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Associations between prenatal maternal urinary concentrations of personal care product chemical biomarkers and childhood respiratory and allergic outcomes in the CHAMACOS study

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Abstract

Background: Personal care product chemicals may be contributing to risk for asthma and other atopic illnesses. The existing literature is conflicting, and many studies do not control for multiple chemical exposures.

Methods: We quantified concentrations of three phthalate metabolites, three parabens, and four other phenols in urine collected twice during pregnancy from 392 women. We measured T helper 1 (Th1) and T helper 2 (Th2) cells in their children's blood at ages two, five, and seven, and assessed probable asthma, aeroallergies, eczema, and lung function at age seven. We conducted linear and logistic regressions, controlling for additional biomarkers measured in this population as selected by Bayesian Model Averaging.

Results: The majority of comparisons showed null associations. Mono-n-butyl phthalate (MnBP) was associated with higher Th2% (RR: 10.40, 95% CI: 3.37, 17.92), and methyl paraben was associated with lower Th1% (RR: -3.35, 95% CI: -6.58, -0.02) and Th2% at borderline significance (RR: -4.45, 95% CI: -8.77, 0.08). Monoethyl phthalate was associated with lower forced expiratory flow from 25–75% of forced vital capacity (FEF_{25–75%}) (RR: -3.22 liters/second, 95% CI: -6.02, -0.34). Propyl paraben (OR: 0.86, 95% CI: 0.74, 0.99) was associated with decreased odds of probable asthma.

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Conclusions: While some biomarkers, particularly those from low molecular weight phthalates, were associated with an atopic cytokine profile and poorer lung function, no biomarkers were associated with a corresponding increase in atopic disease.

Keywords

Asthma; allergy; personal care products; phthalates; cytokines; chemical mixtures

Introduction

Chemicals in personal care products, including some phthalates, parabens, and other phenols, may impact children's immune development and risk for asthma. Low molecular weight phthalates (diethyl phthalate (DEP), dibutyl phthalate (DBP), and diisobutyl phthalate (DiBP)) are used in fragrances, cosmetics, and medications (Kelley et al. 2011; Koniecki et al. 2011). Parabens (e.g., methyl paraben, propyl paraben, butyl paraben) are used as preservatives in cosmetics, pharmaceuticals, and paper products because of their bactericidal and fungicidal properties (Guo and Kannan 2013; Liao and Kannan 2014). Triclosan, an antibacterial found in some toothpastes, deodorants, and antimicrobial fabrics (Dann and Hontela 2011), was recently banned from antibacterial soaps for not being generally recognized as safe and effective (United States Food and Drug Administration 2013). 2,4-Dichlorophenol is an intermediate in pesticide manufacturing, but is also a photo-degradation product of triclosan (Latch et al. 2005). 2,5-Dichlorophenol is used in moth balls and room and toilet deodorizers (Wei et al. 2014). Benzophenone-3, also known as oxybenzone, absorbs ultraviolet rays A and B and is used in sunscreens and other products for skin protection, and in cosmetics to prolong product durability (Han et al. 2016). Exposure to these chemicals or their precursors is widespread: low molecular weight phthalate metabolites were detected in the urine of 97% or greater of 2013–2014 National Health and Nutrition Examination Survey (NHANES) participants, and most phenols were detected in 96% or greater of participants (CDC 2018).

Rates of childhood asthma, aeroallergy, and eczema are high in the U.S. and have been increasing for several decades (Akinbami et al. 2009; Asher et al. 2006; Jackson et al. 2013). In the 2013–2014 NHANES, 15.9% of children had been diagnosed with asthma (CDC 2013–2014), and in the 2005–2006 NHANES (the latest year for which this information is available) self-reported doctor diagnosis of aeroallergy and eczema prevalence were 24.6% and 14.3%, respectively (CDC 2005–2006). Activity of T helper cells lymphocytes may be involved in the biological mechanisms of asthma, inhalant allergies, and other allergic diseases (Barnes 2001; Deo et al. 2010). T helper cells release cytokines that send chemical messages to other immune cells (Zhu and Paul 2008). Of the two main types of T helper cells, T helper 1 (Th1) cells combat intracellular viruses and bacteria through cell-mediated immunity and secrete cytokines such as interferon gamma (IFN- γ), interleukin-2 (IL-2), and IL-3, while T helper 2 (Th2) cells are involved in allergic and inflammatory responses and secrete cytokines such as IL-4, IL-5, and IL-6 (Volcheck 2008). A higher ratio of Th2 cytokines to Th1 cytokines has been associated with increased risks of aeroallergy and asthma (Barnes 2001; Deo et al. 2010), suggesting a possible mechanism of asthma and allergy development.

Some animal and *in vitro* studies suggest exposure to low molecular weight phthalates leads to higher Th2 cytokine concentrations (Li et al. 2014; Maruyama et al. 2007; Shigeno et al. 2009). DEP and DBP also propagate the release of inflammatory cytokines *in vitro* directly from airway epithelial cells which then lead to proliferation and migration of bronchial smooth muscle cells (Kuo et al. 2011). These are key steps in the airway remodeling that characterizes asthma (Glue et al. 2005) and they may occur prenatally (Bousquet et al. 2004; Kumar 2008). Parabens, triclosan, and benzophenone-3 exposure also leads to higher Th2 cytokine concentrations *in vitro* or in animals (Anderson et al. 2012; Hegazy et al. 2015; Jannesson et al. 2004; Kato et al. 2006; Kwon et al. 2013; Marshall et al. 2015), and animal studies suggest that exposure to triclosan leads to reduced lung function (Anderson et al. 2012). Phthalates and parabens are xenoestrogens (Harris et al. 1997; Routledge et al. 1998), which have been shown to lower forced expiratory volume in one second (FEV₁) and forced vital capacity (FVC) and increase airway hyperresponsiveness (Bonds and Midoro-Horiuti 2013), as well as encourage Th2 differentiation and other allergic inflammatory responses (Bonds and Midoro-Horiuti 2013). Exposures to low molecular weight phthalates, parabens, and phenols (or their precursors) are also associated with increased biomarkers of oxidative stress in humans (Ferguson et al. 2014; Ferguson et al. 2011; Holland et al. 2016; Watkins et al. 2015) and *in vitro* (Bukowska 2003; Gao et al. 2013; Lourenço et al. 2015; Xu et al. 2013) which can play a crucial role in asthma development (Kato et al. 2006) by increasing inflammation and promoting airway hyperresponsiveness (Cho and Moon 2010). Maternal oxidative stress in utero can lead to epigenetic effects in pregnancy, the induction of fetal proinflammatory genes (Martino and Prescott 2011), and increased fetal oxidative stress that can affect the development of the fetal immune and respiratory systems (Leon Hsu et al. 2015).

Some epidemiologic studies on prenatal exposure to these chemicals have found an association with atopic outcomes. A study of 154 children found that prenatal maternal urinary concentrations of mono-n-butyl phthalate (MnBP) were associated with increased risk of asthma from ages 5–11 (Whyatt et al. 2014), and in 610 children, prenatal maternal urinary concentrations of monoisobutyl phthalate (MiBP) were associated with dermatitis at age 3 (Herberth et al. 2017). However, prenatal urinary concentrations of low molecular weight phthalates were not associated with asthma, or eczema in 164 children ages 6 and 7 (Buckley et al. 2018). Prenatal parabens were associated with asthma and wheeze in Vernet *et al.*'s study of 587 five-year-olds (Vernet et al. 2017), but not with asthma, wheeze, or aeroallergy in Lee-Sarwar *et al.*'s study of 467 three- and four-year-olds (Lee-Sarwar et al. 2017). In Vernet *et al.*'s study (Vernet et al. 2017), 2,5-dichlorophenol was associated with wheeze, and it was associated with eczema in Lee-Sarwar *et al.*'s study (Lee-Sarwar et al. 2017).

Phthalates, parabens, and some other phenols have been detected in human placentas, furthering the case for studying these exposures prenatally (Mose et al. 2007; Valle-Sistac et al. 2016). Pregnancy also represents a susceptible window of exposure, as much of the immune and respiratory systems develop *in utero*. To further elucidate the relationships between exposures to these chemicals and childhood asthma and atopy, we measured urinary concentrations of three phthalate metabolites, three parabens, and four other phenols in maternal urine collected at two time points during pregnancy and analyzed associations with

asthma, aeroallergies, eczema, and lung function in their children at age seven, and with T helper cells at ages two, five, and seven. We analyzed data from a unique study population and employed Bayesian Model Averaging to control for confounding by co-exposure to pollutants, addressing gaps in previous literature.

Methods

CHAMACOS Study.

Participants were enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), a longitudinal cohort study examining the effects of *in utero* and childhood environmental exposures on growth, neurodevelopment, respiratory disease, and pubertal development in the Salinas Valley, California, an agricultural community. English or Spanish-speaking mothers who were eligible for low-income health insurance (MediCal), at least 18 years old, less than 20 weeks' pregnant, and who were planning on delivering at the county hospital were recruited to participate in the study in 1999–2000 from prenatal care clinics serving the Latino, farmworker population in the Salinas Valley. A total of 601 mothers were enrolled and 531 were followed through the birth of a live infant. Of those, 517 had at least one prenatal measurement of low molecular weight phthalates, parabens, or other phenols and 392 children additionally had information on respiratory or allergy symptoms, spirometry, or cytokine measurements. These 392 children did not differ substantially in demographics or in maternal urinary biomarker concentrations compared to children who were lost to follow-up and did not have outcome data. Research protocols were approved by the Office for the Protection of Human Subjects (OPHS) at UC Berkeley. The Centers for Disease Control and Prevention (CDC) deferred to OPHS. Mothers provided written informed consent and children provided verbal assent at age seven.

Exposure assessment.

Spot urine samples were collected from mothers at the time of the two pregnancy interviews in 1999–2000. Samples were collected in phthalate-free polypropylene urine cups, aliquoted into glass vials, and stored at -80°C until shipment to the CDC in Atlanta, Georgia for analysis. Urinary specific gravity was measured using a hand-held refractometer (National Instrument Company Inc., Baltimore, MD). We corrected for urinary dilution using the formula: $(\text{analyte concentration} * (1.024 - 1)) / (\text{sample specific gravity} - 1)$ (Cone et al. 2009).

Missing specific gravity values were imputed for 81 participants by regressing specific gravity on creatinine and multiplying the coefficient by existing creatinine values to generate missing specific gravity values, and sensitivity analyses were run excluding the women with imputed specific gravity.

Solid phase extraction coupled with isotope dilution high performance liquid chromatography-tandem mass spectrometry was used to quantify concentrations of the following three phthalate metabolites and eight phenols: monoethyl phthalate [MEP, a metabolite of DEP]; MnBP, a metabolite of DBP; and MiBP, a metabolite of DiBP; methyl paraben; propyl paraben; butyl paraben; triclosan; benzophenone-3; 2,4-dichlorophenol; and 2,5-dichlorophenol. Butyl paraben was dropped from analyses because it was only detected

in 67% of measurements at the first pregnancy visit and 71% of measurements at the second pregnancy visit. Analytic methods have been published previously both for phthalates (Silva MJ 2007) and phenols (Ye et al. 2005). Phthalate metabolite and phenol concentrations were reported in ng/mL of urine. Limits of detection (LOD) ranged from 0.2 ng/mL – 2.3 ng/mL, depending on the analyte. If concentrations were below the LOD, we assigned the instrumental reading values, if available, or we used maximum likelihood estimation to imputed a randomly selected value below the LOD from the log-normal distribution (Lubin et al. 2004). Biomarker concentrations were above the highest calibration standard in a number of samples (methyl paraben: 39 measurements [23 at baseline, 16 at 26 weeks]; propyl paraben: 41 measurements [5 at baseline, 36 at 26 weeks]; triclosan: 18 measurements [14 at baseline, 4 at 26 weeks]; 2,4-dichlorophenol: 34 measurements [14 at baseline, 20 at 26 weeks]; 2,5-dichlorophenol: 111 measurements [55 at baseline, 56 at 26 weeks]; benzophenone-3: 66 measurements [34 at baseline, 32 at 26 weeks]) because the objective at the time of measurement was to only quantify bisphenol A concentrations. For this work, concentrations of these other biomarkers were replaced with the highest calibrator concentration used (100 ng/mL for 2,4-dichlorophenol, 1000 ng/mL for all other parabens and phenols). We chose to present low molecular weight phthalates, parabens, and other phenols together in this paper because they come from similar sources (mainly personal care products). A separate paper explores concentrations of high molecular weight phthalates and bisphenol A with the same outcomes as in this study.

Questionnaire data collection.

Interviews were conducted with the mothers in English or Spanish using structured questionnaires at two times during pregnancy (mean 13 and 26 weeks gestation). Information was gathered on demographic characteristics, including their age, family income, parity, if they had a family history of asthma, whether or not they smoked at all during pregnancy, how many hours per day they were around someone else who smoked, and whether or not they had furry pets in their home. Home interviews were also conducted prenatally, during which housing density (number of people living in the home per number of rooms in the home) was measured. Mothers were also interviewed at delivery, and when the child was six months, one year, two years, three and a half years, five years, and seven years old. Respiratory and allergy symptoms were collected at each time point, with the most detailed information collected at age seven.

Mothers were asked at all visits if their child had ever been diagnosed with asthma by a doctor or nurse. We coded a child as having been diagnosed with asthma if their mother responded positively to these questions at any age.

When the child was seven, mothers were asked whether her child was currently taking any asthma medication. Mothers were also asked about the following respiratory symptoms in the previous 12 months via questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Asher et al. 1995): tightness in the chest and difficulty breathing; wheezing or whistling in the chest; trouble sleeping, speaking, or running or playing because of wheezing, whistling, shortness of breath, or coughing without a cold; or an asthma attack.

At the seven year visit, mothers were asked if their child had been diagnosed with “eczema or an allergic skin rash” or “hay fever or allergic rhinitis” in the previous 12 months. Mothers were also asked if their child had aeroallergy symptoms (“runny or itchy eyes apart from colds” or “sneezing or a runny nose apart from colds”) in the previous 12 months.

Spirometry methods.

Children underwent a clinical examination at age seven that included spirometry, which was administered by two specifically trained bilingual and bicultural technicians. Three identical EasyOne spirometers were used in the study and routine calibration was performed every morning. All maneuvers were reviewed and verified by two physicians with experience in pediatric spirometry, and the best verified maneuver was used for analyses. The following were measured from each maneuver: FEV₁, FVC, FEV₁/FVC, and forced expiratory flow from 25–75% of FVC (FEF_{25%–75%}). FEV₁ measurements were obtained for 300 children and had a mean volume of 1.8 liters (SD: 0.5). Other measurements were obtained for 270 children.

If mothers reported that their child had respiratory symptoms on the seven year visit questionnaire, the child repeated spirometry 20 minutes after administration of an inhaled bronchodilator (albuterol). If children improved by 12% or more from their best measurement of FEV₁ or FVC before the bronchodilator to their best measurement after, they were classified as having a positive bronchodilator test.

Cytokine detection.

Intracellular Th1 and Th2 cytokines were detected in unfrozen pediatric whole blood, collected at ages two, five, and seven, using flow cytometry. Methods have been described previously and validated for epidemiologic studies (Duramad et al. 2004). Briefly, 500 µL of whole blood was activated for 4 hours with phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO) and ionomycin (Sigma) for CD4⁺. Cells were stained to detect monoclonal antibodies specific for IFN-γ (Becton Dickinson) or IL-4 (Becton Dickinson) cytokines. Cells that stained positive for IFN-γ only were classified as Th1 cells and the percentage of Th1 cells (Th1%) was defined as the number of Th1 positive cells divided by the total number of CD4⁺ cells. A similar procedure was used to detect Th2 cells and define the percentage of Th2 cells (Th2%) by staining for IL-4 (Becton Dickinson) cytokines. Th1:Th2 cell ratio was defined as Th1% divided by Th2%. Overall, 101 children had cytokine data for all three time points, with 239 children having data for age two, 231 for age five, and 254 for age seven.

Outcome variable definitions.

We classified children as having “probable asthma” at age seven if they were currently taking asthma medication or had two or more of the following criteria: any current respiratory symptom, doctor diagnosis of asthma at any age, or a positive bronchodilator test. We classified children as having eczema if their mother reported they had been diagnosed with eczema or an allergic skin rash in the last year. We defined having aeroallergies as maternal report of any of the following in the last year: 1) a diagnosis of hay

fever/rhinitis, 2) runny or itchy eyes apart from colds, or 3) sneezing or a runny nose apart from colds.

Statistical analysis.

We conducted multivariable logistic regression models to assess the odds of probable asthma, aeroallergy, and eczema, and we conducted linear regression models to assess spirometry performance associated with prenatal maternal urinary biomarker concentrations. Associations were considered significant if the P-value was below 0.05, and borderline significant if the P-value was below 0.10 and above or equal to 0.05. Urinary biomarker concentrations were averaged across pregnancy and were log-normally distributed and were examined continuously as \log_2 -transformed variables. Separate models were constructed for each chemical. Probable asthma, aeroallergies, and eczema were analyzed as binary variables. Lung function parameters were analyzed as continuous variables. FEV₁ and FVC were normally distributed but FEV₁/FVC and FEF_{25-75%} were log-normally distributed and were \log_{10} -transformed for analysis. Regression coefficients thus represent mean (FEV₁ and FVC) or percent change (FEV₁/FVC and FEF_{25-75%}) in outcomes for each two-fold increase in urinary biomarker concentration. We first conducted analyses on children who had at least one verified maneuver, as determined by the two physicians conducting quality control after data collection. Regressions on spirometry were then performed only on children who had at least two verified maneuvers in sensitivity analyses (n =276 for FEV₁ and 234 for other spirometry measurements), and on children who were not currently taking any asthma medication (n=283 for FEV₁ and 257 for other spirometry measurements).

Th1%, Th2%, and Th1:Th2 cell ratio at ages two, five, and seven were all log-normally distributed and were thus \log_{10} -transformed and analyzed as continuous variables. For each two-fold increase in urinary biomarker concentration, regression coefficients thus represent percent change in outcomes. We conducted generalized estimating equations (GEEs) with Gaussian specification to determine the longitudinal association of cytokine variables with urinary biomarker concentrations across ages. Associations were considered significant if the P-value was below 0.05, and borderline significant if the P-value was below 0.10 and above or equal to 0.05. Additionally, we obtained p-values for interaction by age by conducting GEE models with interaction terms for child age. For longitudinal associations indicating interaction by age (p-value for interaction below 0.10) we then conducted linear regression models to assess associations between the indicated biomarker and outcome at individual ages (ages two, five, and seven).

We controlled for maternal age at birth, parity, household income as a proportion of poverty at baseline, family history of asthma (mother, father, or siblings), season of birth, passive and active smoking during pregnancy, furry pets in the home during pregnancy, and housing density during pregnancy as confounders in demographically adjusted models. These were determined via directed acyclic graphs *a priori*. Household income as a proportion of poverty at baseline and season of birth were analyzed categorically; family history of asthma, active smoking during pregnancy, and furry pets in the home during pregnancy were analyzed as binary variables; and maternal age at birth, parity, passive smoking during

pregnancy (hours per day around a smoker), and housing density were analyzed continuously.

Modeling exposure to one biomarker at a time unrealistically represents exposure to these chemicals, which are often found in products together, and we chose to control for additional chemical exposure in fully adjusted models. To reduce dimensions while modeling exposure to other chemicals measured in this cohort, we used Bayesian Model Averaging (BMA) to determine which additional chemical exposures to include in the fully adjusted regression models. BMA estimates models for all possible combinations of the specified biomarkers with an outcome and weights different models by their posterior model probability, adjusted for specified demographic covariates, to determine the influence of different biomarkers on a health outcome relative to other biomarkers (Madigan et al. 1996), measured as Posterior Inclusion Probabilities (PIPs). We decided to employ BMA because unlike other variable selection models, BMA constructs models with all possible combinations of the given covariates and averages these models, taking uncertainty into account appropriately, to produce easily evaluable PIPs that indicate how likely each covariate is to be included in the final model. We included the following biomarkers, measured in the same urinary samples from this cohort, in the BMA models in addition to the personal care product biomarkers focused on in this paper: other phthalates (monobenzyl phthalate, monocarboxy-isononyl phthalate, monocarboxy-octyl phthalate, mono-(3-carboxypropyl) phthalate) and phenols (bisphenol A). Overall there was only weak to moderate evidence that certain other biomarkers were possible risk factors and/or confounders, as evidenced by the fact that few exposure-outcome combinations resulted in PIPs >0.5. Hence we selected the top three ranked biomarkers (i.e. those with the highest PIPs in the BMA model) to add to fully adjusted regression models as a conservative approach to co-exposure confounding. In sensitivity analyses, we included the top five and the top seven ranked biomarkers. Additionally, we chose not to control for 2,4-DCP or 2,5-DCP in regression models of dichlorophenols because they are highly correlated (0.90, see Supplemental Figure 1). For example, in models of FEV₁/FVC, 2,5-DCP is controlled for in all models, as chosen by BMA analysis, but not in the regression of FEV₁/FVC on 2,4-DCP. This applied only to the regressions on FEV₁/FVC and Th1%.

Because the mothers in this study were mostly farmworkers or lived with farmworkers, and because we previously found associations of between respiratory symptoms, spirometry, and the sum of urinary concentrations of dialkyl phosphate metabolites (DAPs) (Raanan et al. 2016), which reflect exposure to organophosphate pesticides, and measures of elemental sulfur sprayed near participants' homes (Raanan et al. 2017), we chose to include these two additional covariates in sensitivity analyses for probable asthma and spirometry. We also conducted sensitivity analyses stratified by sex.

Descriptive statistics and regressions were conducted in Stata 14 (College Station, TX) and BMA analyses were conducted in R (Team 2013) (Vienna, Austria) with the BMS package (Zeugner 2011).

Results

As seen in Table 1, mothers were mostly under 30 (76%), living below 100% of the federal poverty index (62%), and multiparous (61%). Most mothers did not smoke during pregnancy (96%) and were not around a smoker during pregnancy (89%), and most of their homes during pregnancy did not have furry pets (98%) and housed more than one person per room (75%). Most children did not have a family history of asthma (90%) and more children were born in winter (28%) than in any other season.

All personal care product biomarkers were detected in over 95% of samples, with the exception of butyl paraben and of triclosan (Table 2); triclosan was detected in 84% of samples at the first pregnancy visit and 88% of samples at the second pregnancy visit. Correlations ranged from 0.90 (2,4-dichlorophenol and 2,5-dichlorophenol) to 0.09 (methyl paraben and triclosan) (Supplemental Figure 1). Compared with Mexican-American women aged 18–45 in NHANES' samples at the closest years to CHAMACOS data collection (1999–2000 for phthalates, 2003–2004 for other phenols, 2005–2006 for parabens), our study population had higher MEP, MP, and 2,4-DCP concentrations, but comparable concentrations of other chemicals, suggesting our population may differ in ways other than age and race (data not shown) (CDC 2018).

As reported by the child's mother, 22 children (6%) were taking medication for asthma at age seven, 54 (15%) had respiratory symptoms at age seven, and 70 children (16%) had received an asthma diagnosis from a doctor at any age. Of the 42 children who took the bronchodilator test, 10 (24%) improved by 12% or greater on any spirometry measurement. We categorized 37 children (11%) as having probable asthma, 87 (25%) as having inhalant allergies, and 23 (7%) as having eczema. The mean FEV1 volume was 1.8 liters (SD: 0.5), the mean FVC volume was 2.1 liters (SD: 0.6), FEV₁/FVC measurements had a mean of 0.9 (SD: 0.1), and the mean FEF_{25–75} volume was 2.5 liters (SD: 0.9). Descriptive information was similar when restricted to children with 2 or more valid maneuvers (data not shown). Th1:Th2 cell ratios increased with age, corresponding with an increase in Th1% with age and a stable Th2% (Supplemental Table S1).

Supplemental Table S2 shows the PIPs for each phthalate metabolite, paraben, and other phenol included in the BMA analysis. The top three ranked biomarkers for each outcome were included as covariates in fully adjusted regression models for those outcomes.

Tables 3, 4, and 5 show crude models, demographically adjusted models, and fully adjusted models. As shown in Table 3 in demographically adjusted models, prenatal urinary concentrations of triclosan were associated with increased odds of probable asthma (OR: 1.13, 95% CI: 0.99, 1.29) and concentrations of propyl paraben were associated with decreased odds of probable asthma (OR: 0.89, 95% CI: 0.78, 1.02), both at borderline significance. In fully adjusted models, prenatal urinary concentrations of propyl paraben (OR: 0.86, 95% CI: 0.74, 0.99) were associated with decreased odds of probable asthma. There were no significant associations with aeroallergies or eczema.

Table 4 shows that MEP concentrations were associated with lower FEF_{25–75}% (Percent difference: –3.10 liters/second, 95% CI: –5.84, –0.28) in demographically and fully adjusted

models. Results were similar in children with two or more forced expiratory maneuvers judged to be adequately performed by the physician reviewers, and when excluding children who were currently using asthma medication (data not shown).

In sensitivity analyses, we also included DAPs and elemental sulfur in models for probable asthma and spirometry because we have detected associations between them in previous papers. Models with these chemicals included yielded similar results (data not shown).

GEE results modeling cytokine associations across ages are shown in Table 5, as are p-values for interaction by age. In fully adjusted models, MiBP concentrations were associated with lower Th1:Th2 cell ratio (Percent difference: -5.28, 95% CI: -10.56, 0.31) at borderline significance. Methyl paraben concentrations were associated with lower Th1% in fully adjusted models (Percent difference: -3.35, 95% CI: -6.58, -0.02), and were associated with lower Th2%, but with borderline significance (Percent difference: -4.45, 95% CI: -8.77, 0.08). MnBP concentrations were associated with higher Th2% (Percent difference: 10.40, 95% CI: 3.37, 17.92). P-values for age interaction in GEE models indicate that results differed across ages for the associations between MnBP concentrations and Th1:Th2 cell ratio and Th2%. Table 6 shows associations between MnBP concentrations and these outcomes for individual ages (ages two, five, and seven). The strong age two association with a lower Th1:Th2 cell ratio (Percent difference: -19.54, 95% CI: -28.26, -9.76) is not apparent at ages five and seven. There is also a strong association between MnBP concentrations and higher Th2% at ages two (Percent difference: 16.97, 95% CI: 6.33, 28.67) and five (Percent difference: 20.75, 95% CI: 7.25, 35.95), but not at age seven.

In sensitivity analyses of fully adjusted models stratified by sex (Supplemental Tables S3-5), boys only showed an association between MEP concentrations and lowered odds of probable asthma and in girls only triclosan concentrations were associated with an increased odds of probable asthma. The nonstratified association between MEP concentrations and lower FEF_{25-75%} persisted in girls only. Boys only showed an association between MEP concentrations and a higher Th1:Th2 cell ratio, as well as associations between triclosan and MEP concentrations and higher Th1%, and an association between MnBP concentrations and higher Th2%. The nonstratified associations between MP and lower Th1% and Th2% did not persist.

Sensitivity analyses removing the 81 women with imputed specific gravity yielded similar results (data not shown). Sensitivity analyses including the top five and seven biomarkers ranked by PIP value showed similar results (data not shown).

Discussion

Though most of our results were null, we did find some consistent associations. The findings that show the most consistency across models are the association between MEP concentrations and lower FEF_{25-75%}, the association between MnBP concentrations and higher Th2%, the association between MP concentrations and lower Th1% and Th2%, and the association between PP concentrations and lower odds of probable asthma.

In contrast to previous studies, we did not find an association between biomarkers of low molecular weight phthalates and atopic diseases, although we did show an association with an atopic cytokine profile. In contrast to our findings, the Columbia Center for Children's Environmental Health study found that prenatal urinary concentrations of MnBP were associated with asthma at ages 5–11 (Whyatt et al. 2014). However, in accordance with our findings, other longitudinal prenatal studies found no association between *in utero* exposures to low molecular weight phthalates and risk for asthma: the Spanish Infancia y Medio Ambiente-Sabadell study of 657 children ages six months to seven years (Gascon et al. 2015), the Polish Mother and Child Cohort study of 144 children age two (Stelmach et al. 2015), the U.S. Mount Sinai Children's Environmental Health study of 164 children ages six and seven (Buckley et al. 2018), and a French cohort study of 587 children age five (Vernet et al. 2017).

To our knowledge, no previous studies have examined the relationship between low molecular weight phthalates and FEF_{25–75%} or FEV₁/FVC. However, one cross-sectional study of 3,147 6–79 year olds analyzed low molecular weight phthalates in relation to FEV₁ and FVC and, unlike our study, they did not find an association with MEP but did find an association between MnBP and lower FEV₁ and FVC (Cakmak et al. 2014).

The Lifestyle and environmental factors and their Influence on Newborns Allergy (LINA) risk study, a longitudinal study of 610 children, found prenatal MiBP concentrations were associated with dermatitis at age three (Herberth et al. 2017), and a French cohort study found prenatal MEP and MiBP were associated with eczema in 604 children (Soomro et al. 2018). However, several other longitudinal studies, like ours, did not find an association between prenatal exposures to low molecular phthalates and aeroallergies or eczema (Gascon et al. 2015; Herberth et al. 2017; Stelmach et al. 2015; Wang et al. 2014), or found null results in addition to their positive results (Herberth et al. 2017). Additionally, in contrast to our findings, two previous studies of exposures to low molecular weight phthalates found no associations with cytokine concentrations: The Canadian Maternal-Infant Research on Environmental Chemicals study measured urinary biomarkers of phthalates and of cytokines in the cord blood of 1,121 infants (Ashley-Martin et al. 2015), and the Puerto Rico Testsite for Exploring Contamination Threats study measured urinary biomarkers of phthalates and of cytokines in the plasma of 120 pregnant women (Ferguson et al. 2014). However, these studies were cross-sectional and did not measure cytokines past infancy. Our study was in agreement with animal studies that show DBP exposure leads to increased Th2 cytokines (Li et al. 2014; Shigeno et al. 2009), but did not affect lung function (Chen et al. 2015). Overall, our findings confirm those of many previous longitudinal studies suggesting that exposures to low molecular weight phthalates are not associated with atopic diseases, but our Th1:Th2 cell findings suggest there may still be an increased risk for immune dysregulation.

Only one other study has examined the relationship between parabens and cytokine levels, and found no association between urinary methyl, propyl, or butyl paraben concentrations and the Th2 cytokines IL-1, IL-6, and TNF- α nor the Th1 cytokine IL-10 in a study of 106 pregnant women (Watkins et al. 2015). This study was cross-sectional and examined cytokines in the pregnant women only and it is therefore difficult to compare to our findings

of an association between prenatal MP concentrations and lower Th1% and higher Th2% cells in childhood.

Our unexpected finding that propyl paraben concentrations were associated with lowered odds of probable asthma is in contrast to a study that found prenatal paraben concentrations were associated with asthma and wheeze in early childhood (Vernet et al. 2017), and to a study that found null results (Lee-Sarwar et al. 2017). Taken together with these other studies, our results suggest that *in utero* exposures to parabens have inconsistent associations with components of atopic diseases in childhood, possibly due to differences in asthma classification, geographic areas, ages, and racial/ethnic makeup of the populations studied.

Our sex-specific results tended to show adverse associations in girls only, though findings were not consistent. However, the population size of the stratified models was substantially lower and may affect the ability to detect associations, and the validity of those detected. Previous studies have also found sex-specific results: a study of 171 children that found prenatal urinary MEP concentrations were related to increased asthma risk at age eight in boys only (Ku et al. 2015). In 182 children ages 6–12, DBP in house dust was associated with increased risk for rhinoconjunctivitis in girls only (Bamai et al. 2016).

Many of the biomarkers quantified in this study represent co-exposures in personal care products. For example, a single product may have artificial scents, preservatives, and sunblock, containing phthalates, parabens, and benzophenone-3 at once. Exploring associations of one biomarker of these chemicals at a time does not accurately model people's exposures. One strength of our study is we implemented BMA to identify additional biomarkers measured in this population that may confound relationships between other biomarkers and atopic outcomes. This has allowed us to include the most relevant potentially confounding biomarkers in the model and isolate the associations that one chemical may have with an atopic outcome to a greater extent than previous studies.

Other strengths of our study include that we followed children from before birth until age seven because *in utero* chemical exposure, a time of respiratory and immune development, may be more influential than later exposure in relation to health outcomes (Duijts 2012; Pinkerton and Joad 2000; Warner 2004). At age seven, the first age we were able to obtain reliable spirometry, we performed a thorough assessment of respiratory symptoms. In addition to assessing aeroallergies and eczema, we used several sources of data to classify children as having probable asthma, including a bronchodilator test. We required children to meet two or more of the criteria we used, or to currently be using asthma medication, in order to eliminate those who may have respiratory symptoms that are not related to asthma and those who may have resolved asthma that was diagnosed at an earlier age. Another strength of our study is that to further characterize atopic immune status in childhood, we measured Th1 and Th2 cells throughout this period of early childhood to analyze relationships with the rapidly developing immune system. Our method of measuring Th1 and Th2 concentrations identified the number of cells that predominantly produced Th1 and Th2 cytokines, unlike typical methods that measure concentrations of cytokines in the blood.

A limitation of our study, however, is that while aeroallergies and eczema are both atopic diseases, we were not able to determine if cases of probable asthma were atopic or of another etiology. Nonatopic childhood asthma can be related to exposure to air pollution, exercise-induced stress, obesity, tobacco smoke, or respiratory infections (Noutsios and Floros 2014). A significant percentage of asthmatic patients are non-atopic (Romanet-Manent et al. 2002). Another limitation is that the concentrations of biomarkers studied here all have relatively low intraclass correlation coefficients (low molecular weight phthalates range from 0.19 to 0.39, parabens range from 0.41 to 0.46, and the other phenols range from 0.46 to 0.56, calculated from the current cohort between the first and second pregnancy measures [over a median of 13 weeks]), which demonstrates relatively high variability in exposures. While we do have two urinary biomarker concentration measurements during pregnancy, even two measures may not adequately capture representative exposure to chemicals whose biomarkers show high temporal variability. Another limitation is that BMA does not account well for multicollinearity. Although few of the chemicals we analyzed were highly correlated (namely the dichlorophenols), several of them are often found in the same product or may be metabolites of the same parent compound and BMA is not able to account for this in its analysis. Also of note is the differing number of chemical covariates included in our models. We chose to include in regressions the three chemicals with the highest PIPs for each outcome. Because of this, most models are adjusted for three chemical covariates but some are only adjusted for two (for example, the regression of FEV₁ on MEP is adjusted only for MCOP and MnBP because MEP was itself one of the chosen covariates). This varying precision should be taken into consideration when interpreting our results. Additionally, we have chosen not to control for multiple comparisons (Rothman 1990), and caution the interpretation of our results.

In addition to our null results, our study found that prenatal maternal urinary concentrations of biomarkers of low molecular weight phthalates often found in personal care products are associated with increased Th2% and lower lung function measurements, and that *in utero* exposures to parabens are associated with lower Th1% and Th2% and are unexpectedly associated with decreased odds of probable asthma. This is the first study to evaluate the association between *in utero* exposure to prenatal personal care product chemicals and childhood cytokine expression, and the first study to examine the association between prenatal maternal urinary paraben concentrations and childhood cytokine levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- LMW phthalates were related to more Th2 producing cells and lower lung function
- No biomarkers were associated with increased odds of asthma, allergies, or eczema
- Methyl paraben was associated with fewer Th1 producing cells
- Bayesian Model Averaging was used to control for multiple chemical exposure

Table 1.

Demographic characteristics of the study population, CHAMACOS Study, Salinas, CA

Characteristics at time of pregnancy	N	(%)
Maternal age		
18–24	174	(44.7)
25–29	123	(31.6)
30–34	62	(15.9)
35+	30	(7.7)
Household income as a proportion of poverty		
<100%	240	(61.2)
>100%	152	(38.8)
Maternal education		
6th grade or less	174	(44.7)
7th–12th grade	136	(35.0)
High school graduate or greater	79	(20.3)
Maternal country of birth		
United States	48	(12.3)
Mexico	334	(85.9)
Other	7	(1.8)
Years mother has lived in the United States		
Five or fewer	195	(50.1)
Six to ten	96	(24.7)
Eleven or more	101	(26.0)
Parity		
First child	126	(32.4)
Second child	117	(30.1)
Third child or greater	146	(37.5)
Child's mother, father, or siblings have asthma history		
No	354	(90.3)
Yes	38	(9.7)
Housing density (number of people per number of rooms)		
<=0.5	6	(1.6)
0.51–1.00	86	(23.6)
1.01–1.50	138	(37.8)
>=1.51	135	(37.0)
Furry pets in home during pregnancy		
Yes	9	(2.3)
No	379	(97.7)

Characteristics at time of pregnancy	N	(%)
Smoked at all during pregnancy		
Yes	15	(3.8)
No	377	(96.2)
Number of hours per day around a smoker during pregnancy		
0	343	(88.6)
0.5	32	(8.3)
1–2	6	(1.6)
3+	6	(1.6)
Season of birth		
Winter	111	(28.4)
Spring	104	(26.6)
Summer	84	(21.5)
Fall	92	(23.5)

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Table 2.

Distribution of specific gravity corrected biomarkers in maternal prenatal urine collected at two time points in pregnancy

Biomarker	N	% > LOD		% > Highest calibration standard		Average of two measurements								
		Early Pregnancy	Late Pregnancy	Early Pregnancy ¹	Late Pregnancy ²	Early Pregnancy ¹	Late Pregnancy ²	10th%	25th%	50th%	75th%	90th%	95th%	
MEP (ng/mL)	361	100.0%	347	100.0%	99.7%	0.0%	0.0%	233.3	46.5	108.5	223.6	490.2	934.7	1574.2
MnBP (ng/mL)	361	99.4%	347	99.4%	100.0%	0.0%	0.0%	27.8	9.5	15.9	26.4	47.5	91.3	119.5
MtBP (ng/mL)	361	95.2%	347	95.2%	97.5%	0.0%	0.0%	3.4	1.1	1.9	3.3	6.1	10.9	16.3
MP (ug/mL)	381	100.0%	364	100.0%	100.0%	0.3%	3.6%	152.8	29.5	75.5	168.9	365.0	631.3	767.1
PP (ug/mL)	381	98.5%	351	98.5%	98.7%	0.8%	9.4%	39.6	2.6	11.6	48.0	166.4	446.5	636.6
BP (ug/mL)	381	66.5%	364	66.5%	71.0%	0.0%	0.0%	0.5	0.1	0.1	0.3	1.7	12.6	23.5
TCS (ug/mL)	381	83.5%	364	83.5%	88.1%	2.4%	1.4%	22.4	1.6	6.0	20.8	112.1	363.3	593.2
2,4-DCP (ug/mL)	381	100.0%	364	100.0%	100.0%	2.6%	4.7%	5.8	1.5	2.3	3.8	14.6	47.8	69.3
2,5-DCP (ug/mL)	377	99.7%	363	99.7%	100.0%	11.1%	11.9%	74.5	6.4	16.3	68.4	451.3	859.9	1109.1
BP3 (ug/mL)	376	99.7%	363	99.7%	99.5%	6.7%	7.2%	27.1	2.5	5.2	18.3	152.1	564.8	944.4

¹Mean = 13 weeks gestation

²Mean = 26 weeks gestation

LOD = Limit of detection

Table 3. Association of log₂ prenatal urinary biomarker concentrations and asthma outcomes

Biomarker	Probable asthma			Aerovallergies			Eczema		
	Crude OR (95% CI)	Demographically adjusted ¹ OR (95% CI)	Fully adjusted ² OR (95% CI)	Crude OR (95% CI)	Demographically adjusted ¹ OR (95% CI)	Fully adjusted ³ OR (95% CI)	Crude OR (95% CI)	Demographically adjusted ¹ OR (95% CI)	Fully adjusted ⁴ OR (95% CI)
MEP	n=336-346 0.89 (0.73, 1.10)	n=297-307 0.87 (0.69, 1.10)	n=292-294 0.88 (0.69, 1.14)	n=327-334 1.10 (0.95, 1.27)	n=296-303 1.10 (0.94, 1.29)	n=291-293 1.05 (0.89, 1.23)	n=326-331 1.01 (0.78, 1.31)	n=283-290 0.94 (0.71, 1.26)	n=279-283 1.01 (0.75, 1.37)
MnBP	1.01 (0.76, 1.33)	1.06 (0.78, 1.44)	0.93 (0.65, 1.32)	1.08 (0.88, 1.32)	1.09 (0.87, 1.35)	0.88 (0.68, 1.15)	0.79 (0.55, 1.13)	0.72 (0.48, 1.07)	0.90 (0.56, 1.45)
MIBP	1.06 (0.83, 1.35)	1.11 (0.86, 1.45)	1.02 (0.77, 1.36)	1.03 (0.87, 1.23)	1.03 (0.85, 1.26)	0.90 (0.72, 1.12)	0.81 (0.60, 1.10)	0.72 (0.51, 1.02) [#]	0.80 (0.53, 1.19)
MP	0.94 (0.77, 1.15)	0.96 (0.77, 1.20)	1.17 (0.85, 1.60)	1.06 (0.91, 1.23)	1.03 (0.88, 1.21)	1.00 (0.84, 1.18)	1.04 (0.80, 1.35)	0.97 (0.74, 1.27)	1.04 (0.77, 1.40)
PP	0.89 (0.79, 1.01) [#]	0.89 (0.78, 1.02) [#]	0.86 (0.74, 0.99) [*]	1.02 (0.93, 1.11)	0.99 (0.90, 1.09)	0.96 (0.87, 1.07)	1.01 (0.86, 1.18)	0.98 (0.83, 1.15)	0.98 (0.82, 1.17)
TCS	1.14 (1.01, 1.29) [*]	1.15 (0.99, 1.29) [#]	1.10 (0.95, 1.27)	1.00 (0.92, 1.08)	0.97 (0.88, 1.06)	0.96 (0.87, 1.06)	1.10 (0.95, 1.28)	1.06 (0.90, 1.24)	1.08 (0.92, 1.28)
2,4-DCP	1.10 (0.92, 1.32)	1.12 (0.92, 1.37)	1.10 (0.89, 1.36)	1.01 (0.88, 1.15)	1.03 (0.89, 1.20)	1.00 (0.86, 1.16)	0.98 (0.77, 1.25)	0.95 (0.73, 1.24)	1.02 (0.78, 1.34)
2,5-DCP	0.98 (0.86, 1.12)	1.00 (0.86, 1.15)	0.98 (0.84, 1.14)	0.99 (0.90, 1.09)	0.99 (0.89, 1.10)	0.97 (0.87, 1.08)	0.94 (0.80, 1.11)	0.93 (0.77, 1.11)	0.97 (0.81, 1.17)
BPS	1.02 (0.91, 1.15)	1.01 (0.89, 1.15)	1.03 (0.90, 1.19)	1.04 (0.95, 1.13)	1.07 (0.97, 1.17)	1.05 (0.95, 1.16)	1.02 (0.88, 1.18)	1.01 (0.87, 1.18)	1.04 (0.89, 1.22)

[#] p < 0.1;
^{*} p < 0.05;
^{**} p < 0.01

Separate models created for each biomarker

- ¹ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, and housing density during pregnancy
- ² Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, monocarboxyisooctyl phthalate, propyl paraben, monocarboxyisononyl phthalate
- ³ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono(3-carboxypropyl) phthalate, monocarboxyisooctyl phthalate, bisphenol A
- ⁴ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-isobutyl phthalate, mono-isobutyl phthalate, mono-n-butyl phthalate

Table 4. Association of log₂ prenatal urinary biomarker concentrations and spirometry outcomes

Biomarker	FEV ₁			FVC		
	Crude β (95% CI) n=294–296	Demographically adjusted ¹ β (95% CI) n=261–267	Fully adjusted ² β (95% CI) n=257–261	Crude β (95% CI) n=260–266	Demographically adjusted ¹ β (95% CI) n=237–243	Fully adjusted ³ β (95% CI) n=233–237
MEP	-0.03 (-0.06, 0.00) [#]	-0.03 (-0.06, 0.01)	-0.02 (-0.06, 0.02)	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.02)	-0.02 (-0.06, 0.02)
MnBP	0.02 (-0.03, 0.06)	0.01 (-0.03, 0.06)	0.05 (-0.01, 0.10)	0.01 (-0.04, 0.07)	0.01 (-0.04, 0.07)	0.02 (-0.05, 0.09)
MiBP	0.01 (-0.03, 0.05)	0.01 (-0.04, 0.05)	0.03 (-0.02, 0.08)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.07)	0.03 (-0.03, 0.08)
MP	0.00 (-0.03, 0.03)	-0.01 (-0.04, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)	0.00 (-0.04, 0.05)
PP	0.01 (-0.01, 0.03)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)
TCS	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.03)
2,4-DCP	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.03)	-0.03 (-0.07, 0.00) [#]	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.02)
2,5-DCP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	-0.01 (-0.04, 0.01)	-0.01 (-0.03, 0.02)	0.00 (-0.03, 0.02)
BP3	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.03)

Biomarker	FEV ₁ /FVC			FEF _{25–75%}		
	Crude Percent difference (95% CI) n=260–266	Demographically adjusted ¹ Percent difference (95% CI) n=237–243	Fully adjusted ⁴ Percent difference (95% CI) n=233–235	Crude Percent difference (95% CI) n=260–266	Demographically adjusted ¹ Percent difference (95% CI) n=237–243	Fully adjusted ⁵ Percent difference (95% CI) n=233–235
MEP	-0.29 (-0.78, 0.21)	-0.23 (-0.72, 0.26)	-0.21 (-0.72, 0.30)	-3.68 (-6.32, -0.96) ^{**}	-3.32 (-6.11, -0.44) [*]	-3.22 (-6.02, -0.34) [*]
MnBP	0.23 (-0.44, 0.90)	0.37 (-0.30, 1.04)	0.77 (-0.01, 1.56) [#]	0.02 (-3.74, 3.92)	0.15 (-3.83, 4.29)	2.96 (-1.77, 7.92)
MiBP	0.16 (-0.42, 0.75)	0.21 (-0.38, 0.81)	0.20 (-0.51, 0.91)	0.68 (-2.62, 4.08)	0.34 (-3.19, 4.00)	1.46 (-2.31, 5.38)
MP	-0.09 (-0.57, 0.40)	-0.03 (-0.51, 0.45)	-0.02 (-0.51, 0.47)	-0.13 (-2.83, 2.64)	-0.72 (-3.54, 2.19)	1.41 (-1.82, 4.74)
pp	-0.06 (-0.36, 0.24)	-0.13 (-0.42, 0.17)	-0.07 (-0.39, 0.24)	-0.20 (-1.89, 1.52)	-0.71 (-2.47, 1.09)	0.17 (-1.78, 2.16)
TCS	0.04 (-0.25, 0.33)	-0.07 (-0.37, 0.22)	-0.08 (-0.38, 0.22)	0.99 (-0.65, 2.66)	0.58 (-1.19, 2.38)	1.08 (-0.71, 2.90)
2,4-DCP	0.15 (-0.30, 0.61)	0.00 (-0.45, 0.44)	-0.09 (-0.54, 0.37)	-0.93 (-3.44, 1.64)	-0.91 (-3.52, 1.78)	-0.73 (-3.38, 1.99)
2,5-DCP	0.00 (-0.32, 0.31)	-0.10 (-0.41, 0.21)	-0.18 (-0.49, 0.14)	-0.61 (-2.35, 1.17)	-0.65 (-2.49, 1.23)	-0.55 (-2.41, 1.33)
BP3	-0.03 (-0.31, 0.25)	0.10 (-0.18, 0.38)	0.08 (-0.22, 0.37)	-0.49 (-2.05, 1.09)	-0.16 (-1.83, 1.54)	0.40 (-1.33, 2.17)

- # p<0.1;
- * p<0.05;
- ** p<0.01

Separate models created for each biomarker

- 1 Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, and housing density during pregnancy
- 2 Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, monocarboxyisooctyl phthalate, the sum of di(2-ethylhexyl) phthalate metabolites, monobenzyl phthalate
- 3 Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, monocarboxyisooctyl phthalate, mono-isobutyl phthalate, the sum of di(2-ethylhexyl) phthalate metabolites
- 4 Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-n-butyl phthalate, 2,5 dichlorophenol, monobenzyl phthalate [Regressions of 2,4-dichlorophenol did not control for 2,5-dichlorophenol due to high collinearity.]
- 5 Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, monocarboxyisooctyl phthalate, monoethyl phthalate, the sum of di(2-ethylhexyl) phthalate metabolites

Longitudinal association of log 2 prenatal urinary biomarker concentrations and Th cell parameters at various ages, with p-values for age interaction

Table 5.

Biomarker	The:Th2 cell ratio				Th1%				Th2%			
	Crude Percent difference (95% CI)	Demographically adjusted Percent difference (95% CI)	Fully adjusted ² Percent difference (95% CI)	p-int	Crude Percent difference (95% CI)	Demographically adjusted Percent difference (95% CI)	Fully adjusted ³ Percent difference (95% CI)	p-int	Crude Percent difference (95% CI)	Demographically adjusted Percent difference (95% CI)	Fully adjusted ⁴ Percent difference (95% CI)	p-int
MEP	n=341-362 3.18 (-1.59, 8.17)	n=309-329 2.25 (-2.38, 7.10)	n=304-306 3.48 (-1.35, 8.54)	0.37	n=341-362 -0.07 (-3.53, 3.52)	n=309-329 0.06 (-3.49, 3.73)	n=304-306 1.76 (-1.97, 5.63)	0.16	n=341-362 -3.21 (-7.25, 1.00)	n=309-329 -2.17 (-6.16, 1.99)	n=304-306 -2.36 (-6.90, 2.41)	0.96
MnBP	n=341-362 -5.58 (-11.40, 0.63) [#]	n=309-329 -5.75 (-11.90, 0.82) [#]	n=304-306 -2.92 (-10.16, 4.90)	0.01	n=341-362 0.83 (-4.70, 6.69)	n=309-329 2.53 (-3.10, 8.49)	n=304-306 5.89 (-0.90, 13.15) [#]	0.29	n=341-362 6.54 (0.53, 12.91) [*]	n=309-329 8.50 (1.90, 15.52) [*]	n=304-306 10.40 (3.37, 17.92) ^{**}	0.02
MIBP	n=341-362 -6.70 (-11.58, -1.54) [*]	n=309-329 -6.33 (-11.13, -1.27) [*]	n=304-306 -5.28 (-10.56, 0.31) [#]	0.31	n=341-362 -2.82 (-7.01, 1.55)	n=309-329 -1.01 (-4.64, 2.76)	n=304-306 0.38 (-3.73, 4.66)	0.89	n=341-362 4.14 (-0.94, 9.49)	n=309-329 5.71 (0.55, 11.12) [*]	n=304-306 2.50 (-2.96, 8.25)	0.51
MP	n=341-362 1.07 (-3.17, 5.50)	n=309-329 1.45 (-2.81, 5.91)	n=304-306 1.85 (-2.31, 6.19)	0.80	n=341-362 -4.13 (-7.32, -0.82) [*]	n=309-329 -3.54 (-6.78, -0.18) [*]	n=304-306 -3.35 (-6.58, -0.02) [*]	0.32	n=341-362 -5.17 (-8.96, -1.23) [*]	n=309-329 -4.84 (-8.76, -0.74) [*]	n=304-306 -4.45 (-8.77, 0.08) [#]	0.62
PP	n=341-362 -1.01 (-3.73, 1.79)	n=309-329 -0.22 (-3.01, 2.65)	n=304-306 0.18 (-2.74, 3.19)	0.49	n=341-362 -1.76 (-3.83, 0.36)	n=309-329 -0.59 (-2.56, 1.41)	n=304-306 1.11 (-1.70, 3.99)	0.39	n=341-362 -0.76 (-3.14, 1.67)	n=309-329 -0.32 (-2.98, 2.41)	n=304-306 2.17 (-1.79, 6.28)	0.65
TCS	n=341-362 1.51 (-1.24, 4.33)	n=309-329 1.59 (-1.22, 4.47)	n=304-306 2.33 (-0.56, 5.30)	0.79	n=341-362 0.16 (-1.97, 2.33)	n=309-329 0.97 (-1.16, 3.14)	n=304-306 0.96 (-1.22, 3.20)	0.96	n=341-362 -1.45 (-3.88, 1.05)	n=309-329 -0.76 (-3.32, 1.86)	n=304-306 -1.39 (-3.96, 1.24)	0.64
2,4-DCP	n=341-362 1.61 (-2.79, 6.21)	n=309-329 0.37 (-3.83, 4.74)	n=304-306 0.29 (-4.01, 4.80)	0.97	n=341-362 2.98 (-0.29, 6.37) [#]	n=309-329 2.27 (-0.96, 5.61)	n=304-306 2.90 (-0.38, 6.28) [#]	0.31	n=341-362 1.32 (-2.56, 5.36)	n=309-329 1.90 (-2.04, 6.00)	n=304-306 2.17 (-1.83, 6.33)	0.28
2,5-DCP	n=341-362 0.18 (-2.91, 3.37)	n=309-329 -0.59 (-3.58, 2.49)	n=304-306 -0.26 (-3.34, 2.91)	0.75	n=341-362 1.90 (-0.42, 4.26)	n=309-329 1.15 (-1.02, 3.37)	n=304-306 1.51 (-0.66, 3.72)	0.11	n=341-362 1.65 (-1.04, 4.42)	n=309-329 1.70 (-1.08, 4.56)	n=304-306 1.54 (-1.31, 4.47)	0.48
BP3	n=341-362 -0.79 (-3.24, 1.71)	n=309-329 -1.19 (-3.79, 1.48)	n=304-306 -1.41 (-4.08, 1.33)	0.85	n=341-362 -1.38 (-3.30, 0.57)	n=309-329 -1.34 (-3.25, 0.61)	n=304-306 -0.55 (-2.59, 1.52)	0.27	n=341-362 -0.58 (-2.88, 1.79)	n=309-329 -0.09 (-2.56, 2.44)	n=304-306 0.50 (-2.17, 3.23)	0.43

[#] p < 0.1;

^{*} p < 0.05;

^{**} p < 0.01

Separate models created for each biomarker

¹ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, and housing density during pregnancy

² Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, and child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-isobutyl phthalate, mono-n-butyl phthalate, triclosan

³ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, and child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, methyl paraben, the sum of di(2-ethylhexyl) phthalate m etabolites, 2,4-dichlorophenol [Regressions of 2,5-dichlorophenol did not control for 2,4-dichlorophenol due to high collinearity.]

⁴ Models control for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, and child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-n-butyl phthalate, methyl paraben, monoethyl phthalate

Table 6.

Association of log 2 prenatal mono-n-butyl phthalate concentrations and Th cell parameters by age

	Fully adjusted Percent change (95% CI)	
	Th1:Th2 cell ratio	Th2%
	n=194–223	n=194–223
Age two	-19.54 (-28.26, -9.76)** ¹	16.97 (6.33, 28.67)** ²
Age five	-6.61 (-18.19, 6.62) ³	20.75 (7.25, 35.95)** ⁴
Age seven	7.14 (-2.53, 17.77) ⁵	-0.97 (-9.58, 8.46) ⁶

¹ Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-n-butyl phthalate, monoethyl phthalate, monocarboxyisooctyl phthalate

² Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-n-butyl phthalate, benzophenone-3, 2,4-dichlorophenol

³ Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-isobutyl phthalate, triclosan, mono-n-butyl phthalate

⁴ Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, mono-n-butyl phthalate, triclosan, monocarboxyisononyl phthalate

⁵ Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, monocarboxyisooctyl phthalate, monocarboxyisononyl phthalate, mono-n-butyl phthalate

⁶ Model controls for maternal age, parity, season of birth, household income as a proportion of poverty at baseline, child's family history of asthma, active and passive smoking during pregnancy, furry pets in the home during pregnancy, housing density during pregnancy, methyl paraben, propyl paraben, 2,5-dichlorophenol