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Prenatal exposure to persistent organic pollutants and emotional and behavioral outcomes from early childhood to adolescence

Rhea Cohort Study in Crete, Greece

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Background: Persistent organic pollutants (POPs) are widespread, hazardous chemicals, but their impact on emotional and behavioral development is not well understood. This study aimed to investigate whether prenatal exposure to POPs influences internalizing, externalizing, and attention deficit hyperactivity disorder (ADHD) symptoms from early childhood to adolescence.

Methods: We utilized longitudinally collected data from 467 mother–child pairs in the Rhea study. Concentrations of hexachlorobenzene, dichlorodiphenyldichloroethylene, and six polychlorinated biphenyl congeners (PCBs) were determined in maternal serum samples collected during early pregnancy. Mothers reported their children's internalizing, externalizing, and ADHD symptoms at ages 4 (Strengths and Difficulties Questionnaire, ADHD Test), 6, 11, and 15 years (Child Behavior Checklist, Conners' Parent Rating Scale). The associations between prenatal pollutant exposure and longitudinally studied outcomes were assessed using generalized estimating equation models.

Results: In utero exposure to hexachlorobenzene and dichlorodiphenyldichloroethylene was not associated with emotional or behavioral outcomes. Prenatal exposure to PCBs was associated with decreased internalizing symptoms from childhood through adolescence and reduced ADHD symptoms at age 4 (adjusted β [95% confidence interval]: –0.17 [–0.29, –0.05], and –0.16 [–0.30, –0.02], per doubling of exposure, respectively). Sensitivity analyses confirmed these findings, though the association between PCB exposure and internalizing symptoms was not observed in women with sufficient gestational weight gain.

Conclusions: Our findings suggest that prenatal POP exposure does not adversely affect emotional and behavioral development from preschool age through adolescence. Further research is warranted to elucidate the potential impact of gestational POP exposure on developmental trajectories.

Keywords: persistent organic pollutants; prenatal exposure; children; adolescents; internalizing symptoms; externalizing symptoms; attention deficit hyperactivity disorder; longitudinal assessment

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Introduction

Recent epidemiological data indicate that mental disorders affect a significant number of children and adolescents[1,2] and high prevalence rates are reported especially for internalizing and externalizing disorders.[3] Mental disorders during childhood and adolescence are linked to poor academic achievement,[4,5] an increased risk for future unemployment,[6] and mental health issues in adult life,[7,8] highlighting the need for

What this study adds

Several studies have explored the potential impact of prenatal exposure to persistent organic pollutants (POPs) on internalizing and externalizing problems, yielding inconclusive results. Notably, existing research has primarily focused on child outcomes at single time points, rather than examining them longitudinally. Addressing this gap, our study investigated the influence of prenatal POP exposure on emotional and behavioral development over an extended period, assessing outcomes at four different time points from early childhood to adolescence. Our results did not indicate any adverse effects, as null associations were observed for hexachlorobenzene and dichlorodiphenyldichloroethylene, while PCB exposure was associated with fewer internalizing symptoms.

further research into their potential causes. The Developmental Origins of Health and Disease hypothesis posits that the factors influencing an individual's susceptibility to physical and psychological disorders can be traced back to intrauterine life.[9] The fetal period is a critical developmental window, during which environmental insults can have programming and lasting effects on the growing biological systems,[10] particularly the highly plastic and complex nervous system, potentially impacting neurodevelopment.[11,12]

Persistent organic pollutants (POPs) are a group of hazardous chemicals prevalent in the environment. The term "POPs" encompasses substances used in industrial processes and applications, such as polychlorinated biphenyls (PCBs), and pesticides utilized in agriculture, including hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDT), and its metabolite dichlorodiphenyldichloroethylene (DDE). Although the Stockholm Convention has banned or restricted the use of POPs,[13] human exposure remains a concern[14,15] due to their high resistance to degradation and their tendency to bioaccumulate in fatty tissue, which leads to higher concentrations at higher levels of the food chain.[16] Consequently, humans are exposed to POPs primarily through diet, especially by consuming food with high-fat content, such as fatty fish, meat, eggs, and dairy products.[16–18] Importantly, these toxic chemicals can be transferred from the mother to the placenta and ultimately to the developing fetus.[19]

Inconclusive evidence exists on the effect of POP exposure on emotional and behavioral development. Various studies have demonstrated that early-life exposure to POPs is linked to elevated behavioral symptoms in toddlerhood,[20,21] conduct problems,[22] emotional[23] and total difficulties[24] during childhood, and increased anxiety in emerging adulthood.[25] However, several studies have not identified any effect of prenatal POP exposure on internalizing and externalizing symptoms in childhood[26–28] and depression up to early adulthood.[29] Importantly, inverse associations between chemical exposure and symptomatology have been also observed. For instance, Oulhote et al[30] reported that maternal PCB levels were linked to fewer child difficulties at 7 years of age. Additionally, an exposome-based study, including a subsample of the Rhea cohort, found that exposure to organochlorine compounds was associated with decreased externalizing problems in children aged 3 to 7 years, but this was mainly observed among women with insufficient gestational weight gain (GWG).[31] Another study that followed participants until adolescence showed that early-life exposure to HCB and DDE seemed to have a protective effect against anxiety.[32]

Similar to internalizing and externalizing problems, heterogeneous findings have been reported regarding prenatal exposure to POPs and attention deficit hyperactivity disorder (ADHD) symptoms. Some evidence suggests that POP exposure is positively associated with ADHD symptomatology, particularly for HCB[33] and PCB exposure.[34–36] Notably, children from a Canadian mother–child study who were prenatally exposed to higher concentrations of POPs were found to have elevated ADHD symptoms and altered neural activity in brain areas implicated in inhibitory control, an executive function important in ADHD.[37] Neugebauer et al[38] linked higher maternal PCB levels with increased attention deficits, but also observed associations with fewer parent-reported ADHD behaviors in school-aged children. In contrast, null associations have been previously reported in the Rhea cohort between prenatal POP levels and ADHD symptoms in preschool-aged children.[28] Additionally, a pooled analysis of seven European birth cohorts found no evidence of an association between POP exposure and ADHD diagnosis in children aged 3 to 10 years,[39] with similar findings also observed in studies conducted at older ages.[29,40]

Various POPs are recognized as neurotoxic to humans, with some also causing developmental neurotoxicity.[11] These chemicals can interfere with brain development, potentially leading to neurobehavioral and emotional problems. While the exact molecular mechanisms are not fully understood, several potential mechanisms have been proposed, including disruption of thyroid hormone signaling, oxidative stress, impaired dopamine neurotransmission, and alterations in calcium signaling.[41–43] These biological mechanisms highlight the complex ways in which POP exposure may be associated with adverse emotional and behavioral outcomes.

The significant heterogeneity in research findings regarding the effects of prenatal POP exposure on emotional and behavioral development prompts the need for further investigation. Taking into account that symptoms manifest within a developmental context, studies that evaluate neurodevelopmental outcomes longitudinally, rather than at single time points, can provide more comprehensive insights.[44] To address this, we aimed to investigate whether gestational exposure to POPs (i.e., HCB, DDE, and PCBs) influences the developmental trajectories of emotional and behavioral symptoms from early childhood to adolescence (ages 4, 6, 11, and 15 years), utilizing data from the Rhea mother–child cohort in Crete, Greece.

Methods

Study population

Participants in the present study were part of the Rhea cohort, a longitudinal mother–child study based in Crete, Greece. Pregnant women over 16 years old, residing in the prefecture of Heraklion, Crete, with a good understanding of the Greek language, were invited to take part in the study from February 2007 to February 2008. Initially, 1610 pregnant women enrolled in the study at the time of the first major ultrasound examination, and 1363 singleton pregnancies were followed up until delivery. A comprehensive cohort profile is detailed by Chatzi et al.[45] Assessments were carried out twice during pregnancy, at birth admission, and subsequently at several developmental stages: infancy (18 months), early childhood (4 years), middle childhood (6 years), preadolescence (11 years), and adolescence (15 years). In brief, evaluations included biological sample collection, medical records, neurodevelopmental assessments, and questionnaire administration. The present study was conducted according to the principles of the Helsinki Declaration. The Rhea Study received approval from the Ethics Committee of the University Hospital of Heraklion (reference number: 96/6 February 2007), and the most recent follow-up (IntExt Trajectories project) was approved by the Ethics Committee of the University of Crete (reference number: 43/16 March 2022). Written informed consent was obtained from all parents and from the children themselves at age 15 before each assessment.

To examine the developmental trajectories of internalizing, externalizing, and ADHD symptoms, we used data from four follow-up assessments conducted at ages 4, 6, 11, and 15 years. A total number of 997 children were assessed with respect to

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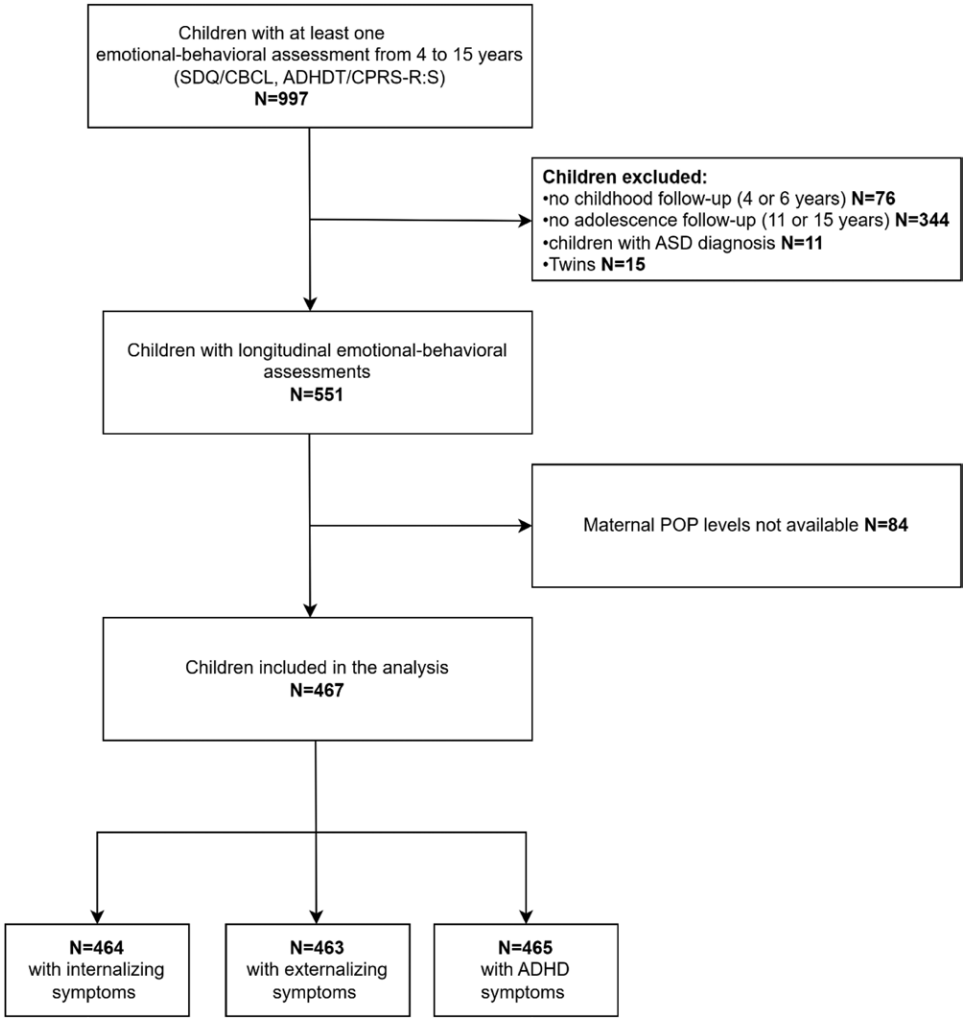

Figure 1. Flowchart of the study population. ASD indicates autism spectrum disorders.

emotional–behavioral development at least once from 4 to 15 years. To ensure that both childhood and adolescence were represented, we set the criterion that participants should have data from at least two assessments: one conducted during childhood (at ages 4 or 6 years) and another during adolescence (at ages 11 or 15 years). Twins (n = 15) and children with an autism spectrum disorder diagnosis (n = 11) were excluded from analyses. For 84 children, the maternal POP levels were not available. Thus, the final sample consisted of 467 children (Figure 1). The total number of observations was 1,524. Data from two time points were available for 89 participants (19.1%), data from three time points were available for 166 children (35.6%), and all four time points were available for 212 children (45.4%). Compared with nonparticipating mothers, mothers in our sample were more likely to have higher educational levels, household disposable income, and higher prepregnancy body mass index (BMI). Participating children had slightly higher birthweight and were more likely to have attended nursery before the age of 2 years (Table S1; http://links.lww.com/EE/A336).

Measures

Biological sample collection and exposure assessment

During the third and fourth months of pregnancy, maternal serum samples were collected in 10 ml silicone gel separator vacutainer tubes (Becton Dickinson, Cowley, Oxford, United Kingdom). Within 2 hours of blood collection, the tubes were centrifuged at 2500 rpm for 10 minutes, and the resulting serum aliquots were stored at −80 °C until analysis. The analyses of POPs were conducted at the Finnish Institute for Health and Welfare, Chemicals Risks Team, Kuopio, Finland, using an Agilent 7000B gas chromatograph triple quadrupole mass spectrometer (GC–MS/MS). The pretreatment of serum samples for GC–MS/MS analysis has been previously described by Koponen et al.[46] This process determined the serum concentrations of HCB, DDE, and six individual PCB congeners (IUPAC numbers: 118, 138, 153, 156, 170, and 180). All concentrations were reported on wet-weight and expressed in pg/ml serum. Samples below the limit of quantification (LOQ) were assigned a value of LOQ/2 (LOQ = 6 pg/ml for PCB118 and PCB156; LOQ = 10 pg/ml for HCB, DDE, PCB138, PCB153, PCB170, and PCB180). As quality control, two blank samples were analyzed in each batch of samples (n = 34). The average mass of blanks in each batch was subtracted from the mass of studied serum samples in the same batch. Also, two samples of Standard Reference Material SRM 1589a from National Institute of Standards and Technology (NIST, Gaithersburg, MD) that have certified concentrations for organochlorine pesticides and PCBs were analyzed in each sample batch. Average concentrations of POPs measured from SRM 1589a varied from 94% to 102% of certified concentrations and coefficient variation was from 2.7% to 7.4% depending on the compound, respectively. Detectability levels were high (97.4%–100%) for all chemicals, except for PCB156, where 57.2% of the samples showed levels above the LOQ. We opted to utilize wet-weight POP levels

and adjust for maternal serum cholesterol and triglycerides as continuous variables in all multivariable models to minimize potential biases associated with automatic lipid adjustment.[47] The total PCB concentration was calculated by summing the concentrations of the six individual PCB congeners and was used in all analyses. Maternal POP concentrations were treated as continuous variables on a $\log_2$ scale.

Outcome assessment

Children's internalizing and externalizing symptoms were assessed based on maternal reports, using the Strengths and Difficulties Questionnaire (SDQ)[48] at age 4 and the Child Behavior Checklist (CBCL)[49] at ages 6, 11, and 15 years. The SDQ is a 25-item psychometric tool designed to evaluate emotional and behavioral strengths and difficulties, adapted to the Greek population.[50] The CBCL/6–18, part of the Achenbach System of Empirically Based Assessment, is a widely used parent-report questionnaire consisting of 113 items evaluating adaptive and maladaptive functioning in children aged 6 to 18 years. It has been adapted to the Greek population.[51] In the present analyses, two broad-band scales were used from both instruments: (1) internalizing problems and (2) externalizing problems, with higher scores indicating greater difficulties.

Children's ADHD symptomatology was evaluated through maternal reports using the ADHD test (ADHDT)[52] at age 4 and the Conners' Parent Rating Scale-Revised: Short Form (CPRS-R:S)[53] at ages 6, 11, and 15 years. The ADHDT is a psychometric tool comprising 36 items designed to evaluate ADHD-related symptoms, and it has been adapted to the Greek population.[54] The total score, used in the present analyses, represents an index for total ADHD difficulties. The CPRS-R:S is a 27-item tool for assessing ADHD symptoms. The Rhea cohort team conducted the translation and cross-cultural adaptation of the CPRS-R:S, following the recommended methodology.[55] The total ADHD symptoms index was utilized in the current analyses. For both indices, higher scores reflect a greater severity and frequency of ADHD symptoms.

Statistical analysis

Descriptive statistics of the basic characteristics, the exposures, and the outcomes of the study population are presented in terms of means and standard deviations (SD) for continuous variables and frequencies with percentages for categorical variables. Pearson correlation coefficients were calculated to examine the correlation among chemicals.

To allow for some missing items, the scores were prorated (if missing items were less than 25%) in all analyzed scales. Although different instruments were used at the 4-year follow-up, both SDQ and CBCL assess internalizing and externalizing symptoms and both ADHDT and CPRS-R:S capture the core symptoms of ADHD (i.e., inattention and hyperactivity). Thus, similar constructs are evaluated, ensuring comparability. However, since the instruments differ in their scoring scales, we also performed statistical harmonization. To harmonize internalizing, externalizing, and ADHD scores obtained from different instruments, we calculated z scores (mean = 0, SD = 1) of all prorated scales at each timepoint[56] and the z scores were used in all further analyses.

Due to the relatively high percentage of missing covariate data (≈80% of the participants had complete data) and to increase sample size and reduce bias, we imputed missing covariate data using multiple imputations with chained equations and generated 20 imputed datasets.[57,58] Exposure and outcome data were not imputed. The imputation model included exposures, outcomes, and covariates under study, as well as additional auxiliary variables.[58] Distributions in imputed datasets were similar to those observed (Table S2; http://links.lww.com/EE/A336). Effect estimates from the imputed data sets were combined with the use of Rubin's rules.[57]

Generalized estimating equation (GEE) models with an unstructured correlation matrix were applied to estimate β coefficients and 95% confidence intervals (CIs) for the associations of prenatal exposure to the studied chemicals with internalizing, externalizing and ADHD symptoms z scores across 4 to 15 years of age. Potential confounders included maternal and child characteristics with an established or potential association with environmental exposures and children's internalizing, externalizing, or ADHD symptoms. Further, we used the approach of directed acyclic graphs for confounder selection (Figure S1; http://links.lww.com/EE/A336). Child sex, child age at assessment, and maternal serum lipids were a priori included in all models. Thus, for each exposure–outcome association we constructed three models: (1) basic model: adjusted for child sex (male, female), exact age (years) at the time of outcome assessment, follow-up (categorical with four levels for 4, 6, 11, and 15 years), and maternal total lipids (cholesterol and triglycerides); (2) covariate-adjusted model: additionally adjusted for maternal age at delivery (years), maternal education (low level: ≤9 years of mandatory schooling, medium level: >9 years of mandatory schooling up to attending post-secondary school education, high level: attending university or having a university/technical college degree), parity (nulliparous, multiparous), log-equivalized disposable household income in tertiles,[59] prepregnancy BMI (kg/m^2), and smoking during the first trimester of pregnancy (never, ever); (3) coexposure-adjusted model: same as the covariate-adjusted model and including all the studied exposures in the same model. To assess potential multicollinearity among POPs in this model, the variance inflation factor was calculated. Values of variance inflation factor were below 2.5, indicating that multicollinearity was not a concern.

To test potential age-varying effects, we constructed models that included an interaction term between the exposure variable and the follow-up (4, 6, 11, and 15 years). From these models, we derived effect estimates for each time point as well as a P value for the interaction with follow-up. Additionally, we examined potential effect modifications by sex, as sex differences are observed in emotional and behavioral problems.[60–62] We introduced a multiplicative interaction term between each exposure and child sex in the models, calculating the respective effect estimates for each sex and a P value for the interaction. We also investigated the interaction between exposure and GWG, based on evidence suggesting that weight changes may alter chemical concentrations between the bloodstream and adipose tissue[63–65] and on previous indications from Jedynak et al[31] for a moderating role of GWG. Total GWG was examined as categorical variable (insufficient, sufficient, and excessive), defined according to the Institute of Medicine guidelines of 2009 based on prepregnancy BMI (for prepregnancy BMI < 18.5 kg/m^2 recommended total weight gain was 12.5–18.0 kg, for BMI = 18.5–24.9 kg/m^2: 11.5–16.0 kg, for BMI = 25.0–29.9 kg/m^2: 7.0–11.5 kg and for BMI ≥ 30.0 kg/m^2: 5.0–9.0 kg).[66] An effect estimate for each GWG category was obtained from these models, along with a P value for the interaction between exposure and GWG.

To assess the robustness of our findings we repeated analyses: (1) including only those participants with no missing covariate data (complete case analysis, n = 365), (2) excluding preterm-born children (<37 gestational weeks, n = 59), (3) excluding children with a diagnosis of learning disability or ADHD (n = 42), and (4) excluding data from the 4-year follow-up assessment and analyzing only participants with at least two available outcome assessments at ages 6, 11, and 15 years (n = 400).

All hypothesis testing was performed assuming a 0.05 significance level and a two-sided alternative hypothesis. All statistical analyses were conducted using Stata software, version 13.0 (Stata Corp, College Station, TX).

Table 1.

Characteristics of the study population, Rhea mother–child cohort, Crete, Greece (n = 467)

	n (%)/mean (SD)
Maternal characteristics	
Age at delivery (years)	30.1 (4.7)
Missing	2 (0.4)
Ethnicity	
Non-Greek	19 (4.1)
Greek	447 (95.7)
Missing	1 (0.2)
Education	
Low	49 (10.5)
Medium	236 (50.5)
High	181 (38.8)
Missing	1 (0.2)
Smoking (12th week)	
No	388 (83.1)
Yes	71 (15.2)
Missing	8 (1.7)
Parity	
Nulliparous	208 (44.5)
Multiparous	257 (55.0)
Missing	2 (0.4)
BMI prepregnancy (kg/m^2)	25.0 (5.1)
Missing	10 (2.1)
Total cholesterol (mg/dl)	210.9 (43.0)
Missing	60 (12.8)
Triglycerides (mg/dl)	129.5 (54.5)
Missing	60 (12.8)
Gestational weight gain	
Sufficient	336 (28.7)
Insufficient	244 (20.8)
Excessive	475 (40.5)
Missing	117 (10.0)
Household income (€, median [25th–75th percentile])	1047.2 (817.6–1335.3)
First tertile (<892€)	137 (29.3)
Second tertile (892€–1223€)	137 (29.3)
Third tertile (>1223€)	138 (29.6)
Missing	55 (11.8)
Neonatal characteristics	
Sex	
Male	250 (53.5)
Female	217 (46.5)
Delivery type	
Vaginal	236 (50.5)
C-section	231 (49.5)
Birthweight (g)	3198.9 (441.4)
Gestational age (weeks)	38.2 (1.6)
Missing	2 (0.4)
Breastfeeding duration (months)	4.2 (4.1)
Missing	16 (3.4)
Child characteristics	
Nursery before 2 years	
No	357 (76.4)
Yes	109 (23.3)
Missing	1 (0.2)
Exact age at follow-up	
4 years	4.2 (0.2)
6 years	6.6 (0.3)
11 years	11.0 (0.3)
15 years	14.9 (0.4)

Results

Description of the study population

The characteristics of the participants are presented in Table 1. The mean (±SD) age of the participating mothers was 30.1 years (±4.7) and the vast majority were Greek (95.7%). Most mothers had acquired medium (50.5%) or high educational levels (38.8%) and did not smoke during pregnancy (83.1%). There was a slightly higher representation from multiparous (55.0%) relative to nulliparous (44.5%) mothers. Regarding neonatal characteristics, 53.5% of the participating children were male, while 46.5% were female. Half of the children (50.5%) were vaginally delivered. The mean birthweight was 3198.9 g (±441.4) and the mean gestational age at delivery was 38.2 weeks (±1.6). The mean duration of breastfeeding was found 4.2 months (±4.1). Finally, the mean age of the participating children at each of the four follow-up assessments was 4.2, 6.6, 11.0, and 14.9 years, respectively.

Table 2 presents maternal concentrations of POPs. Among all POPs, the highest concentration was found for DDE, and among the examined PCBs, the highest concentration was found for PCB153. POPs were significantly correlated to each other (*P* values < 0.001) (Figure 2). Pearson correlation coefficients among individual PCBs ranged from 0.60 to 0.96. We observed lower correlations between HCB and DDE (coefficient = 0.49), between HCB and PCBs (coefficients ranged from 0.48 to 0.60), and between DDE and PCBs (coefficients ranged from 0.37 to 0.57). The mean scores of the outcomes at each follow-up assessment are reported in Table 3. Males had higher scores on internalizing symptom scales at ages 4 and 6, while females exhibited more internalizing problems at the age of 15 years. In contrast, males were reported to have greater externalizing difficulties at ages 4, 6, and 11 and more ADHD-related symptoms at each follow-up assessment.

Prenatal exposure to persistent organic pollutants and internalizing, externalizing, and attention deficit hyperactivity disorder symptoms

The associations between prenatal exposure to POPs and internalizing, externalizing, and ADHD symptoms from 4 to 15 years of age estimated by GEE models are presented in Table 4.

In the basic model, HCB and DDE were associated with fewer externalizing problems and ΣPCB_6 was associated with fewer internalizing, externalizing, and ADHD symptoms from 4 to 15 years of age. However, after adjusting for covariates, only the association between ΣPCB_6 and internalizing symptoms remained significant. Each doubling of maternal serum ΣPCB_6 was associated with a decrease of 0.17 (95% CI: −0.29, −0.05, *P* value = 0.005) units of internalizing symptom *z* scores. The association of ΣPCB_6 with externalizing and ADHD symptoms was in the same direction albeit not significant (β [95% CI]: −0.11 [−0.23, 0.02], *P* value = 0.090 and −0.05 [−0.17, 0.07], *P* value = 0.395, respectively). In the coexposure-adjusted models, simultaneously including all three exposures, the association between ΣPCB_6 and internalizing symptoms was similar (β [95% CI]: −0.18 [−0.31, −0.05], *P* value = 0.006). When we examined individual PCB congeners, we observed that all six PCBs were similarly associated with internalizing symptoms, whereas only PCB118 was associated with externalizing symptoms (Table S3; http://links.lww.com/EE/A336).

Assessing effect modification by age

The age-specific associations derived from GEE models with an exposure–follow-up interaction term are presented in Figure 3 and Table S4; http://links.lww.com/EE/A336. We observed that the associations with internalizing and externalizing symptoms did not vary over time (all *P*-interaction with follow-up > 0.05). However, this analysis revealed a significant association between ΣPCB_6 and ADHD symptoms only at the age of 4 years (β [95% CI]: −0.16 [−0.30, −0.02], *P*-interaction with follow-up = 0.018).

Assessing effect modification by sex

In sex-specific analysis (Table S5; http://links.lww.com/EE/A336), a significant interaction between DDE exposure and sex was detected for externalizing symptoms (*P*-interaction

Table 2.

Distribution of maternal POP levels (pg/ml, n = 467), Rhea mother–child cohort, Crete, Greece

Chemical	GM (95% CI)	Mean	SD	Min	Percentiles			Max	LOQ	%>LOQ
					25th	50th	75th			
HCB	92.7 (88.2, 97.4)	111.1	99.7	28.2	64.9	85.9	123.3	1330.5	10	100.0
DDE	2080.0 (1930.0, 2242.0)	2941.0	2902.0	182.0	1236.0	2044.0	3558.0	23175.0	10	100.0
PCB118	18.2 (17.4, 19.2)	20.9	11.4	3.0	13.3	18.6	25.9	105.2	6	98.1
PCB138	70.5 (67.1, 74.2)	81.8	47.2	11.3	48.8	71.3	104.4	411.3	10	100.0
PCB153	130.7 (124.3, 137.5)	152.4	95.5	22.9	91.4	129.9	192.9	853.9	10	100.0
PCB156	6.3 (5.9, 6.7)	8.3	7.2	3.0	3.0	6.9	11.2	81.5	6	57.2
PCB170	35.5 (33.4, 37.7)	44.5	37.3	5.0	23.7	36.2	55.0	386.0	10	97.4
PCB180	71.1 (67.1, 75.3)	87.7	73.0	5.0	48.0	70.7	106.8	755.4	10	99.8
ΣPCB_6[a]	337.0 (320.3, 354.6)	395.7	259.0	53.7	237.4	336.9	500.8	2397.4		

[a]ΣPCB_6: sum of PCBs 118, 138, 153, 156, 170, 180.
GM indicates geometric mean.

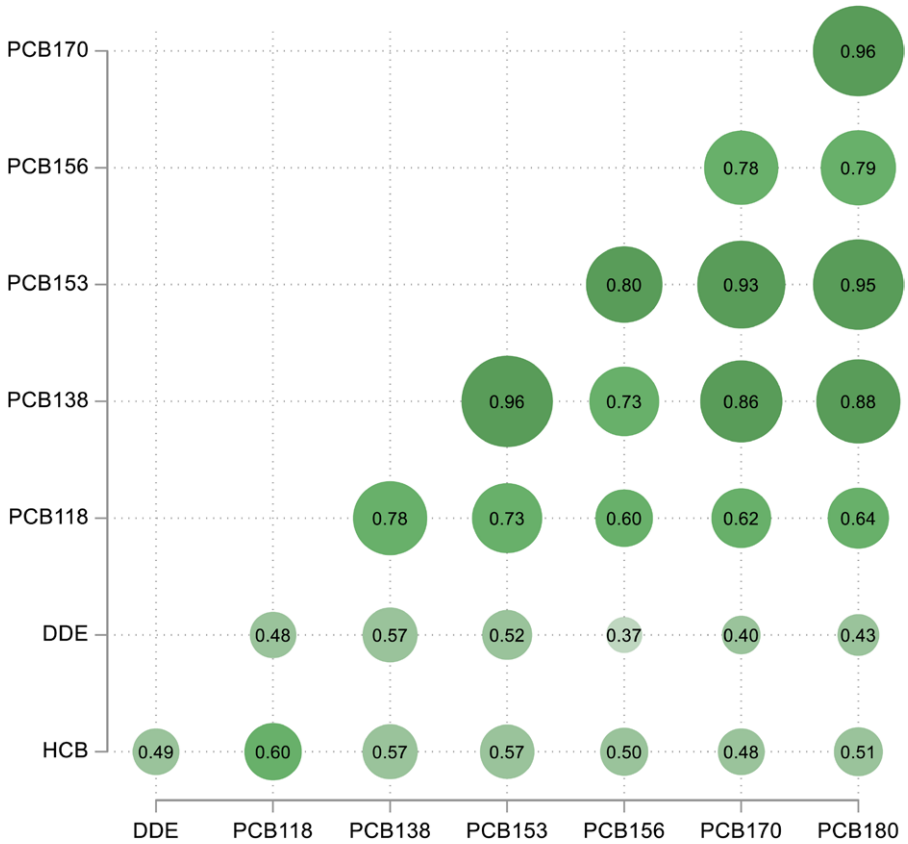

Figure 2. Plot of Pearson correlation coefficients among POPs (n = 467). POP levels are $\log_2$ transformed. Color and size of each circle identify the magnitude of the correlation. All shown coefficients are statistically significant (all *P* values <0.001).

with sex = 0.046). Although effect estimates were in the opposite direction in boys and girls, none of them reached statistical significance (β [95% CI]: −0.07 [−0.16, 0.03] for boys and 0.06 [−0.03, 0.15] for girls). In addition, the effect estimates for the association between ΣPCB_6 and externalizing symptoms were more pronounced and significant only in boys (β [95% CI]: −0.19 [−0.34, −0.03] compared with −0.05 [−0.22, 0.13] in girls, *P*-interaction with sex = 0.156). The association between ΣPCB_6 and internalizing symptoms was similar between sexes (β [95% CI]: −0.19 [−0.34, −0.05] compared with −0.18 [−0.34, −0.01] in girls, *P*-interaction with sex = 0.850).

Assessing effect modification by gestational weight gain

In GEE models including interaction terms with GWG (Table 5), no significant interactions were found (all *P*-interaction with GWG > 0.05). However, we observed that the negative association between prenatal exposure to ΣPCB_6 and internalizing symptoms ceased to be statistically significant for children whose mothers had gained sufficient weight during pregnancy, but remained significant in the cases of insufficient and excessive weight gain (β [95% CI]: −0.28 [−0.51, −0.06] and −0.31 [−0.48, −0.14], respectively, *P*-interaction with GWG = 0.069).

Sensitivity analysis

Sensitivity analyses restricted to participants with no missing covariate data (complete case analysis, n = 365) yielded comparable results for the association between ΣPCB_6 and internalizing symptoms, with a slightly magnified effect estimate, while also indicating an association between ΣPCB_6 and fewer externalizing symptoms. Excluding preterm-born children (n = 59)

Table 3.

Outcome distribution (raw scores; n = 467), Rhea mother–child cohort, Crete, Greece

	Overall		Males		Females		
	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	*P* value
Internalizing symptoms							
SDQ—4 years	436	3.2 (2.5)	228	3.4 (2.6)	208	2.9 (2.3)	0.030
CBCL—6 years	389	5.9 (4.3)	212	6.3 (4.6)	177	5.4 (3.8)	0.029
CBCL—11 years	289	7.0 (5.6)	160	7.4 (5.8)	129	6.5 (5.3)	0.173
CBCL—15 years	404	6.8 (5.7)	210	5.9 (5.2)	194	7.7 (6.1)	0.002
Externalizing symptoms							
SDQ—4 years	435	5.3 (3.2)	228	5.8 (3.4)	207	4.8 (2.8)	0.001
CBCL—6 years	391	8.5 (6.3)	213	9.7 (6.7)	178	7.0 (5.5)	<0.001
CBCL—11 years	289	7.1 (6.3)	160	8.0 (7.2)	129	5.9 (4.8)	0.007
CBCL—15 years	403	6.5 (6.2)	210	6.4 (6.2)	193	6.6 (6.3)	0.777
ADHD symptoms							
ADHDT—4 years	434	14.8 (12.3)	227	16.9 (13.5)	207	12.6 (10.3)	<0.001
CPRS-R:S—6 years	386	8.7 (5.4)	211	9.6 (5.7)	175	7.7 (4.8)	<0.001
CPRS-R:S—11 years	291	8.3 (5.6)	161	9.2 (6.2)	130	7.2 (4.8)	0.003
CPRS-R:S—15 years	404	7.9 (5.6)	210	8.7 (5.8)	194	7.0 (5.3)	0.003

Bold font indicates $P < 0.05$.

Table 4.

Associations of in utero exposure to POPs with child internalizing, externalizing, and ADHD symptoms from childhood to adolescence (n = 467), Rhea mother–child cohort, Crete, Greece

			Basic model[a]		Covariate-adjusted model[b]		Coexposure-adjusted model[c]	
Outcome	Exposure	n	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
Internalizing symptoms	HCB	464	−0.09 (−0.18, 0.00)	0.052	−0.04 (−0.15, 0.07)	0.438	0.00 (−0.11, 0.12)	0.958
z score	DDE	464	−0.05 (−0.11, 0.00)	0.070	−0.02 (−0.08, 0.05)	0.605	0.01 (−0.05, 0.08)	0.720
	ΣPCB_6	464	−0.19 (−0.28, −0.10)	<0.001	−0.17 (−0.29, −0.05)	0.005	−0.18 (−0.31, −0.05)	0.006
Externalizing symptoms	HCB	463	−0.11 (−0.21, −0.02)	0.021	−0.01 (−0.12, 0.10)	0.818	0.02 (−0.10, 0.14)	0.759
z score	DDE	463	−0.08 (−0.14, −0.02)	0.014	−0.01 (−0.08, 0.05)	0.705	0.00 (−0.07, 0.07)	0.934
	ΣPCB_6	463	−0.21 (−0.30, −0.12)	<0.001	−0.11 (−0.23, 0.02)	0.090	−0.11 (−0.25, 0.02)	0.091
ADHD symptoms	HCB	465	−0.03 (−0.12, 0.07)	0.569	0.07 (−0.04, 0.18)	0.202	0.10 (−0.02, 0.22)	0.095
z score	DDE	465	−0.05 (−0.11, 0.01)	0.091	−0.01 (−0.07, 0.06)	0.864	−0.01 (−0.08, 0.06)	0.758
	ΣPCB_6	465	−0.13 (−0.22, −0.04)	0.006	−0.05 (−0.17, 0.07)	0.395	−0.08 (−0.21, 0.05)	0.219

Internalizing, externalizing, and ADHD symptoms are expressed as *z* scores. All exposures are $\log_2$ transformed. Values were derived from GEE analyses with child measures at 4, 6, 11, and 15 years. Bold font indicates $P < 0.05$.

[a]Basic model: adjusted for child sex, exact age at assessment, follow-up (categorical with four levels for 4, 6, 11, and 15 years), and maternal lipids (total cholesterol and triglycerides).

[b]Covariate adjusted model: The basic model additionally adjusted for maternal age at delivery, maternal education, parity, household income, prepregnancy BMI, and smoking during pregnancy.

[c]Coexposure adjusted model: Covariate-adjusted model additionally adjusted for exposure to DDE and ΣPCB_6 (models for HCB), to HCB and ΣPCB_6 (models for DDE), or to HCB and DDE (models for ΣPCB_6).

and those diagnosed with learning disabilities or ADHD (n = 42) did not meaningfully alter the results in terms of magnitude and direction. Finally, to assess the potential impact of data harmonization, we repeated the analysis including only participants with at least two follow-up assessments at ages 6, 11, and 15 years (n = 400), since outcome assessment at age 4 was conducted using different instruments. The results were consistent with our findings (Table S6; http://links.lww.com/EE/A336).

Discussion

In this study, we aimed to investigate the effect of prenatal exposure to POPs on the longitudinal course of internalizing, externalizing, and ADHD symptoms from childhood through adolescence. According to our findings, higher gestational exposure to PCBs was associated with decreased internalizing symptoms across ages, and with decreased ADHD symptoms only at 4 years of age. Prenatal HCB and DDE exposure was not associated with emotional and behavioral development.

Prior studies have supported that early-life exposure to PCBs is linked to increased behavioral or emotional difficulties,[21–23] while other studies have not detected any associations.[24,26–29] In contrast, our findings showed associations between maternal PCB levels and fewer emotional symptoms in children. Additionally, PCB exposure was linked to reduced externalizing symptoms in the minimally adjusted model, though this association remained negative but became nonsignificant after adjusting for all covariates. Similarly, Oulhote et al[30] reported that maternal levels of PCBs were associated with decreased SDQ scores at age 7, although they suggested that this finding might be affected by other factors not accounted for in their analyses, like omega-3 polyunsaturated fatty acids. Moreover, an exposome-based analysis across five European cohorts, including the Rhea cohort, demonstrated that organochlorine compounds (i.e., DDT and PCB138) were inversely associated with externalizing problems, while this was mainly observed among women with insufficient GWG.[31] Notably, in our models assessing interaction, the association between higher PCB levels and lower emotional symptoms was primarily observed among women with insufficient and excessive weight gain, while this association ceased to be statistically significant among women with adequate weight gain during pregnancy. Insufficient weight gain may increase pollutant transfer both in utero and through breastfeeding,[63,65] while excessive weight gain leads to greater POP storage in adipose tissue, lowering blood concentrations.[64] However, stored pollutants may be released into the bloodstream after childbirth due to rapid weight loss, increasing exposure of the infant through breastfeeding. These physiological changes

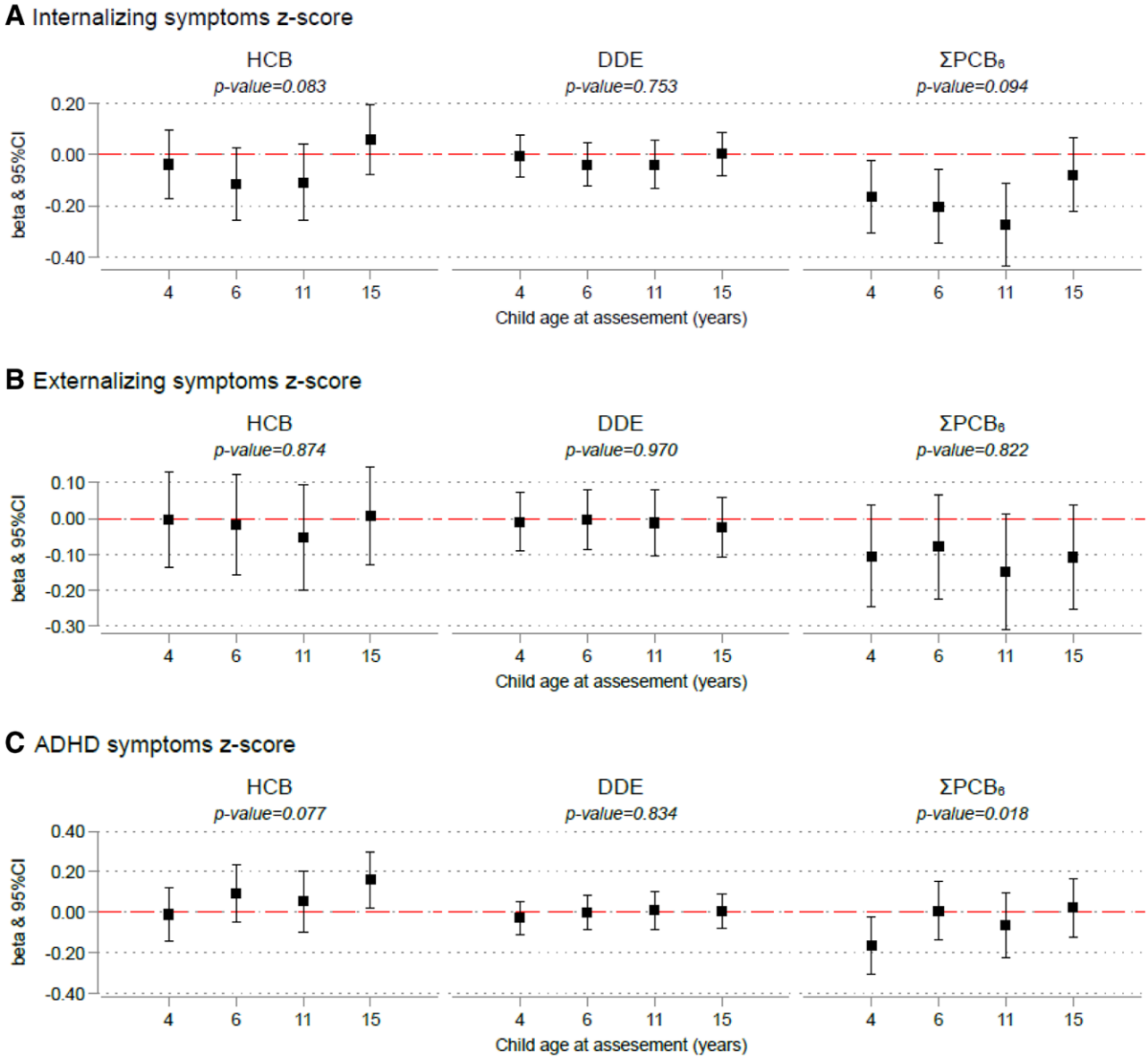

Figure 3. Associations of in utero exposure to POPs with child internalizing, externalizing, and ADHD symptoms at 4, 6, 11, and 15 years (n = 467) estimated by GEE analyses, Rhea mother–child cohort, Crete, Greece. Internalizing, externalizing, and ADHD symptoms are expressed as *z* scores. All exposures are $\log_2$ transformed. All models are adjusted for child sex, exact age at assessment, follow-up (categorical with four levels for 4, 6, 11, and 15 years), maternal lipids (total cholesterol and triglycerides), maternal age at delivery, maternal education, parity, household income, prepregnancy BMI, and smoking during pregnancy and include a multiplicative interaction term between exposure and follow-up (4, 6, 11, and 15 years). Shown *P* values correspond to the exposure–follow-up interaction term.

during and after pregnancy likely modulate exposure levels, potentially affecting the magnitude and direction of PCB effects. Given this, a nutritional factor associated with weight gain may have confounded our results. Thus, the findings regarding PCB exposure should be interpreted with caution and further research is needed to clarify the mechanisms.

Regarding ADHD-related symptoms, we observed an inverse longitudinal association with prenatal exposure to PCBs; however, this association disappeared after adjusting for confounding variables and the effect was evident only at age 4. While several studies have suggested a link between in utero exposure to PCBs and ADHD symptomatology,[34–37,67] null associations have also been reported in the literature.[28,29,38–40,68] Notably, there is considerable variation in outcome assessment across studies, both in terms of the neuropsychological instruments used and whether the evaluation is based on parent or teacher reports or a clinical diagnosis. Therefore, this variation limits comparability between findings. In addition, most findings supporting adverse effects of PCB exposure on ADHD-related outcomes come from studies conducted in mothers living near PCB-contaminated areas, such as New Bedford[35,36] and Lake Michigan[34] with higher levels of exposure. Given that ADHD is a neurodevelopmental disorder with diverse symptoms and a multifactorial etiology, evidence suggests that PCB exposure may disproportionately impair specific functions, such as response inhibition,[37,67,69] which our study, focusing on an overall ADHD index, might have failed to capture.

Animal studies have shown that exposure to PCBs can elicit endocrine-disrupting effects,[70] alterations in dopamine and glutamate neurotransmission,[42,70] calcium imbalance and changes in dendritic growth,[43] and oxidative stress and apoptosis.[71] Regarding behavioral effects, Elnar et al[72] showed that PCB exposure in mice can have anxiogenic effects, possibly through the disruption of calcium signaling, whereas they did not find

Table 5.

Associations of in utero exposure to POPs with child internalizing, externalizing, and ADHD symptoms from childhood to adolescence, estimated by GEE analyses, according to GWG, Rhea mother–child cohort, Crete, Greece

			Insufficient GWG (n = 77, 19.9%)		Sufficient GWG (n = 133, 34.5%)		Excessive GWG (n = 176, 45.6%)		
Outcome	**Exposure**	**n**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	**β (95% CI)**	***P* value**	***P*-interaction**
Internalizing	HCB	384	−0.05 (−0.31, 0.22)	0.734	−0.07 (−0.24, 0.10)	0.428	−0.12 (−0.27, 0.03)	0.119	0.829
symptoms	DDE	384	−0.03 (−0.17, 0.11)	0.640	−0.01 (−0.11, 0.10)	0.917	−0.06 (−0.17, 0.04)	0.226	0.725
z score	ΣPCB_6	384	−0.28 (−0.51, −0.06)	0.015	−0.07 (−0.26, 0.11)	0.447	−0.31 (−0.48, −0.14)	<0.001	0.069
Externalizing	HCB	383	−0.05 (−0.31, 0.21)	0.728	−0.04 (−0.21, 0.13)	0.683	−0.08 (−0.23, 0.07)	0.280	0.900
symptoms	DDE	383	−0.04 (−0.18, 0.10)	0.601	−0.06 (−0.17, 0.05)	0.278	0.01 (−0.10, 0.11)	0.896	0.649
z score	ΣPCB_6	383	−0.19 (−0.42, 0.04)	0.098	−0.14 (−0.33, 0.05)	0.139	−0.18 (−0.35, −0.01)	0.038	0.912
ADHD	HCB	384	−0.10 (−0.36, 0.16)	0.454	0.07 (−0.10, 0.24)	0.420	0.01 (−0.14, 0.16)	0.885	0.511
symptoms	DDE	384	−0.11 (−0.25, 0.03)	0.117	−0.07 (−0.18, 0.04)	0.192	−0.00 (−0.11, 0.10)	0.931	0.406
z score	ΣPCB_6	384	−0.23 (−0.46, −0.00)	0.048	−0.07 (−0.26, 0.12)	0.460	−0.04 (−0.21, 0.14)	0.687	0.298

Internalizing, externalizing, and ADHD problems are expressed as *z* scores. All exposures are $\log_2$ transformed. Models are adjusted for child sex, exact age at assessment, follow-up (categorical with four levels for 4, 6, 11, and 15 years), maternal lipids (total cholesterol & triglycerides), maternal age at delivery, maternal education, parity, household income, prepregnancy BMI, and smoking during pregnancy and include a multiplicative interaction term between exposure and GWG (insufficient/sufficient/excessive). The three categories of GWG were defined according to the Institute of Medicine (IOM) guidelines of 2009 based on prepregnancy BMI (for prepregnancy BMI < 18.5 kg/m^2 recommended total weight gain was 12.5–18.0 kg, for BMI = 18.5–24.9 kg/m^2: 11.5–16.0 kg, for BMI = 25.0–29.9 kg/m^2: 7.0–11.5 kg, and for BMI ≥ 30.0 kg/m^2: 5.0–9.0 kg). The *P*-interaction values correspond to the exposure-GWG interaction term. Bold font indicates $P < 0.05$.

evidence of depressive-like behaviors. In contrast, another animal study demonstrated that PCB exposure can impair *N*-methyl-D-aspartate receptor binding in various brain regions and indicated that it may have anxiolytic effects.[73] Importantly, an animal study supported that the effect of PCB153 exposure depends on both the animal strain—suggesting an important interaction with the genetic background—and the exposure levels, with low doses decreasing ADHD-like behaviors and higher doses increasing such behaviors.[74] It should be noted that PCB levels found in mothers of the Rhea cohort are considerably lower compared with other European birth cohorts, reflecting regional differences in environmental pollution and historical use of PCBs.[75,76] Consequently, we cannot rule out the possibility that the relatively narrow range of concentrations examined might have limited our ability to fully capture the effects of PCBs on neurodevelopment. Furthermore, there is evidence in the literature supporting nonmonotonic relationships between endocrine-disrupting chemicals, such as PCBs, and health outcomes, suggesting that these chemicals may produce varying effects at different doses.[77] The complex biological mechanisms underlying the dose-specific effects of chemicals remain unclear,[41] highlighting the need for further research on the effects of PCB exposure.

Regarding HCB and DDE, we did not find associations with internalizing, externalizing, and ADHD symptoms. Findings in the literature remain inconsistent, with studies reporting negative,[22,24,25,33] positive,[32] or null associations.[28,29,39,40] The heterogeneity found in the literature may arise from variations in the type of examined samples (e.g., maternal serum, cord blood, and breast milk), the timing of sample collection (different gestational periods, at childbirth), the analytical techniques employed, and the outcome evaluation. Importantly, we assessed the trajectory of children's symptoms from age 4 to 15 years, whereas all the aforementioned studies investigated the effects of chemical exposure at specific, discrete stages of development. Therefore, direct comparisons between findings should be made with caution. It is, also, important to note that the concentrations of DDE and HCB in our study are considerably higher than those reported in pregnant women in Athens, Greece.[15] Compared with international levels, DDE exposure is significantly higher in the Rhea cohort, while HCB levels are comparable or lower than those observed in cohorts from other countries.[18,76,78] Despite the exposure levels, our results did not show any association between prenatal exposure to HCB and DDE and emotional or behavioral development.

Despite the known neurotoxic potential of POPs,[11] we did not observe adverse effects of prenatal exposure to these chemicals on developmental trajectories. Our results were null for HCB and DDE, while inverse associations were observed for PCB exposure. We adjusted our models for several confounding variables, such as maternal age and education, both associated with increased serum concentrations of POPs,[79] and more favorable child outcomes.[80,81] However, residual confounding may explain the unexpected associations of PCB exposure with fewer internalizing symptoms. Breastfeeding could also have influenced the observed association, as it is a primary route of exposure to POPs after birth, while also being associated with beneficial effects on child development.[82] In addition, a study, following participants until emerging adulthood, stressed the moderating role of nonchemical risk factors, indicating that the effect of organochlorine compounds on internalizing symptoms varied based on the quality of the home environment during adolescence.[25] Given that our study extended into adolescence, a complex and turbulent developmental stage, family, and parenting factors might have further confounded our findings. Future longitudinal studies should rigorously account for contextual factors throughout the development to better clarify these relationships.

Strengths and limitations

Among the strengths of this study is the prospective design, which enabled us to comprehensively investigate the potential effects of chemical exposures during the critical fetal period on developmental trajectories from early childhood through adolescence, thereby addressing important developmental stages for the manifestation of internalizing and externalizing symptoms. Exposures were evaluated using biomarkers and the procedures for collecting biological samples and measuring concentration levels were performed using state-of-the-art laboratory techniques, ensuring the reliability of the data. Additionally, the assessment of children's emotional and behavioral development was conducted using reliable and valid psychometric tools, and our sample came from a well-established birth cohort, the Rhea mother–child cohort in Crete.

However, our study is characterized by several limitations. The study used a subsample of the population with available data, which may have led to selection bias. However, no major differences were found between participants and nonparticipants, and especially POP exposure was not found to predict inclusion in the final analytic sample. We used the same questionnaires

to assess neurodevelopmental outcomes, apart from the age of 4 years. The use of different instruments to assess outcomes at different ages could lead to inconsistencies in measurement, which may result in misclassification and potentially inaccurate estimates of associations. Although there is evidence supporting the correlation between these questionnaires[83] and we performed data harmonization using established techniques, we cannot exclude the possibility that data harmonization might have affected our results, which should be interpreted with caution. Furthermore, the outcome assessment relied exclusively on maternal reports, which might have distorted our findings. Although the overall sample size of the study was sufficient, we acknowledge that the statistical power may not be adequate to detect small or nuanced effects, particularly those related to sex- and time-varying factors. Finally, we adjusted our models for a series of confounding variables that were associated with exposure and outcome. However, residual confounding could still arise from unmeasured variables.

Conclusions

Our findings showed no associations of HCB and DDE exposure with the developmental trajectories of internalizing and externalizing symptoms. Surprisingly, however, in utero exposure to PCBs was associated with reduced emotional difficulties throughout development. To advance our understanding of the long-term neurodevelopmental effects of POPs, it is crucial to conduct further research on the impact of prenatal pollutant exposure on symptom trajectories. Future studies should prioritize unraveling the complex biological pathways and mechanisms underlying POP exposure. Such efforts are essential for developing effective strategies to prevent the exposure of pregnant women, and consequently their unborn children, to hazardous chemicals during the critical period of fetal development.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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