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

Mireia Gascon, MSc,[a,b,c] Maribel Casas, PhD,[a,b,c] Eva Morales, PhD,[a,b,c,d] Damaskini Valvi, MSc,[a,b,c] Ana Ballesteros-Gómez, PhD,[e] Noelia Luque, PhD,[e] Soledad Rubio, PhD,[e] Núria Monfort, PhD,[d] Rosa Ventura, PharmD,[b,d] David Martínez, BSc,[a,b,c] Jordi Sunyer, PhD,[a,b,c,d] and Martine Vrijheid, PhD[a,b,c]

Barcelona and Córdoba, Spain

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 2015;135:370-8.)

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

From [a]the Centre for Research in Environmental Epidemiology (CREAL), Barcelona; [b]Universitat Pompeu Fabra (UPF), Barcelona; [c]CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona; [d]IMIM (Hospital del Mar Medical Research Institute), Barcelona; and [e]Departamento de Química Analítica, Universidad de Córdoba.

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.

Available online October 30, 2014.

Corresponding author: Mireia Gascon, MSc, Parc de Recerca Biomèdica de Barcelona (PRBB)–Centre for Research in Environmental Epidemiology (CREAL), Doctor Aiguader, 88 | 08003 Barcelona, Catalonia, Spain. E-mail: mgascon@creal.cat.

0091-6749/$36.00

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

allergy outcomes in children at various ages from birth to 7 years in a longitudinal birth cohort study.

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