Prenatal exposure to a wide range of environmental chemicals and child behaviour between 3 and 7 years of age – An exposome-based approach in 5 European cohorts

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HIGHLIGHTS
• We studied simultaneous effect of prenatal exposure to several compounds on child behaviour.
• 47 exposure biomarkers from 8 chemical exposure families were studied in 5 European cohorts.
• Bisphenol A and mono-n-butyl phthalate were associated with increased behavioural problems in children.
• Copper was associated with decreased behavioural problems.

GRAPHICAL ABSTRACT

ABSTRACT

Background: Studies looking at associations between environmental chemicals and child behaviour usually consider only one exposure or family of exposures.

Objective: This study explores associations between prenatal exposure to a wide range of environmental chemicals and child behaviour.

Methods: We studied 708 mother-child pairs from five European cohorts recruited in 2003–2009. We assessed 47 exposure biomarkers from eight chemical exposure families in maternal blood or urine collected during pregnancy. We used the Strengths and Difficulties Questionnaire (SDQ) to evaluate child behaviour between three and seven years of age. We assessed associations of SDQ scores with exposures using an adjusted least absolute
1. Introduction

Child neurodevelopmental disorders are associated with long-term functional impairments which cause substantial social and financial costs for the affected individuals, their families and society as a whole. The annual cost (including medical and non-medical costs) of child neurodevelopment disorders in Europe has been estimated at €21 billion (Gustavsson et al., 2011). This makes the identification of modifiable risk factors for these disorders a priority target for public health. The root causes of most childhood neurodevelopmental disorders are multifactorial and only partly understood. In addition to genetic factors, exposure to environmental contaminants during periods of high sensitivity of the brain, such as pregnancy and early childhood, is suspected to play a role in the origin of neurodevelopmental disorders (Bellinger, 2009; Grandjean and Landrigan, 2014). In a review focusing on human studies, Grandjean and Landrigan identified 12 environmental chemicals or families of chemicals as neurodevelopmental toxicants (Grandjean and Landrigan, 2006, 2014), including several metals and inorganic compounds (lead, methylmercury, inorganic arsenic, manganese, fluoride), polychlorinated biphenyls (PCBs), some solvents (toluene, ethanol), certain pesticides [organophosphate (OP) pesticides] and polybrominated diphenyl ethers (PBDEs). The authors listed over 200 additional chemicals, including some phthalates, bisphenols, and cotinine, that are potentially neurotoxic in humans based on data from the US National Library of Medicine, the US Agency for Toxic as well as thallium and diethyl dithiophosphate due to their low detection using a quantile regression approach for the imputation of left-censored weight gain during pregnancy. Internalizing score worsen in association with exposure to diethyl thiophosphate (DETP, IRR: 1.11, 95%CI: 1.00;1.24) but the effect was driven by the smallest cohort. Internalizing score improved with increased concentration of perfluoroundecanoic acid (PFUnDA, IRR: 0.92, 95%CI: 0.85;1.00), however the association was driven by the two smallest cohorts with the lowest PFOS concentrations.

Discussion: This study added evidence on deleterious effects of prenatal exposure to BPA and MnBP on child behaviour. Other associations should be interpreted cautiously since they were not consistent with previous studies or they have not been studied extensively.

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2. Methods

2.1. Study design and population

This study is a part of the HELIX project which includes six European mother-child cohorts: Born in Bradford (Bib, UK), Etude des Déterminants Pré et Postnataux du Développement et de la Santé de l’Enfant (EDEN, France), Infancia y Medio Ambiente (INMA, Spain), Kaunas Cohort (KANC, Lithuania), Norwegian Mother, Father and Child Cohort Study (MoBa, Norway) and Mother-Child Cohort in Crete (RHEA, Greece). The study design is described in detail elsewhere (Maitre et al., 2018; Vrijheid et al., 2014). Out of the 1301 children originally included in the HELIX sub-cohort (Maitre et al., 2018; Vrijheid et al., 2014), we relied on a sub-sample of 708 mother-child pairs for which child behaviour was assessed using the Strengths and Difficulties Questionnaire (SDQ) at three to seven years of age (Appendix Fig. 1). Children from the MoBa cohort were not included because the SDQ was not implemented in this group.

2.2. Assessment of prenatal exposure to environmental chemicals

We assessed 54 biomarkers of exposure to a broad spectrum of environmental chemicals (Appendix Table 1, Appendix Table 2). Briefly, in blood we assessed biomarkers of exposure to eight organochlorine compounds (OCs), two PBDEs, five per- and polyfluoroalkyl substances (PFASs) and 15 metals and non-metals (essential and toxic elements). In urine, we assessed biomarkers of exposure to 10 phthalate metabolites, seven phenols, six OP pesticide metabolites, and cotinine. Out of those, we excluded five essential elements not considered to be neurotoxic as well as thallium and diethyl dithiophosphate due to their low frequency of detection (1.5% and 2.1%, respectively). This left 47 biomarkers for further analyses. Methods of biomarker assessment and descriptive statistics and correlation patterns between the biomarkers are described elsewhere (Haug et al., 2018; Tamayo-Uria et al., 2019).

2.3. Behavioural outcomes

We evaluated child behaviour using the SDQ (Goodman, 1997), which was completed by the mothers between three and seven years of child’s age. SDQ scores were collected as part of the individual cohort initiatives and harmonized and pooled a posteriori. In this analysis we relied on the combined externalizing and internalizing scores only, since they have been shown to be more consistent across informants (e.g., parents, teachers) and more discriminant with respect to clinical disorders in low-risk community samples, like the one examined in our study, compared to the five sub-scales (Goodman et al., 2010) (Appendix Table 3). Moreover, given our limited sample size and the large number of studied exposure biomarkers, combining the SDQ sub-scales limited the number of performed tests.

2.4. Statistical analysis

We singly imputed biomarker concentrations below the limit of detection using a quantile regression approach for the imputation of left-
censored missing data (Nadarajah and Kotz, 2006). We divided urinary biomarker concentrations by creatinine concentration. Haemal lipophilic biomarker concentrations were standardized and expressed in ng/g of total lipids in serum or plasma. Concentrations were then ln-transformed (cotinine) or log_{2}-transformed (all other biomarkers) to approach normality and standardized for the interquartile range (IQR) by dividing biomarker concentration observed for each individual for a given exposure by the IQR calculated for this exposure.

We selected the following adjustment factors based on a priori knowledge: cohort, season of conception, child’s sex and age at the SDQ assessment, parity, maternal age and education level, maternal working and active smoking status during pregnancy and maternal pre-pregnancy body mass index (see Appendix Table 4 for details). Missing data for exposure biomarker concentrations (see Appendix Table 5) and adjustment factors were multiply imputed (100 imputed datasets) via a chained equations algorithm (White et al., 2011). To explore the associations between 47 biomarkers and externalizing and internalizing behaviour scores we applied two statistical approaches. First, we used a least absolute shrinkage and selection operator (LASSO) algorithm with log link function. LASSO considers all exposures simultaneously (Tibshirani, 1996) and performs variable selection through estimates’ shrinkage (i.e., the lowest regression coefficients corresponding to the least informative predictors are assigned a zero value). We determined the range of penalty parameter $\lambda$ by maximizing the prediction log-likelihood using 10-fold cross-validation. To prevent overfitting, we defined the optimal $\lambda$ as the one providing the sparsest model (as measured by the number of nonzero regression coefficients) among those yielding a log-likelihood within one standard error of the maximum log-likelihood (Krstajic et al., 2014). To stabilise estimates, LASSO was fitted on each of the 100 imputed datasets and an exposure was retained only if it was selected in at least 50% of runs (Wood et al., 2008). Second, to compare with previous single-pollutant studies, we also performed an exposome-wide association study (ExWAS): we fit a negative binomial regression model on each of the 100 imputed datasets for each exposure biomarker and SDQ score, then aggregated the results using Rubin’s rule for multiple imputed data (Patel et al., 2010). To control for multiple comparisons, we applied a family-wise error rate (FWER) correction to the p value threshold. The correction uses a Bonferroni procedure extended to handle correlated tests: the actual number of exposures being tested ($M$) is replaced by a smaller value called the effective number of independent exposures ($M_e$). $M_e$ is estimated by $\sum_{i=1}^{M} I(\lambda_i > 1) (\lambda_i - 1)$, where $I(x)$ is an indicator function and $\lambda_i$ are the eigenvalues of the matrix of correlations between M exposures. The p value threshold to control FWER to $\alpha$, using $M_e$ in a Bonferroni procedure, is then $\alpha / M_e$ (adapted from Li et al., 2012).

To test the robustness of the associations between SDQ scores and exposure biomarkers identified by the LASSO (selected in at least 50% of runs) and ExWAS (those with uncorrected p values <0.05) we performed further sensitivity analyses. We evaluated the linearity of the associations using generalized additive model (GAM) with restricted cubic splines function. Then we ran a regression simultaneously adjusted for all biomarkers associated with the SDQ scores in the main ExWAS (p values <0.2). We additionally adjusted our main model for breastfeeding and fish and seafood consumption during pregnancy (since fish and seafood may accumulate persistent organic contaminants and heavy metals). We explored sex-specific effects by adding an interaction term between each biomarker of exposure and child sex and performed an ExWAS restricted to the participants with no missing biomarker concentrations. For the biomarkers associated with the SDQ externalizing score we ran an ExWAS after exclusion of the BiB cohort, as we had noted that children from this population had markedly lower externalizing score (median = 0.5) compared to the other cohorts (medians ≥5, Table 1). Apart from the mentioned analyses, for all measured exposure biomarkers we evaluated the between-cohort heterogeneity of the adjusted association using the $I^2$ statistic (Higgins and Thompson, 2002). We relied on the following threshold for the $I^2$ interpretation: $I^2 < 0.3$: low heterogeneity, $0.3 \leq I^2 < 0.6$: moderate heterogeneity, $I^2 \geq 0.6$: substantial to high heterogeneity (Deeks et al., 2019). Finally, because excessive maternal weight gain during pregnancy could lead to decreased blood concentrations of lipophilic compounds due to their storage in the adipose tissue (Kim et al., 2011; Lee et al., 2014; Verner et al., 2013) and to behavioural problems in the offspring (Pugh et al., 2016), we ran an additional analysis stratified on gestational weight gain for all the biomarkers from the OCs family.

All analyses were conducted using R v. 4.0.2 (R Core Team and R Foundation for Statistical Computing, 2020) and RStudio v. 1.3.1056 (RStudio Team, 2020) using packages: mice (van Buuren and Groothuis-Oudshoorn, 2011) for multiple imputation, mpath (Wang et al., 2015) to fit LASSO, MASS (Venables and Ripley, 2002) for the ExWAS analysis, metaplus (Beath, 2016) to estimate between-cohort heterogeneity and gam (Hastie, 2020) and rms (Harrell Jr, 2020) to evaluate linearity of associations between biomarkers of exposure and SDQ scores.

Data used in this study is confidential and can only be provided upon request and after approval of the HELIX consortium. The code is available in the public repository of the Team of Environmental Epidemiology applied to Reproduction and Respiratory Health (https://gricad-gdlab.univ-grenoble-alpes.fr/lab-env-epi).

3. Results

3.1. Characteristics of the study population and prenatal exposure to environmental contaminants

Characteristics of the study population and exposure biomarker distributions are detailed in Table 1 and Appendix Table 5, respectively. Median child age at the SDQ assessment was 5.6 years. Median SDQ externalizing and internalizing scores were 5 and 3 points, respectively. Heterogeneity was observed between cohorts for most covariates as well as for the SDQ scores, with parents from the BiB cohort reporting behaviour scores of their children to be better than of those from other cohorts (p value of the Kruskal-Wallis test <0.001, Table 1). High frequency of detection was observed for most of the 47 exposure biomarkers, with 39 detected in at least 89% of the tested samples (Appendix Table 5). Heterogeneity was observed between cohorts for most exposures (p values of the Kruskal-Wallis test <0.05, Appendix Table 5).

3.2. Association between prenatal chemical exposure and SDQ scores

3.2.1. Externalizing score

Among the 47 exposures studied, the adjusted LASSO for the externalizing score selected only copper (Cu). Cu was also detected in the ExWAS analysis as associated with lower externalizing score, meaning decreased risk of behavioural problems [Incidence rate ratio (IRR): 0.90, 95% confidence interval (CI): 0.82;0.98 for an IQR change in the transformed Cu concentration, Table 2]. In addition to Cu, the ExWAS identified five other associations. Bisphenol A (BPA, IRR: 1.06, 95%CI: 1.01;1.12) and mono-n-butyl phthalate (MnBP, IRR: 1.06, 95% CI: 1.00;1.13) were positively associated with the externalizing score, while perfluoroundecanoate (PFUnDA, IRR: 0.92, 95%CI: 0.84;0.99) and two OCs [dichlorophenyltrichloroethane (DDT, IRR: 0.92, 95%CI: 0.84;1.00) and PCB-138 (IRR: 0.88, 95%CI: 0.79;0.99)] were negatively associated with this score. While not significant (p values ranged between 0.065 for PCB-153 to 0.253 for PCB-180), all the other compounds from the OCs family tended to be negatively associated with the externalizing score (Appendix Table 6).

3.2.2. Internalizing score

The adjusted LASSO did not retain any exposure biomarker as being associated with the internalizing score, while the ExWAS identified a positive association with diethyl thiophosphate (DETP) concentration.
The total weight gain was recommended to be 12.5-18.0 kg for BMI = 18.5-24.9 kg/m², 11.5-16.0 kg for BMI = 25.0-29.9 kg/m², 7.0-11.5 kg for BMI = 30.0-34.9 kg/m², and 5.0-9.0 kg for BMI > 34.9 kg/m².

Analysis were linear (Appendix Fig. 2). Compared to the main ExWAS analyses, children in the main analyses were included, while in the sensitivity analyses, children from the TUC cohort were additionally included. All exposure-SDQ score associations detected in our main analysis were significant at the 0.05 level (IRR: 1.11, 95%CI: 1.00;1.24) and a negative association with perfluorooctane sulfonate concentration (PFOS, IRR: 0.92, 95%CI: 0.85;1.00, Table 2 and Appendix Table 7).

### Table 2
Population characteristics for the mother-child pairs included in the study: overall and by cohort.

| Characteristic                      | BiB 46 (6.5%) | EDEN 193 (27.3%) | INMA 218 (30.8%) | KANC 83 (11.7%) | RHEA 168 (23.7%) | p value of equality between cohorts<sup>a</sup> |
|-------------------------------------|---------------|------------------|------------------|-----------------|------------------|---------------------------------------------|
| Overall distribution                |               |                  |                  |                 |                  | <0.001                                      |
| Season of conception                | ...           | ...              | ...              | ...             | ...              | <0.001                                      |
| January-March                       | 208 (29.4%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| April-June                          | 159 (22.5%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| July-September                      | 174 (24.6%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| October-December                    | 164 (23.2%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Missing                              | 3 (0.4%)      | ...              | ...              | ...             | ...              | <0.001                                      |
| Active smoking during pregnancy     | ...           | ...              | ...              | ...             | ...              | <0.001                                      |
| No                                  | 553 (78.1%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Yes                                 | 145 (20.5%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Missing                              | 10 (1.4%)     | ...              | ...              | ...             | ...              | <0.001                                      |
| Parity                              | ...           | ...              | ...              | ...             | ...              | <0.001                                      |
| Nulliparous                         | 317 (44.8%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| 1 child                             | 268 (37.9%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| ≥ 2 children                        | 114 (16.1%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Maternal level of education         | 9 (1.3%)      | ...              | ...              | ...             | ...              | <0.001                                      |
| Primary school                      | 89 (12.6%)    | ...              | ...              | ...             | ...              | <0.001                                      |
| Secondary school                    | 292 (41.2%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| University degree or higher         | 317 (44.8%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Maternal age (years)                | 30.9 (5.5%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Maternal pre-pregnancy BMI<sup>b</sup> | ...         | ...              | ...              | ...             | ...              | <0.001                                      |
| Underweight                         | 28 (4.0%)     | ...              | ...              | ...             | ...              | <0.001                                      |
| Normal weight                       | 426 (60.2%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Overweight                          | 158 (22.3%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Obesity                             | 87 (12.3%)    | ...              | ...              | ...             | ...              | <0.001                                      |
| Maternal pre-pregnancy BMI<sup>b</sup> | ...         | ...              | ...              | ...             | ...              | <0.001                                      |
| Gestational weight gain on maternal | ...           | ...              | ...              | ...             | ...              | <0.001                                      |
| pre-pregnancy BMI<sup>b</sup>       | ...           | ...              | ...              | ...             | ...              | <0.001                                      |
| Insufficient                        | 186 (26.3%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Adequate                            | 199 (28.1%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Excessive                           | 263 (37.1%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Missing                              | 60 (8.5%)     | ...              | ...              | ...             | ...              | <0.001                                      |
| Child sex<sup>c</sup>               | ...           | ...              | ...              | ...             | 0.860            | <0.001                                      |
| Female                              | 313 (44.5%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Male                                | 395 (55.5%)   | ...              | ...              | ...             | ...              | <0.001                                      |
| Missing                              | 0 (0.0%)      | ...              | ...              | ...             | ...              | <0.001                                      |
| Child age at SDQ assessment (years)<sup>d</sup> | 5.6 [4.2;6.4] | ...              | ...              | ...             | <0.001                                      |
| Maternal age (years)<sup>e</sup>    | 30.9 [27.7;34.1] | ... | ... | ... | <0.001                                      |
| Missing                              | 4 [0.6]       | 0 (0.0%)         | 0 (0.0%)         | 0 (0.0%)        | <0.001                                      |
| SDQ externalizing score<sup>f</sup> | 5.0 [2.8;7.0] | 0.5 [0.0;3.8]    | 5.0 [2.0;7.0]    | 5.0 [3.0;8.0]   | 0.012                                      |
| Maternal age (years)<sup>e</sup>    | 30.9 [27.7;34.1] | ... | ... | ... | <0.001                                      |
| Missing                              | 0 (0.0%)      | 0 (0.0%)         | 0 (0.0%)         | 0 (0.0%)        | 0 (0.0%)         | <0.001                                      |
| SDQ internalizing score<sup>f</sup> | 3.0 [1.0;5.0] | 2.0 [0.0;4.0]    | 3.0 [1.0;5.0]    | 3.0 [1.0;4.0]   | 0.012                                      |

Distributions are reported as number and percentage for categorical variables and as median, 1st and 3rd quartiles for continuous variables. All values are before imputation.

<sup>a</sup> Kruskal-Wallis test was applied on continuous variables and χ² or exact Fisher test was applied on categorical variables.

<sup>b</sup> Gestational weight gain on maternal pre-pregnancy BMI was categorized into 3 categories: insufficient, adequate and excessive according to the recommendations of the US Institute of Medicine (Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy Weight Guidelines 2009). For BMI < 18.5 kg/m² recommended total weight gain was 12.5-18.0 kg, for BMI = 18.5-24.9 kg/m²: 11.5-16.0 kg, for BMI = 25.0-29.9 kg/m²: 7.0-11.5 kg and for BMI = 30.0-34.9 kg/m² recommended weight gain was 5.0-9.0 kg.

<sup>c</sup> Child sex and child age at the SDQ assessment and SDQ scores were not imputed. Abbreviations: BiB = Born in Bradford. EDEN = Étude des Déterminants Pré et Postnatals du Développement et de la Santé de l’Enfant. INMA = Infancia y Medio Ambiente. KANC = Kaunas Cohort. RHEA = Mother-Child Cohort in Crete. BMI = body mass index. SDQ = Strengths and Difficulties Questionnaire.

Close to the significance level (IRR: 1.11, 95%CI: 1.00;1.24) and a negative association with perfluorooctane sulfonate concentration (PFOS, IRR: 0.92, 95%CI: 0.85;1.00, Table 2 and Appendix Table 7).

### 3.2.3 Sensitivity analyses

After correction for multiple testing (corrected p value of 0.0017), none of the exposure-SDQ score associations passed the significance threshold. All exposure-SDQ score associations detected in our main analysis were linear (Appendix Fig. 2). Compared to the main ExWAS where each exposure biomarker was studied separately, adjustment for coexposures (i.e., exposures associated with the SDQ scores with a p value below 0.2) led to similar effect estimates except for PCB-138 (IRR: 0.95, 95%CI: 0.82;1.11) and PFOS (IRR: 0.94, 95%CI: 0.81;1.08) for which the negative association with SDQ scores was attenuated and the confidence intervals widened (Table 2). Effect estimates for analyses additionally adjusted for fish and seafood consumption (not shown) and breastfeeding (not shown) were similar to those observed in the main analysis. For the complete case analysis, while the effect...
the prenatal exposure to environmental contaminants and SDQ externalizing and internalizing scores (between the prenatal exposure to environmental contaminants and SDQ externalizing and internalizing scores (Adjusted associations) for complete case analysis, ExWAS after exclusion of the BiB cohort).

| Exposure | Model simultaneously adjusted for covariates | ExWAS for complete case analysis | ExWAS after exclusion of the BiB cohort |
|----------|---------------------------------------------|---------------------------------|--------------------------------------|
|          |                                             |                   |                                      |
| BPA (μg/g of creatinine) | 1.06 (1.01; 1.12) | 0.028 | 0.842 | 1.05 (0.99; 1.11) | 0.105 | 1.07 (1.01; 1.13) | 0.013 |
| Phenol  | 1.06 (1.01; 1.12) | 0.026 | 662 |
| Organochlorine compound | 0.92 (0.85; 1.00) | 0.037 | 1 |
| Phthalate | 1.06 (1.00; 1.13) | 0.048 | 1 |
| PCB-118 | 0.92 (0.84; 0.99) | 0.034 | 1 |
| PCB-138 | 0.94 (0.87; 1.02) | 0.164 | 0.381 |
| PFUnDA | 0.93 (0.86; 1.01) | 0.091 | 646 |
| PrPA | 1.06 (1.00; 1.13) | 0.041 | 662 |
| MnBP | 1.06 (1.01; 1.12) | 0.026 | 662 |
| BUPA | 1.06 (1.00; 1.13) | 0.058 | 1 |
| DDE | 0.92 (0.84; 1.00) | 0.045 | 1 |
| DDT | 1.11 (1.00; 1.24) | 0.034 | 1 |
| DETP | 0.91 (0.83; 1.00) | 0.081 | 1 |
| HCB | 0.92 (0.84; 0.99) | 0.032 | 1 |
| PCB-153 | 1.11 (0.99; 1.23) | 0.060 | 0.212 | 0.93 (0.86; 1.01) | 0.091 |
| DETP | 0.91 (0.83; 1.00) | 0.081 | 1 |
| PERPA | 1.06 (1.00; 1.13) | 0.051 | 1 |
| PFOS | 1.06 (1.01; 1.12) | 0.028 | 0.842 | 1.05 (0.99; 1.11) | 0.105 | 1.07 (1.01; 1.13) | 0.013 |

4. Discussion

Among the 47 exposures tested, only seven were associated (uncorrected p values <0.05) with either externalizing or internalizing SDQ score in children between three and seven years of age. Association with one additional exposure biomarker was close to significance (uncorrected p value = 0.053). Cautious interpretation of the results is required since none passed the significance threshold after the FWER correction of the p values obtained in the ExWAS. For this reason, in the discussion we focused on the associations that were detected by both the LASSO and the ExWAS or that were consistent with previous human literature. The other associations should be treated as hypothesis generating.

Cu was detected by LASSO and by ExWAS as negatively associated with SDQ externalizing score, suggesting lower risk of behavioural problems. Cu is essential for many biological processes, including brain development during the foetal period (Scheiber et al., 2014), and an excess or insufficiency of Cu may lead to health problems (Gaetke et al., 2014). Our finding of a negative association between Cu and SDQ score (suggesting decreased risk of behavioural problems) needs to be replicated as, to our knowledge, the only study that assessed prenatal Cu and externalizing behaviour relied on the older children of the HELIX cohort (6–11 years) and did not report any effect (IRR: 1.00, 95%CI: 0.91;1.09, [Maire et al. submitted to journal]). Cu concentrations in our study population (geometric mean = 1440 μg/L of blood, 95%CI = 1410;1471) were slightly higher than those reported among non-pregnant females in the most recent US NHANES study (geometric mean = 1270 μg/L, 95%CI = 1240;1300, Centers for Disease Control and Prevention, 2019). However, this may be due to the fact that serum Cu concentrations tend to increase during pregnancy (Vukelic et al., 2012).

Prenatal BPA urinary concentration was associated with higher (worse) scores on the externalizing behaviour sub-scale. Such association has also been suggested by another study assessing behaviour at older age on a similar population (IRR: 1.07, 95%CI: 0.99;1.16, Maire et al. submitted to journal), suggesting that the association we observed between three and seven years of age might persist when the children get older. Previous studies coherently reported positive associations between prenatal BPA and externalizing behaviour scores (Braun et al., 2009, 2017b; Evans et al., 2014; Li et al., 2020; Perera et al., 2012; Philippat et al., 2017; Roen et al., 2015; Stacy et al., 2017) or the hyperactivity-inattention score (Casas et al., 2015), an item included
in our externalizing SDQ score sub-scale. All the mentioned studies, except for those relying on the HOME mother-child cohort (Braun et al., 2009, 2017b; Stacy et al., 2017), reported these associations among boys, while we did not observe a sex-specific effect. Previous studies also reported higher scores on the internalizing behaviour sub-scale in association with the prenatal exposure to bisphenol A (Braun et al., 2011, 2017a; Evans et al., 2014; Grohs et al., 2019; Harley et al., 2013; Li et al., 2020; Perera et al., 2012, 2016; Philippat et al., 2017; Roen et al., 2015). While not significant (p value = 0.21), effect estimate for our study population also suggested a positive association between BPA and internalizing SDQ score (IRR: 1.04, 95%CI: 0.98;1.12). The animal research literature is also consistent here: numerous studies in

| Substance                          | I² | IRR  | 95%CI          |
|-----------------------------------|----|------|----------------|
| Bisphenol A (BPA)                 | 0.348 | 1.05 [0.73, 1.37] |
| Copper (Cu)                       | < 0.001 | 0.63 [0.19, 1.06] |
| Dichlorodiphenyltrichloroethane (DDT) | 0.417 | 0.73 [0.51, 0.95] |
| Mono-n-butyl phthalate (MnBP)     | < 0.001 | 0.88 [0.42, 1.34] |
| Polychlorinated biphenyl-138 (PCB-138) | < 0.001 | 0.60 [0.12, 1.07] |
| Perfluoroundecanoate (PFUnDA)     | < 0.001 | 1.04 [0.62, 1.76] |
| Diethyl thiophosphate (DETP)      | 0.612 | 2.04 [1.61, 2.46] |
| Perfluorooctane sulfonate (PFOS)  | 0.569 | 0.68 [0.40, 0.97] |

Fig. 1. Sensitivity analysis (n = 708, BiB n = 46; EDEN n = 193; INMA n = 218; KANC n = 83; RHEA n = 168). Cohort-specific associations between prenatal exposures and SDQ externalizing (A) and internalizing (B) scores detected by the ExWAS (p value of association <0.05 except of diethyl thiophosphate for which the p value = 0.053). Regression models were adjusted for cohort, season of conception, child sex and age at SDQ assessment, parity, maternal: education level, work status, age, pre-pregnancy BMI and prenatal active smoking status. The “All cohorts” estimates are those obtained in the main ExWAS. IRRs are reported with 95%CIs and correspond to the change in the probability of the SDQ scores increasing by one unit for an IQR change in the log2 of the biomarker concentration in maternal blood or urine. We relied on the following threshold for I² interpretation: I² < 0.3 low heterogeneity, 0.3 ≤ I² < 0.6 moderate heterogeneity, I² ≥ 0.6 substantial to high heterogeneity. The black squares display the IRRs (size of the square reflects the relative size of each cohort) and the horizontal lines their 95%CIs. Abbreviations: BiB = Born in Bradford. EDEN = Étude des Déterminants Pré et Postnatals du Développement et de la Santé de l’Enfant. INMA = Infancia y Medio Ambiente. KANC = Kaunas Cohort. RHEA = Mother-Child Cohort in Crete. CI = confidence interval of the IRR estimate. ExWAS = exposome-wide association study. IQR = inter-quartile range. IRR = incidence rate ratio. BMI = body mass index. SDQ = Strengths and Difficulties Questionnaire.
rodents have reported a link between exposure to BPA and behaviour (Anderson et al., 2013; Ishido et al., 2011; Komada et al., 2014; Nakagami et al., 2009; Palanza et al., 2008; Rochester et al., 2018; Tian et al., 2010). Moreover, in vitro and in vivo studies provide evidence that BPA can affect biological pathways crucial for normal brain development by binding oestrogen receptors or interacting with the thyroid hormone and hypothalamic-pituitary-adrenal axis (Mustieles et al., 2015; Mustieles and Fernández, 2020; Nesan et al., 2018).

Maternal urinary MnBP concentration was associated with worse externalizing behaviour score. MnBP is a metabolite of dibutyl phthalate (DBP), a compound that exerts anti-androgenic activity (National Academies of Sciences, Engineering, and Medicine, 2017). Two previous human studies of prenatal MnBP concentration and child behaviour reported an association with externalizing behaviour among boys (Engel et al., 2010; Lien et al., 2015) and one reported an association with internalizing behaviour among older children (Palanza et al., 2011; Forns et al., 2015). The negative association between MnBP and PCBs on child behaviour (Forns et al., 2016; Rosenquist et al., 2017) and the associations we observed between OCs and SDQ scores should be interpreted with caution.

We found a negative association between two PFASs (PFOS and PFUnDA) and child behaviour. The association with PFOS was only observed in the two cohorts with the smallest sample size (BiB and KANC with n = 46 and 83, respectively) and the lowest median PFOS concentrations. The association with PFOS was not expected as previous human studies reported either increased behavioural problems linked to this exposure (Høyer et al., 2015) or no association at all (Fei and Olsen, 2011; Forns et al., 2015). The negative association between PFUnDA and child behaviour was also observed among older children of the HELIX cohorts (IRR: 0.89, 95%CI: 0.80:0.98, Maidet al. submitted to journal) and needs further investigation.

5. Strengths and limitations

Our study is among the first to simultaneously consider a large number of exposures (n = 47) from multiple families in relation to externalizing and internalizing behaviour scores in children. Its strengths include the longitudinal design, which allows prospectively assessing exposure during pregnancy (a critical period for brain development), and the use of a standardized tool (SDQ) to evaluate child behaviour. We relied on two complementary statistical approaches: ExWAS produces effect estimates that are comparable to previous studies and can be used in meta-analyses, while LASSO considers all exposures simultaneously, performs variable selection, and is on average less likely to generate false positives (spurious associations) than ExWAS (Barrera-Gómez et al., 2017). Moreover, we investigated potential confounding: the associations for BPA, Cu, DDT, DETP, MnBP and PFUnDA remained after adjusting for other exposures. Finally, relying on five cohorts with differing confounding structure (e.g., women from the BiB cohort had overall lower education levels) might improve causal inference: an association seen in multiple heterogeneous cohorts is less likely to result from residual confounding than an association seen in only one or a few homogeneous cohorts (Richmond et al., 2014). On the other hand, since the cohorts were recruited before the start of the HELIX project, collection of biological samples during pregnancy was not harmonized leading to different timings (i.e., different trimester) for exposure assessment across cohorts. Additionally, for some cohorts the same exposure biomarker was sometimes assessed by different laboratories (see Appendix Table 2), which may partly explain the between-cohort heterogeneity.
of the results observed for some exposures. This should not have a strong impact on our results since the interlaboratory comparisons performed in the framework of the HELIX protocol suggested a high correlation between assessments performed in different laboratories. For instance, correlation coefficients between phenol urinary concentrations measured by the Norwegian Institute of Public Health and Centers for Disease Control and Prevention in 12 maternal samples of the EDEN cohort were ≥ 0.90 (Supplementary of Tamayo-Uria et al., 2019). Due to limited availability of biological samples, some biomarkers of exposure were not assessed in all cohorts (e.g., metals and semi-metals were not assessed in INMA, see Appendix Table 5 for details). We used multiple imputation on the missing values as it has been shown to generate less bias than exclusion of a variable or a stratum (e.g., exclusion of an entire cohort for which an exposure biomarker concentration was fully missing, Held et al., 2016; Jolani et al., 2015). Nevertheless, for the exposures with many missing values (metals, PBDEs) multiple imputation may have widened the confidence intervals of our effect estimates, limiting the ability to detect associations. Moreover, we relied on spot urine samples to assess exposure to compounds whose urinary concentration has moderate to high intra-individual variability during pregnancy (reviewed by Casas et al., 2018). This can potentially lead to exposure misclassification, attenuation bias and power reduction (Perrier et al., 2016). It has been shown that the measurement error and resulting attenuation of the effect estimates varies across exposures (exposures with the most intra-individual variability have the highest errors, Perrier et al., 2016). Therefore, we must be cautious when comparing exposure-SDQ associations across exposures with differing intra-individual variability. We decided not to assess all possible second-order interactions between exposures because, given our limited sample size and large number of exposures, it could have substantially decreased the power and increased the false positive rate (Barrera-Gómez et al., 2017). Finally, we focused only on prenatal exposure and did not assess exposure in early postnatal life, a period also recognized as crucial for brain development.

6. Conclusion

In line with previous epidemiological studies, our results suggested a deleterious association between prenatal exposure to bisphenol A, MnBP (a metabolite of DBP) and child behaviour. According to the toxicological literature, the association observed for BPA is biologically plausible. DETP was also associated with worse behavioural scores, however this result should be interpreted with caution since it was driven by the smallest cohort. Cu, DDT, PCB-138, PFOS and PFUnDA were associated with lower risk of behavioural problems. These associations were not reported previously and for lipophilic compounds (DDT and PCB-138) could even result from changes in body composition during pregnancy.

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CRediT authorship contribution statement

Jedynak had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Vrijheid. Supervision of all aspects of study design and data collection: Vrijheid. Cohort data collection: Gütskow, Guxens, Casas, McEachan, Gražulevičienė, Chatzi, Kampouri, Vafeiadi, Vrijheid, Slama, Mon-Williams, Wright. Acquisition, analysis, or interpretation of data: Jedynak, Philippat, Julvez, López-Vicente, Tamayo, Sunyer, Casas, Thomsen, Vrijheid, Maire. Drafting of the manuscript: Jedynak, Philippat. Statistical analysis: Jedynak, Basagaña, Philippat. Obtained funding: Thomsen, Slama, Chatzi, Wright, Gražulevičienė, Vrijheid.

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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.

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Appendix A. Supplementary data

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