture effects on internalizing behaviours remain unexplored. EDs do not only interfere with endocrine function, but also with immune function and inflammatory processes. Based on this body of evidence, we hypothesized that inflammation at birth is a plausible biological pathway through which prenatal exposure to EDs in mixture could operate to influence offspring internalizing behaviours. Based on the EDEN birth cohort, we investigated whether exposure to a mixture of EDs increased the odds of internalizing disorders in 459 boy infants at age 3, and whether the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α measured at birth are mediators of this effect. To determine both the joint and individual associations of prenatal exposure to EDs with infant internalizing behaviours and the possible mediating role of cytokines, we used the counterfactual hierarchical Bayesian Kernel Machine Regression (BKMR) regression-causal mediation analysis. We show that prenatal exposure to a complex mixture of EDs has limited effects on internalizing behaviours in boys at age 3. We also show that IL-1 β , IL-6, and TNF- α are unlikely mediators or suppressors of ED mixture effects on internalizing behaviours in boys at age 3. Further studies on larger cohorts are warranted to refine the deleterious effects of EDs in mixtures on internalizing behaviours and identify possible mediating pathways.

Keywords

Endocrine disruptors; mixture; cytokines; internalizing behaviour; neurodevelopment; mother-child birth cohort; hierarchical BKMR; mediation

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ETHICS APPROVAL

The EDEN cohort promoted by INSERM has received approval from the CCPPRB (Comité consultatif de protection des personnes dans la recherche biomédicale, the French Institutional Ethic Board for human subjects biomedical research) of Kremlin Bicêtre and from the CNIL (Commission Nationale Informatique et Liberté, the French national agency on data privacy) for biological samplings and data collection. Informed written consent was obtained from parents at the time of enrolment and after delivery. The use of EDEN samples and data in the present study has been approved by the EDEN steering committee. The analysis of blinded urine specimens at CDC laboratories was determined not to constitute engagement in human subjects' research.

BACKGROUND

Alterations in early neurodevelopmental processes can enhance a child's vulnerability to psychological and emotional distress, and lead to inwardly focused negative behaviours such as internalizing disorders (Liu et al., 2011). Internalizing disorders altogether affect up to 20% of pre-schoolers and are associated with childhood depression and anxiety disorders, peer relationship problems, and somatic symptoms (Liu et al., 2011). Psychosocial, genetic, and environmental factors are potential contributors to internalizing disorders (Tien et al., 2020). Notably, antenatal exposure to environmental pollutants (present in air, food, water, personal care, and household goods) can impair neurodevelopment and lead to internalizing behaviours (Grandjean and Landrigan, 2014). Some of these pollutants are known as endocrine disruptors (ED) since they interfere with hormonal systems. ED are also suspected to exert neurodevelopmental toxicity, with consequences on offspring behavioural outcomes and psychopathology (Grandjean and Landrigan, 2014).

Numerous epidemiological studies have focussed on single ED prenatal exposures and identified candidate EDs, mostly phenols, parabens, and phthalates, associated with internalizing behaviours (Roen et al., 2015) or associated symptoms (emotional and peer relationship problems, somatic complaints) (Daniel et al., 2020; Engel et al., 2010; Philippat et al., 2017). Notably, we have previously shown that mono-n-butyl-phthalate (mnBP) and monobenzyl phthalate (mBzP) were positively associated with internalizing behaviour at age 3 in boys (Philippat et al., 2017). It is worth noting that, although there is a publication bias in the literature towards reports of positive associations, weak or lack of effects of ED exposure on internalizing behaviours in boys can also be observed, as discussed in exhaustive reviews/meta-analysis for BPA or phthalates (Ejaredar et al., 2017; Radke et al., 2020).

ED are rarely present as single compounds, but rather co-occur in mixtures. An emerging literature is now addressing the joint effects of ED prenatal exposure on neurodevelopmental disorders (e.g. developmental delay, language delay, or autism spectrum disorders (ASD)) or psychopathology associated with internalizing behaviours). Associations between exposure to a phenol/paraben/phthalate mixtures with developmental delay were reported (Barkoski et al., 2019; Bennett et al., 2022). Also, exposure to organophosphorus pesticides in mixture increased the odds of autism spectrum disorders (ASD) (Bennett et al., 2022). Another study showed that exposure to a mixture of EDs comprising bisphenol A (BPA), triclosan (Tc), and phthalates, increased the odds of language delay in 30-month-old infants (Caporale et al., 2022). Exposure to mixtures of phthalates, bisphenols, and organophosphorus pesticides was associated with lower nonverbal intellectual quotient at age 6, but not with internalizing

behaviour in 782 children from the Generation R cohort (van den Dries et al., 2021). Finally, one study found associations between *in utero* exposure to mixtures of phenols and phthalates and child's internalizing behaviours, but mainly in girls (Guilbert et al., 2021). These studies suggest a possible effect of prenatal exposure to EDs mixtures on internalizing behaviour and related neurodevelopmental disorders in infants.

To reinforce causal inferences from epidemiological association studies and identify targets for prevention, mediation studies could help refine the biological pathways through which mixtures of EDs operate. *In vitro* and animal studies have shown that ED can directly interfere with the neuroendocrine system, in particular thyroid or sexual hormones signalling or metabolism, causing disruption of neuronal differentiation and maturation (Lucaccioni et al., 2021). Also, mounting evidence from *in vitro* and animal studies support the notion that ED can also impact immune function. Notably, bisphenols/phenols, phthalates, Tc, and parabens impact the development and functions of most immune cell types (including monocytes, neutrophils, lymphocytes, dendritic cells), with direct effects on the balance in proinflammatory and anti-inflammatory cytokines secreted by immune cells (Nowak et al., 2019). ED are thought to either suppress or enhance immune responses and ED exposure is associated with inflammation, allergies, or autoimmune disorders (Bansal et al., 2018). Cytokines produced by immune cells are key modulators of inflammatory processes and in particular the pro-inflammatory cytokines interleukin (IL)-1 β , IL-6, and Tumor Necrosis Factor (TNF)- α . Besides their role in inflammation, these cytokines also regulate essential neurodevelopmental processes, including neurogenesis, synaptogenesis, and myelination (Zengeler and Lukens, 2021). This supports the notion that cytokines can influence the risk of psychopathology, including internalizing behaviours, later in life.

Based on the above, we hypothesised that inflammation at birth, using the cytokines IL-1 β , IL-6, and TNF- α measured in cord blood as proxies, is a plausible biological pathway through which ED prenatal exposure could operate to influence offspring's neurodevelopment, and in particular internalizing behaviours. Relying on the EDEN mother-child cohort, we used Bayesian Kernel Machine Regression-Causal Mediation Analysis (BKMR-CMA) to investigate at once whether antenatal exposure to mixtures of 20 EDs increased the odds of internalizing behaviours in boy infants at age 3 and whether IL-1 β , IL-6, and TNF- α at birth are mediators of this effect.

2. MATERIAL & METHODS

2.1. Study sample

The present study is nested within the EDEN mother-child cohort (Heude et al., 2016). Pregnant women were recruited before 24 weeks of gestation in the French University Hospitals of Nancy and Poitiers. Clinical and psychosocial data were gathered from medical records, interviews with the mother and auto-questionnaires. Assessment of phenols, phthalates, and parabens in the EDEN cohort was done as part of a previous project to study associations between phenols, phthalates and male genital anomalies such as hypospadias and cryptorchidism (Chevrier et al., 2012), explaining why only males were included. Given that sex-specific differences in the effects of EDs have been reported on neurodevelopmental outcomes (Hilz and Gore, 2022), we also focussed only on boys in the present study. *In fine*, our study sample consisted of 459 mother-boy pairs and 400 pairs for which cord blood sera, maternal urinary levels of ED during pregnancy, and internalizing behaviour scores at age 3 were available (Tab. 1).

2.2. Outcome: child's internalizing behaviour at age 3

Child's behaviour was assessed by the Strengths and Difficulties Questionnaire (SDQ) completed by the mother (Goodman, 1997). The SDQ is a widely used psychometric instrument to evaluate psychopathology in children (Goodman, 2001). This questionnaire includes 25 items scored on a 3-point scale (0: not true; 1: somewhat true; 2: certainly true) that are combined into 4 difficulties sub-scores: conduct problems, hyperactivity-inattention, peer relationship problems and emotional symptoms, and 1 strength sub-score: prosocial behaviour. Each sub-score ranges from 0 to 10. The internalizing behaviour scale, refers to problems of withdrawal, somatic complaints, and anxiety/depression. The internalizing behaviour score is computed by summing the peer relationship problems and emotional symptoms sub-scores (Goodman, 2001).

2.3. Exposure

Women were asked to collect first morning urine void at home just before the prenatal study visit between 22 and 29 gestational weeks. Using solid phase extraction-high-performance liquid chromatography-isotope dilution tandem mass spectrometry, we measured 20 ED in maternal urine (Tab. 2): five phenols (2,4-dichlorophenol (2,4-DC); 2,5-dichlorophenol (2,5-DC); BPA; benzophenone-3 (Bzp-3); Tc), four parabens (butyl-paraben (BP); ethyl-paraben (EP); propyl-paraben (PP); methyl-paraben (MP)), seven phthalates (monoethyl phthalate (mEP); mono-n-butyl phthalate (mnBP); mono-isobutyl phthalate (miBP); monobenzyl

	Nb (%)	NA (%)	Min.	Q25	Mean	Median	Q75	Max.	SD
Center : Nancy	212 (46)	0							
Center : Poitiers	247 (54)								
Pre-pregnancy BMI (kg/m ²)	454 (99)	1.1	15.8	20	23	22	25.2	44.6	4.4
Maternal dietary pattern during pregnancy : Healthy	390 (85)	15	-1.8	-0.6	0.02	-0.06	0.5	5.5	0.9
Maternal dietary pattern during pregnancy : Western	390 (85)	15	-3.6	-0.7	-0.06	-0.1	0.5	4.1	1
Smoking (nb cigarettes/day)	450 (98)	2	0	0	1.1	0	0	16.7	2.7
Caffeine consumption during pregnancy (mg/day)	448 (98)	2.4	0	14.2	94.4	60.8	118.5	537.3	107.3
Alcohol drinking during pregnancy (mean nb glasses/week)	390 (85)	15	0	0	0.49	0	0.3	14	1.2
Maternal depression during pregnancy (yes)	102 (22)	0.7							
Maternal depression during pregnancy (no)	354 (77)								
Maternal prenatal anxiety (STAI scale)	458 (99)	0.2	0	3	9.972	7	14	53	9.7
Age at delivery (years)	459 (100)	0	17	26	29.7	29	33	44	4.9
Delivery mode : Vaginal (Reference)	348 (76)								
Delivery mode : Forceps	50 (11)	0							
Delivery mode : C-section	61 (13)								
Trimester of birth : 1 st	114 (25)								
Trimester of birth : 2 nd	129 (28)	0							
Trimester of birth : 3 rd	120 (26)								
Trimester of birth : 4 th	96 (21)								
Sex: Male	459 (100)	0							
Sex: Female	0								
Parental Separation : yes	38 (8)	9.6							
Parental Separation : no	377 (82)								
Maternal education (years)	456 (99)	0.7	9	12	13.9	14	17	17	2.6
Paternal education (years)	416 (91)	9.4	9	11	13.2	12	17	17	2.6
House Income at age 3	395 (86)	13.9	2	4	5.3	5	6	8	1.5
Number of siblings	397 (86)	13.5	0	0	0.9	1	1	5	0.9
Breastfeeding : No	110 (24)								
Breastfeeding : ≤ 6 months	255 (56)	0							
Breastfeeding : > 6 months	94 (20)								
Child's BMI at age 3 (kg/m ²)	396 (86)	13.7	12.9	15.1	15.8	15.8	16.4	21.5	1.1
Child's dietary pattern at age 3 : Processed and fast foods	400 (87)	12.9	-1.8	-0.6	0.08	-0.1	0.5	12	1.2
Child's dietary pattern at age 3 : Recommended guidelines	400 (87)	12.9	-4.1	-0.7	-0.08	-0.02	0.5	3.6	1.1
Age at internalizing behaviour score assessment (years)	403 (88)	12.2	1061	1135	1153.3	1148	1163.5	1411	30.5
Internalizing behaviour score	400 (87)	12.9	0	1	3	3	4	15	2.4

Tab. 1. Study sample characteristics. BMI, body mass index; SD, standard deviation; Min., minimum; Max., maximum; N/A, non-available (N/A) values; Min., minimum; Max., maximum.

phthalate (MBzP); monocarboxynonyl phthalate (mCNP); monocarboxyoctyl phthalate (mCOP), mono(3-carboxypropyl) phthalate (mCPP)), and four di(2-ethylhexyl) phthalate (DEHP) metabolites (mono(2-ethylhexyl) phthalate (mEHP); mono(2-ethyl-5-hydroxy-hexyl) phthalate (mEHHP); mono(2-ethyl-5-oxohexyl) phthalate (mEOHP); mono(2-ethyl-5-carboxypentyl) phthalate (mECPP)). Urinary creatinine was used as an internal standard for normalization. Measures were performed at the Centers for Disease Control and Prevention laboratory (Atlanta, Georgia, USA), according to established procedures and we used instrumental reading values to calculate concentrations (Silva et al., 2007; Ye et al., 2005). Instrumental reading values equal to 0 (i.e., indicative of no signal) were replaced by the lowest instrumental reading value divided by the square root of 2 (Harley et al., 2013; Philippat et al., 2014a). Prior to statistical analysis, concentrations were standardized for creatinine, sampling conditions (e.g., hour of sampling, gestational age at sampling), and analysis year (2008 or 2011) using a two-step standardization approach (Mortamais et al., 2012). To account for high within-group correlation but relatively lower across-group correlations across ED (Fig. S1), ED were grouped as follow in subsequent analysis: Group 1 consisted of 2,4-DC and 2,5-DC, group 2 of the 4 parabens (MP, EP, BP, PP), Group 3 of the four DEHP metabolites (mEHP, mEHHP, mEOHP, mECPP), while the remaining 10 ED were considered individually (Tab. 2).

2.4. Cytokine measures in cord blood samples

Venous cord blood was sampled immediately after birth and allowed to clot. Blood samples were centrifuged within 24 h post collection. Serum was collected, aliquoted, and stored at -80°C . Cord blood sera were assessed for the levels of 28 cytokines (Barbosa et al., 2020), using a multiplex electrochemiluminescence-based immunoassay and according to the manufacturer's recommendations (Meso Scale Diagnostics, Rockville, USA). Details related to procedures for measurement, quality control, and coefficient of variations are provided in (Barbosa et al., 2020). As proxies for inflammation at birth, we considered the emblematic pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α (Tab. 3). IL-6 and TNF- α levels were above LLOD in all samples. For IL-1 β concentrations below the lower limit of detection (LLOD), half the LLOD value was imputed as recommended for immunological measurements constrained by detection limits (Uh et al., 2008).

	Compound ID	Abbreviation	Concentration (µg/L)									Nb (%) ND
			Min.	Q25	Mean	Median	Q75	Max.	SD	SE		
Phenols	2,4-Dichlorophenol	2,4-DC	Group 1	0.09	0.61	2.98	1.04	1.85	139.43	10.42	0.52	1 (0.25)
	2,5-Dichlorophenol	2,5-DC		0.74	4.53	83.04	10.35	31.39	4733.36	378.31	18.92	0 (0)
	Bisphenol A	BPA		0.37	1.52	3.61	2.39	3.99	95.39	5.67	0.28	0 (0)
	Benzophenone-3	BzP-3		0.05	1.15	42.70	2.30	6.66	3645.04	274.23	13.71	13 (3.25)
	Triclosan	Tc		0.08	3.80	158.59	25.38	192.13	2479.07	294.54	14.73	28 (7)
Parabens	butyl-paraben	BP	Group 2	0.03	0.49	12.61	2.26	11.88	344.35	30.52	1.53	15 (3.75)
	ethyl-paraben	EP		0.02	0.71	16.47	3.90	17.21	299.89	32.60	1.63	49 (12.25)
	propyl-paraben	PP		0.11	3.33	63.02	14.73	51.78	2062.92	179.58	8.98	1 (0.25)
	methyl-paraben	MP		1.92	33.58	476.12	113.42	303.99	22865.18	1666.50	83.32	0 (0)
Phthalates	monoethyl phthalate	mEP	Group 3	7.92	51.34	222.04	97.60	203.64	4093.38	454.97	22.75	0 (0)
	mono-n-butyl phthalate	mnBP		2.87	30.03	127.87	45.07	75.64	2418.86	314.38	15.72	0 (0)
	mono-isobutyl phthalate	miBP		1.54	24.76	61.98	41.95	71.94	689.95	69.93	3.50	0 (0)
	monobenzyl phthalate	mBzP		1.61	10.82	37.68	18.93	35.52	970.43	79.09	3.95	0 (0)
	monocarboxynonyl phthalate	mCNP		0.14	0.85	3.15	1.32	2.28	90.70	7.46	0.37	0 (0)
	monocarboxyoctyl phthalate	mCOP		0.19	2.37	6.79	3.82	6.33	375.31	20.04	1.00	0 (0)
	mono(3-carboxypropyl) phthalate	mCPP		0.34	1.35	3.37	2.04	3.61	32.03	4.04	0.20	0 (0)
	mono(2-ethylhexyl) phthalate	mEHP		0.08	4.47	12.34	7.89	15.06	193.43	16.26	0.81	3 (0.75)
	mono(2-ethyl-5-hydroxy-hexyl) phthalate	mEHHP		2.00	15.54	41.84	28.99	46.91	1565.80	86.06	4.30	0 (0)
	mono(2-ethyl-5-oxohexyl) phthalate	mEOHP		1.78	13.65	34.20	23.89	38.34	1141.13	64.60	3.23	0 (0)
mono(2-ethyl-5-carboxypentyl) phthalate	mECP	4.76	25.18	61.07	39.70	63.86	1827.33	108.17	5.41	0 (0)		

Tab. 2. ED concentrations in maternal urine. Min., minimum; Max., maximum; Min., minimum; Max., maximum; SD, standard deviation; SE, standard error; Nb (%) ND, number and percentage of samples for which instrumental reading values were at 0.

Cytokine	Min.	Mean	Median	Max.	SD	SE	Nb (%) below LLOD
IL-1 β (pg/ml)	0.02	1.66	0.20	456.20	21.33	1.00	34 (8.50)
IL-6 (pg/ml)	0.16	23.89	2.77	2756.37	167.15	7.80	0 (0)
TNF- α (pg/ml)	1.44	4.77	3.20	631.53	29.34	1.37	0 (0)

Tab. 3. Pro-inflammatory cytokines concentrations in cord blood serum. LLOD, Lower Limit of Detection; Min., minimum; Max., maximum; Min., minimum; Max., maximum; SD, standard deviation; Nb (%), number and percentage of samples for which the concentration was below LLOD.

2. 5. Covariates

Previous studies have identified maternal, perinatal and psychosocial variables associated with behavioural abnormalities in children (de La Rochebrochard and Joshi, 2013; Gumusoglu and Stevens, 2019; Melchior et al., 2015; Noonan et al., 2018; Polanska et al., 2015; van der Waerden et al., 2015). Based on these studies, and considering that many of these variables can also influence cytokine production or ED exposure, we selected the following variables (Fig. 1). Maternal variables were: maternal age at delivery (years), maternal pre-pregnancy body mass index (BMI in kg/m²), alcohol drinking during pregnancy (mean number of glasses/week), smoking during pregnancy (number of cigarettes/day), caffeine intake during pregnancy (mg caffeine/day), maternal dietary pattern during pregnancy (Healthy, vs. Western), record of depression during pregnancy (yes/no), and symptoms of prenatal anxiety. Maternal dietary patterns were constructed based on frequency food diaries validated in previous studies led in the EDEN cohort (Collet et al., 2021; Galera et al., 2018; Yuan et al., 2017). Maternal depression was assessed at 24-28 weeks of amenorrhea, using the Center for Epidemiological Studies Depression questionnaire (CES-D (Radloff, 1991)) and women presenting a CES-D score above a cutoff of 17 were considered as depressed. Symptoms of maternal anxiety were assessed at 24-28 weeks of amenorrhea, using the State-Trait Anxiety Inventory (STAI) (Spielberger and Vagg, 1984) and the continuous STAI score was used to assess the severity of anxiety symptoms. Perinatal variables were: delivery mode (vaginal, forceps, C-section), birth trimester, and recruitment center (Nancy or Potiers). Gestational age or birth weight were not included as covariates as they could be on the path from ED exposure to internalizing behaviours and contribute to lower estimated effects. There are indeed several studies suggesting associations between prenatal exposure to these compounds and birth weight, as well as gestational age (Welch et al., 2022; Zhong et al., 2020). Postnatal socioeconomic variables were: house income at age 3 (in €), maternal and paternal education duration (years), parental separation (yes/no), and breastfeeding (no, < 6 months, > 6 months). Child's variables at age 3 were: BMI (kg/m²) and child's diet (Processed and fast foods vs.

Recommended food guidelines, according to a food frequency diary validated in previous studies led in EDEN cohort (Deschamps et al., 2009; Lioret et al., 2015; Yuan et al., 2017)), Multiparity (number of older siblings) and age at SDQ assessment (years) were also included.

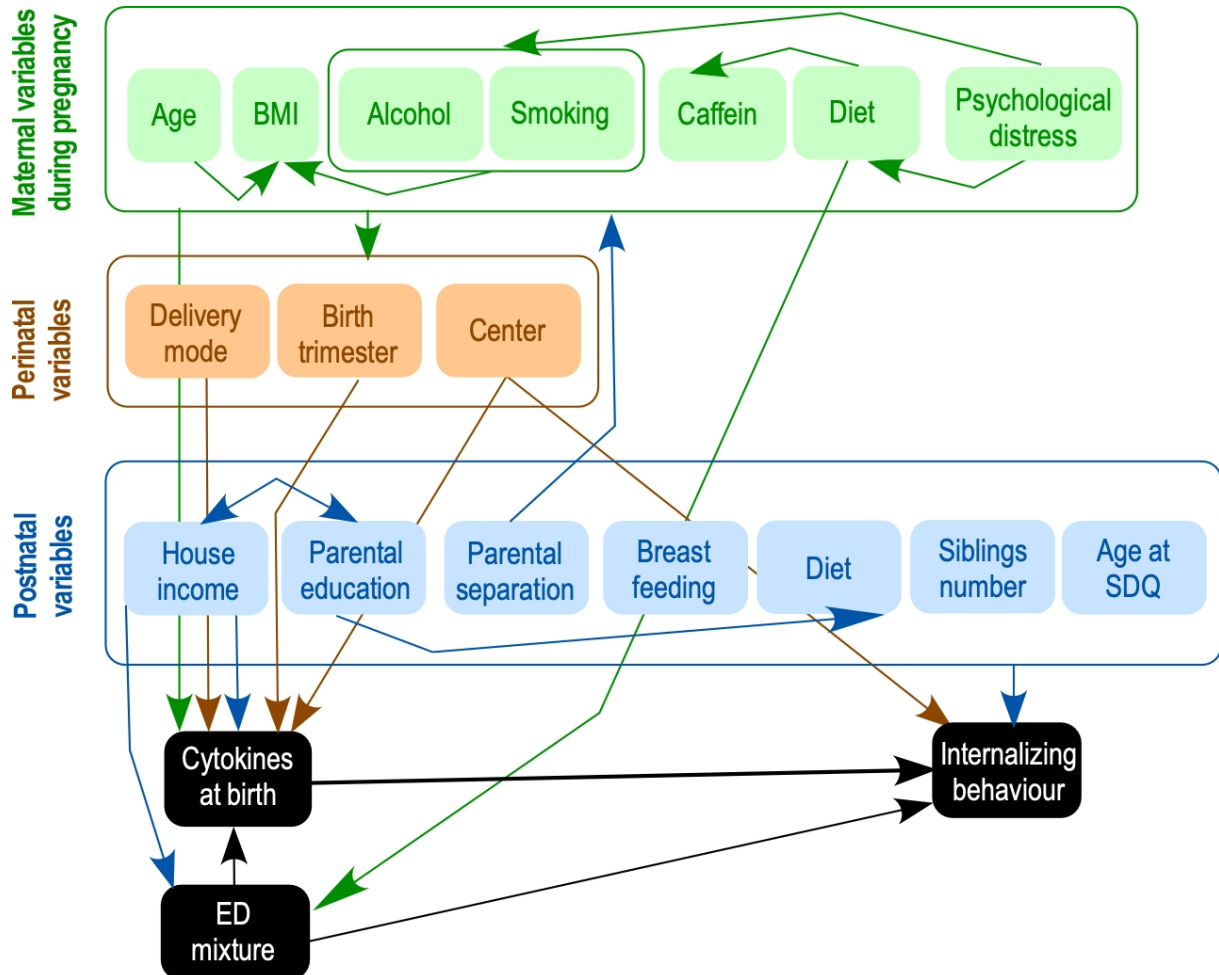


Fig. 1. Relationships between covariates, confounders, exposure (mixtures of EDs), mediators (cytokines at birth), and outcome (internalizing behaviour score).

2.6. Statistical analyses

Multiple imputation (MI) of missing data. To overcome the issue of missing covariate data, we used the mice package in R to generate 30 multiple-imputed datasets (van Buuren and Groothuis-Oudshoorn, 2011). The following analyses were performed over these 30 multiple-imputed datasets.

Bayesian Kernel Machine Regression causal mediation analysis (BKMR-CMA) framework. Until recently, epidemiological studies to study ED effects in complex mixtures were limited by available statistical methodologies to handle i) multiple and correlated ED exposures, ii) non-

linear effects of ED exposures on the outcome, iii) regression instability as the number of exposures and covariates increases (Demeinex and Slama, 2019; Tanner et al., 2020). Here we relied on the BKMR-CMA framework which now allows to simultaneously estimate the total effect of exposure to mixtures of EDs and mediating effects (Devick et al., 2022). BKMR appears currently as the least biased and most accurate in estimating independent and cumulative effects of chemicals, as well as dose-response relationships and interaction effects (Bobb et al., 2018). The BKMR-CMA approach defines natural direct effects (NDE) and natural indirect effects (NIE) that sum up to the total effect (TE) (Fig. 2).

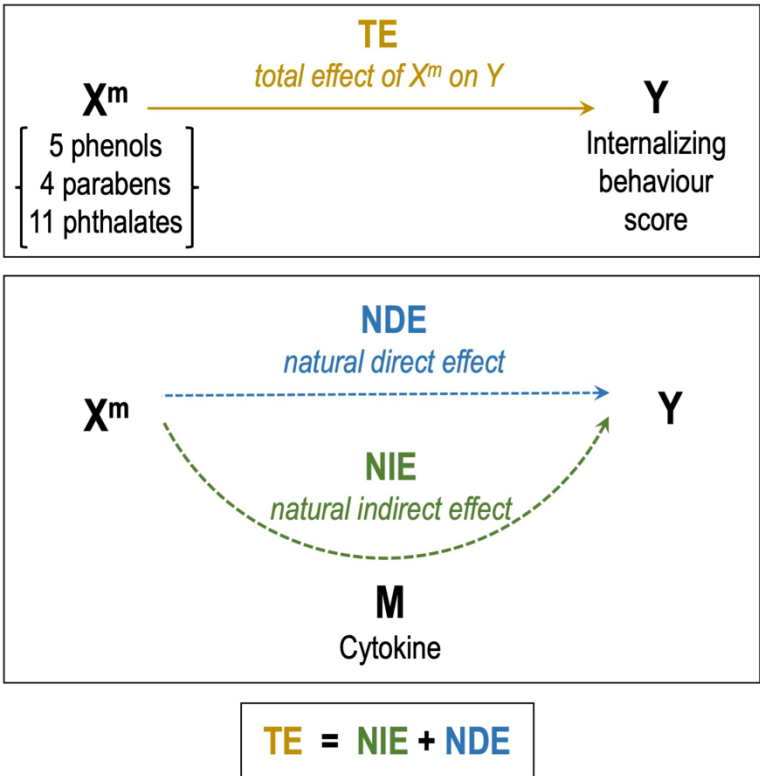


Fig. 2. BKMR causal mediation analysis counterfactual framework. Relationships between mixture exposure (X^m), outcome (Y), and mediator (M).

In a counterfactual framework, the individual causal effect of the exposure on the outcome is defined as the hypothetical contrast between the outcomes that would be observed in the same individual at the same time with or without the exposure, or in the presence of two different levels of the exposure. Let Y_{am} denote the counterfactual outcome Y if the exposure X was set to level x and mediator M was set to level m. Let M_x be the counterfactual mediator M that would have been observed if the exposure X was set to level x. Accordingly, $Y_{xM_x^*}$ represents the counterfactual outcome Y if the exposure X was set to level x and the mediator M was set to the level it would have taken if the exposure X was set to level x^* . The mediated effects of

interest, the total effect (TE), the natural direct effect (NDE) and the natural indirect effect (NIE) are formally defined as: $TE = E[Y_x - Y_{x^*}]$; $NDE = E[Y_x M_{x^*} - Y_{x^*} M_{x^*}]$; $NIE = E[Y_x M_x - Y_x M_{x^*}]$ (Devick et al., 2022).

Because the BKMR-CMA framework does not allow for considering multiple mediators in the same model, we conducted the mediation analysis using each cytokine independently. We simulated counterfactuals to estimate TE, NDE and NIE for a change in the exposure from z^* to z , where each ED is set at the 25th and 75th percentile, while each cytokine is set at the 25th, 50th or 75th percentile. BKMR-CMA analysis was performed according to (Devick et al., 2022), using the R statistical analysis software using the 'bkmr' package version 0.2.0 (Bobb, 2022).

All numerical variables were scaled and the exposure and mediators were also log-transformed. To account for high within-group correlation but relatively lower across-group correlations across ED (Fig. S1), we used the hierarchical variable selection option in the bkmr package. Group 1 consisted of 2,4-DC and 2,5-DC, group 2 of the 4 parabens (MP, EP, BP, PP), Group 3 of the four DEHP metabolites (mEHP, mEHHP, mEOHP, mECP), while the remaining 10 ED were considered individually (Tab. 2). For each MI dataset, we conducted 30,000 iterated BKMR runs and produced estimates for each MI dataset with 95% credible intervals (CI). The 95% CI determines the distribution of possible estimate values between its upper and lower boundaries with the subjective probability of 95%. We derived from BKMR-CMA runs the Posterior Inclusion Probabilities (PIP), the full posterior distribution of TE, NDE, and NIE estimates and 95% CI, the predictor-response functions, and the contribution of individual predictors to the response.

2.7. Ethics

The EDEN cohort received approval from the Ethical Research Committee (CCPPRB) of Bicêtre Hospital and from the French National Data Protection Agency (CNIL). Informed written consent was obtained from parents at the time of enrolment and after delivery. The analysis of blinded urine specimens at CDC laboratories was determined not to constitute engagement in human subjects' research.

3. RESULTS

Our study sample consisted of 459 mother-boy pairs recruited in two maternity centres (Tab.1). As for exposure, we considered 20 ED metabolites in mixtures. Among these 20 ED, ten (BPA, Tc, mEP, mnBP, mBzP, mEHP, mEHHP, mEOHP, mECP, mCOP) were present in mixtures of EDs recently shown to be associated with language delay in young infants (Caporale et al., 2022). Our mixture also contains parabens known to co-occur with phenols in mixtures of EDs (Bennett et al., 2022). All 20 ED were measured in maternal urinary samples collected during pregnancy among mothers who delivered a boy (Philippat et al., 2017) (Table 2). Tc, MP, mEP, and mnBP presented the highest mean concentrations ($> 100 \mu\text{g/L}$), while 2,4-DC, BPA, mCNP, and mCPP presented the lowest ($<5 \mu\text{g/L}$) (Tab. 2). Medium to strong correlations were observed among ED from the same classes (Fig. S1), in particular among the two dichlorophenols (2,4-DC, 2,5-DC), the four parabens (MP, EP, PP, BP), and four DEHP metabolites (mEHP, mEHHP, mEOHP, mECP). Weak correlations were observed across ED classes, with Tc correlating with 2,4-DC, parabens and one phthalate metabolite mEP, and BPA correlating with phthalates and DEHP metabolites (Fig. S1). As mediators, we considered the cytokines IL-1 β , IL-6, and TNF- α which were measured in cord blood serum samples collected at birth (Barbosa et al., 2020) (Tab. 3). These cytokines are induced during inflammation and are commonly used as proxies to measure inflammation (Khalfallah et al., 2022). IL-1 β , IL-6, and TNF- α levels correlated in cord blood serum samples supporting they may be involved in common inflammatory processes (Fig. S2).

We used hierarchical BKMR-CMA to model at once the effects of the 20 EDs in mixture on internalizing behaviour and the possible mediating effects of cytokines. Hierarchical BKMR allowed to group ED metabolites from the same class showing strong correlations (see Methods and Fig. S1), while uncorrelated ED metabolites were considered independently. When addressing the total effect (TE) of EDs mixture across BKMR runs, a joint change in the EDs mixture from the 25th percentile value to its 75th percentile value was associated with an increase of 1.018 on the internalizing behaviour score (Tab. S1). However, the wide CI comprising zero [-1.822; 3.858] (Tab. S1) was indicative of an instability in the estimation.

The PIP provided further insights on the ED selection across BKMR runs and therefore of the importance of each ED metabolite or metabolite group in defining the exposure-outcome association (Fig. 3). In general, the estimated individual PIP were small (PIP <0.5), with the phthalate miBP exhibiting the highest value (PIP >0.2). PIP inspection also highlighted possible minor contribution of mEP and mnBP in defining the association between ED exposure and internalizing behaviour scores at age 3. The importance of miBP and mnBP was also

confirmed by examining individual dose-response associations for each ED (Fig. 4), when all the other ED were fixed at their median. miBP somehow presented with a non-linear negative relationship with the outcome, while mnBP exhibited a positive linear relationship with the outcome. The associations of the other ED with internalizing behaviour scores were mainly unchanged while holding the other compounds at their median, indicating no synergistic or multiplicative interactions.

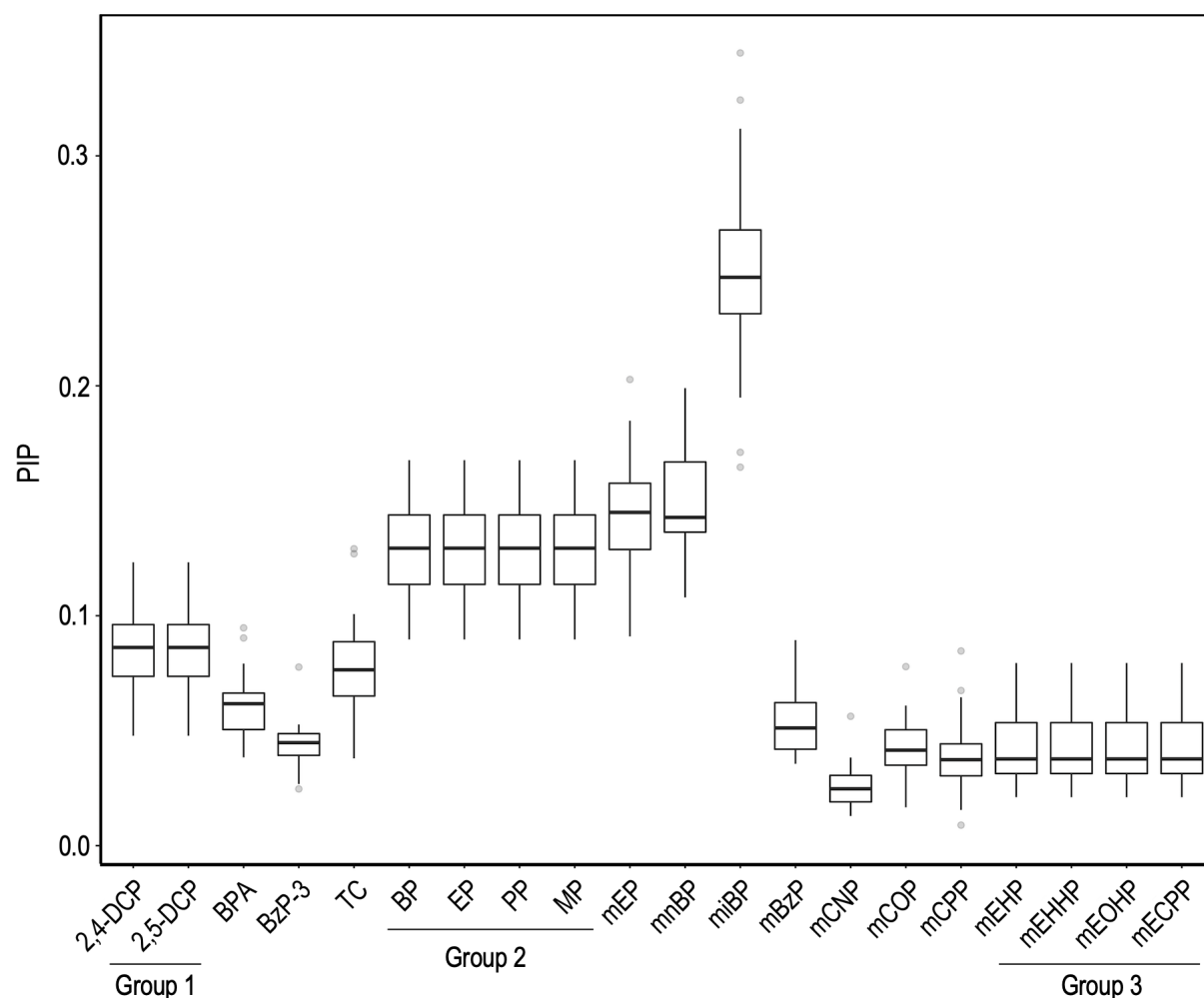


Fig. 3. Individual posterior inclusion probabilities (PIP) for each ED metabolite or ED metabolites groups from BKMR. Abbreviations: Group 1 (2,4-dichlorophenol (2,4-DC), 2,5-dichlorophenol (2,5-DC)); bisphenol-A (BPA); benzophenone-3 (BzP-3); triclosan (TC); Group 2 (butyl-paraben (BP), ethyl-paraben (EP); propyl-paraben (PP); methyl-paraben (MP)); monoethyl phthalate (mEP); mono-n-butyl phthalate (mnBP); mono-isobutyl phthalate (miBP); monobenzyl phthalate (mBzP); monocarboxynonyl phthalate (mCNP); monocarboxyoctyl phthalate (mCOP), mono(3-carboxypropyl) phthalate (mCPP)), Group 3 (mono(2-ethylhexyl) phthalate (mEHP), mono(2-ethyl-5-hydroxy-hexyl) phthalate (mEHHP), mono(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (mECPP)).

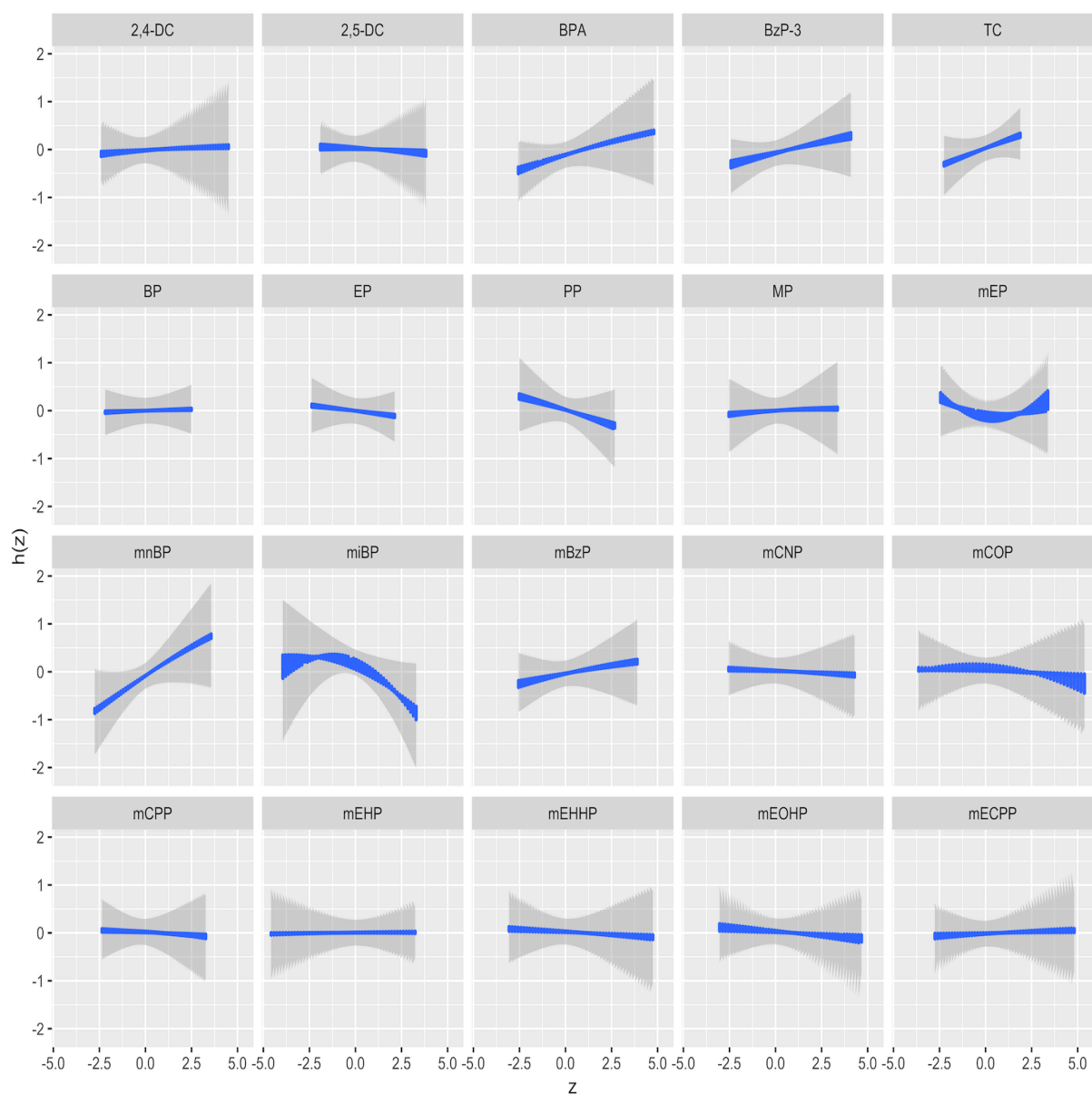


Fig. 4. Univariate dose-response associations from BKMR. Plots assess the adjusted association between each ED exposure and the internalizing behaviour, while fixing the other ED exposures to their median. The estimate for each of the 30 multiply-imputed datasets are plotted and $CI_{95\%}$ are shown in grey to depict variability.

To better characterize the contribution of individual exposures to the overall effect, single-exposure effect estimates were computed (Fig. S3). Again, CI for each compound estimate were wide across quantiles, indicative of an instability in the estimation. Increases in individual exposure to 2,5-DC, Tc, parabens (BP, PP, MP), and a subset of phthalates (mnBP, miBP, mBzP) exhibited a trend for association with higher internalizing behaviour scores, when all of the remaining ED were fixed at their 75th percentile as compared to when they were fixed at their 25th percentile (Fig. S3). On the other hand, 2,4-DC, BPA, mCNP, and mCPP appeared not to contribute to the total effect on internalizing behaviour, as their mean TE estimates approached zero across the quantiles of concentrations (Fig. S3).

Despite the weak TE of EDs in mixture on internalizing scores and arguing that a suppressive effect of cytokines could have contributed to lower TE estimates, we decomposed the total effect of exposure to EDs in mixture on internalizing behaviours into the NDE of EDs in mixture and the NIE mediated by each cytokine (Fig. 5, Tab. S1). For the different models, in the presence of either IL-1 β , IL-6, or TNF- α , NDE effects were systematically lower than the TE (Fig. 5, Tab. S1). The NIE for each model including either IL-1 β , IL-6, or TNF- α , were respectively 0.101, 0.1, and 0.102 (Fig. 5, Tab. S1), indicating that cytokines contribute to explain 10% of EDs in mixture TE on internalizing behaviours. However, CI_{95%} were very wide, again limiting the conclusions to be drawn. Our data therefore do not support a credible mediating or suppressive effect of cytokines between EDs mixture exposure and internalizing behaviours.

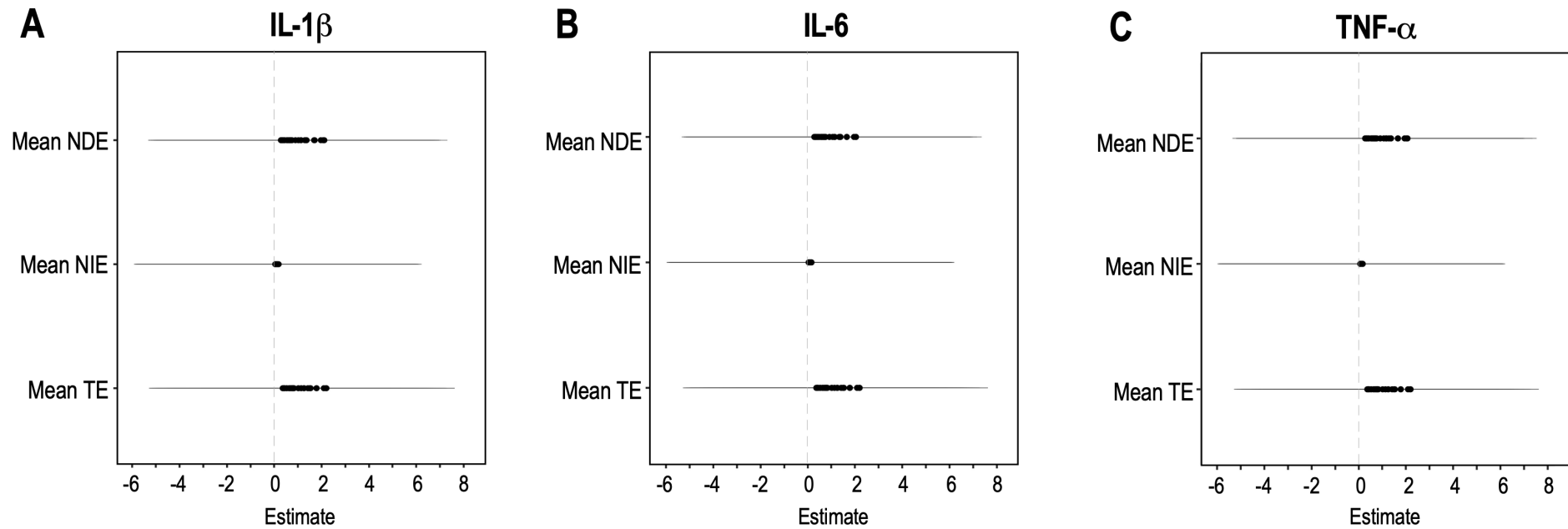


Fig. 5. Natural direct effect of multiple ED exposure on internalizing behaviour and indirect effects of each cytokine. The total effect (TE) of multiple ED exposure was decomposed into the natural direct effect (NDE) and the natural indirect effect (NIE) for each cytokine of interest: IL-1 β (A), IL-6 (B), and TNF- α (C). Individual dots represent the estimates for each of the 30 multiply-imputed datasets and the corresponding CI_{95%}.

4. DISCUSSION

Effects of prenatal exposure to EDs in mixture on internalizing behaviour at age 3 in boys

In this prospective birth cohort study and applying BKMR-CMA modelling, we found limited evidence to indicate that prenatal exposure to a complex mixture of 20 EDs (5 phenols, 4 parabens, 11 phthalates and metabolites) negatively impacts neurodevelopment assessed by internalizing behaviours in boys at age 3. Furthermore, the overall mixture effect was weak, with weak individual effect estimates and wide CI. The phthalates mnBP, miBP, and mEP appeared nevertheless to have the strongest influence, but their relatively modest PIP, as well as the wide CI of our estimates calls for caution in the interpretation of these results. Using a classical single pollutant modelling strategy in the same study samples comprising boys only, we previously found that individual exposure to mnBP was significantly associated with increased internalizing behaviour scores. Of note, none of the other 19 ED considered was significantly associated with increased internalizing behaviour score. Moreover, mnBP effects were weak (OR=1.06; CI_{95%} [1.01;1.11]) and did not survive multiple testing (Philippat et al., 2017). This argues in favour of a generally weak effect of these EDs in mixture on internalizing behaviour in this study sample, possibly driven by the phthalate mnBP.

It remains difficult to compare our results to other studies, as scarce studies have addressed the effects of EDs mixtures on internalizing behaviours in the general population. Also, non-overlapping lists of ED are generally considered in mixture. For example, we show that increased exposure to EDs in mixture (consisting of 12 phenols, 13 phthalates, 2 non-phthalate plasticizer metabolites) was associated with higher internalizing behaviour scores, applying Weighted Quantile Sum (WQS) regression in a study sample of 416 mother-child pairs from the French SEPAGES cohort (Guilbert et al., 2021). The highest weights were observed for mEP, mBzP, and mnBP. This is partially in agreement with our BKMR analysis in the EDEN cohort as PIP, univariate dose-response associations and single-exposure effect pointed towards mnBP as a possible driver of EDs mixture effect on internalizing behaviour score. However, the EDs in mixture considered by Guilbert was different from our study and ED levels were measured in within-subject pools of multiple urine samples collected at two distinct time points during pregnancy (>20 samples/individual pool), resulting in a more accurate exposure measure. Still, the score increase in girls was 2.47 points (CI_{95%} [0.6;4.33]) for an increase of one tertile in the WQS index, compared with 1.17 points (CI_{95%} [-0.5;2.84]) in boys. Considering the wide CI encompassing zero in boys, this study suggests a weak or absence of effects of this mixture of EDs in boys. Therefore, focusing only in boys and relying only on a single measure point of exposure to the EDs in mixture may have contributed to weaken the effects

we observe in the present study. It is also possible that these mixtures of EDs interfere with female hormonal pathways (mainly via binding to oestrogen receptors (Borrow and Handa, 2017)) during neurodevelopment leading to exacerbated internalizing behaviours, and less with male hormonal pathways, contributing to weaken the observed effects in boys (Hilz and Gore, 2022). Also internalizing behaviours are more frequent in females, at least in adult population (McLean et al., 2011), suggesting that females may be more susceptible to environmental risk factors associated with internalizing behaviours.

We cannot exclude the fact that our results may actually reflect a lack of effects of ED mixture exposure to these EDs in mixture on internalizing behaviours. This would agree with a previous report of a lack of association between prenatal exposure to a mixture of phthalates, bisphenol, and organophosphorus pesticides with internalizing or autistic behaviours at age 6 in 782 children from the Generation R cohort (van den Dries et al., 2021). Also, no association between prenatal mixture exposure to the phthalates mnBP, mBzP, miBP, mEP, and four DEHP metabolites and internalizing behaviours or anxious-shy behaviours was observed among 411 mother-child pairs (Daniel et al., 2020). Quite interestingly, an association with anxious-shy behaviours was observed only when considering mixture exposure to a specific subset of DEHP metabolites (Daniel et al., 2020). As stressed by the authors, the latter results advocate that the inclusion of compounds with distinct biological activity in the same models (for example DEHP and non-DEHP metabolites) may bias towards the null hypothesis and may call for favouring mixtures of EDs with common biological activities. In our case, we included 20 EDs from three main families of compounds known to act on distinct biological pathways. This may have contributed to hinder the true biological effect of specific EDs present in our mixture on internalizing behaviours.

Exposure to EDs in mixture and other neurodevelopmental outcomes

When considering other outcomes related to neurodevelopment, mixed reports coexist in the literature. One recent study reported that prenatal exposure to a mixture of EDs comprising BPA, Tc, phthalates, and perfluorinated compounds (partly overlapping with our mixture of 20 EDs), was associated with higher odds of language delay in a sample of more than 2000 infants (Caporale et al., 2022). Also, higher prenatal exposure to a mixture of phthalates, bisphenols, and organophosphorus pesticides was associated with lower nonverbal intellectual quotient at age 6 in 782 children from the Generation R cohort (van den Dries et al., 2021). Another study found evidence that prenatal exposure to a mixture of organophosphorus pesticides increased the odds of ASD in a cohort of 237 typically developing and 224 children with ASD (Bennett et al., 2022). On the other hand, another study provided limited evidence for adverse relationship between prenatal exposure to a mixture of

dioxins and polychlorinated biphenyls with psychomotor development or mental development at 6 months of age in a sample of 259 infants (Yim et al., 2022). These studies suggest that depending on the mixture of EDs considered in terms of complexity, diversity, and measuring method, the psychopathological outcome, or the population sample (clinical, general population, boys only), distinct conclusions can be drawn related to the adverse effects of exposure to EDs in mixture on child's behaviour in general.

Mediation or suppression of the effects of EDs in mixture on internalizing behaviour by cytokines

Since an association between a specific mixture of EDs and health outcome does not necessarily mean that the relationship is causative, identifying possible intermediary pathways underlying the effects of EDs in mixture would be one move towards causality or at least towards an understanding of the underlying biological pathways. The recent development of BKMR-CMA provides methodological framework to address this. To our knowledge, no other study has addressed whether perinatal inflammation could be a plausible biological pathway which could modulate the effects of EDs in mixture on neurodevelopment. Although our results indicated that the total effect of EDs in mixture on internalizing behaviours was at best weak or absent, we considered the mediation analysis could uncover a suppressive effect of cytokines, which could have contributed to lower the total effect estimates. This was not the case and given the wide credible intervals for the mediation estimates, our analyses suggest that cytokines elevated in inflammatory conditions (IL-1 β , IL-6, TNF- α) are unlikely mediators of the deleterious effects of EDs in mixture on internalizing behaviours.

Limitations of the study

Although our study sample was well characterized in terms of covariates which could influence either the exposure, outcome or mediators, this study also presents some limitations. First, although the window of sampling was narrow (25-29 weeks amenorrhea), ED were measured at a single time point in maternal urines, this might not reflect accurately long-term ED foetal exposure as placental barrier permeability to the different classes of ED may vary. Second, as statistical strategies to model the effects of exposure to EDs in mixture complexify, improving power by relying on a larger population sample may be critical to grasp complex effects of EDs in mixture. Third, we have only addressed the effects of exposure to EDs in mixture on internalizing behaviours and have not considered neuropsychopathological outcomes more proximal to the exposure window. Fourth, we have only considered boys and our results have not been replicated yet in an independent cohort, precluding generalization of our findings.

How to improve future studies?

Recent development of powerful analytical strategies now allows estimation of the effects of EDs exposure in mixture on human health in general, and on neurodevelopment in particular. It may be timely to set high standards for such analyses to improve reliability of this emerging literature and to leave room for reports of weak or lack of effects. In terms of standards, relying on population samples of larger sample size, contrasting results obtained from boys and girls, improving the accuracy of ED exposure measures by multiple sampling appear critical to capture the adverse effects of EDs exposure in mixture on neurodevelopment. Also, to guide accurate hypothesis formulation as well as select the EDs present in the mixture, it is important to account for prior reports of weak or low association and *a priori* knowledge related to each ED biological activity. Indeed, it is plausible that considering small sample size and ED compounds within the mixture with distinct biological activity in the same models may bias towards the null hypothesis. These considerations are in line with a recent review (Joubert et al., 2022), which advocates for the use of the most recent statistical advancements for data analysis to improve exposure-response estimation, for increased accuracy of exposure measures, this to globally enable more informed analyses of environmental mixtures effects. Finally, identifying biological pathways through mediation studies should highlight targets for prevention. Pursuing in this direction should improve risk assessment of exposures to EDs in mixture on child's neurodevelopment to reduce exposure and improve public health.

5. CONCLUSIONS

Our study provides limited evidence supporting a deleterious impact of prenatal exposure to a mixture of phenols, parabens, and phthalates on internalizing behaviours in boys at age 3. Also, the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α are unlikely mediators of the detrimental effect of prenatal exposure to EDs in mixture. Although this could be interpreted as a weak or null effect of exposure to this mixture of EDs and cytokines on neurodevelopment, it is possible that our study design (sample size, single exposure and mediator assessment, considering boys only) may have contributed to hinder the actual true effect of EDs in mixture on internalizing behaviour. As mixture and mediation methods are continuously evolving, future studies should set best practices both in terms of biostatistical strategy but most importantly in terms of study design to reliably estimate the actual true effect of prenatal ED mixture exposure on neurodevelopment and guide public health policies to regulate exposures to EDs in mixture.

6. REFERENCES

- Bansal, A., Henao-Mejia, J., and Simmons, R.A. (2018). Immune System: An Emerging Player in Mediating Effects of Endocrine Disruptors on Metabolic Health. *Endocrinology* 159, 32-45.
- Barbosa, S., Khalfallah, O., Forhan, A., Galera, C., Heude, B., Glaichenhaus, N., and Davidovic, L. (2020). Immune activity at birth and later psychopathology in childhood. *Brain Behav Immun Health* 8, 100141.
- Barkoski, J.M., Busgang, S.A., Bixby, M., Bennett, D., Schmidt, R.J., Barr, D.B., Panuwet, P., Gennings, C., and Hertz-Picciotto, I. (2019). Prenatal phenol and paraben exposures in relation to child neurodevelopment including autism spectrum disorders in the MARBLES study. *Environ Res* 179, 108719.
- Bennett, D.H., Busgang, S.A., Kannan, K., Parsons, P.J., Takazawa, M., Palmer, C.D., Schmidt, R.J., Doucette, J.T., Schweitzer, J.B., Gennings, C., *et al.* (2022). Environmental exposures to pesticides, phthalates, phenols and trace elements are associated with neurodevelopment in the CHARGE study. *Environ Int* 161, 107075.
- Bobb, J. (2022). *bkmr: Bayesian Kernel Machine Regression*. R package version 0.2.2
- .
- Bobb, J.F., Claus Henn, B., Valeri, L., and Coull, B.A. (2018). Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ Health* 17, 67.
- Borrow, A.P., and Handa, R.J. (2017). Estrogen Receptors Modulation of Anxiety-Like Behavior. *Vitam Horm* 103, 27-52.
- Caporale, N., Leemans, M., Birgersson, L., Germain, P.L., Cheroni, C., Borbely, G., Engdahl, E., Lindh, C., Bressan, R.B., Cavallo, F., *et al.* (2022). From cohorts to molecules: Adverse impacts of endocrine disrupting mixtures. *Science* 375, eabe8244.
- Chevrier, C., Petit, C., Philippat, C., Mortamais, M., Slama, R., Rouget, F., Calafat, A.M., Ye, X., Silva, M.J., Charles, M.A., *et al.* (2012). Maternal urinary phthalates and phenols and male genital anomalies. *Epidemiology* 23, 353-356.
- Collet, O.A., Heude, B., Forhan, A., Delcourt, C., Orri, M., Van der Waerden, J., Melchior, M., Cote, S., Lioret, S., de Lauzon-Guillain, B., *et al.* (2021). Prenatal Diet and Children's Trajectories of Anxiety and Depression Symptoms from 3 to 8 Years: The EDEN Mother-Child Cohort. *J Nutr* 151, 162-169.
- Daniel, S., Balalian, A.A., Insel, B.J., Liu, X., Whyatt, R.M., Calafat, A.M., Rauh, V.A., Perera, F.P., Hoepner, L.A., Herbstman, J., *et al.* (2020). Prenatal and early childhood exposure to phthalates and childhood behavior at age 7 years. *Environ Int* 143, 105894.
- de La Rochebrochard, E., and Joshi, H. (2013). Siblings and child development. *Longitudinal and Life Course Studies* 4, 276-287.
- Demeinex, B., and Slama, R. (2019). Endocrine Disruptors: from Scientific Evidence to Human Health Protection (Policy Department for Citizens' Rights and Constitutional Affairs Directorate General for Internal Policies of the Union PE 608.866).

- Deschamps, V., de Lauzon-Guillain, B., Lafay, L., Borys, J.M., Charles, M.A., and Romon, M. (2009). Reproducibility and relative validity of a food-frequency questionnaire among French adults and adolescents. *Eur J Clin Nutr* 63, 282-291.
- Devick, K.L., Bobb, J.F., Mazumdar, M., Claus Henn, B., Bellinger, D.C., Christiani, D.C., Wright, R.O., Williams, P.L., Coull, B.A., and Valeri, L. (2022). Bayesian kernel machine regression-causal mediation analysis. *Stat Med* 41, 860-876.
- Ejaredar, M., Lee, Y., Roberts, D.J., Sauve, R., and Dewey, D. (2017). Bisphenol A exposure and children's behavior: A systematic review. *J Expo Sci Environ Epidemiol* 27, 175-183.
- Engel, S.M., Miodovnik, A., Canfield, R.L., Zhu, C., Silva, M.J., Calafat, A.M., and Wolff, M.S. (2010). Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environ Health Perspect* 118, 565-571.
- Galera, C., Heude, B., Forhan, A., Bernard, J.Y., Peyre, H., Van der Waerden, J., Pryor, L., Bouvard, M.P., Melchior, M., Lioret, S., *et al.* (2018). Prenatal diet and children's trajectories of hyperactivity-inattention and conduct problems from 3 to 8 years: the EDEN mother-child cohort. *J Child Psychol Psychiatry* 59, 1003-1011.
- Goodman, R. (1997). The Strengths and Difficulties Questionnaire: a research note. *Journal of child psychology and psychiatry, and allied disciplines* 38, 581-586.
- Goodman, R. (2001). Psychometric properties of the strengths and difficulties questionnaire. *J Am Acad Child Adolesc Psychiatry* 40, 1337-1345.
- Grandjean, P., and Landrigan, P.J. (2014). Neurobehavioural effects of developmental toxicity. *Lancet Neurol* 13, 330-338.
- Guilbert, A., Rolland, M., Pin, I., Thomsen, C., Sakhi, A.K., Sabaredzovic, A., Slama, R., Guichardet, K., and Philippat, C. (2021). Associations between a mixture of phenols and phthalates and child behaviour in a French mother-child cohort with repeated assessment of exposure. *Environ Int* 156, 106697.
- Gumusoglu, S.B., and Stevens, H.E. (2019). Maternal Inflammation and Neurodevelopmental Programming: A Review of Preclinical Outcomes and Implications for Translational Psychiatry. *Biological psychiatry* 85, 107-121.
- Heude, B., Forhan, A., Slama, R., Douhaud, L., Bedel, S., Saurel-Cubizolles, M.J., Hankard, R., Thiebaugeorges, O., De Agostini, M., Annesi-Maesano, I., *et al.* (2016). Cohort Profile: The EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. *International journal of epidemiology* 45, 353-363.
- Hilz, E.N., and Gore, A.C. (2022). Sex-specific Effects of Endocrine-disrupting Chemicals on Brain Monoamines and Cognitive Behavior. *Endocrinology* 163.
- Joubert, B.R., Kioumourtoglou, M.A., Chamberlain, T., Chen, H.Y., Gennings, C., Turyk, M.E., Miranda, M.L., Webster, T.F., Ensor, K.B., Dunson, D.B., *et al.* (2022). Powering Research through Innovative Methods for Mixtures in Epidemiology (PRIME) Program: Novel and Expanded Statistical Methods. *Int J Environ Res Public Health* 19.

- Khalfallah, O., Barbosa, S., Martinuzzi, E., Davidovic, L., Yolken, R., and Glaichenhaus, N. (2022). Monitoring inflammation in psychiatry: Caveats and advice. *Eur Neuropsychopharmacol* 54, 126-135.
- Lioret, S., Betoko, A., Forhan, A., Charles, M.A., Heude, B., de Lauzon-Guillain, B., and Group, E.M.-C.C.S. (2015). Dietary patterns track from infancy to preschool age: cross-sectional and longitudinal perspectives. *J Nutr* 145, 775-782.
- Liu, J., Chen, X., and Lewis, G. (2011). Childhood internalizing behaviour: analysis and implications. *J Psychiatr Ment Health Nurs* 18, 884-894.
- Lucaccioni, L., Trevisani, V., Passini, E., Righi, B., Plessi, C., Predieri, B., and Iughetti, L. (2021). Perinatal Exposure to Phthalates: From Endocrine to Neurodevelopment Effects. *Int J Mol Sci* 22.
- McLean, C.P., Asnaani, A., Litz, B.T., and Hofmann, S.G. (2011). Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res* 45, 1027-1035.
- Melchior, M., Hersi, R., van der Waerden, J., Larroque, B., Saurel-Cubizolles, M.J., Chollet, A., Galera, C., and Group, E.M.-C.C.S. (2015). Maternal tobacco smoking in pregnancy and children's socio-emotional development at age 5: The EDEN mother-child birth cohort study. *Eur Psychiatry* 30, 562-568.
- Mortamais, M., Chevrier, C., Philippat, C., Petit, C., Calafat, A.M., Ye, X., Silva, M.J., Brambilla, C., Eijkemans, M.J., Charles, M.A., *et al.* (2012). Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals. *Environ Health* 11, 29.
- Noonan, K., Burns, R., and Violato, M. (2018). Family income, maternal psychological distress and child socio-emotional behaviour: Longitudinal findings from the UK Millennium Cohort Study. *SSM - population health* 4, 280-290.
- Nowak, K., Jablonska, E., and Ratajczak-Wrona, W. (2019). Immunomodulatory effects of synthetic endocrine disrupting chemicals on the development and functions of human immune cells. *Environ Int* 125, 350-364.
- Philippat, C., Nakiwala, D., Calafat, A.M., Botton, J., De Agostini, M., Heude, B., Slama, R., and Group, E.M.-C.C.S. (2017). Prenatal Exposure to Nonpersistent Endocrine Disruptors and Behavior in Boys at 3 and 5 Years. *Environ Health Perspect* 125, 097014.
- Polanska, K., Jurewicz, J., and Hanke, W. (2015). Smoking and alcohol drinking during pregnancy as the risk factors for poor child neurodevelopment - A review of epidemiological studies. *Int J Occup Med Environ Health* 28, 419-443.
- Radke, E.G., Braun, J.M., Nachman, R.M., and Cooper, G.S. (2020). Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence. *Environ Int* 137, 105408.
- Radloff, L.S. (1991). The use of the Center for Epidemiologic Studies Depression Scale in adolescents and young adults. *Journal of youth and adolescence* 20, 149-166.

- Roen, E.L., Wang, Y., Calafat, A.M., Wang, S., Margolis, A., Herbstman, J., Hoepner, L.A., Rauh, V., and Perera, F.P. (2015). Bisphenol A exposure and behavioral problems among inner city children at 7-9 years of age. *Environ Res* 142, 739-745.
- Silva, M.J., Samandar, E., Preau, J.L., Jr., Reidy, J.A., Needham, L.L., and Calafat, A.M. (2007). Quantification of 22 phthalate metabolites in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci* 860, 106-112.
- Spielberger, C.D., and Vagg, P.R. (1984). Psychometric properties of the STAI: a reply to Ramanaiah, Franzen, and Schill. *Journal of personality assessment* 48, 95-97.
- Tanner, E., Lee, A., and Colicino, E. (2020). Environmental mixtures and children's health: identifying appropriate statistical approaches. *Curr Opin Pediatr* 32, 315-320.
- Tien, J., Lewis, G.D., and Liu, J. (2020). Prenatal risk factors for internalizing and externalizing problems in childhood. *World J Pediatr* 16, 341-355.
- Uh, H.W., Hartgers, F.C., Yazdanbakhsh, M., and Houwing-Duistermaat, J.J. (2008). Evaluation of regression methods when immunological measurements are constrained by detection limits. *BMC Immunol* 9, 59.
- van Buuren, S., and Groothuis-Oudshoorn, K. (2011). mice: Multivariate Imputation by Chained Equations in R. *Journal of Statistical Software* 45, 1-67.
- van den Dries, M.A., Ferguson, K.K., Keil, A.P., Pronk, A., Spaan, S., Ghassabian, A., Santos, S., Jaddoe, V.W.V., Trasande, L., Tiemeier, H., *et al.* (2021). Prenatal Exposure to Nonpersistent Chemical Mixtures and Offspring IQ and Emotional and Behavioral Problems. *Environ Sci Technol* 55, 16502-16514.
- van der Waerden, J., Galera, C., Larroque, B., Saurel-Cubizolles, M.J., Sutter-Dallay, A.L., Melchior, M., and Group, E.M.-C.C.S. (2015). Maternal Depression Trajectories and Children's Behavior at Age 5 Years. *J Pediatr* 166, 1440-1448 e1441.
- Welch, B.M., Keil, A.P., Buckley, J.P., Calafat, A.M., Christenbury, K.E., Engel, S.M., O'Brien, K.M., Rosen, E.M., James-Todd, T., Zota, A.R., *et al.* (2022). Associations Between Prenatal Urinary Biomarkers of Phthalate Exposure and Preterm Birth: A Pooled Study of 16 US Cohorts. *JAMA Pediatr* 176, 895-905.
- Ye, X., Kuklennyik, Z., Needham, L.L., and Calafat, A.M. (2005). Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 383, 638-644.
- Yim, G., Minatoya, M., Kioumourtzoglou, M.A., Bellavia, A., Weisskopf, M., Ikeda-Araki, A., Miyashita, C., and Kishi, R. (2022). The associations of prenatal exposure to dioxins and polychlorinated biphenyls with neurodevelopment at 6 Months of age: Multi-pollutant approaches. *Environ Res* 209, 112757.
- Yuan, W.L., Nicklaus, S., Lioret, S., Lange, C., Forhan, A., Heude, B., Charles, M.A., and de Lauzon-Guillain, B. (2017). Early factors related to carbohydrate and fat intake at 8 and 12 months: results from the EDEN mother-child cohort. *Eur J Clin Nutr* 71, 219-226.

- Zengeler, K.E., and Lukens, J.R. (2021). Innate immunity at the crossroads of healthy brain maturation and neurodevelopmental disorders. *Nat Rev Immunol* 21, 454-468.
- Zhong, Q., Peng, M., He, J., Yang, W., and Huang, F. (2020). Association of prenatal exposure to phenols and parabens with birth size: A systematic review and meta-analysis. *Sci Total Environ* 703, 134720.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

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

The data will be made available upon reasonable request.

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