Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy

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Background: There is growing concern that prenatal exposure to bisphenol A (BPA) and phthalates, which are widely used in consumer products, might affect susceptibility to infections and the development of allergy and asthma in children, but there are currently very few prospective studies.

Objective: We sought to evaluate whether prenatal exposure to BPA and phthalates increases the risk of respiratory and allergic outcomes in children at various ages from birth to 7 years.

Methods: We measured BPA and metabolites of high-molecular-weight phthalates, 4 di-(2-ethylhexyl) phthalate (DEHP) metabolites (Σ_4DEHP) and mono-benzyl phthalate (MBzP), and 3 low-molecular-weight phthalate (LMWP) metabolites (Σ_3LMWP) in urine samples collected during the first and third trimesters in pregnant women participating in the Infancia y Medio Ambiente–Sabadell birth cohort study. The occurrence of chest infections, bronchitis, wheeze, and eczema in children was assessed at ages 6 and 14 months and 4 and 7 years through questionnaires given to the mothers. Atopy (specific IgE measurement) and asthma (questionnaire) were assessed at ages 4 and 7 years, respectively.

Results: The relative risks (RRs) of wheeze (RR, 1.20; 95% CI, 1.03-1.40; *P* = .02), chest infections (RR, 1.15; 95% CI, 1.00-1.32; *P* = .05), and bronchitis (RR, 1.18; 95% CI, 1.01-1.37; *P* = .04) at any age increased for each doubling in concentration of maternal urinary BPA. Σ_4DEHP metabolites were associated with the same outcomes (wheeze: RR, 1.25; 95% CI, 1.04-1.50, *P* = .02; chest infections: RR, 1.15; 95% CI, 0.97-1.35; *P* = .11; bronchitis: RR, 1.20; 95% CI, 1.01-1.43; *P* = .04). MBzP was associated with higher risk of wheeze (RR, 1.15; 95% CI, 1.00-1.33; *P* = .05). The risk of asthma at age 7 years was also increased with increasing prenatal BPA, Σ_4DEHP, and MBzP exposure. There were no other exposure-outcome associations.

Conclusions: Prenatal exposure to BPA and high-molecular-weight phthalates might increase the risk of asthma symptoms and respiratory tract infections throughout childhood. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Bisphenol A, phthalates, eczema, wheeze, chest infections, bronchitis, asthma, specific IgE, atopy, children*

The increasing prevalence of asthma and allergic diseases over a relatively short period of time[1] has raised concerns about the potential role of environmental pollutants.[2] Certain pollutants have been suggested to affect susceptibility to infections and development of allergy and asthma during the first years of life, including compounds commonly used in plastic manufacture.[1,2] In recent years, researchers have focused on bisphenol A (BPA) and phthalates because of their potential immunomodulatory capacities[3,4] and the possible effects of these compounds on the development of the respiratory system during fetal life.[5] BPA and phthalates are produced and used in large quantities worldwide and are present in a wide range of consumer products, including cosmetics, plastics, carpets, building materials, toys, and cleaning products.[6-8] The main routes of exposure for the general population are diet (for BPA and high-molecular-weight phthalates) and personal care products (for low-molecular-weight phthalates [LMWPs]).[8-11]

The prenatal period is critical in the development of the immune and respiratory systems, and potential harmful effects of toxic pollutants during this period might result in long-lasting impaired capacity to fight infections and increased risk of allergic manifestations later in life.[12-16] Although there is some evidence of the immunomodulatory properties of both BPA and phthalates in animal and *in vitro* models,[17-19] there is limited evidence of their health effects in susceptible human populations, such as children. Results of previous studies have been inconsistent, mainly because of the use of cross-sectional or retrospective study designs or the use of environmental rather than biomarker-assessed exposure estimates.[11,20-25] In fact, only 3 prospective birth cohort studies have assessed prenatal BPA[26,27] or phthalate[28] exposure in biological samples (maternal urine).

The aim of the present study was to evaluate whether urine biomarker measurements of BPA and phthalates during pregnancy are associated with increased risks of respiratory and allergy outcomes in children at various ages from birth to 7 years in a longitudinal birth cohort study.

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Supported by a research grant from the RecerCaixa (2010ACUP 00349). The Infancia y Medio Ambiente (INMA) project received further funds from the Instituto de Salud Carlos III (Red INMA G03/176, CB06/02/0041), the Spanish Ministry of Health (FIS-PI041436 and FIS-PI081151), Generalitat de Catalunya (CIRIT 1999SGR 00241), the Agency for Management of University and Research Grants (AGAUR; FI-DGR 2012), and Fundació "La Marató de TV3" (090430).

Disclosure of potential conflict of interest: M. Gascon has received funding from the Agency for Management of University and Research Grants. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication April 1, 2014; revised September 17, 2014; accepted for publication September 22, 2014.

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0091-6749/$36.00

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http://dx.doi.org/10.1016/j.jaci.2014.09.030

Abbreviations used

BPA: Bisphenol A
DAG: Directed acyclic graph
DEHP: Di-(2-ethylhexyl) phthalate
INMA: Infancia y Medio Ambiente
LMWP: Low-molecular-weight phthalate
LOD: Limit of detection
MBzP: Mono-benzyl phthalate
MECPP: Mono-(2-ethyl-5-carboxypentyl) phthalate
MEHHP: Mono-(2-ethyl-5-hydroxyhexyl) phthalate
MEHP: Mono-(2-ethylhexyl) phthalate
MEOHP: Mono-(2-ethyl-5-oxohexyl) phthalate
MEP: Mono-ethyl phthalate
MiBP: Mono-isobutyl phthalate
MnBP: Mono-n-butyl phthalate
RR: Relative risk

METHODS

Study population

Pregnant women from the general population were recruited into the Infancia y Medio Ambiente (Environment and Childhood; INMA) birth cohort set up in Sabadell (Catalonia, Spain) between 2004 and 2008 (n = 657). Protocol details are described elsewhere.[29] Briefly, women were recruited during the first trimester's routine antenatal care visit in the main public hospital or health center of reference if they fulfilled the inclusion criteria: age of 16 years or greater, intention to deliver in the reference hospital, singleton pregnancy, no assisted conception, and no problems with communication. The study was conducted with the approval of the hospital ethics committee, and written informed consent was obtained from the parents of all children.

Respiratory and allergy outcomes

Interviewer-led questionnaires given to the mothers collected information on the occurrence of wheeze, chest infections, and eczema in the offspring at ages 6 and 14 months and 4 and 7 years. The questionnaire was the Spanish or Catalan version of the validated International Study of Asthma and Allergies in Childhood questionnaire, depending on the primary language of the mother.[30,31] Information on bronchitis was obtained at 6 and 14 months and 4 years of age. The occurrence of chest infection (or bronchitis, respectively) was defined as a positive answer to the following question: "In the last 6 months (or 12 months if asked at ages 4 or 7 years), has the doctor told you that your child has had a chest infection (or bronchitis, respectively)?" Wheeze was defined as a positive answer to the following question: "Has your child ever experienced whistling or wheeze from the chest, but not noisy breathing from the nose in the last 6 (or 12) months?" At age 7 years, wheeze was defined as a positive answer to the following question: "Has your child ever experienced whistling or wheeze from the chest in the last 12 months?" At 6 and 14 months and 4 years of age, the occurrence of eczema was defined as a positive answer to the following question: "In the last 6 (or 12) months, did your child have atopic eczema?" At age 7 years, eczema was defined as a positive answer to the following question: "Has your child ever had any itchy rash which was intermittently coming and going at any time in the past 12 months?" In the 7-year questionnaire, mothers were also asked about the asthma status of their children with the following questions: "Has your child ever been diagnosed by a doctor as having asthma?" and "Has your child ever taken medication for asthma or respiratory difficulties (chest tightness, shortness of breath) in the last 12 months? If yes, please specify which treatment/s."

In this study we classified a child as asthmatic if the mother reported: (1) ever doctor-diagnosed asthma, (2) asthma treatment in the last 12 months, or (3) wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.[32] At age 4 years, we measured specific IgE levels in children by using the RAST in 2 solid phases (IMMULITE; Siemens, Munich, Germany). Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.

Exposure variables

Spot urine samples of mothers were collected at 12 and 32 weeks' gestation and stored in 10-mL polypropylene tubes at −20°C. Creatinine levels were determined at the Echevarne Laboratory in Barcelona (Spain) by using the Jaffé method (kinetic with target measurement, compensated method) with a Beckman Coulter (Fullerton, Calif) reactive in AU5400 (IZASA, Barcelona, Spain).

BPA concentrations in urine were determined in the Department of Analytical Chemistry, University of Cordoba (Spain), as previously described.[9] Total BPA (free plus conjugated) was quantified by means of liquid chromatography mass spectrometry with a limit of detection (LOD) of 0.1 μg/L. A subset of samples (n = 10) was analyzed for free BPA without enzymatic hydrolyses to rule out external contamination or degradation of the conjugates. Free BPA, if detected at all, represented less than 10% of total BPA in these samples, indicating that external contamination was unlikely.[33,34] Therefore we regard the total BPA level in urine as a valid biomarker of BPA exposure (see Casas et al[9] for further information). Urine concentrations of a total of 8 phthalate metabolites were quantified in the Bioanalysis Research Group at Hospital del Mar Medical Research Institute (IMIM, Barcelona, Spain): mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono-(2-ethyl-hexyl) phthalate (MEHP); mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP); mono-benzyl phthalate (MBzP); mono-ethyl phthalate (MEP); mono-isobutyl phthalate (MiBP); and mono-n-butyl phthalate (MnBP). The determination of concentrations of total (free plus glucuronoconjugated) phthalate metabolites consisted of sample preparation by using enzymatic hydrolysis with β-glucuronidase enzymes and solid-phase extraction, followed by ultraperformance liquid chromatography coupled to tandem mass spectrometry. The LOD for the different congeners ranged from 0.5 to 1 μg/L.

Both BPA and phthalate concentrations were adjusted for creatinine (in micrograms per gram of creatinine) to control for urine dilution. We used the average of the first- and third-trimester concentrations as our exposure variable in the main analyses to provide a better estimate of exposure throughout pregnancy. This has been recommended by other studies of these compounds because they have particularly short biological lives in the range of hours to days.[6,9,10,35] Phthalate metabolites were then grouped based on the common parent of the metabolites (the sum of di-[2-ethylhexyl] phthalate [Σ_4DEHP] metabolites: MEHP, MEHHP, MEOHP, and MECPP), MBzP metabolite, or the type of phthalates (the sum of LMWP [Σ_3LMWP] metabolites: MEP, MiBP, and MnBP) because these are thought to have different physicochemical properties.[20]

Covariates

Information on the following covariates was obtained through questionnaires answered by mothers during the first and third trimesters of pregnancy and at the child's age of 14 months: maternal age, education and country of origin, maternal smoking during pregnancy, secondhand smoke exposure during pregnancy and at the age of 4 years, presence of pets at home during pregnancy, number of older siblings, day care attendance during the first year of life, duration of exclusive breast-feeding, maternal consumption of canned tuna, and maternal and paternal history of asthma/allergy symptoms. Parents were classified as allergic if they reported having allergic asthma, atopic dermatitis, eczema, or rhinitis in the third-trimester health questionnaire. Maternal prepregnancy body mass index, gestational age, weight at birth, season of birth, and child's sex were collected from clinical records or reported by mothers.

Statistical methods

Missing values in covariates (between 0% and 0.8%) were imputed by using multiple imputation methods to avoid loss of participants in the study.[36] The same method was used to impute BPA and phthalate concentrations of less than the LOD (between 0% and 0.8% of the samples) by defining the range of

imputed values between 0 and the LOD value for each compound. Detailed information on the imputation process can be found in Table E1 in this article's Online Repository at www.jacionline.org. Results presented in this study are averaged over the 100 imputations performed.

Of the 657 pregnant women initially recruited in the INMA-Sabadell cohort, 608 provided outcome information on wheeze, chest infections, bronchitis, and eczema in at least 1 period of follow-up between birth and age 7 years. Of these, 462 had information on prenatal BPA exposure, and 391 had information on prenatal phthalates exposure. Also, 474 children had information on asthma status at age 7 years; of these, 361 had prenatal BPA or phthalate exposure information. Finally, specific IgE levels were measured in 311 children, and 175 and 176 children, respectively, had information on BPA and phthalate exposure.

Because distributions of pollutant concentrations were skewed, the exposure variables were $\log_2$ transformed. The $\log_2$ transformation means that all risk estimates are expressed per doubling of pollutant concentration; an RR of 1.20 thus means that for every doubling of the biomarker concentration, the risk of having the symptom increases by 20%. To assess the association between BPA and phthalate exposure and the risk of respiratory or immune outcomes from birth to age 7 years, we used generalized estimating equations with an unstructured correlation matrix and an interaction term between child's age at follow-up and the exposure variable. This interaction term was used to test for a difference in the exposure effect between the different ages of children at each follow-up: 6 months, 14 months, 4 years, and 7 years. In longitudinal studies generalized estimating equations allow estimation of the parameters of a generalized linear model with a possible unknown correlation of the outcomes collected at different time points.[37] They further allow the inclusion of subjects with incomplete information at some point during the follow-up period. Logistic regression models were used to evaluate associations between the exposures of interest and the dichotomous outcome variables assessed at a single time point (asthma at age 7 years and atopy at age 4 years). To determine the covariates to be included in multivariate models for each exposure variable, we applied directed acyclic graphs (DAGs)[38] using DAGity software.[39] Covariates included in the respective DAGs if they were described to be associated with the exposure or the outcome in previous literature, and such associations were shown in bivariate analyses[9,40] of our data ($P \leq .1$, see Table E2 in this article's Online Repository at www.jacionline.org). According to the DAGs, final multivariate models for BPA included maternal education, number of siblings, and maternal smoking during pregnancy, and multivariate models for phthalate metabolites additionally included maternal prepregnancy body mass index and maternal history of asthma, allergy, or both (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org).

Linearity of the association between the different exposure variables and outcomes was assessed by using generalized additive models. Because there was no evidence of nonlinearity, BPA and phthalate concentrations were treated as continuous variables.

Sensitivity analyses were performed for each single phthalate metabolite (MEHP, MEHHP, MEOHP, MECPP, MBzP, MEP, MiBP, and MnBP). To differentiate the role and importance of each exposure assessed, we also performed a multipollutant model in which the 4 main exposure variables (BPA, Σ_4DEHP, MBzP, and Σ_3LMWP) were included. Because only one study previously evaluated the effects of BPA exposure in different trimesters of pregnancy to identify specific time windows of susceptibility,[27] we further conducted sensitivity analyses for the 2 trimesters of exposure separately. Finally, because previous studies have suggested that associations can differ by child's sex,[11,41] we tested this interaction. Analyses were conducted with STATA software, version 12.0 (StataCorp, College Station, Tex), and R statistical package version 3.0.2.

RESULTS

Study population characteristics

In the current study population the prevalence of wheeze and chest infections decreased from birth until 7 years of age, and the prevalence of bronchitis and eczema was lowest at 6 months of age (Table I). The prevalence of asthma at age 7 years was around 14%, and 7.4% of the study population was classified as atopic (Table I). The median BPA concentration was 2.4 μg/g creatinine (Table II). Among phthalate metabolites, the LMWP metabolite MEP had the highest median concentration (405.3 μg/g creatinine), followed by Σ_4DEHP metabolites (101.7 μg/g creatinine), and the concentration of MBzP was the lowest (11.9 μg/g creatinine; Table II). Moderate correlations were found between the different groups of compounds, with Pearson correlation coefficients ranging from 0.15 to 0.31. The highest correlations were observed between Σ_4DEHP and MBzP ($r = 0.31$) and Σ_4DEHP and BPA ($r = 0.21$, results not shown).

Children not included in the present study because of lacking outcome or exposure data had a lower prevalence of wheeze and chest infections at the age of 14 months compared with included children. Also, their mothers were younger, more likely to be primiparous, and had lower education levels (results not shown).

BPA and respiratory and allergy outcomes

Each doubling in concentration of maternal urinary BPA was associated with an increase of 20% in the adjusted relative risk (RR) of wheeze (RR, 1.20; 95% CI, 1.03-1.40; $P = .02$), 15% in the adjusted RR of chest infection (RR, 1.15; 95% CI, 1.00-1.32; $P = .05$), and 18% in the adjusted RR of bronchitis (RR, 1.18; 95% CI, 1.01-1.37; $P = .04$) at any age during the study period (Table III). There was no increase in the risk of eczema in relation to maternal urinary BPA concentrations (Table III). The risk of asthma at the age of 7 years increased with increasing prenatal BPA exposure (RR, 1.21; 95% CI, 0.94-1.57), but this association was not statistically significant ($P = .14$). Atopy at the age of 4 years was not associated with prenatal BPA exposure (RR, 1.07; 95% CI, 0.65-1.77; $P = .78$; Table III). Adjustment for confounding factors had little influence on risk estimates (Table III). When associations between BPA and wheeze were assessed separately at each age at follow-up, the CIs became somewhat wider, but risk estimates were consistent across the different ages (P for age at follow-up interaction = .80; Fig 1, *A*). For chest infections and bronchitis, RRs were consistent across the ages at follow-up until the age of 4 years ($P > .5$; Fig 1, *B* and *C*). RRs for wheeze, respiratory tract infections (including chest infections and bronchitis), and asthma tended to be higher in girls than in boys, but there was no statistical evidence that BPA associations differed between the sexes (P for interaction > .08, data not shown).

Phthalates and respiratory and allergy outcomes

Each doubling in concentration of maternal urinary Σ_4DEHP was associated with an increase in the adjusted RRs of wheeze (RR, 1.25; 95% CI, 1.04-1.50; $P = .02$), chest infections (RR, 1.14; 95% CI, 0.97-1.35; $P = .11$), and bronchitis (RR, 1.20; 95% CI, 1.01-1.43; $P = .04$) at any age during the study period (Table III). The risk of asthma at age 7 years also increased with increasing Σ_4DEHP concentrations (RR, 1.38; 95% CI, 1.05-1.82; $P = .02$). Secondary metabolites (MEHHP, MECPP, and MEOHP) showed the strongest associations, whereas no associations were found for the primary metabolite MEHP (see Table E3 in this article's Online Repository at www.jacionline.org). MBzP concentrations were associated with an increased risk of wheeze at any age during the study period (RR, 1.15; 95% CI, 1.00-1.33; $P = .05$); smaller and nonsignificant increases

TABLE I. Prevalence of respiratory outcomes and eczema

	BPA		Phthalates	
Age at follow-up	No.	Percent	No.	Percent
Wheeze				
6 mo	437	19.7	370	20.8
14 mo	424	30.4	382	30.1
4 y	385	21.8	387	22.2
7 y	361	11.1	361	11.4
Chest infections				
6 mo	445	22.0	377	23.3
14 mo	423	33.8	381	32.3
4 y	385	2.9	387	2.6
7 y	361	7.5	361	8.3
Bronchitis				
6 mo	445	16.0	377	17.2
14 mo	423	25.3	387	24.4
4 y	385	24.4	370	24.8
Eczema				
6 mo	437	11.7	371	12.9
14 mo	421	17.8	380	18.7
4 y	385	23.6	387	23.8
7 y	361	18.3	361	17.7
Asthma at age 7 y*	361	13.6	361	14.1
Atopy at age 4 y†	175	7.4	176	7.4

*Children were classified as asthmatic if the mother reported ever doctor-diagnosed asthma at age 7 years, asthma treatment in the last 12 months (at age 7 years), or wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.
†Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.

TABLE II. BPA (n = 462) and phthalate (n = 391) metabolite levels (in micrograms per gram of creatinine)*

	Median	25th-75th Percentile	Minimum-maximum
BPA	2.4	1.7-3.7	0.3-69.4
Phthalates			
Σ_4DEHP metabolites	101.7	69.5-147.9	26.5-1670.0
MEHP	11.0	7.3-17.2	1.8-266.9
MEHHP	28.0	17.9-41.5	5.3-503.4
MEOHP	20.9	14.3-30.3	4.1-378.3
MECPP	39.5	27.2-59.8	7.7-718.9
MBzP	11.9	7.2-20.1	1.5-405.1
Σ_3LMWP metabolites	483.0	265.3-866.6	65.2-10003
MEP	405.3	199.4-804.0	34.0-9379.8
MiBP	31.4	21.7-48.2	5.1-334.2
MnBP	30.7	19.9-47.3	5.8-835.7

*Average of measurements at 2 time points in the first and third trimesters of pregnancy.

were found for chest infections (RR, 1.08; 95% CI, 0.95-1.23; P = .24) and bronchitis (RR, 1.06; 95% CI, 0.92-1.22; P = .43). Increasing maternal urinary MBzP concentrations were associated with asthma at age 7 years (RR, 1.26; 95% CI, 1.01-1.82; P = .02). Concentrations of prenatal Σ_3LMWP metabolites in maternal urine were not associated with any of the outcomes assessed (Table III). None of the phthalate metabolites were associated with eczema at any age or atopy at age 4 years (Table III). As with BPA, adjustment for confounding factors had little influence on the risk estimates obtained (Table III). Associations between Σ_4DEHP and wheeze were consistent over the ages at follow-up (P for age at follow-up interaction = .43; Fig 1, *A*). Associations with chest infections and bronchitis were consistent up to the age of 4 years (P for interaction = .09 [Fig 1, *B*] and P for interaction = 0.81 [Fig 1, *C*]). For MBzP, increased wheeze risks were mainly seen at age 7 years (P for age at follow-up interaction = .11, Fig 1). RRs for the associations between MBzP concentration and wheeze were greater in girls than boys (girls: RR, 1.28; 95% CI, 1.03-1.58; boys: RR, 0.96; 95% CI, 0.81-1.13; interaction P = .02), and a similar pattern was observed for chest infections (P for interaction = .06) and asthma at age 7 years (P for interaction = .15). DEHP associations with wheeze, respiratory tract infections, and asthma tended to be higher in girls than in boys, but these differences were not statistically significant (P for interaction > .10, data not shown).

Including all 4 main groups of pollutants (BPA, Σ_4DEHP, MBzP, and Σ_3LMWP) in one multipollutant model led to small reductions of between 1% and 8% in the RR estimates compared with results with the single-pollutant model (see Table E4 in this article's Online Repository at www.jacionline.org). When we evaluated separately the associations with first- and third-trimester pollutant concentrations, risk estimates were higher for third-trimester than first-trimester BPA concentrations and higher for first-trimester than third-trimester Σ_4DEHP and MBzP concentrations (see Table E5 in this article's Online Repository at www.jacionline.org).

DISCUSSION

In the present study higher concentrations of BPA and high-molecular-weight phthalates (Σ_4DEHP and MBzP) in maternal urine during pregnancy were associated with an increased risk of wheeze, respiratory tract infections, and asthma in offspring during childhood. Although effect sizes were moderate (RR increases between 10% and 40% per doubling of exposure) and sometimes of modest statistical significance, results were relatively consistent across outcomes and ages at follow-up and robust to the inclusion of other chemical exposures and other confounding factors.

Urine BPA concentrations in the present study were of similar magnitude as those reported previously in 2 birth cohort studies on BPA and respiratory health in children from the United States.[26,27] In one of these studies mean prenatal BPA concentrations were associated with higher risk of wheeze at age 6 months, although the association was not observed at age 3 years.[27] The other study reported that higher urinary BPA concentrations measured during the third trimester of pregnancy were associated with a reduced risk of wheeze at age 5 years and, on the contrary, that higher postnatal urinary levels were related to an increased risk of wheeze and asthma at 5 to 7 years of age.[26] In the present study we found an increased risk of wheeze and respiratory tract infections during childhood, and this was consistent over the ages at follow-up to age 7 years for wheeze and up to age 4 years for respiratory tract infections. The risk of asthma at age 7 years was also increased, although the association was not statistically significant.

Several studies have reported a potential relationship between phthalates and allergic symptoms, including asthma and related symptoms, in children.[11,20-25] However, most of these studies used a case-control[20-23] or cross-sectional[11,24,25] design or assessed phthalate levels in dust as a marker of phthalate exposure[20-23]; this limits the conclusions that can be drawn. In the

TABLE III. Associations between maternal urinary BPA and phthalate metabolite levels* and occurrence of respiratory and allergy outcomes during childhood

	BPA, RR (95% CI)	*P* value	Σ_4DEHP,† RR (95% CI)	*P* value	MBzP, RR (95% CI)	*P* value	Σ_3LMWP,‡ RR (95% CI)	*P* value
Wheeze, chest infections, bronchitis, and eczema from birth until age 7 y§								
No.	462		391		391		391	
Unadjusted								
Wheeze	1.16 (1.00-1.35)	.05	1.29 (1.08-1.54)	.01	1.15 (1.00-1.32)	.05	0.99 (0.86-1.14)	.92
Chest infections	1.10 (0.96-1.26)	.18	1.18 (1.00-1.39)	.04	1.07 (0.95-1.22)	.27	1.02 (0.90-1.16)	.79
Bronchitis	1.12 (0.97-1.30)	.13	1.22 (1.03-1.46)	.02	1.06 (0.92-1.22)	.40	0.96 (0.84-1.11)	.61
Eczema	0.99 (0.84-1.16)	.91	1.05 (0.88-1.26)	.60	1.07 (0.93-1.23)	.37	0.94 (0.82-1.08)	.94
Adjusted								
Wheeze	1.20 (1.03-1.40)	.02	1.25 (1.04-1.50)	.02	1.15 (1.00-1.33)	.05	0.95 (0.82-1.10)	.49
Chest infections	1.15 (1.00-1.32)	.05	1.14 (0.97-1.35)	.11	1.08 (0.95-1.23)	.24	1.00 (0.87-1.13)	.96
Bronchitis	1.18 (1.01-1.37)	.04	1.20 (1.01-1.43)	.04	1.06 (0.92-1.22)	.43	0.95 (0.82-1.09)	.45
Eczema	1.00 (0.85-1.18)	.99	1.00 (0.83-1.20)	.99	1.05 (0.91-1.21)	.51	0.91 (0.79-1.05)	.21
Asthma at age 7 y‖								
No.	361		361		361		361	
Unadjusted	1.22 (0.95-1.56)	.12	1.38 (1.05-1.81)	.02	1.25 (1.02-1.55)	.04	1.07 (0.85-1.35)	.57
Adjusted	1.21 (0.94-1.57)	.14	1.38 (1.05-1.82)	.02	1.26 (1.01-1.82)	.02	1.06 (0.83-1.35)	.63
Atopy at age 4 y¶								
No.	175		176		176		176	
Unadjusted	1.04 (0.64-1.70)	.86	1.07 (0.60-1.90)	.81	0.97 (0.61-1.54)	.89	1.08 (0.69-1.68)	.74
Adjusted	1.07 (0.65-1.77)	.78	1.13 (0.60-2.11)	.71	0.97 (0.61-1.56)	.91	1.11 (0.68-1.81)	.66

BPA models were adjusted for maternal education, number of siblings, and maternal smoking during pregnancy, and phthalate models were additionally adjusted for maternal history of asthma/allergy and maternal body mass index.

*RR per doubling concentration (levels were $\log_2$ transformed).

†The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, and MECPP.

‡The Σ_3LMWP metabolites include MEP, MiBP, and MnBP.

§Wheeze, chest infections, and eczema were assessed at ages 6 and 14 months and 4 and 7 years, and bronchitis was assessed at ages 6 and 14 months and 4 years.

‖Children were classified as asthmatic if the mother reported ever doctor-diagnosed asthma at age 7 years, asthma treatment in the last 12 months (at age 7 years), or wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.

¶Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.

present study the risk of wheeze, respiratory tract infections, and asthma increased with increasing concentrations of Σ_4DEHP in maternal urine during pregnancy. MBzP concentrations were associated with wheeze and asthma but less consistently with respiratory tract infections. No associations between Σ_3LMWP concentrations and the assessed outcomes were observed. Also, we found that secondary rather than primary DEHP metabolites were more likely to be associated with risk of respiratory outcomes in the child. Differences in results between congeners and metabolites might be due to their different physicochemical properties[20] and hormonal activity action.[42,43] A previous birth cohort study observed an association between maternal urinary MBzP concentrations during pregnancy and eczema in offspring before 24 months of age but not at older ages (5 years).[28] We did not observe any association between the phthalate metabolites and eczema or atopy.

BPA[17] and, to a lesser extent, phthalates[42,43] mimic the activity of estrogen, a female hormone that can play an active role in immunomodulation in women.[44] Additionally, the incidence and severity of allergic disorders are higher in adult female subjects.[44] Two studies reported a higher risk of allergic asthma in female than male subjects in relation to BPA[41] and DEHP metabolite[11] exposure. The present study suggests that girls are at a somewhat higher risk of respiratory tract infections, wheeze, and asthma in relation to BPA, Σ_4DEHP, and MBzP exposure. However, differences between the sexes only reached statistical significance for MBzP and were not always consistent across outcomes for the same exposure. This lack of consistency might be partly explained by our small sample size after stratification. Larger studies are warranted to study the role of sex in the potential health effects of these compounds. In addition, we cannot rule out the possibility that before 7 years of age, sex differences in allergic symptoms/diseases have not completely manifested, which means that studies evaluating the long-term respiratory and allergy health effects of these compounds are needed.

The mechanisms by which BPA and phthalates affect the immune and respiratory systems are not completely understood.[3-5,19,45-47] In animal and *in vitro* models BPA has been observed to increase the production of the proallergic cytokine IL-4 and serum IgE and to promote eosinophilic inflammation in the airways.[4,45] Results of a study with mice also suggest that prenatal BPA can affect the innate immune system but not the antiviral adaptive immune response.[46] For phthalates, suggested mechanisms include their capacity to act as adjuvants, which promote T_H2 differentiation and influence antibody response.[3,19] Also, DEHP has been described to alter airway cell differentiation and surfactant protein production in the lungs.[5] Our study did not observe any associations between prenatal BPA or phthalate exposure and atopy at age 4 years measured based on specific IgE levels. This is in accordance with the results obtained in another birth cohort assessing prenatal and postnatal BPA exposure and specific IgE levels at the age of 7 years.[26] In our study information on atopy was available for only part (38% for BPA and 49% for phthalates) of the study population, and therefore the results should be interpreted with caution. In fact, in this part of the study population the associations between prenatal BPA and phthalate exposure and respiratory outcomes were somewhat attenuated and no longer statistically

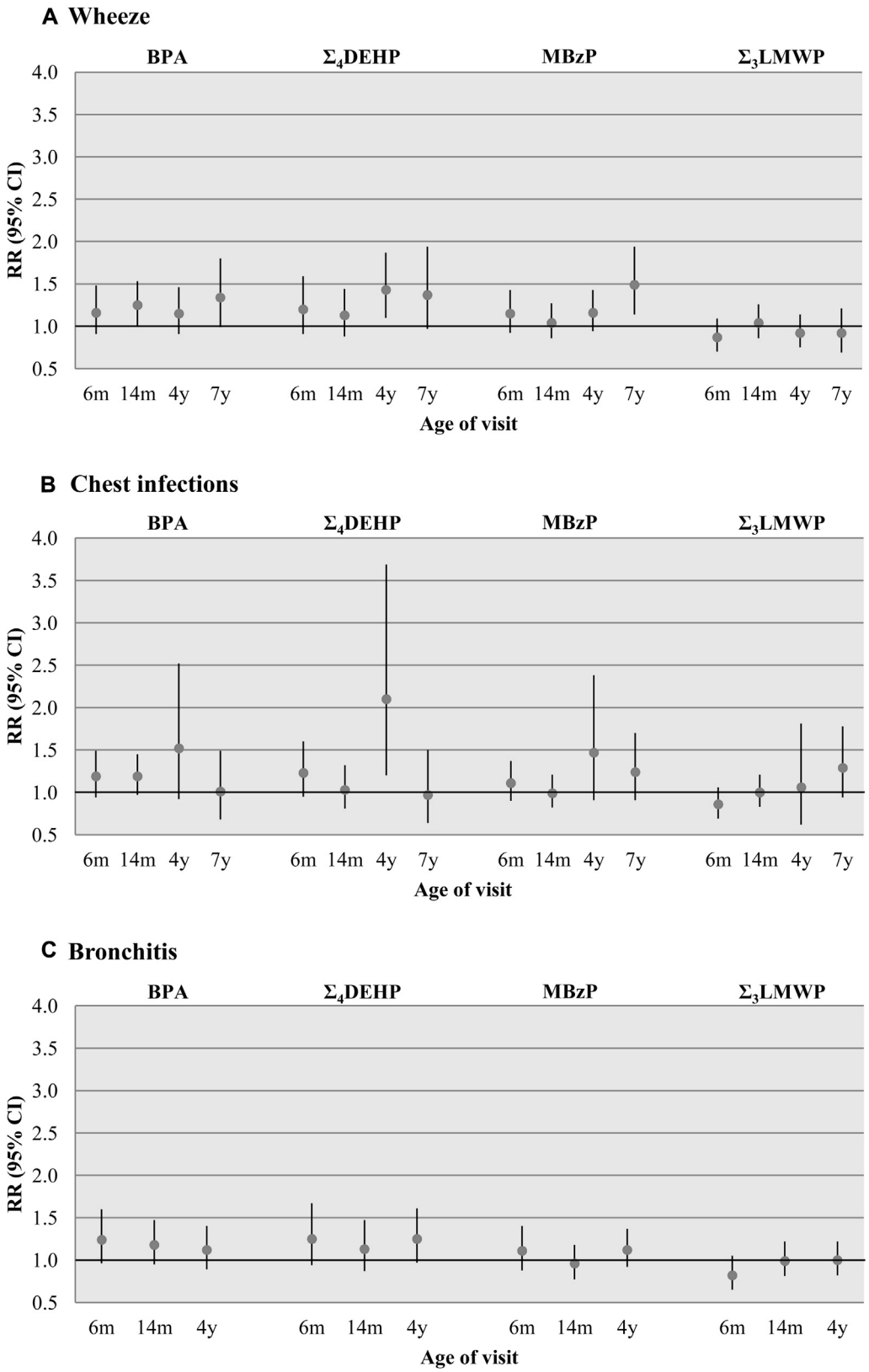

FIG 1. Adjusted associations between maternal urinary BPA and phthalate metabolite concentrations (RR per doubling concentration [levels were $\log_2$ transformed]) and occurrence of wheeze **(A)**, chest infections **(B)**, and bronchitis **(C)** at each age at follow-up from birth until age 7 years. BPA models were adjusted for maternal education, number of siblings and maternal smoking during pregnancy, and phthalate models were adjusted additionally for maternal history of asthma/allergy and maternal body mass index. The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, and MECPP. The Σ_3LMWP metabolites include MEP, MiBP, and MnBP.

significant (results not shown). Researchers, to explain the mechanisms behind the occurrence of allergy- and asthma-related outcomes, mainly focuses on the T_H2 cell pathway promotion.[48] However, it has been observed that a suppressive effect on both T_H1 and T_H2 cells can take place in the causation of these outcomes[16] and that diseases related to both cell types coexist more frequently than might be expected by chance.[49] Furthermore, it seems that BPA and phthalates, as described above, could be affecting not only the adaptive immune system but also the innate immune system.[3-5,19,45-47] Thus to improve our understanding of the mechanistic pathways underlying the potential effects of BPA and phthalates on the developing respiratory and immune systems, inclusion of both humoral and cellular immunity markers is recommended in prospective birth cohort studies.[50]

We found moderate correlations between the different compounds, and these are in accordance with previous study populations.[51-53] These results indicate that these compounds do not completely or always share the same sources of exposure.[51-53] BPA and phthalates have a very short half-life (of hours) and are rapidly excreted from the body. Thus a single measurement is not representative of long-term exposure, such as over the entire pregnancy.[6,9,10,35,54] We averaged the concentrations determined in 2 pregnancy trimesters to obtain a better estimate of prenatal BPA and phthalate exposure during the entire pregnancy. The use of 2 measurements might still not be enough to avoid exposure misclassification,[6,9,10,35,54] but this type of misclassification is likely to be nondifferential (ie, not related to the outcome of interest). Nondifferential misclassification of exposure is likely to result in a dilution of risk estimates,[55] which means that in our study it is more likely that risks were underestimated than overestimated. Future studies can improve exposure estimation by analyzing biomarkers at multiple time points during pregnancy. Although recognizing the limitations of one spot urine measurement, we evaluated associations for the first and third trimesters separately as a sensitivity analysis. Σ_4DEHP and MBzP exposure in the first trimester seemed to show stronger associations with respiratory outcomes than exposure in the third trimester, whereas for BPA, stronger associations were found in the third trimester. A study with monkeys reported alterations in the maturation of secretory cells in the proximal conducting airways after exposure to BPA during late gestation but not during early prenatal life.[56] This could be one mechanism explaining the results obtained in the present study for BPA, however, and as discussed above, these results need to be taken with much caution.

Some previous studies have suggested an association between postnatal BPA and phthalate exposure and respiratory and allergic diseases.[11,20-22,24-26,57] In our study population we did not measure postnatal exposure to BPA and phthalates. However, correlations between maternal and child BPA and phthalate urine concentrations have been described as very low or nonexistent in our study population for BPA with a subset of 130 children,[9] as well as in other studies.[51,58] Therefore it is unlikely that postnatal exposures acted as confounding variables in the associations observed between prenatal exposures and the respiratory and allergy outcomes assessed. An important strength of the present study is that this is the first birth cohort study to include prenatal BPA and phthalate exposures in one analysis; our results show that the compounds more strongly associated with wheeze and respiratory tract infections were BPA and Σ_4DEHP.

The use of questionnaires to assess the occurrence of respiratory symptoms or diseases is well known to be prone to reporting errors by study participants. However, reporting bias would only occur if the errors in responses were related to the level of exposure to phthalates or BPA, which we think is unlikely to have been the case. Furthermore, we had objective information on the atopic status of the child (specific IgE levels measured at age 4 years), and it moderately correlated with asthma at age 7 years ($r = 0.42$, $P = .01$), as well as with eczema at age 4 years ($r = 0.31$, $P = .09$) and 7 years ($r = 0.46$, $P = .01$), as expected.[59,60] Also, the prevalence of asthma at age 7 years in our study population was similar to that reported by a study evaluating asthma symptom prevalence in several regions of Spain,[31] which provides confidence in the information reported by the mothers.

Finally, approximately one third of our initial study population was lost to follow-up, and this part included less educated and younger mothers. This has resulted in an underrepresentation of these groups in our sample, but it is unlikely that this has led to spurious associations between pollutant concentrations and health effects.

The present study suggests that prenatal exposure to BPA and high-molecular-weight phthalates (Σ_4DEHP and MBzP) might increase the risk of asthma symptoms and respiratory tract infections throughout childhood. Future studies should focus on improving prenatal and postnatal exposure estimates and including larger study populations. Also, mechanisms underlying the suggested associations warrant further investigation. In the meantime and where possible, policies to reduce exposure to such compounds should be advocated.

We thank all the participants for their generous collaboration and Silvia Fochs, Anna Sànchez, Maribel López, Nuria Pey, and Muriel Ferrer for their assistance in contacting families and administering questionnaires. A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html.

Key messages

- **There is growing concern that widely used chemicals, such as BPA and phthalates, can affect the child's susceptibility to infections and the development of allergy and asthma through their potential immunomodulatory properties.**
- **The study suggests that prenatal exposure to BPA and high-molecular-weight phthalates (Σ_4DEHP and MBzP) can increase the risk of asthma symptoms and respiratory tract infections throughout childhood.**
- **Policies to reduce exposure to these compounds should be advocated.**

REFERENCES

1. Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, et al. MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. Allergy 2011;66:596-604.
2. Winans B, Humble MC, Lawrence BP. Environmental toxicants and the developing immune system: a missing link in the global battle against infectious disease? Reprod Toxicol 2011;31:327-36.
3. Bornehag CG, Nanberg E. Phthalate exposure and asthma in children. Int J Androl 2010;33:333-45.

4. Kwak ES, Just A, Whyatt R, Miller RL. Phthalates, pesticides, and bisphenol-a exposure and the development of nonoccupational asthma and allergies: how valid are the links? Open Allergy J 2009;2:45-50.
5. Miller MD, Marty MA. Impact of environmental chemicals on lung development. Environ Health Perspect 2010;118:1155-64.
6. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr 2013;25:247-54.
7. Dodson RE, Nishioka M, Standley LJ, Perovich LJ, Brody JG, Rudel RA. Endocrine disruptors and asthma-associated chemicals in consumer products. Environ Health Perspect 2012;120:935-43.
8. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol 2007;24:139-77.
9. Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin A-E, Fernandez MF, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int 2013;56:10-8.
10. Hoppin JA, Brock JW, Davis BJ, Baird DD. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ Health Perspect 2002;110:515-8.
11. Bertelsen RJ, Carlsen KC, Calafat AM, Hoppin JA, Haland G, Mowinckel P, et al. Urinary biomarkers for phthalates associated with asthma in Norwegian children. Environ Health Perspect 2013;121:251-6.
12. Dietert RR, DeWitt JC, Germolec DR, Zelikoff JT. Breaking patterns of environmentally influenced disease for health risk reduction: immune perspectives. Environ Health Perspect 2010;118:1091-9.
13. Pinkerton KE, Joad JP. The mammalian respiratory system and critical windows of exposure for children's health. Environ Health Perspect 2000;108(suppl):457-62.
14. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. Lancet 2010;376:826-34.
15. Bisgaard H, Bonnelykke K. Long-term studies of the natural history of asthma in childhood. J Allergy Clin Immunol 2010;126:187-97.
16. Warner JO. The early life origins of asthma and related allergic disorders. Arch Dis Child 2004;89:97-102.
17. Rogers JA, Metz L, Yong VW. Review: endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. Mol Immunol 2013;53:421-30.
18. Konkel L. BPA and altered airway cells: association seen in rhesus macaques after third-trimester exposure. Environ Health Perspect 2013;121:a254.
19. Kimber I, Dearman RJ. An assessment of the ability of phthalates to influence immune and allergic responses. Toxicology 2010;271:73-82.
20. Bornehag CG, Sundell J, Weschler CJ, Sigsgaard T, Lundgren B, Hasselgren M, et al. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case-control study. Environ Health Perspect 2004;112:1393-7.
21. Kolarik B, Naydenov K, Larsson M, Bornehag CG, Sundell J. The association between phthalates in dust and allergic diseases among Bulgarian children. Environ Health Perspect 2008;116:98-103.
22. Hsu NY, Lee CC, Wang JY, Li YC, Chang HW, Chen CY, et al. Predicted risk of childhood allergy, asthma, and reported symptoms using measured phthalate exposure in dust and urine. Indoor Air 2012;22:186-99.
23. Callesen M, Bekö G, Weschler CJ, Sigsgaard T, Jensen TK, Clausen G, et al. Associations between selected allergens, phthalates, nicotine, polycyclic aromatic hydrocarbons, and bedroom ventilation and clinically confirmed asthma, rhinoconjunctivitis, and atopic dermatitis in preschool children. Indoor air 2014;24:136-47.
24. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, et al. Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort. Am J Respir Crit Care Med 2012;186:830-7.
25. Hoppin JA, Jaramillo R, London SJ, Bertelsen RJ, Salo PM, Sandler DP, et al. Phthalate exposure and allergy in the U.S. population: results from NHANES 2005-2006. Environ Health Perspect 2013;121:1129-34.
26. Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, et al. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol 2013;131:736-42.
27. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, et al. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. Environ Health Perspect 2012;120:916-20.
28. Just AC, Whyatt RM, Perzanowski MS, Calafat AM, Perera FP, Goldstein IF, et al. Prenatal exposure to butylbenzyl phthalate and early eczema in an urban cohort. Environ Health Perspect 2012;120:1475-80.
29. Guxens M, Ballester F, Espada M, Fernandez MF, Grimalt JO, Ibarluzea J, et al. Cohort profile: the INMA—INfancia y Medio Ambiente—(Environment and Childhood) Project. Int J Epidemiol 2012;41:930-40.
30. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. Eur Respir J 1995;8:483-91.
31. Carvajal-Urueña I, García-Marcos L, Busquets-Monge R, Morales Suárez-Varela M, García de Andoin N, Batlles-Garrido J, et al. [Geographic variation in the prevalence of asthma symptoms in Spanish children and adolescents. International Study of Asthma and Allergies in Childhood (ISAAC) Phase 3, Spain]. Arch Bronconeumol 2005;41:659-66.
32. Van Wonderen KE, Van Der Mark LB, Mohrs J, Bindels PJE, Van Aalderen WMC, Ter Riet G. Different definitions in childhood asthma: how dependable is the dependent variable? Eur Respir J 2010;36:48-56.
33. Koch HM, Kolossa-Gehring M, Schröter-Kermani C, Angerer J, Brüning T. Bisphenol A in 24 h urine and plasma samples of the German Environmental Specimen Bank from 1995 to 2009: a retrospective exposure evaluation. J Expo Sci Environ Epidemiol 2012;22:610-6.
34. Völkel W, Kiranoglu M, Fromme H. Determination of free and total bisphenol A in urine of infants. Environ Res 2011;111:143-8.
35. Chevrier J, Gunier RB, Bradman A, Holland NT, Calafat AM, Eskenazi B, et al. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ Health Perspect 2013;121:138-44.
36. Royston P. Multiple imputation of missing values: Update of ice. Stata J 2005;5:527-36.
37. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 1986;73:13-22.
38. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol 2008;8:70.
39. Textor J, Hardt J, Knüppel S. DAGitty: a graphical tool for analyzing causal diagrams. Epidemiology 2011;22:745.
40. Gascon M, Vrijheid M, Martinez D, Ballester F, Basterrechea M, Blarduni E, et al. Pre-natal exposure to dichlorodiphenyldichloroethylene and infant lower respiratory tract infections and wheeze. Eur Respir J 2012;39:1188-96.
41. Vaidya SV, Kulkarni H. Association of urinary bisphenol A concentration with allergic asthma: results from the National Health and Nutrition Examination Survey 2005-2006. J Asthma 2012;49:800-6.
42. Okubo T, Suzuki T, Yokoyama Y, Kano K, Kano I. Estimation of estrogenic and anti-estrogenic activities of some phthalate diesters and monoesters by MCF-7 cell proliferation assay in vitro. Biol Pharm Bull 2003;26:1219-24.
43. Christen V, Crettaz P, Oberli-Schrämmli A, Fent K. Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro. Chemosphere 2010;81:1245-52.
44. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. Curr Opin Allergy Clin Immunol 2013;13:92-9.
45. Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal bisphenol a exposure promotes the development of experimental asthma in mouse pups. Environ Health Perspect 2010;118:273-7.
46. Roy A, Bauer SM, Lawrence BP. Developmental exposure to bisphenol A modulates innate but not adaptive immune responses to influenza A virus infection. PLoS One 2012;7:e38448.
47. Clayton EMR, Todd M, Dowd JB, Aiello AE. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect 2011;119:390-6.
48. Antó JM, Pinart M, Akdis M, Auffray C, Bachert C, Basagaña X, et al. Understanding the complexity of IgE-related phenotypes from childhood to young adulthood: a Mechanisms of the Development of Allergy (MeDALL) seminar. J Allergy Clin Immunol 2012;129:943-54.e4.
49. Simpson CR, Anderson WJA, Helms PJ, Taylor MW, Watson L, Prescott GJ, et al. Coincidence of immune-mediated diseases driven by Th1 and Th2 subsets suggests a common aetiology. A population-based study using computerized general practice data. Clin Exp Allergy 2002;32:37-42.
50. Tryphonas H. Approaches to detecting immunotoxic effects of environmental contaminants in humans. Environ Health Perspect 2001;109(suppl):877-84.
51. Frederiksen H, Nielsen JKS, Mørck TA, Hansen PW, Jensen JF, Nielsen O, et al. Urinary excretion of phthalate metabolites, phenols and parabens in rural and urban Danish mother-child pairs. Int J Hyg Environ Health 2013;216:772-83.
52. Becker K, Göen T, Seiwert M, Conrad A, Pick-Fuss H, Müller J, et al. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int J Hyg Environ Health 2009;212:685-92.
53. Saravanabhavan G, Guay M, Langlois É Giroux S, Murray J, Haines D. Biomonitoring of phthalate metabolites in the Canadian population through the Canadian Health Measures Survey (2007-2009). Int J Hyg Environ Health 2013;216:652-61.
54. Frederiksen H, Kranich SK, Jørgensen N, Taboureau O, Petersen JH, Andersson AM. Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies. Environ Sci Technol 2013;47:958-67.
55. Pollack AZ, Perkins NJ, Mumford SL, Ye A, Schisterman EF. Correlated biomarker measurement error: an important threat to inference in environmental epidemiology. Am J Epidemiol 2013;177:84-92.

56. Van Winkle LS, Murphy SR, Boetticher MV, VandeVoort CA. Fetal exposure of rhesus macaques to bisphenol A alters cellular development of the conducting airway by changing epithelial secretory product expression. Environ Health Perspect 2013;121:912-8.
57. Callesen M, Bekö G, Weschler CJ, Langer S, Brive L, Clausen G, et al. Phthalate metabolites in urine and asthma, allergic rhinoconjunctivitis and atopic dermatitis in preschool children. Int J Hyg Environ Health 2014;217:645-52.
58. Lewis RC, Meeker JD, Peterson KE, Lee JM, Pace GG, Cantoral A, et al. Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children. Chemosphere 2013;93:2390-8.
59. Pearce N, Pekkanen J, Beasley R. How much asthma is really attributable to atopy? Thorax 1999;54:268-72.
60. Schäfer T. The impact of allergy on atopic eczema from data from epidemiological studies. Curr Opin Allergy Clin Immunol 2008;8:418-22.

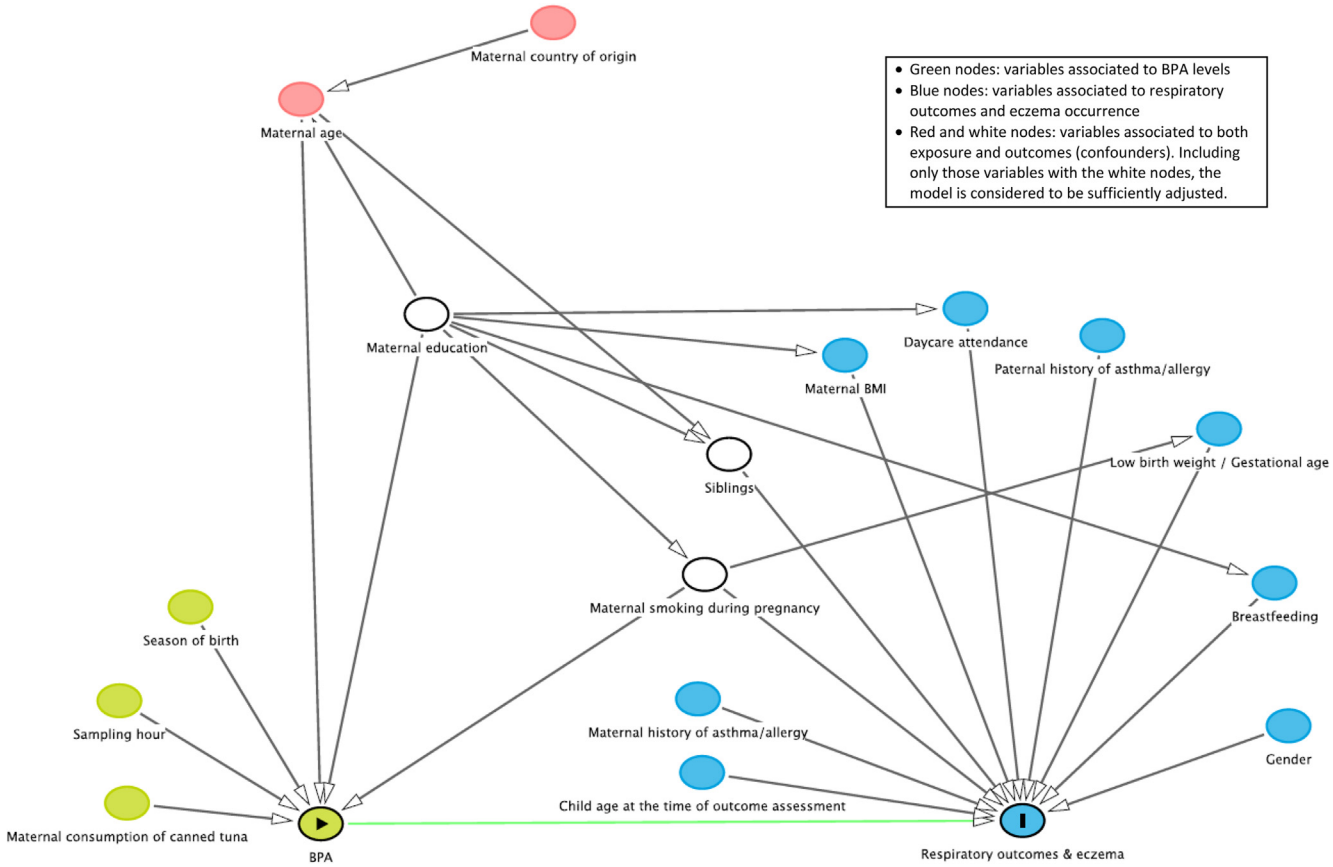

FIG E1. DAG for BPA.

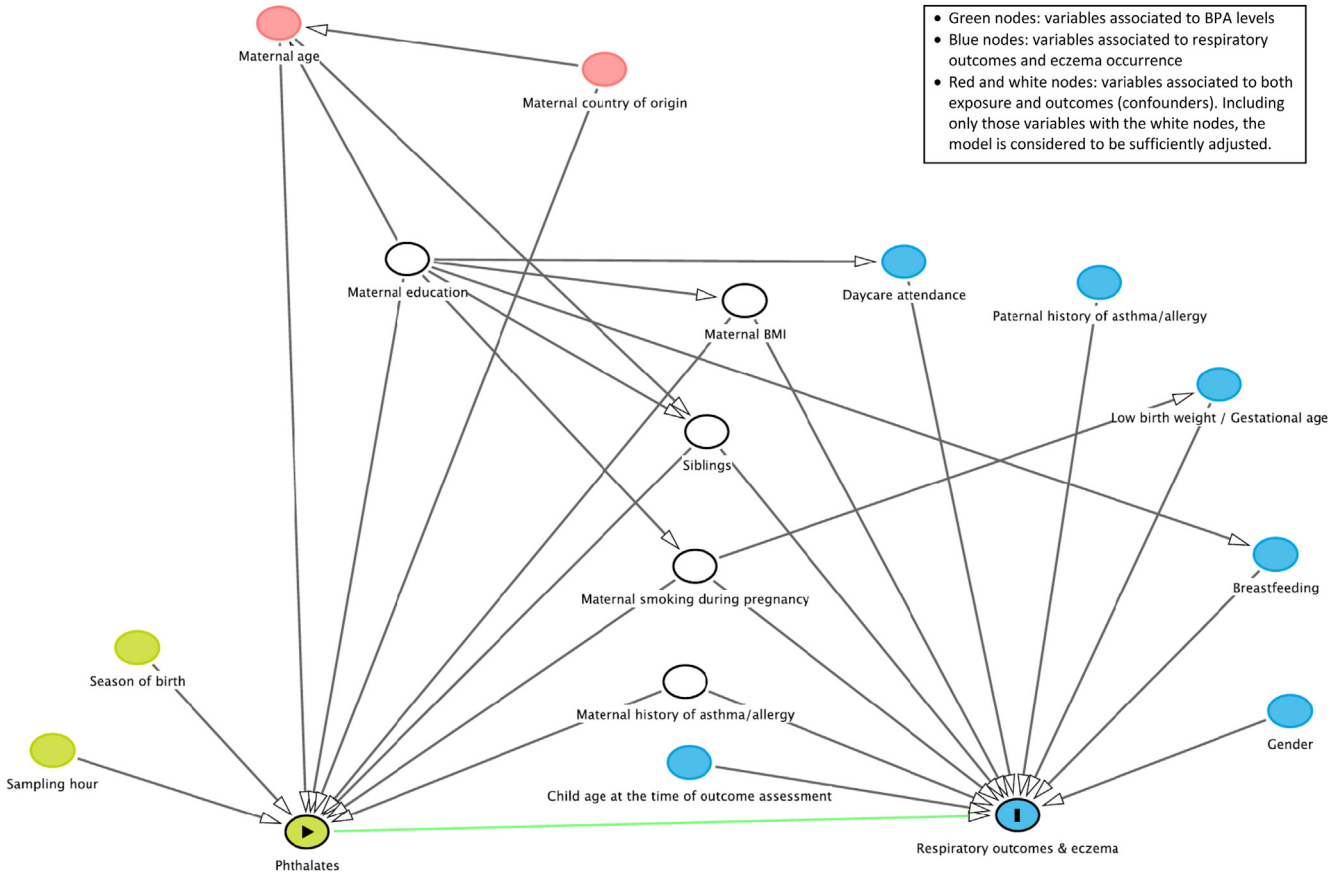

FIG E2. DAG for phthalates.

TABLE E1. Description of the imputation procedure

Software used and key setting: STATA 12 software (Stata Corporation, College Station, Tex): *ice* command (with 100 cycles)

Number of imputed data sets created: 100

- Variables included in the imputation procedure:
  - Child's variables: sex, gestational age, birth weight, weeks of breast-feeding, season of birth, siblings, day care attendance
  - Parental variables: maternal age, prepregnancy maternal weight and height, maternal and paternal asthma, rhinitis, eczema and atopy, maternal education, maternal smoking during pregnancy and first year of life, maternal total IgE level, maternal country of origin, parity
  - Other variables: time of sample collection (first and third trimesters), concentrations of BPA and all phthalates measured in maternal urine (first and third trimesters), consumption of canned tuna (first and third trimesters)
- Treatment of nonnormally distributed variables: $\log_2$ transformed
- Treatment of binary/categorical variables: logistic, ordinal, and multinomial models
- Statistical interactions included in imputation models: imputations were done separately for each study population analyzed (BPA [n = 462], phthalates [n = 391], and all compounds [n = 359])
- Percentage of samples less than the LOD:
  - BPA: 0% of the first-trimester samples and 0.6% of the third-trimester samples
  - Phthalates: 0% to 0.5% of first-trimester samples and 0% to 0.8% of third-trimester samples, depending on the metabolite

TABLE E2. Characteristics of the study population and median levels of BPA and phthalate metabolites (in micrograms per gram of creatinine) in maternal urine samples

				Phthalate metabolites							
	Percent (n = 462)	BPA	Percent (n = 391)	MEHHP	MEHP	MEOHP	MECPP	MBzP	MEP	MiBP	MnBP
Child characteristics											
Sex											
Girls	48.4	2.45	48.1	28.02	10.82	20.99	39.70	12.32	443.12	33.20	30.97
Boys	51.6	2.46	51.9	27.92	11.06	20.83	39.48	11.66	350.01	30.54	29.03
Birth season											
Winter	26.5	2.57*	26.3	28.72*	11.16	21.17*	39.35*	12.71	491.87	31.37	31.31*
Spring	26.7	2.14	25.6	23.85	10.26	18.86	34.91	12.12	419.42	27.31	26.51
Summer	25.4	2.99	26.9	28.37	11.81	22.15	43.45	11.51	401.04	37.44	36.47
Autumn	21.5	2.32	21.2	30.00	10.04	21.98	39.96	10.90	306.49	30.57	25.35
Siblings											
None	55.2	2.76*	57.6	26.26	10.27*	20.21	37.51	11.91	393.72	31.83	32.77
1	37.8	2.23	37.1	31.42	11.72	23.15	43.81	11.35	423.86	31.39	27.99
≥2	7.0	2.00	5.4	26.02	8.50	22.67	41.31	13.03	458.24	27.29	25.35
Maternal characteristics											
Age (y)											
≤25	11.3	3.04*	10.0	30.96	11.06	20.33	45.27	10.20*	317.82	31.65	33.73
>25 to 30	39.9	2.46	40.8	26.26	11.18	20.49	38.47	13.39	408.71	33.51	32.34
>30 to 35	36.3	2.36	36.2	28.36	10.66	21.17	40.30	10.78	437.60	30.21	26.93
>35	12.6	2.08	13.1	26.02	11.34	21.17	39.81	10.80	347.76	30.84	32.77
Education											
Primary school	26.8	2.74*	22.4	29.80	12.59*	21.74	43.90	12.71	536.68*	35.60*	31.04
Secondary school	41.0	2.34	42.7	26.24	9.80	20.21	38.84	10.77	405.28	29.87	29.03
University or higher degree	32.2	2.56	34.9	27.37	11.06	21.17	38.49	12.70	328.51	31.98	32.65
BMI (kg/m^2)											
≤18.5	5.8	1.94	5.6	27.81	10.02	20.19	36.66*	11.51*	351.56	27.54*	26.21
>18.5 to 25	66.7	2.59	67.8	26.26	11.06	20.53	38.42	11.01	393.72	31.46	30.80
>25 to 30	18.8	2.40	18.2	32.02	11.58	22.67	48.07	13.82	458.24	34.48	36.18
>30	8.7	2.22	8.4	28.36	9.11	20.48	43.50	11.91	543.8	27.93	27.54
Prenatal smoking											
No	84.6*	2.37*	85.1	27.14	10.96*	20.60	39.35	11.89	373.45*	30.57*	30.09
Yes	15.4	3.05	14.9	31.40	12.37	22.49	39.70	11.33	562.73	35.65	38.15
Maternal allergy†											
No	68.7	2.63	67.9	26.06*	10.96	20.10*	38.46*	11.99	401.04	31.98	30.78*
Yes	31.3	2.14	32.1	32.02	11.06	23.46	44.62	11.56	474.63	30.79	30.71

BMI, Body mass index.
*$P \leq .1$.
†Mothers were classified as allergic if they answered positively to having allergic asthma, atopic dermatitis, eczema, or rhinitis during the health questionnaire of the third trimester of pregnancy.

TABLE E3. Associations between maternal urinary single DEHP metabolite levels* and occurrence of respiratory and allergy outcomes during childhood

	MEHP,† RR (95% CI)	*P* value	MEHHP,‡ RR (95% CI)	*P* value	MEOHP,‡ RR (95% CI)	*P* value	MECPP,‡ RR (95% CI)	*P* value
Wheeze, chest infections, bronchitis, and eczema from birth until age 7 y (n = 391)§								
Wheeze	1.07 (0.90-1.26)	.45	1.22 (1.04-1.45)	.01	1.27 (1.07-1.51)	.01	1.25 (1.05-1.49)	.01
Chest infections	0.96 (0.82-1.12)	.60	1.16 (1.00-1.34)	.06	1.18 (1.01-1.38)	.04	1.16 (0.99-1.37)	.06
Bronchitis	1.02 (0.87-1.21)	.78	1.22 (1.04-1.43)	.02	1.22 (1.03-1.45)	.02	1.20 (1.01-1.43)	.04
Eczema	0.93 (0.78-1.10)	.40	1.03 (0.88-1.21)	.70	1.01 (0.84-1.20)	.92	1.01 (0.85-1.20)	.90
Asthma at age 7 y (n = 361)‖								
	1.23 (0.93-1.61)	.15	1.27 (0.99-1.64)	.06	1.40 (1.08-1.81)	.01	1.42 (1.08-1.87)	.01
Atopy at age 4 y (n = 176)¶								
	1.20 (0.67-2.13)	.54	1.02 (0.58-1.81)	.94	1.08 (0.59-1.96)	1.08	1.20 (0.66-2.19)	.55

Models were adjusted for maternal education, number of siblings, maternal smoking during pregnancy, maternal history of asthma/allergy, and maternal body mass index.
*RR per doubling concentration (levels were $\log_2$ transformed).
†Primary (MEHP) and ‡secondary (MEOHP and MECPP) DEHP metabolites.
§Wheeze, chest infections, and eczema were assessed at ages 6 and 14 months and 4 and 7 years, and bronchitis was assessed at ages 6 and 14 months and 4 years.
‖Children were classified as asthmatic if the mother reported ever doctor-diagnosed asthma at age 7 years, asthma treatment in the last 12 months (at age 7 years), or wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.
¶Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.

TABLE E4. Associations between maternal urinary BPA and phthalate metabolite levels* and occurrence of respiratory and allergy outcomes during childhood in a multipollutant model

	BPA, RR (95% CI)	*P* value	Σ_4DEHP,† RR (95% CI)	*P* value	MBzP, RR (95% CI)	*P* value	Σ_3LMWP,‡ RR (95% CI)	*P* value
One-pollutant model								
Wheeze, chest infections, bronchitis, and eczema from birth until age 7 y (n = 373)§								
Wheeze	1.18 (1.00-1.40)	.05	1.25 (1.03-1.51)	.02	1.11 (0.95-1.28)	.19	0.90 (0.78-1.05)	.20
Chest infections	1.17 (1.00-1.36)	.04	1.16 (0.98-1.38)	.08	1.07 (0.94-1.23)	.30	1.01 (0.89-1.16)	.86
Bronchitis	1.17 (0.99-1.38)	.07	1.22 (1.01-1.47)	.04	1.03 (0.89-1.20)	.66	0.94 (0.82-1.09)	.43
Eczema	1.00 (0.84-1.19)	.98	1.02 (0.84-1.24)	.84	1.06 (0.92-1.23)	.41	0.91 (0.79-1.06)	.22
Asthma at age 7 y (n = 346)‖								
	1.18 (0.91-1.53)	.21	1.35 (1.01-1.80)	.04	1.25 (0.99-1.56)	.06	1.05 (0.82-1.34)	.71
Atopy at age 4 y (n = 168)¶								
	1.03 (0.62-1.72)	.90	1.12 (0.58-2.19)	.73	0.84 (0.49-1.43)	.52	0.98 (0.59-1.65)	.95
Four-pollutants model								
Wheeze, chest infections, bronchitis, and eczema from birth until age 7 y (n = 373)§								
Wheeze	1.16 (0.97-1.37)	.10	1.21 (0.99-1.48)	.07	1.06 (0.91-1.23)	.48	0.87 (0.74-1.01)	.07
Chest infections	1.14 (0.98-1.34)	.10	1.11 (0.93-1.34)	.25	1.03 (0.89-1.19)	.69	0.98 (0.86-1.13)	.80
Bronchitis	1.14 (0.96-1.36)	.13	1.20 (0.98-1.47)	.07	0.98 (0.84-1.15)	.82	0.91 (0.79-1.06)	.23
Eczema	1.01 (0.84-1.20)	.95	1.00 (0.82-1.24)	.96	1.08 (0.92-1.26)	.35	0.90 (0.78-1.05)	.18
Asthma at age 7 y (n = 346)‖								
	1.15 (0.87-1.51)	.33	1.25 (0.92-1.70)	.16	1.18 (0.93-1.49)	.18	1.00 (0.77-1.49)	1.00
Atopy at age 4 y (n = 168)¶								
	0.80 (0.42-1.53)	.50	1.25 (0.62-2.52)	.53	0.81 (0.46-1.44)	.47	1.03 (0.61-1.74)	.91

All models were adjusted for maternal education, number of siblings, maternal smoking during pregnancy, maternal history of asthma/allergy, and maternal body mass index to have the same adjusted models.

*RR per doubling concentration (levels were $\log_2$ transformed).

†The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, and MECPP.

‡The Σ_3LMWP metabolites include MEP, MiBP, and MnBP.

§Wheeze, chest infections, and eczema were assessed at ages 6 and 14 months and 4 and 7 years, and bronchitis was assessed at ages 6 and 14 months and 4 years.

‖Children were classified as asthmatic if the mother reported ever doctor-diagnosed asthma at age 7 years, asthma treatment in the last 12 months (at age 7 years), or wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.

¶Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.

TABLE E5. Adjusted associations between maternal urinary BPA and phthalate metabolite levels† measured in the first and third trimesters of pregnancy and occurrence of respiratory and allergy outcomes during childhood‡

	BPA, RR (95% CI)	*P* value	Σ_4DEHP,‡ RR (95% CI)	*P* value	MBzP, RR (95% CI)	*P* value	Σ_3LMWP,§ RR (95% CI)	*P* value
Wheeze, chest infections, bronchitis, and eczema from birth until age 7 y‖								
No.	462		391		391		391	
First trimester								
Wheeze	1.09 (0.96-1.25)	.20	1.21 (1.04-1.41)	.02	1.15 (1.02-1.30)	.02	1.00 (0.88-1.13)	.99
Chest infections	1.06 (0.94-1.19)	.37	1.17 (1.02-1.35)	.02	1.07 (0.96-1.19)	.24	1.00 (0.89-1.12)	.96
Bronchitis	1.09 (0.96-1.24)	.20	1.15 (0.99-1.34)	.06	1.04 (0.92-1.16)	.56	0.98 (0.87-1.11)	.76
Eczema	0.98 (0.86-1.13)	.79	1.17 (1.01-1.36)	.04	1.13 (1.00-1.27)	.04	0.97 (0.86-1.09)	.61
Third trimester								
Wheeze	1.15 (1.01-1.31)	.03	1.04 (0.90-1.20)	.59	1.04 (0.92-1.18)	.50	0.93 (0.83-1.05)	.26
Chest infections	1.10 (0.98-1.24)	.11	0.99 (0.86-1.13)	.84	1.04 (0.93-1.16)	.53	1.02 (0.91-1.13)	.77
Bronchitis	1.17 (1.03-1.33)	.02	1.07 (0.92-1.23)	.39	1.06 (0.94-1.20)	.36	0.98 (0.87-1.10)	.72
Eczema	0.97 (0.85-1.11)	.65	0.86 (0.74-1.00)	.05	0.91 (0.80-1.03)	.12	0.89 (0.79-1.00)	.06
Asthma at age 7 y¶								
No.	361		361		361		361	
First trimester	1.03 (0.81-1.31)	.81	1.33 (1.04-1.69)	.02	1.25 (1.04-1.51)	.02	1.07 (0.87-1.31)	.55
Third trimester	1.21 (0.97-1.50)	.09	1.13 (0.88-1.44)	.33	1.06 (0.86-1.31)	.56	1.01 (0.82-1.23)	.95
Atopy at age 4 y#								
No.	175		176		176		176	
First trimester	0.94 (0.59-1.50)	.80	1.26 (0.73-2.20)	.41	1.07 (0.72-1.59)	.74	1.10 (0.74-1.64)	.65
Third trimester	0.94 (0.60-1.49)	.81	1.06 (0.65-1.71)	.83	0.89 (0.59-1.33)	.89	1.10 (0.71-1.70)	.67

BPA models were adjusted for maternal education, number of siblings, and maternal smoking during pregnancy, and phthalate models were adjusted additionally for maternal history of asthma/allergy and maternal body mass index.

*$P \leq .05$.

†RR per doubling concentration (levels were $\log_2$ transformed).

‡The Σ_4DEHP metabolites include MEHHP, MEHP, MEOHP, and MECPP.

§The Σ_3LMWP metabolites include MEP, MiBP, and MnBP.

‖Wheeze, chest infections, and eczema were assessed at ages 6 and 14 months and 4 and 7 years, and bronchitis was assessed at ages 6 and 14 months and 4 years.

¶Children were classified as asthmatic if the mother reported ever doctor-diagnosed asthma at age 7 years, asthma treatment in the last 12 months (at age 7 years), or wheeze in the last 12 months at the age of 7 years plus wheeze in at least 1 of the other previous follow-ups.

#Children were classified as atopic if they had IgE levels of 2 kU/L or greater to any of the following common allergens: *Dermatophagoides pteronyssinus*, cat epithelium, and *Phleum pratense*.