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Sex specific associations between in utero exposure to persistent organic pollutants and allergy-related outcomes in childhood: The Rhea Mother–Child Cohort (Crete, Greece)

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Original Article

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Abstract

Accumulating evidence suggests that in utero exposures can influence the development of the immune system. Few studies have investigated whether prenatal exposure to persistent organic pollutants (POPs) is associated with allergy-related phenotypes in childhood, nor explored sex differences. We examined the association between prenatal exposure to POPs and offspring allergic outcomes in early and mid-childhood. We included 682 mother–child pairs from the prospective birth cohort Rhea. We measured dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB) and 6 polychlorinated biphenyl (PCB) congeners in maternal first trimester serum. Parents completed the questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC) for allergy-related phenotypes when their children were 4 and 6 years old. We used Poisson regression models to estimate Risk Ratios. Prenatal HCB was associated with increased risk for rhinoconjunctivitis at 6 years (RR (95% CI): 2.5; (1.3, 4.8) for a doubling in the exposure). Among girls, prenatal DDE was associated with increased risk for current wheeze, current asthma and current rhinoconjunctivitis at 4 years (RR (95%CI): 1.4 (0.8, 2.6), 1.6 (1.1, 2.4) and 1.8 (1.0, 3.3) and p-interaction = 0.035, 0.027 and 0.059, respectively), with increased risk for current rhinoconjunctivitis at 6 years (RR (95%CI): 1.7 (0.7, 3.8) and p-interaction = 0.028) and total PCBs were associated with increased risk for current eczema at 4 years (RR (95%CI): 2.1 (1.1, 4.2) and p-interaction = 0.028). In boys, prenatal DDE was associated with decreased risk for current wheeze and current asthma at 4 years. Our findings suggest that even low levels of exposure to POPs prenatally may affect the development of childhood allergy-related outcomes in a sex and age-specific manner.

Introduction

Persistent organic pollutants (POPs) are a class of anthropogenic carbon-based organic chemicals that were widely used as pesticides [e.g., dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyl dichloroethene (DDE), hexachlorobenzene (HCB)] and in industrial processes [polychlorinated biphenyls (PCBs)],[1] through most of the 20th century until they were banned (PCBs, HCB) or tightly restricted (DDT).[2] These chemicals constitute a major health concern because they have long half-lives, they exhibit resistance to metabolic degradation, they accumulate in fatty tissues, and they exert toxic effects on humans and the environment.[1,3–5] POPs enter the human body via inhalation or dermal contact but the main route of exposure is through ingestion of contaminated food, especially meat, dairy, fruits, and vegetables.[1,6] Exposure to POPs can take place as early as in utero, since these pollutants can be transferred to the embryo and the fetus through the placenta.[4] Because of their overall estrogenic, anti-estrogenic, and anti-androgenic effects, POPs have the potential to disrupt the endocrine system in the human body.[7–9] Health hazards and adverse health effects of exposure to POPs are established by many studies[10–12] and their effects have been suggested to differ by sex.[13]

The prevalence of allergy-related diseases, such as asthma, rhinitis and eczema, increased substantially in the second half of the twentieth century and have become a global concern in many developed and developing countries.[14] These diseases are the most common noncommunicable diseases among children worldwide,[15] often begin in early childhood and generally persist into adulthood with high individual and socio-economic burdens.[16] Risk factors for asthma and other allergic diseases are suggested to be genetic, lifestyle and/or environmental.[17–20]

Prenatal POPs exposure has been suggested to exert immunotoxic effects since they have been associated with markers of immune function, such as white blood cell counts and numbers of lymphocyte subsets[21] as well as inflammatory markers.[22] However, the evidence regarding potential associations between in utero exposure to POPs with asthma and allergic diseases remains inconclusive. DDE, PCBs and HCB, have been associated with increased risk for asthma and wheezing, in infancy or early childhood[19,22–26] and a pooled meta-analysis of European studies has shown a positive association between DDE and wheeze in early childhood.[18] Conversely, a South African study reported positive but non-significant associations between DDE and symptoms of asthma, rhinitis and eczema at 3.5 years[24] and a Danish study found no association between maternal DDE concentrations during pregnancy and asthma in their children at age 20 years.[27] Also, prenatal organochlorines were negatively associated with eczema among school-age children from Greenland and Ukraine.[28] Finally, a recent study that investigated a wide array of early life exposures using an exposome approach, did not report associations between prenatal POP exposure and the development of rhinitis or eczema in children 6–11 years of age.[29]

Further, exposure to POPs in utero has been shown to interfere with sex hormones in both boys and girls even as early as in infancy[30–32] while sex hormones have been shown to determine immune response.[33] Also taking into account that sex-related effects are common with developmental immunotoxicity[34] it is plausible to hypothesize that there might be different allergic disease responses of males and females following prenatal exposure to POPs.

Our aim was to investigate the associations between prenatal DDE, HCB and PCBs exposure and wheezing, asthma, rhinitis, and eczema at 4 and 6 years of age leveraging data from the Rhea mother-child cohort in Crete, Greece. Given the estrogenic, anti-estrogenic, and anti-androgenic effects that DDE, HCB, and PCBs may exhibit,[7–9] we also hypothesized that there may be differences in response to these endocrine disruptors between the two sexes because of differences in the natural androgen–estrogen balance during critical windows of fetal development.

Materials and methods

Study population

The Rhea birth cohort included 1363 mother-child pairs living in the prefecture of Heraklion, Crete, Greece.[35] Briefly, pregnant women were recruited during the first major ultrasound examination (mean ± SD, 11.96 ± 1.49 weeks) in 2007–2008 and several contacts followed. Eligibility criteria included a good understanding of the Greek language, maternal age at least 16 years, and singleton pregnancy.

A total of 1110 blood samples provided by the study participants were analyzed for POP exposure. During the follow-up visits, information about allergy-related outcomes were collected for 701 children at 4 years of age and for a subset of 464 children at 6 years. We further excluded mother-child pairs that were missing key covariate information, thus retaining a total of 682 and 454 children at 4 and 6 years of age respectively.

The study was approved by the ethics committee of the University Hospital in Heraklion, Crete, Greece, and all participants provided written informed consent after complete description of the study.

POP exposure

Maternal serum samples were collected at the first prenatal visit around the 3rd and 4th month of pregnancy in 10-mL silicone gel separator vacutainers (Becton Dickinson), were centrifuged within 2 hours from blood collection at 2500 rpm for 10 min, and were then stored in aliquots at –80°C until assayed. The POP analyses were performed in the National Institute for Health and Welfare, Chemical Exposure Unit, Kuopio, Finland, with an Agilent 7000B gas chromatograph triple quadrupole mass spectrometer (GC-MS/MS). Pretreatment of serum samples for GC-MS/MS analysis has been described elsewhere.[36] As quality control, two samples of SRM 1589a from NIST that have certified concentrations for OCPs, PCBs and PBDEs were analysed in each batch of samples (n = 34). Average concentrations of POPs measured from SRM 1958a varied from 92% to 102% of certified concentrations and co-efficient variation was from 2.5% to 8.1% depending on the compound, respectively. Serum concentrations of six individual PCB congeners (118, 138, 153, 156, 170, and 180), HCB, DDT and DDE, and BDE-47 (polybrominated diphenyl ether) were determined. All the results were reported on whole weight and expressed in picograms per milliliter serum, whereas samples below the limit of quantification (LOQ) were assigned the value 0.5 × LOQ. LOQ was 6 pg/mL for PCB-118 and PCB-156; 10 pg/mL for HCB, DDE, PCB-138, PCB-153, PCB-170, PCB-180, and BDE-47; and 50 pg/mL for DDT. We chose to use wet-weight levels for the POPs but we conducted sensitivity analysis adjusting for fasting maternal serum triglycerides and cholesterol as continuous variables in all multivariable models to assess potential biases associated with automatic lipid adjustment.[37] The percentage of samples with levels of DDT above the LOQ was 35.3%. For PCB-156 and BDE-47, 53.7% and 22.4% were above the LOQ, respectively. Because of high percentages of samples below the LOQ (>50%), DDT and BDE-47 were not used in the statistical analyses. PCB-156 also may raise concern due to the high percentage of samples below the LOQ, but in previous published work we have demonstrated that exclusion of PCB-156 from the sum of all PCBs does not meaningfully change the results.[38] POPs were treated as continuous variables on a log2 scale. We calculated total PCB concentrations by summing the concentrations of the six individual PCB congeners.

Allergy-related phenotypes

Information on wheeze, asthma, eczema, and rhinitis occurrence was obtained by questionnaires adapted from the International Study on Asthma and Allergy in Childhood (ISAAC).[39] The health outcomes were defined following the phenotypic definitions proposed by the MeDALL consortium.[40,41] Current wheeze was defined as positive answer to the question "Has your child had breathing difficulties (chest tightness, shortness of breath) in the past 12 months?". Current eczema was defined as positive answers to both: "Has your child had an itchy rash which was coming and going (intermittently) at any time in the past 12 months?"; and "Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind the knees, in front of the ankles,

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under the buttocks, or around the neck, ears or eyes.". Current rhinoconjunctivitis was defined as positive answers to both "In the past 12 months, has your child had problems with sneezing or a runny or blocked nose when he/she did not have a cold or flu?", and "If yes, in the past 12 months, has this nose problem been accompanied by itchy-watery eyes?". Current asthma was defined as a positive answer to at least two of the three following questions: "Has your child had wheezing or whistling in the chest in the past 12 months?", "Has your child ever been diagnosed by a doctor as having asthma?", and "Has your child taken any medicines for asthma or breathing difficulties (wheezing, chest tightness, shortness of breath) in the last 12 months".

Statistical analysis

First, we calculated descriptive statistics for the study population characteristics including outcomes, exposures and covariates. Comparison of these characteristics between boys and girls were performed using t-tests and chi-square tests for continuous and categorical variables respectively. Differences in the exposures between cases and controls were evaluated using t-tests for all children and separately in boys and girls.

Multivariable Poisson models with log link and robust standard errors were used to estimate Risk Ratios (RRs) and 95% confidence intervals (CIs) for the associations between the POP exposures and binary outcome variables. Covariates were selected a priori based on recent ESCAPE and MeDALL studies[42,43] thus all models were adjusted for child age (years) and sex (male/female), maternal age (years), history of maternal and paternal atopy (asthma, rhinitis and eczema; yes/no), maternal BMI pre-pregnancy (kg/m^2), maternal level of education (<6 years/6–12 years/>12 years) and parity (nulliparous/multiparous).

In order to evaluate the sex-specific associations a multiplicative interaction term between each exposure and child sex was introduced in the models and consequently stratified analyses were conducted. We additionally applied generalized additive models (GAMs), using smoothing spline functions to fit the relationship between the prenatal POP exposures and the immune related outcomes. Further, to assess the robustness of our findings, we performed several sensitivity analyses: (1) adjusting for maternal serum cholesterol and triglycerides levels, (2) adjusting for birth weight, which may lie in the causal pathway, (3) excluding those born prematurely (<37 weeks gestational age, N = 76) who may be at increased risk, (4) removing the adjustment for parental allergy to minimize the potential for over-adjustment and (5) adjusting for parental smoking at home, presence of mold or dampness at home, and furry pets in the home at the time of outcome measurement as important precision variables.

The level of significance was set at $p < 0.05$ (two-sided) for all analyses.

Analyses were conducted using Stata software, Version 13.0 (StataCorp LLC, College Station, Texas) and for GAMs we used R 4.0.5 and the mgcv package.[44]

Results

Table 1 presents various characteristics of mothers enrolled in this study and their children, overall and by child sex. Participating mothers had a mean ±SD age of 29.9 ± 4.9 years at delivery and a mean ±SD prepregnancy BMI of 24.8 ± 4.8 kg/m^2. Most of them had a medium education level (6–12 years) and were multiparous. A total of 5.9%, 24.4% and 15.9% of the mothers had a medical history of asthma, rhinitis, and eczema, respectively, and 5.1%, 17.2%, and 4.2% of the fathers had a medical history of asthma, rhinitis, and eczema respectively.

The mean ±SD age at the 4-year follow-up was 4.2 ± 0.2 and 4.4%, 7.9%, 4.6% and 9.9% had current wheeze, asthma, rhinoconjunctivitis, and eczema, respectively. Current asthma and rhinoconjunctivitis were more prevalent among boys (9.7% and 6.2% in boys and 5.9% and 2.8% in girls). With regards to the 6-year follow up, the mean ±SD age was 6.6 ± 0.3 years and 8.1%, 11.0%, 3.5%, and 9.9% of the participating children had current wheeze, asthma, rhinoconjunctivitis and eczema, respectively. At 6 years, we did not observe any differences in the rates of these phenotypes among the two sexes.

Maternal POP concentrations are presented in Table 2. The highest concentrations were found for DDE (geometric mean (95%CI): 2023.1 (1899.9, 2154.2) pg/mL), followed by total PCBs (geometric mean (95%CI): 315.6 (301.7, 330.1) pg/mL) and HCB (geometric mean (95%CI): 89.4 (85.6, 93.3) pg/mL). Levels of maternal POPs were similar for boys and girls (data not shown). In all children, prenatal total PCBs concentrations were lower among children with current wheeze at 6 years while prenatal HCB concentrations were higher among children with current rhinoconjuctivitis at 6 years (Table S1). The differences in exposures' levels separately for boys and girls are presented in Figure 1. Prenatal DDE concentrations were lower among boys with current wheeze at 4 and 6 years and prenatal total PCBs concentrations were lower among boys with current wheeze at 6 years. On the other hand, we observe higher prenatal HCB concentrations among girls with current rhinoconjuctivitis at 4 and 6 years, higher prenatal DDE concentrations among girls with current asthma at 4 years and with current rhinoconjuctivitis at 4 and 6 years and higher prenatal total PCBs concentrations among girls with current eczema at 4 years (mean ± sd values and p-values for these comparisons are provided in Table S1).

The adjusted associations of POPs and allergy-related outcomes at the ages of 4 and 6 years are presented in Tables 3 and 4, respectively. Overall we found no associations with any of the outcomes at 4 years of age but we detected a significant association between HCB and rhinoconjunctivitis at 6 years, where a 2-fold increase of in utero exposure to HCB was associated with a 2.5 times increased risk for rhinoconjunctivitis [RR (95%CI): 2.5 (1.3, 4.8)]. We also detected some statistically significant interaction terms between sex and POPs exposure (p-interaction < 0.05) thus we further conducted separate analysis for boys and girls. At 4 years, we observed a consistent pattern of positive associations of all POPs in girls while in boys associations were predominantly negative. At 6 years, the directions of the observed associations were not homogenous among both boys and girls. Among girls, prenatal DDE was associated with increased risk for current wheeze, current asthma and current rhinoconjunctivitis at 4 years (RR (95%CI): 1.4 (0.8, 2.6), 1.6 (1.1, 2.4) and 1.8 (1.0, 3.3) and p-interaction = 0.035, 0.027 and 0.059 respectively), with increased risk for current rhinoconjunctivitis at 6 years (RR (95%CI): 1.7 (0.7, 3.8) and p-interaction = 0.028). Total PCBs were associated with a 2-fold increase for current eczema at 4 years (RR (95%CI): 2.1 (1.1, 4.2) and p-interaction = 0.028) in girls with similar associations for all PCB congeners (Table S1). In boys, prenatal DDE was associated with decreased risk for current wheeze and current asthma at 4 years. Finally, the association between HCB and current rhinoconjunctivitis at 6 years was more pronounced among girls [RR (95%CI): 4.4 (1.3, 15.4)] compared to boys [RR (95%CI): 2.6 (1.1, 1.8)] but the interaction did not reach significance (p-interaction = 0.162).

In sensitivity analysis, the smooth effect curves generated from GAMs (Table S1 and Figures S1 and S2) indicated no significant

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Table 1. Maternal and child characteristics, mother–child cohort "Rhea" in Crete, Greece (n = 682)

Characteristics	Overall, N = 682 n (%) or Mean ± SD	Boys, N = 360 n (%) or Mean ± SD	Girls, N = 322 n (%) or Mean ± SD	p-value*
Maternal age	29.9 ± 4.9	29.7 ± 4.9	30.1 ± 5.1	0.306
Pre-pregnancy BMI (Kg/m2)	24.8 ± 4.8	24.9 ± 4.8	24.7 ± 4.9	0.629
Maternal education				
Low	111 (16.3)	55 (15.3)	56 (17.4)	0.186
Medium	343 (50.3)	193 (53.6)	150 (46.6)	
High	228 (33.4)	112 (31.1)	116 (36.0)	
Parity, multiparous	378 (55.4)	200 (55.6)	178 (55.3)	0.942
Maternal history of asthma	40 (5.9)	24 (6.7)	16 (5.0)	0.346
Maternal history of allergic rhinitis	166 (24.4)	84 (23.4)	82 (25.5)	0.530
Maternal history of eczema	110 (15.9)	68 (19.3)	37 (11.9)	0.009
Paternal history of asthma	35 (5.1)	15 (4.2)	11 (3.5)	0.608
Paternal history of allergic rhinitis	116 (17.2)	19 (5.3)	16 (5.0)	0.855
Paternal history of eczema	29 (4.2)	72 (20.2)	44 (13.8)	0.030
Child characteristics				
Birth weight (g)	3220 ± 448	3293 ± 453	3139 ± 429	<0.001
ETS at home (4 years)	307 (46.3)	154 (44.8)	153 (48.0)	0.410
ETS at home (6 years)	166 (36.2)	88 (34.8)	78 (37.9)	0.494
Dampness in the house (4 years)	220 (31.0)	113 (31.7)	97 (30.0)	0.707
Mold in the house (4 years)	202 (29.6)	104 (28.9)	98 (30.4)	0.659
Mold in the house (6 years)	179 (39.3)	109 (43.4)	70 (34.3)	0.048
Pet ownership (4 years)	61 (8.9)	30 (8.3)	31 (9.6)	0.554
Pet ownership (6 years)	62 (13.6)	30 (12)	32 (15.7)	0.248
Outcomes in the fourth year of life				
Age at assessment (years)	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	0.876
Current wheeze	30 (4.4)	16 (4.4)	14 (4.3)	0.951
Current asthma	54 (7.9)	35 (9.7)	19 (5.9)	0.065
Current rhinoconjunctivitis	31 (4.6)	22 (6.2)	9 (2.8)	0.035
Current eczema	69 (9.9)	41 (11.4)	27 (8.4)	0.196
Outcomes in the sixth year of life				
Age at assessment (years)	6.6 ± 0.3	6.6 ± 0.3	6.5 ± 0.3	0.256
Current wheeze	37 (8.1)	23 (9.2)	14 (6.7)	0.365
Current asthma	50 (11.0)	31 (12.3)	19 (9.3)	0.310
Current rhinoconjunctivitis	16 (3.5)	11 (4.4)	5 (2.5)	0.267
Current eczema	46 (9.9)	30 (12.0)	16 (7.9)	0.145

ETS: Environmental tobacco smoke.
*p-values were calculated using t-test and chi-square.

departures from the overall conclusions of the primary analyses in terms of the existence and the direction of the associations, with the exception of a significant a J-shaped pattern for the association between DDE concentrations and current wheeze at 6 years that was observed only among boys (p-value = 0.006). Results also remained similar across all other sensitivity analyses (Tables S2–S7), with the exception of the interaction between DDE and gender for current wheeze and asthma at 4 years that became non-significant after the exclusion of children that were born prematurely.

Discussion

In our analysis, prenatal exposure to POPs had no overall effect on children's allergy-related outcomes at 4 years, while a significant

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Table 2. First-trimester maternal serum POP levels (pg/mL, n = 682), Rhea mother–child cohort, Crete, Greece

Chemical	GM (95% CI)	Minimum	Maximum	Percentiles			LOQ	%>LOQ
				25th	50th	75th		
HCB	89.4 (85.6, 93.3)	19.5	1331	61.6	82.3	117.2	10	100
DDE	2023 (1900, 2154)	182	22,714	1191	1955	3509	10	100
PCB-118	17.1 (16.3, 17.8)	3.00[a]	144	12.0	17.5	25.3	6	96.9
PCB-153	123 (118, 129)	13.0	1349	85.5	124	186	10	100
PCB-138	66.1 (63.1, 69.1)	5.0[a]	743	45.6	67.7	102	10	99.7
PCB-156	5.98 (5.66, 6.31)	3.0[a]	77.9	3.0[a]	6.43	10.6	6	53.7
PCB-180	66.2 (63.0, 69.6)	5.0[a]	980	44.5	66.2	104	10	99.7
PCB-170	32.8 (31.1, 34.6)	5.0[a]	542	21.7	33.4	53.0	10	96.0
Total PCBs	316 (302, 330)	34.0	3758	217	318	479		

GM: Geometric mean; LOQ: Limit of quantification.
[a]Value is LOQ/2.

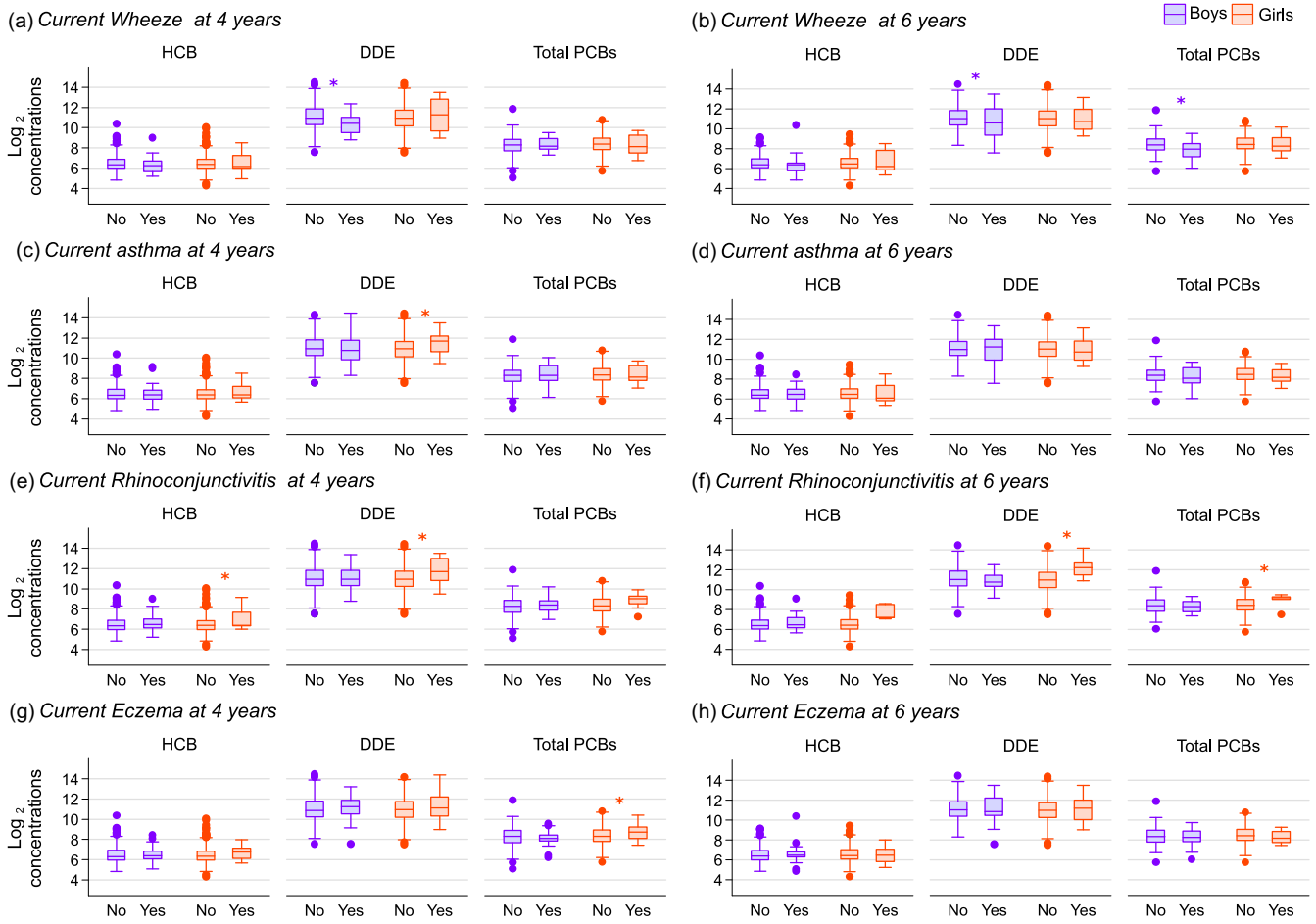

Fig. 1. Differences in POPs concentrations (log2) in relation to wheeze, asthma, rhinoconjuctivitis and eczema at 4 and 6 years of age separately in boys and girls. Asterisks indicate significant difference in exposure levels based on t-tests ($p < 0.05$).

increase in the risk for rhinoconjuctivitis was observed at 6 years for all children prenatally exposed to higher concentrations of HCB.

Further, our results support a sex-specific association between in utero exposure to DDE and rhinoconjuctivitis for 4 and 6-year-old girls, and asthma-related outcomes (current wheeze and current asthma) for 4-year-old girls. Finally, girls prenatally exposed to higher levels of PCBs had an increased risk for current eczema at 4 years of age. To our knowledge, this is the first

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Table 3. Sex-stratified associations of in utero POP exposures allergy-related outcomes at 4 years of age, Rhea mother–child cohort, Crete, Greece

		All		Boys		Girls		
Exposures	Outcome	Cases/ controls	RR (95% CI)	Cases/ controls	RR (95% CI)	Cases/ controls	RR (95% CI)	p-interaction
HCB	Current wheeze[a]	30/652	0.8 (0.5, 1.3)	16/344	0.5 (0.2, 1.2)	14/308	1.2 (0.7, 1.8)	0.707
	Current asthma[a]	54/628	0.8 (0.5, 1.1)	35/325	0.6 (0.3, 1.0)	19/303	1.1 (0.7, 1.7)	0.628
	Current rhinoconjunctivitis[b]	31/639	1.4 (0.9, 2.2)	152/179	1.2 (0.7, 2.0)	9/309	**2.2 (1.3, 3.7)**	0.147
	Current eczema[c]	66/587	1.2 (0.9, 1.6)	40/306	1.1 (0.8, 1.6)	26/281	1.3 (0.8, 1.9)	0.338
DDE	Current wheeze[a]	30/652	0.8 (0.5, 1.1)	16/344	**0.4 (0.3, 0.6)**	14/308	1.4 (0.8, 2.6)	**0.035**
	Current asthma[a]	54/628	0.9 (0.7, 1.2)	35/325	**0.7 (0.5, 0.9)**	19/303	**1.6 (1.1, 2.4)**	**0.027**
	Current rhinoconjunctivitis[b]	31/639	1.1 (0.8, 1.5)	152/179	0.9 (0.6, 1.3)	9/309	1.8 (1.0, 3.3)	**0.059**
	Current eczema[c]	66/587	**1.2 (1.0, 1.5)**	40/306	1.2 (0.9, 1.5)	26/281	1.3 (0.9, 1.8)	0.438
Total PCBs	Current wheeze[a]	30/652	0.8 (0.5, 1.4)	16/344	0.7 (0.4, 1.3)	14/308	1.0 (0.4, 2.4)	0.625
	Current asthma[a]	54/628	0.9 (0.6, 1.3)	35/325	0.8 (0.4, 1.3)	19/303	1.1 (0.5, 2.1)	0.552
	Current rhinoconjunctivitis[b]	31/639	1.2 (0.7, 2.0)	152/179	0.9 (0.5, 1.9)	9/309	1.9 (0.7, 4.9)	0.223
	Current eczema[c]	66/587	1.1 (0.7, 1.5)	40/306	0.8 (0.6, 1.2)	26/281	**2.1 (1.1, 4.2)**	**0.028**

Models are adjusted for maternal age, pre pregnancy BMI (kg/m^2) maternal education level, parity, child sex and age at 4 years. Bold values indicate statistically significant differences at $p < 0.05$.
[a]Further adjusted for maternal and paternal history of asthma.
[b]Further adjusted for maternal and paternal history of allergic rhinitis.
[c]Further adjusted for maternal and paternal history of eczema.

Table 4. Sex-stratified associations of in utero POP exposures allergy-related outcomes at 6 years of age, Rhea mother–child cohort, Crete, Greece

		All		Boys		Girls		
Exposures	Outcome	Cases/ controls	RR (95% CI)	Cases/ controls	RR (95% CI)	Cases/ controls	RR (95% CI)	p-interaction
HCB	Current wheeze[a]	37/417	1.1 (0.7, 1.9)	23/227	1.2 (0.6, 2.6)	14/190	1.1 (0.5, 2.2)	0.694
	Current asthma[a]	50/406	0.9 (0.6, 1.3)	31/221	1.0 (0.6, 1.5)	19/185	0.8 (0.4, 1.4)	0.720
	Current rhinoconjunctivitis[b]	15/431	**2.5 (1.3, 4.8)**	10/236	**2.6 (1.1, 6.1)**	5/195	**4.4 (1.3, 15.4)**	0.162
	Current eczema[c]	42/393	1.0 (0.6, 1.5)	27/213	1.1 (0.6, 1.8)	15/180	0.8 (0.4, 1.7)	0.956
DDE	Current wheeze[a]	37/417	0.8 (0.6, 1.1)	23/227	0.7 (0.4, 1.3)	14/190	1.0 (0.7, 1.3)	0.212
	Current asthma[a]	50/406	0.9 (0.7, 1.2)	31/221	1.0 (0.6, 1.4)	19/185	0.9 (0.7, 1.2)	0.858
	Current rhinoconjunctivitis[b]	15/431	1.1 (0.7, 1.7)	10/236	0.9 (0.6, 1.4)	5/195	1.7 (0.7, 3.8)	**0.028**
	Current eczema[c]	42/393	1.0 (0.8, 1.4)	27/213	1.0 (0.7, 1.4)	15/180	1.2 (0.8, 1.8)	0.688
Total PCBs	Current wheeze[a]	37/417	0.7 (0.4, 1.2)	23/227	0.7 (0.3, 1.4)	14/190	0.7 (0.4, 1.4)	0.266
	Current asthma[a]	50/406	0.8 (0.5, 1.2)	31/221	0.9 (0.5, 1.6)	19/185	0.6 (0.4, 1.0)	0.560
	Current rhinoconjunctivitis[b]	15/431	1.1 (0.6, 2.2)	10/236	1.5 (0.7, 3.1)	5/195	0.6 (0.3, 1.4)	0.285
	Current eczema[c]	42/393	0.7 (0.5, 1.1)	27/213	0.9 (0.5, 1.5)	15/180	0.5 (0.3, 0.9)	0.699

Models are adjusted for maternal age, pre pregnancy BMI (kg/m^2), maternal education level, parity, child sex and age at 6 years. Bold values indicate statistically significant differences at $p < 0.05$.
[a]Further adjusted for maternal and paternal history of asthma.
[b]Further adjusted for maternal and paternal history of allergic rhinitis.
[c]Further adjusted for maternal and paternal history of eczema.

study to report that prenatal exposure to POPs might be related to allergy-related phenotypes in childhood in a sex-specific manner.

In our study, HCB was not associated with asthma/wheezing or eczema. These results generally fall in line with previous studies that report no association of prenatal exposure to HCB and risk of wheeze, asthma, and eczema.[23,26,45] On the contrary, results from a Spanish birth cohort indicated a positive association between prenatal exposure to HCB and the risk of wheeze for 10-year-old children, although no associations to other allergy related outcomes were found.[22] To our knowledge, this is the first study to report an association between prenatal HCB and rhinoconjuctivitis.

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A few studies have investigated the relationship between prenatal DDE and allergy-related phenotypes in childhood. According to a systematic review, the existing, although limited evidence points to a positive association between prenatal DDE and wheeze and/or asthma in children.[19] Prenatal exposure to p,p'-DDE was positively associated with wheezing and/or asthma at 12–14 months,[23] 4 years,[23,26] and 6.5 years.[25] However, such associations were not observed at after that age in Menorca, Spain.[22] Further, a Danish study found no association between maternal p,p'-DDE concentrations during pregnancy and diagnosis of asthma or allergy in their children at 20 years of age.[27] In a meta-analysis that included seven European birth cohorts examining prenatal exposure to DDE and PCB-153 and bronchitis or wheeze at early childhood, a positive association was found between wheeze at early childhood (until 18 months of age) and increased DDE, although there were no associations after that age.[18] A principal component characterized by high prenatal exposure to organochlorines (namely PCB-153 and p,p´-DDE) was inversely associated to "ever-wheezing" for 6–8-year-old children from Greenland, whose mothers participated in the INUENDO birth cohort.[28] Finally a recent study of 3.5 year old children from the South African VHEMBE cohort reported positive but unsignificant associations between DDE and wheezing and asthma.[24] The last study is the only one that examined sex interactions and reported that the estimated associations were similar in boys and girls.

Prenatal exposure to total PCBs increased risk for current eczema in 4-year-old girls in the present study. Previous human studies examining the association between prenatal PCBs and allergic phenotypes remain scarce and report mixed findings. PCBs have been previously associated with increased odds for asthma in one study[46] but not in a second one among children 5–7 years old[47] and PCB-153 was not associated with the risk of wheezing in a European pooled analysis of young children.[18] Further, PCBs have been associated with higher risk for eczema/hay fever[46] but the opposite associations were reported by two studies that associated total PCBs and PCB-153 with lower risk for eczema.[28,47]

The observed sex-specific associations could be explained by the endocrine disrupting properties of the chemicals under study. DDE and PCBs have been shown to have anti-androgenic action suppressing androgen receptors and facilitating estrogen receptors[7,8] whereas HCB has been suggested to be an androgen receptor and an estrogen-related receptor antagonist.[9] Prenatal exposure to such compounds has been shown to be related to steroid sex hormones in newborns in a sex-specific way. For example, prenatal PCB exposure has been associated with more pronounced testosterone reduction among female and with more pronounced estradiol reduction among male infants.[48] Estrogens play an important role in the etiology of allergies. Estrogens' influences on immune cells favor the allergic response promoting Th2 polarization, encouraging class switching of B cells to IgE production and prompting mast cell and basophil degranulation.[33] Also, early life lung development is different for females and males. Childhood asthma and airway hyper-responsiveness are more common among boys, but around puberty there occurs a gender switch when the incidence becomes substantially higher among females.[49] Thus, it is possible that the observed male and female different responses to organic pollutants with regards to allergic diseases' manifestation are mediated by POPs related steroid hormones' disturbances. However, it is worth noting that these steroid hormones' disturbances are not yet fully understood, including whether they extend into puberty. It is possible that POPs–estrogens associations are age-specific (as well as sex-specific) which might explain why we do not observe any associations after the age of 4 years.[50] Finally, animal studies suggest sex-specific differences in cytokine expression following gestational exposure to POPs,[51,52] which in turn, have been associated with asthma and related symptoms in children.[53,54] The mechanisms described above are to a large degree hypothetical and further studies are essential to shed light in our understanding of these sexually dimorphic effects.

Our study has several strengths including the prospective design, which allows prospective exposure assessement during pregnancy (a critical period for atopy development), and the long period of follow-up. We also had detailed information on potential confounding factors, including demographics and maternal and paternal history of asthma, rhinitis and eczema and POPs levels in first-trimester maternal serum were measured using state-of-the-art laboratory techniques. We also acknowledge a number of limitations. The rates of allergy-related phenotypes in our population were low and the numbers were even more restricted in sex-stratified analysis, thus these associations should be interpreted with caution. Also, the exposure levels are relatively low in our population compared to other pregnant populations,[55–57] so our findings should not be extrapolated to populations with different levels of POPs exposure. Allergic diseases were not physician diagnosed but were based on parent-reported data, which may contribute to the misclassification of allergic diseases, however, the ISAAC questionnaire is internationally recognized as having good reliability and validity. Finally, although we incorporated extensive information on potential social and environmental factors in our analyses, we acknowledge that residual confounding from unmeasured covariates is still possible.

Conclusions

Our findings suggest that while prenatal POPs exposure is not associated with wholesale changes to allergy-related phenotype manifestations, it may affect some aspects, in a sex and age-specific manner. This is the first study to report such sex-specific associations. Further research in larger populations with comparable exposure levels is needed to confirm our results.

Supplementary materials. For supplementary material for this article, please visit https://doi.org/10.1017/S2040174421000660

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Conflicts of interest. None declared.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards laid down in the Helsinki Declaration of

1975, as revised in 2008. The study was approved by the ethics committee of the University Hospital in Heraklion, Crete, Greece, and all participants provided written informed consent after complete description of the study.

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