In utero exposure to personal care product and plasticizing chemicals and childhood immune dysfunction

By

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Abstract

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Recent studies suggest that phthalates, parabens, and other phenols found in personal care products and plastics may be related to immunologic diseases. This dissertation focuses on *in utero* exposure to these chemicals and the development of asthma, aeroallergies, and eczema in childhood, as well as lung function and relative concentrations of immune system biomarkers in childhood. We explored measurements of immune system functioning and atopic disease in children in the Center for the Health Assessment of Mothers and Children in Salinas (CHAMACOS) study, a prospective cohort of pregnant women and their children, from age six months to seven years and describes data collection and data consistency across ages. We determined classifications of cases of probable asthma, aeroallergies, and eczema at age seven in this population and found that, at age seven, 36 children out of 353 with relevant data (10%) had probable asthma, 87 children of 339 with relevant data (26%) had aeroallergies, and 23 children of 338 with relevant data (7%) had eczema. Lung function measurements were similar to national averages, and cytokine measurements changed with age in expected patterns.

We analyzed associations of prenatal urinary concentrations of high molecular weight phthalates and bisphenol A, commonly found in some plastics, with probable asthma, aeroallergies, eczema, and spirometry at age seven, and with blood cytokine measurements at ages two, five, and seven. Logistic and linear regressions were conducted for 392 children, and Bayesian Model Averaging (BMA) was used to identify a select number of additional personal care product and plasticizing chemicals to be controlled for in the models, in order to account for confounding by joint chemical exposure to all phthalates, parabens, and phenols studied in this dissertation. We found that concentrations of monocarboxyisooctyl phthalate (MCOP) were associated with increased odds of

having probable asthma (OR: 1.54, 95% CI: 1.12, 2.12) and poorer lung function (RR for forced expiratory volume in one second [FEV_1]: -0.09, 95% CI: -0.15, -0.03; RR for forced expiratory flow from 25–75% of FVC [$FEF_{25-75\%}$]: -6.98, 95% CI: -10.95, -2.84). MCOP (OR: 1.28, 95% CI: 1.02, 1.62), MCPP (OR: 1.36, 95% CI: 1.07, 1.73), and BPA (OR: 1.35, 95% CI: 1.06, 1.73) were associated with aeroallergies, in crude models and models adjusting for demographic factors, but not in models adjusting further for additional chemical concentrations.

We also examined associations between prenatal urinary concentrations of low molecular weight phthalates, parabens, triclosan, 2,4-dichlorophenol, 2,5-dichlorophenol, and benzophenone-3, all commonly found in personal care products, and the outcomes described above. Similar methods were used, including selection of additional chemical covariates using BMA. We found that concentrations of propyl paraben (OR: 0.84, 95% CI: 0.73, 0.97) and 2,5-dichlorophenol (OR: 0.63, 95% CI: 0.47, 0.86) were associated with decreased odds of having probable asthma. We also found that concentrations of monoethyl phthalate (MEP) were associated with lower spirometry measurements (RR for FEV_1: -0.03, 95% CI: -0.07, 0.00; RR for $FEF_{25-75\%}$: -3.23, 95% CI: -5.87, -0.52), but that concentrations of mono-n-butyl phthalate (MBP) were associated with higher spirometry measurements (RR for FEV_1: 0.06, 95% CI: 0.01, 0.11; RR for $FEF_{25-75\%}$: 4.29, 95% CI: 0.04, 8.71). We also found that MBP concentrations were longitudinally associated with higher T helper cell 2 percentage (Th2%) across ages two, five, and seven (RR: 8.40, 95% CI: 1.95, 15.26) and that mono-isobutyl phthalate (MiBP) concentrations were longitudinally associated with lower T helper 1:T helper 2 cell ratio (RR: -6.19, 95% CI: -11.70, -0.32).

We explored novel methods to analyze joint exposure to the chemicals studied in this dissertation, as people are typically exposed to mixtures of chemicals, rather than to a single chemical. We chose to analyze probable asthma and allergies, but not eczema, with these methods because eczema showed no associations in our regression analyses above. We also included only FEV_1 out of the four spirometry measures, though results for other spirometry measures were similar. We chose not to include cytokine measurements because associations in the above analyses were not consistent and would likely not yield results useful for the demonstration of these new methods. Bayesian Profile Regression was used to cluster children into groups based on their patterns of joint prenatal exposure to phthalates, parabens, and other phenols. Clusters were then evaluated for their relationship to probable asthma, aeroallergies, and FEV_1. We found that participants clustered into seven exposure groups, characterized by high and low levels of chemicals consistent with expected patterns of product use. However, these clusters were not related to the three immune outcomes. Bayesian Kernel Machine Regression (BKMR) was used to determine associations of each chemical, in the context of joint exposure to the other chemicals, with probable asthma, aeroallergies, and FEV_1. We found that BKMR associations were similar to those found via logistic and linear regressions, and that strong associations of some chemicals had additive effects on associations of other chemicals.

We found evidence that *in utero* urinary concentrations of high molecular weight phthalates appear to be associated with asthma and adverse respiratory function in childhood, and possibly with aeroallergies in childhood. *In utero* urinary concentrations of low molecular weight phthalates appear to be associated with altered cytokine levels in early childhood, and concentrations of parabens and other phenols show limited or no association with respiratory or immune function in childhood.

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Chapter 1: Background and significance

Rates of asthma, inhalant allergy, and eczema have been steadily increasing in children since the 1960s[1]. The prevalence of childhood asthma in the U.S. increased from 3.6% to 7.5% to 9.1% from 1980 to 1995 to 2007[2]. In the 2013-2014 National Health and Nutrition Examination Survey (NHANES), 15.9% of children had ever been diagnosed with asthma[3]. Inhalant allergy prevalence in U.S. children increased from 13.4% to 19.1% from 1995 to 2003[4], and 24.6% of children had ever been diagnosed with aeroallergies in the 2005-2006 NHANES[5]. From 1997 to 2011, eczema in U.S. children increased from 7.4% to 12.5%[6], and 14.3% of children had ever been diagnosed with eczema in the 2005-2006 NHANES[5].

There are many factors that could be contributing to the increases in these largely atopic diseases. Respiratory infections, dietary deficiencies, damp or moldy housing, air conditioning, and increased hygiene have all been associated with increases in asthma or inhalant allergies in recent decades[7,8]. Environmental chemicals may also be causing increases in these diseases. Air pollution, environmental tobacco smoke, heavy metals, and pesticides have been hypothesized as affecting asthma or inhalant allergies[7-12]. Studies in animals and humans suggest that exposure to certain environmental chemicals found in personal care products and plastics, such as phthalates, parabens, and phenols, may also be responsible for some of the increased incidence of disease[13,14].

Characteristics of phthalates, parabens, and phenols used in personal care products and plastics

Personal care product chemicals

Low molecular weight phthalates are used in fragrances, cosmetics, and medications[15,16], and include diethyl phthalate (DEP), dibutyl phthalate (DBP), and diisobutyl phthalate (DiBP). These chemicals are short-branched diesters of phthalic acid, and are non-persistent and rapidly metabolized by hydrolysis[17]. Contrary to most chemicals, when a diester phthalate is hydrolyzed into a monoester phthalate metabolite it becomes more bioactive[17]. The monoester metabolites are normally excreted in urine[17].

Methyl paraben (MP), propyl paraben (PP), and butyl paraben (BP) are used as preservatives in cosmetics, pharmaceuticals, and paper products for their bactericidal and fungicidal properties[18,19]. Parabens are alkyl esters of p-hydroxybenzoic acid that are well absorbed into the body, but not always metabolized[20]. The main hydrolysis metabolite of all three parabens is p-hydroxybenzoic acid, which is excreted along with the parent compounds in urine[21].

Triclosan is a readily absorbed polychlorinated binuclear aromatic compound that is metabolized by conjugation and excreted in urine and feces along with metabolites[22,23]. It is an antibacterial agent found in Colgate Total toothpaste, deodorants, and antimicrobial fabrics[24]. Triclosan was recently banned from antibacterial soaps for not being generally recognized as safe and effective[25].

2,4-dichlorophenol (2,4-DCP) is a chlorinated phenol derivative found as an intermediate in pesticide manufacturing and as a photo-degradation product of triclosan[26]. 2,4-DCP is excreted in urine largely without being metabolized[27]. 2,5-dichlorophenol (2,5-DCP) is also a chlorinated phenol derivative and is an isomer of 2,4-DCP. It is the main hydroxylated metabolite of 1,4-dichlorobenzene[28], which is used in moth balls and room and toilet deodorizers[29], and is also excreted in urine[28].

Benzophenone-3 (BP-3), also known as oxybenzone, is an aromatic ketone that metabolizes into several compounds, all of which are excreted in urine[28,30]. It absorbs ultraviolet rays A and B and is used in sunscreens and other products for skin protection, and in cosmetics to prolong product durability[31].

Plasticizing phthalates and BPA

High molecular weight phthalates, such as benzylbutyl phthalate (BBzP), di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), and di(2-ethylhexyl) phthalate (DEHP), are widely used as plasticizers in food packages, building materials, children's toys, medical devices, vinyl flooring, and paints[32,33]. These chemicals differ from low molecular weight phthalates because they are long-branched, with several more carbon atoms in their backbone[17]. These phthalates are also non-persistent and hydrolyzed by the body quickly, but they undergo additional hydroxylation and oxidation before being excreted in urine and feces[17].

Bisphenol A (BPA) is a chemical based on diphenylmethane with two functional hydroxyphenyl groups and an acetate reactant[34,35]. It is metabolized into several compounds by glucuronidation and sulfation, which are excreted along with free BPA in urine[36]. BPA is used in some plastics manufacturing and is found in food and water packaging, dental sealants, and certain receipts[37].

Exposure to these chemicals is nearly ubiquitous, with most phthalate metabolites detected in 90% or greater of 2013-2014 NHANES participants, and most phenols detected in 96% or greater of participants[3].

Characteristics of atopic immunity

Lymphocytes are subtypes of white blood cells that prevent illness by responding to abnormal cells (like cancer cells) and exogenous material (such as bacteria). T lymphocytes, or T cells, bind to cell antigens and can determine if a cell is unhealthy or foreign. If a cell is recognized as damaged or foreign, the T cell must notify other parts of the immune system so the cell can be destroyed. T helper cells are a type of T cell that regulates this communication to other immune cells. T helper cells release cytokines (chemical messenger proteins) to influence other immune cells' development, attract other immune cells, or signal a direction for other immune cells to go in[38,39]. Of the two main types of T helper cells, T helper 1 (Th1) cells release the cytokines interferon-gamma (IFN-y), interleukin 2 (IL-2), and IL-3 and are designed to combat intracellular viruses and bacteria through cell-mediated immunity[40], while T helper 2 (Th2) cells are involved in allergic and inflammatory responses, releasing cytokines such as IL-5 to attract eosinophils, IL-4 to influence antibodies produced by B cells, and also IL-6, IL-9, IL-13, and IL-25 cells, all of which are pro-inflammatory[40-42]. Il-1B is released by macrophages and IL-33 is released by a number of immune cells; both induce release of Th2 cytokines[43-45]. IL-12 is also released by a number of immune cells but stimulates Th1 cell production[46]. Another cytokine, tumor necrosis factor alpha (TNF-a), is involved in several pro-inflammatory immune responses that are associated with autoimmune diseases[47]. Additionally, thymic stromal lymphopoietin (TSLP) is involved in the production of regulatory T cells, which modulate T helper cells and other immune cells, and are associated with a number of atopic diseases, including allergic asthma[48,49].

Asthma is characterized by airway inflammation, which is caused by higher concentrations of Th2 cells, eosinophils, and leukocytes in the lungs[50], and by abnormal activity of Th2 cells[51] and mast cells in the lungs[52]. Asthma is also characterized by airway remodeling, which consists of an enlargement of airway smooth muscle, increased numbers of mucus-secreting cells in the airway

epithelium, and subepithelial fibrosis[50]. Together, these cause symptoms such as shortness of breath, chest tightness, wheeze, and cough. Asthma may result, in part, from activity of T helper cells[51,53]. For example, Th2 cytokines increase eosinophilic inflammation[54], and asthmatics have higher concentrations of Th2 cytokines in their lungs[51]. Th2 cytokines also potentiate mast cell degranulation, B cell activation with subsequent immunoglobulin E (IgE) production, and airway inflammation[55]. In addition, allergic asthma can develop when chemicals promote sensitization of T helper cells to an inhaled allergen: When T helper cells become sensitized to an allergen, Th2 cells stimulate B cells causing them to produce specific IgE antibodies, which in turn activate mast cells and basophils, leading to inflammation[56]. After T helper cells become specifically sensitized against the allergen, inhaling the allergen will then cause an inflammatory response in the nasal and lower airways[57], sometimes resulting in allergic asthma[56]. In non-allergic asthma, airway inflammation and remodeling still occur, but without production of specific IgE antibodies[50]. Allergic and non-allergic asthma have similar symptoms and are characterized by similar biological changes, such as increased immune cell concentrations, airway remodeling, and mucus hypersecretion[50].

There is some evidence that personal care product and plasticizing chemicals, such as phthalates, parabens, and other phenols, may drive the body towards atopy-prone immunity by promoting Th2 cell dominance, resulting in diseases such as allergic asthma, aeroallergies, and eczema, and may act on other biological mechanisms to result in non-allergic asthma.

In vitro **and animal studies on personal care product chemicals in relation to cytokine disruption and asthma-like lung changes**

Personal care product chemicals and cytokines

There is biological evidence that personal care product chemicals may modify immune system pathways involved in cytokine production. *In vitro* studies show DEP and DBP increase levels of Th2 cytokine IL-4 in animal lymph node cells[58]. DEP and DBP also increase gene expression of Th2 cytokine IL-1B and Th1 cytokine IFN-y in zebrafish embryos, suggesting involvement in both Th1 and Th2-related pathways[59]. Effects on T helper cells may also be explained by the influence of these chemicals on regulatory T cells, which modulate T helper cells. The Lifestyle and environmental factors and their Influence on Newborns Allergy risk (LINA) study, a longitudinal study of 610 infants, found that urinary levels of several low molecular weight phthalates during pregnancy were associated with lower levels of regulatory T cells in cord blood and blood at age two[60]. A benzophenone compound also supports Th2-dominant immunity by depleting glutathione in cultured animal antigen-presenting cells which inhibits their production of Th1 cytokines and amplifies their production of Th2 cytokines[61]. In contrast, benzophenone-3 inhibits IL-1B activity, demonstrating its promotion of Th1 action[62]. Triclosan may also promote Th2-dominant immunity by binding to the ryanodine receptor[63] which can cause an increase in T cell stimulation, as well as increases in major histocompatibility complex II molecule expression and dendritic cell stimulation, both of which are involved in inflammation[64,65]. Some low molecular weight phthalates[66] and triclosan[67] also promote TSLP expression, which can then activate inflammatory and Th2-related immune responses[68,69]. Several of the above pathways may involve activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)[70,71], which induces pro-inflammatory cytokine production and has been associated with autoimmune diseases[72]. NF-kB may act synergistically with estrogen receptor agonists, like low molecular weight phthalates or parabens, to stimulate a pro-inflammatory response in macrophages[73]. Low molecular weight phthalates and parabens are xenoestogens[74,75], and may act via estrogenic pathways to influence the immune

system. Estrogens have been shown to encourage the production of Th2 cytokines and other inflammatory biology, including altered antigen-presenting cell activity, increased IgE signaling, and mast cell degranulation[76]. In addition to these more specific pathways, personal care product chemicals have also shown involvement in other biomarkers of inflammation *in vitro*[77,78], in animals[67,79-82], and in humans[83-91]. This *in vitro* literature suggests that low molecular phthalates are associated with Th2 dominant immunity and that low molecular phthalates, benzophenone-3, and triclosan may otherwise disrupt cytokine behavior and inflammation-related immunity.

Personal care product chemicals may be associated with immune dysfunction in animals. Dermal exposure of mice to a 0.5% solution of an allergen and DBP leads to increases in Th2 cytokines IL-4, TNF-a, TSLP, as well as an increase in antigen-presenting cells compared to mice treated with an allergen only[66]. Similarly, dermal exposure of mice to an allergen and DBP at 0.4, 4, or 40 mg/kg leads to increases in Th2 cytokines IL-4, IL-5, IL-13, and IL-17a along with increases in TSLP and inflammatory cell numbers compared to mice treated with an allergen only[82]. Another study, however, found that mice dermally exposed to a 0.5% solution of DBP have decreased IL-4 levels[92] and found no effect of DEP. Only one animal study has looked at immune response with parabens: mouse dams given 200 mg/kg butyl paraben show increases in Th2 cytokines IL-1B, IL-6, and TNF-a[80]. Triclosan animal studies suggest associations with Th2 dominant immunity: Dermal exposure to a 3% triclosan solution in mice leads to increases in Th2 cytokines IL-1B, IL-4, TNF-a, and TSLP, but decreases in Th2 cytokines IL-25 and IL-33[67]. Rat exposure to 10 ug/kg or 1000 ug/kg intratracheal triclosan leads to increased Th2 cytokines IL-6 and TNF-a, though cytokine increases normalize after two weeks[93]. This animal literature suggests that the low molecular phthalate DBP, parabens, and triclosan are associated with Th2-dominated immunity.

Personal care products chemicals and asthma biomechanisms

Personal care product chemicals may act on several biological processes related to asthma. Phthalates, including DEP and DBP, have been found to propagate release of inflammatory cytokines *in vitro* directly from airway epithelial cells which then lead to proliferation and migration of bronchial smooth muscle cells[94]. DEP is also associated with increased fractional exhaled nitric oxide (FE_{NO}), a biomarker for lung inflammation that is associated with increased risk for and exacerbation of asthma symptoms[95]. Phthalates have also been shown to activate nuclear peroxisome proliferator–activated receptors (PPAR)[96], which are involved in airway inflammation and airway remodeling, among a wide range of physiological actions[97]. The estrogenic nature of phthalates and parabens may also play a role: estrogens have been shown to lower forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) and to increase bronchial hyperactivity[76]. Low molecular phthalates, parabens, and phenols are also associated with increased biomarkers of oxidative stress in humans[84,88-90] and *in vitro*[27,59,98,99] which can play a crucial role in asthma origins[61] by increasing inflammation and promoting bronchial hyperresponsiveness and bronchospasm[100], and are interconnected with Th2-dominated immunity[101,102]. These *in vitro* findings suggest that low molecular phthalates and parabens are related to asthma-like lung changes, and that personal care product chemicals may be involved in asthma biomechanisms through oxidative stress.

Personal care product chemicals may also be associated with asthma-like changes in animals. Dermal exposure of mice to an allergen and DBP at 0.4, 4, or 40 mg/kg leads to increases in mast cell alteration and eosinophil activity compared to mice treated with an allergen only[82]. However, intraperitoneal and subcutaneous exposure to DBP at 10mg/kg does not change airway hyperresponsiveness (AHR) in mice[103]. Triclosan animal studies suggest it is associated with

reduced lung function. Dermal exposure to triclosan solutions of 0.75-3.0% increases AHR and the number of eosinophils in the lungs of mice[104]. Rat exposure to 10 ug/kg or 1000 ug/kg intratracheal triclosan leads to decreased lung cell[93]. This animal literature suggests that low molecular weight phthalates may or may not be associated with asthma-like lung changes, but that triclosan is associated with asthma-like lung changes.

Overall, *in vitro* and animal literature suggests that low molecular phthalates, parabens, and triclosan are related to Th2 dominant immunity as well as asthma-like lung changes.

In vitro **and animal studies on plasticizing phthalates and BPA in relation to cytokine disruption and asthma-like lung changes**

Plasticizing phthalates, BPA, and cytokines

There is biological evidence that plasticizing phthalates and BPA may modify cytokine-related immune system pathways. High molecular weight phthalates and BPA have both been shown to increase levels of Th2 cytokines: *In vitro* studies show that dipentyl phthalate and DiNP both increase levels of Th2 cytokine IL-4[58,105] and that DEHP increases IL-6 and IL-8[106,107], and BPA also increases Th2 cytokines including IL-4[108-113], IL-6[114-120], IL-10[73,109,110,112,121], TNF-a[111,113-118,121], and others[110]. There is evidence that BPA's induction of Th2 cytokines acts through Ca2+: BPA increases Ca2+ influx in cells[122], and when Ca2+ influx or calcineurin (a calcium-dependent molecule that activates T cells) is blocked, increases in Th2 cytokines from BPA are completely attenuated[108]. These plasticizing chemicals may also influence regulatory T cells. The longitudinal LINA study of 610 infants described above also found that urinary levels of several high molecular weight phthalates during pregnancy were associated with lower levels of regulatory T cells in cord blood and in blood at age two[60]. BPA has also been shown in animal models to affect regulatory T cell activity[123,124]. Plasticizing phthalates[70,73,78,106,116-118] and BPA[119] may also influence activation of (NF-kB), and BPA may also act via mitogen-activated protein kinase (MAPK)[114,116] and c-Jun N-terminal protein kinase (JNK)[111,115,117], which are similar in function to NF-kB[117]. Additionally, high molecular weight phthalates also promote thymic stromal lymphopoietin (TSLP) protein expression[66,125,126]. Like personal care product chemicals, high molecular weight phthalates and BPA are xenoestogens[75,127], and are associated with increased biomarkers of oxidative stress in humans[84,88-90,128], in animals[129], and *in vitro*[73,109,121]. In addition, high molecular weight phthalates and BPA have also been associated with other biomarkers of inflammation *in vitro*[77,78,110,121,130], in animals[81,108,114,131-138], and in humans[83,84,88-91,139,140]. This *in vitro* literature suggests that high molecular weight phthalates and BPA are associated with Th2 dominant immunity.

Animal studies on plasticizing phthalates and BPA also suggest associations with an increased Th2 immune response. In mice, oral exposure throughout the juvenile period to 30 to 3,000 ug/kg of DEHP leads to adult increases in Th2 cytokine IL-4 during compared to mice given the allergen only[141]. Inhalation of 13mg/m^3 DEHP during adulthood leads to increased levels of Th2 cytokines IL-4 and IL-5, though exposure also leads to increased Th1 cytokines IL-10 and IFN-y[136]. Rat dams whose mothers were exposed to 50 mg/kg DiNP orally show increased Th2 cytokine IL-13, and decreased Th1 cytokine IFN-y, suggesting Th2 dominant immunity[142]. Several other murine studies have shown exposure to DiNP, DiDP, and DEHP leads to Th2-dominated immunity, evidenced by increases in Th2 cytokines and decreases in Th1 cytokines[82,105,107,125,132,142-147]. Adult oral exposure in mice to 1 mg BPA leads increased Th2 cytokines IL-1B, IL-5, and IL-13[148]. Several additional studies have demonstrated that mice exposed to BPA show increased levels of Th2 cytokines[108,123,124,138,149-154], along with other markers of inflammation such as

immunoglobulins[108,114,138,149,151,152,155,156], though some studies show Th1 cytokine increases[123,138,151,153,157,158]. Overall, research suggests that high molecular weight phthalates and BPA are associated with increases in Th2 cytokines, though they may also be associated with increases in Th1 cytokines.

Plasticizing phthalates, BPA, and asthma biomechanisms

These plasticizing chemicals may also act on biological mechanisms involved in asthma. BBzP also encourages release of inflammatory cytokines *in vitro* directly from airway epithelial cells, leading to proliferation and migration of bronchial smooth muscle cells[94]. High molecular weight phthalates and BPA are also associated with increased fractional exhaled nitric oxide (FE_{NO})[95,159] and PPAR[96,97,160]. BPA exposure *in utero* was shown to increase mRNA expression of two proteins, the club cell secretory protein (CC-16) and MUC5B, responsible for secretion of mucus in the lung epithelium[161], suggesting that high molecular weight phthalates and BPA are associated with biological changes related to asthma.

Similarly, animal studies on high molecular weight phthalates and BPA suggest an association with asthma-like lung changes. In rats, prenatal exposure to an allergen and 30 or 300 mg/kg DEHP leads to a dose-dependent increase in inflammatory immune cells in the lungs as juveniles compared with exposure to the allergen alone[147]. In mice, oral exposure to an allergen and 30 to 3,000 ug/kg of DEHP throughout the juvenile period leads to induction of asthma-like lung inflammation, airway hyperresponsiveness (AHR), and lung eosinophils in adulthood compared to mice treated with an allergen only[141]. Juvenile rats orally exposed to 0.7 or 70 mg/kg DEHP also show increased AHR, lung eosinophils, and airway wall thickness as adults[162]. Inhalation of 13mg/m^3 DEHP during adulthood leads to a decreased volume of inspired air and increased respiratory rate in mice, as well as increased lung eosinophils[136]. Rat dams whose mothers were exposed to 50 mg/kg DiNP orally also show increased AHR and increased eosinophils in the lungs[142], and mice exposed to an allergen and 15 mg/kg DiDP showed a dose-dependent increase in AHR and asthma-like lung remodeling compared to mice exposed only to the allergen [145]. *In utero* exposure in mice to 10 ug/mL BPA in drinking water leads to increased AHR[155,163] and increases in eosinophils in lung fluid in childhood[155,163]. Exposure to 5 ug/mL BPA in drinking water also leads to increases in AHR and lung eosinophils when exposure was from birth until adulthood, but not with exposure *in utero*[149]. *In utero* and childhood oral exposure in mice to 50 or 500 ug/kg BPA also leads to asthma-like lung histopathology, but decreased lung eosinophils as adults[156]. In rhesus macaques, gestational exposure to 1.056 mg/kg BPA leads to thicker lung epithelium later in gestation[161]. Adult oral exposure in mice to 1 mg BPA leads to increased eosinophils in the nasal cavity fluid[148]. Thus, animal literature also suggests that high molecular weight phthalates and BPA are associated with asthma-like changes in the lungs.

In terms of allergic responses, one animal study examined oral exposure *in utero* and during lactation to 1, 10, or 100 mg/ml BPA in mice but found no association with juvenile inhalant allergy symptoms[164]. Two mouse studies have looked at plasticizers and allergic dermatitis, and found that adult oral exposure to 2, 20, or 200 mg/kg DiNP[125] or DiDP[126] leads to increased dermatitis symptoms.

Overall, *in vitro* and animal literature suggests that high molecular phthalates and BPA are associated with increases in Th2 cytokines, asthma-like lung changes, and are possibly associated with dermatitis.

Observational studies in humans suggest personal care product chemicals may be related to cytokine levels and risk for asthma, aeroallergy, or eczema

Epidemiologic studies of personal care product chemicals and cytokines

Only two human studies have looked at T helper cells in relation to low molecular weight phthalates, parabens, and other phenols and both found no associations. The Maternal-Infant Research on Environmental Chemicals (MIREC) study of 1,121 infants found no association between *in utero* urinary levels of low molecular weight phthalates and cord blood levels of Th2 cytokine IL-33[165]. The MIREC study also found no association in 1,219 pregnant women between urinary levels of triclosan during pregnancy and cord blood levels of Th2 cytokine IL-33[166]. In the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) study, urinary levels of monoethyl phthalate (MEP, a metabolite of DEP), mono-n-butyl phthalate (MBP, a metabolite of DBP) and mono-isobutyl phthalate (MiBP, a metabolite of DiBP) were not associated cross-sectionally with plasma levels of Th2 cytokines IL-1B or IL-6, or with Th1 cytokine IL-10 in 120 pregnant women[89]. The PROTECT study also found no association between urinary concentrations of MP, PP, BP, triclosan, or BP-3 and these cytokines cross-sectionally in 106 pregnant women[84].

Epidemiologic studies of personal care product chemicals and asthma

Although only a few studies have evaluated the relationship between *in utero* exposure to personal care products and asthma, there is some evidence of a relationship. The Columbia Center for Children's Environmental Health Cohort, a study of 154 children, found that prenatal urinary concentrations of MBP, but not MEP, were associated with increased risk of asthma from ages 5-11[167]. The Taiwan Maternal and Infant Cohort study of 171 eight-year-old children found *in utero* and early childhood urinary concentrations of MEP, but not MBP, were associated with increased risk for wheeze and asthma in childhood in boys only[140]. In 3,147 participants ages 6-79 in the Canadian Health Measures Survey, urinary concentrations of MBP were cross-sectionally associated with lower FEV_1 and FVC[168]. However, *in utero* urinary levels of low molecular weight phthalates were not associated with increased risk for wheeze and asthma at six months, 14 months, four years, and seven years in a cohort study of 657 children in the Infancia y Medio Ambiente (INMA) study[169]. No association was found in the Polish Mother and Child Cohort study between *in utero* urinary concentrations of low molecular phthalates and wheeze at age two in 144 children[170]. Additionally, a French birth cohort of 587 children found that prenatal exposure to parabens and 2,5-DCP, but not low molecular weight phthalates, were associated with wheeze and asthma at several ages in childhood[171]. However, the Vitamin D Antenatal Asthma Reduction Trial study found that prenatal maternal blood concentrations of triclosan and parabens were not associated with asthma and wheeze in 467 three-year-olds[172], and the Mount Sinai Children's Environmental Health Study found that prenatal urinary concentrations of BP-3 were associated with decreased risk of wheeze at ages six and seven in 164 children, with no associations with low molecular weight phthalates, triclosan, or 2,5-DCP[173].

Several cross-sectional studies have examined urinary concentrations of parabens and other phenols in relation to asthma. Results suggest that triclosan and the dichlorophenols may have an association with asthma, but parabens and BP-3 may not. Urinary concentrations of 2,4-DCP and 2,5-DCP were associated with increased asthma morbidity in 2,125 participants ages six and older in the 2005-2006 NHANES[85]. Triclosan was associated with increased risk for asthma attack among 639 asthmatic children aged 6-18 in NHANES from 2005-2010[174]. However, triclosan was

not associated with risk for asthma diagnosis in that population, nor in 623 ten-year-olds in the Environment and Childhood Asthma Study in Norway[175]. Triclosan was associated with increased risk for atopic asthma in one study of 837 children ages 6-18 in the 2005-2006 NHANES when multinomial logistic regression was used to determine odds for atopic asthma, non-atopic asthma, and wheeze[176]; but not in another study of the same population (N=860) when atopic asthma was defined differently and binomial logistic regression was used[177]. Parabens and BP-3 were not associated with increased risk of asthma in either of these studies[176,177]. No associations were also found between triclosan, BP-3 and asthma diagnosis, asthma medication, wheeze, or spirometry outcomes in 661 children aged 6-19 in NHANES from 2007-2010[178].

Epidemiologic studies of personal care product chemicals and inhalant allergies and eczema

Studies that have examined the relationship between concentrations of low molecular weight phthalates and aeroallergies and eczema indicate a possible relationship with eczema but no evidence of a relationship with aeroallergies. In 610 children in the LINA study, prenatal urinary concentrations of MiBP were associated with dermatitis at age three, but not with inhalant allergies[60]. A small Korean study of 18 children ages 3-7 with atopic dermatitis found that MBP in pooled urine collected twice daily from children for 230 days was associated with increased symptoms of atopic dermatitis[179]. A French birth cohort study of 604 children found prenatal urinary concentrations of MEP and MiBP were associated with eczema between eight months and five years of age[180]. However, prenatal urinary concentrations of low molecular weight phthalates were not associated with atopic dermatitis in 161 children in the Taiwan Birth Panel cohort study ages two and five[139], nor in the Polish Mother and Child Cohort study of 144 children age two[170], nor in the Mount Sinai Children's Environmental Health Study of 164 children ages six and seven[173]. The LINA study also found no cross-sectional associations between low molecular weight phthalate urinary concentrations and aeroallergies in 657 children ages six months to seven years[169].

Studies of the relationship of parabens and phenols with inhalant allergy and eczema suggest that parabens and triclosan may be associated with aeroallergy, but most studies have been cross-sectional. The Vitamin D Antenatal Asthma Reduction Trial study found no associations between prenatal urinary concentrations of triclosan and parabens and aeroallergies at ages three and four[172]. Two studies of NHANES 2005-2006 participants aged 6-18 found that MP, PP, and triclosan were associated with increased risk for inhalant allergy, as measured by specific IgEs, in 859 participants[14], and in 837 participants[176]. One of these studies also examined BP-3 and found no association[14]. In NHANES 2003-2006, urinary triclosan concentrations were associated with diagnosis of aeroallergy or hay fever in 3,728 participants ages six and older[181]. Urinary triclosan was also associated with aeroallergies and rhinitis in a study of 623 Norwegian ten-year-old children in the Environment and Childhood Asthma study[175]. A study of 25,805 Korean children ages 0-13 found an increased risk for rhinitis, but not eczema, associated with higher use of antibacterial household products, which is likely a measure of exposure to triclosan[182]. The Mount Sinai Children's Environmental Health study found an association between urinary 2,5-DCP, but not triclosan or BP-3, and eczema in 164 children aged six and seven[173]. Parabens and other phenols were not associated with eczema in NHANES 2005-2006 in 837 participants ages 6-18[176] or in 4,979 participants ages 20-85[183], or in a Danish study of 845 children aged 4-9[184].

Observational studies in humans suggest plasticizing phthalates and BPA may be related to cytokine levels and risk for asthma, aeroallergy, or eczema

Epidemiologic studies of plasticizing phthalates, BPA and cytokines

Few human studies have looked at T helper cells and exposures to plasticizing phthalates and BPA, and results are contrasting. An experimental study in Germany that exposed 16 people to DEHP-containing dust for three hours found increased levels of Th2 cytokines IL-6 and IL-5 in nasal fluid compared to 16 unexposed controls[185]. However, the MIREC study of 1,121 infants found that maternal urinary levels of high molecular weight phthalates or BPA during pregnancy were not associated with cord blood levels of Th2 cytokine IL-33[165]. In 274 infants aged 0-12 months in the Prediction of Allergy in Taiwanese Children study, *in utero* maternal blood levels of BPA were associated with lower cord blood levels of Th2 cytokines IL-6 and TNF-a[186], although measurements of BPA in blood are often criticized as being less valid than urinary measures. Urinary BPA was associated with higher blood levels of Th2 cytokine IL-6, but not Th2 cytokine TNF-a, in an Italian cross-sectional study of 79 adult males[187].

Epidemiologic studies of plasticizing phthalates, BPA, and asthma

Several longitudinal human studies suggest an association between high molecular weight phthalates and asthma. The Dampness in Buildings and Health follow-up study in Sweden of 4,779 children found that PVC flooring (which may contain BBzP, DiNP, DiDP, or DEHP[188]) in the child's or parent's bedroom at ages 1-3 was associated with increased risk of asthma at ages 6-8[189]. A study of 1,024 children in Ukraine and Poland found *in utero* exposure to DEHP metabolites measured in blood was associated with increased risk of wheeze at ages 5-9[190], however phthalates measured in blood are considered less valid than urinary measurements. *In utero* urinary concentrations of DEHP metabolites, but not monobenzyl phthalate (MBzP, a metabolite of BBzP), were associated with increased risk for wheeze and asthma at six months, 14 months, four years, and seven years in 657 children in the INMA study[169]. The Columbia Center for Children's Environmental Health Cohort found that *in utero* urinary concentrations of MBzP, but not a DEHP metabolite, were associated with increased risk of asthma in 154 children from ages 5-11[167]. The Taiwan Maternal and Infant Cohort study of 171 eight-year-old children found *in utero* and early childhood MBzP and DEHP urinary metabolites were associated with increased risk for wheeze and asthma in boys only[140]. Additionally, urinary concentrations of MBzP, MCPP, and DEHP metabolites were cross-sectionally associated with lower FEV_1 and FVC in males only of 3,147 participants in the Canadian Health Measures Survey ages 6-79[168]. However, no association was found with *in utero* urinary concentrations of high molecular phthalates in the Polish Mother and Child Cohort study of 144 children and wheeze at age two[170]. No associations were also found between prenatal high molecular weight phthalates and asthma in a French birth cohort of 587 children aged between eight months and five years[171], nor in the Mount Sinai Children's Environmental Health study of 164 children ages six and seven[173].

BPA studies in humans suggest an association with asthma. The Columbia Center for Children's Environmental Health Cohort study found BPA urinary concentrations at early childhood ages, but not *in utero*, were associated with asthma and wheeze throughout childhood in 727 children followed prenatally until age 12[159]. The same population (with a reduced N of 292) showed urinary concentrations of BPA at ages 3-7 were associated with wheeze and asthma at ages 5-11 only in children who were above the median of MBzP urinary concentrations[191]. This finding suggests an effect of exposure to multiple chemicals at once. The Health Outcomes and Measures of the

Environment study of 365 children followed prenatally until age five found prenatal urinary BPA concentrations were associated with increased risk for wheeze throughout early childhood[192,193]. Another study of the same population showed prenatal BPA was associated with lowered FEV_1 at age four, but not at age five[192]. The INMA study of 657 children found *in utero* exposure to BPA was associated with wheeze, but not asthma, from ages six months to seven years[169]. A longitudinal study of 127 children in Korea found urinary BPA concentrations at age seven were associated with asthma, but not wheeze or spirometry outcomes, at ages 7-12[194]. Cross-sectional associations have also been found in NHANES. In 661 children ages 6-19 in the 2007-2010 studies, BPA was associated with a lowered ratio of forced expiratory volume in the first one second to forced vital capacity (FEV_1/FVC) and forced expiratory flow at 25–75% of forced vital capacity (FEF_{25-75}), but not FEV_1, FVC, asthma diagnosis, asthma medication use, or wheeze[178]. In 2,548 2005-2006 participants ages six and older, BPA was associated with increased risk for allergic asthma in females only[195].

Epidemiologic studies of plasticizing phthalates, BPA, and aeroallergies and eczema

Several longitudinal studies on high molecular weight phthalates and aeroallergy or eczema have been conducted, most of which suggest a lack of association. The Dampness in Buildings and Health study found that the presence of PVC flooring in homes during early childhood was associated with increased rhinitis, but not eczema later in childhood[189]. However, urinary concentrations of MBzP at age two, but not *in utero*, were associated with increased dermatitis in 161 children in the Taiwan Birth Panel study[139]. In the same study, no associations were found with a DEHP metabolite, although prenatal concentrations of a DEHP metabolite were associated with eczema at age five in a French study of 604 children[180]. Additionally, among 18 Korean children ages 3-7 diagnosed with atopic dermatitis, pooled urinary concentrations of DEHP metabolites taken twice a day for 230 days were associated with increased symptoms of atopic dermatitis[179]. However, *in utero* concentrations of DEHP metabolites and MBzP were not associated with eczema in 657 children ages six months to seven years in the INMA study[169]. Urinary concentrations of MBzP and a DEHP metabolite *in utero* were also not associated with atopic dermatitis or hay fever in 610 children age three in the LINA study[60], nor in the Mount Sinai Children's Environmental Health study of 164 children ages six and seven[173]. There was also no association in the Polish Mother and Child Cohort study between *in utero* urinary concentrations of DEHP metabolites or MBzP and inhalant allergy or dermatitis in 144 children aged two[170].

Several epidemiologic studies have looked at BPA's relationship with aeroallergies or eczema and they suggest no association. *In utero* BPA concentrations were associated with cases of combined eczema and wheeze at six months in 412 Chinese infants[196]. In 18 Korean children diagnosed with atopic dermatitis ages 3-7, pooled urinary BPA concentrations taken twice daily for 230 days were associated with symptoms of atopic dermatitis[179]. However, in the longitudinal Childhood Environment and Allergic Diseases cohort study of 453 children, urinary BPA at ages three and six was not associated with allergic rhinitis or atopic dermatitis at ages three or six[197]. In 657 children in the INMA study, *in utero* urinary concentrations of BPA were not associated with eczema at ages six months, 14 months, four years, or seven years[169]. Three studies have looked at BPA in NHANES 2005-2006, all of which found null associations. BPA was not associated with inhalant allergies in 3,728 participants aged six and over[181], or in 859 participants aged 6-18[14]. In 4,979 participants aged 20-85, BPA was not associated with eczema[183].

Human literature summary

Many of the above studies found relationships between childhood immune dysfunction and chemical exposure specifically *in utero*. Much of the immune system develops prenatally[198], and this is likely a critical time period in which to study how the immune system may be affected by exposure to environmental chemicals. The prenatal literature described above indicates that low molecular weight phthalates are possibly associated with asthma and eczema, but not with aeroallergy or Th2 cytokine levels. It also suggests that high molecular weight phthalates and BPA are associated with asthma, but not with aeroallergy, eczema, or Th2 cytokine levels. To my knowledge, there have been no studies relating prenatal exposure to parabens or other phenols with any of the above outcomes. Longitudinal studies that begin after the prenatal period, as well as cross-sectional studies, cannot examine the impact of chemical exposure on crucial *in utero* immune system development, but still provide valuable information. These studies indicate that low molecular weight phthalates and parabens are likely not associated with Th2 cytokine levels or asthma, but that low molecular weight phthalates are associated with eczema and parabens are associated with aeroallergy. There is cross-sectional evidence linking some other phenols to asthma and aeroallergy, while others are not associated with asthma, aeroallergy, eczema, or Th2 cytokine levels. High molecular weight phthalates are cross-sectionally associated with asthma, aeroallergy, and possibly eczema, while BPA is cross-sectionally associated with asthma and Th2 cytokine levels but not with aeroallergy or eczema.

Hypotheses and research questions for this dissertation

Based on existing in vitro, animal, and epidemiologic literature, the four main research questions covered in this dissertation are:

1) Is exposure to personal care product chemicals in utero associated with increased risk of age seven probable asthma, aeroallergies, or eczema; or with spirometry performance; or with cytokine levels?

I hypothesize that, based on animal and *in vitro* literature, low molecular weight phthalates may be associated with increased risk for probable asthma and eczema and lower spirometry performance; that parabens may be associated with increased risk for aeroallergies; that triclosan may be associated with increased risk for aeroallergies, probable asthma and lower spirometry performance; and that the dichlorophenols may be associated with increased risk for probable asthma and lower spirometry performance. I will choose other chemicals to control for using Bayesian Model Averaging to account for additional chemical exposure.

2) Is exposure to plasticizing phthalates and BPA in utero associated with age seven asthma, aeroallergies, or eczema; or with spirometry performance; or with cytokine levels?

I hypothesize that, based on animal and *in vitro* literature, high molecular weight phthalates may be associated with increased risk for probable asthma, aeroallergies, eczema, lower spirometry performance, and Th2 dominant immunity; and that BPA may be associated with increased risk for probable asthma, eczema, lower spirometry performance, and Th2 or Th1 dominant immunity. I will choose other chemicals to control for using Bayesian Model Averaging to account for additional chemical exposure.

3) How is joint exposure to these two categories of chemicals characterized, and how does joint exposure relate to childhood immune outcomes?

I hypothesize that women will differ on joint exposure to personal care product chemicals and plasticizing phthalates and BPA: some women will have lower overall exposure, some women will have higher overall exposure, and some women will have lower or higher concentrations of a selection of chemicals. For example, low molecular weight phthalates and parabens may cluster together because of their use in similar products and the moderate correlation of their urinary concentrations in our population. I also hypothesize that different categories of joint exposure will vary on their associations with childhood immune outcomes: children whose mothers have higher overall exposure will have higher risk for asthma, aeroallergies, and eczema, will have lower spirometry performance, and will have Th2 dominant cytokine levels; children whose mothers have higher concentrations of only some chemicals and lower concentrations of others may also show these trends; and children whose mothers have lower overall exposure will show opposite trends.

References

1. Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart. *Bmj* 1992;304(6831):873-875.
2. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the United States, 1980–2007. *Pediatrics* 2009;123(Supplement 3):S131-S145.
3. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2013-2014.
4. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H, Group IPTS. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet* 2006;368(9537):733-743.
5. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2005-2006.
6. Jackson KD, Howie LD, Akinbami OJ. Trends in allergic conditions among children: United States, 1997-2011. 2013.
7. Dick S, Friend A, Dynes K, AlKandari F, Doust E, Cowie H, Ayres J, Turner S. A systematic review of associations between environmental exposures and development of asthma in children aged up to 9 years. *BMJ open* 2014;4(11):e006554.
8. Ebert CS, Pillsbury HC. Epidemiology of allergy. *Otolaryngologic clinics of North America* 2011;44(3):537-548.
9. Huang S-K, Zhang Q, Qiu Z, Chung KF. Mechanistic impact of outdoor air pollution on asthma and allergic diseases. *Journal of thoracic disease* 2015;7(1):23.
10. Patelarou E, Tzanakis N, Kelly FJ. Exposure to indoor pollutants and wheeze and asthma development during early childhood. *International journal of environmental research and public health* 2015;12(4):3993-4017.
11. Yang S-N, Hsieh C-C, Kuo H-F, Lee M-S, Huang M-Y, Kuo C-H, Hung C-H. The effects of environmental toxins on allergic inflammation. *Allergy, asthma & immunology research* 2014;6(6):478-484.
12. Yoo Y, Perzanowski MS. Allergic sensitization and the environment: latest update. *Current allergy and asthma reports* 2014;14(10):1-9.
13. Robinson L, Miller R. The impact of bisphenol A and phthalates on allergy, asthma, and immune function: a review of latest findings. *Current environmental health reports* 2015;2(4):379-387.
14. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012;130(2):453-60 e7.
15. Kelley KE, Hernández-Díaz S, Chaplin EL, Hauser RB, Mitchell AA. Identification of phthalates in medications and dietary supplement formulations in the United States and Canada. 2011.
16. Koniecki D, Wang R, Moody RP, Zhu J. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. *Environmental research* 2011;111(3):329-336.
17. Frederiksen H, Skakkebaek NE, Andersson AM. Metabolism of phthalates in humans. *Molecular nutrition & food research* 2007;51(7):899-911.

18. Liao C, Kannan K. Concentrations and composition profiles of parabens in currency bills and paper products including sanitary wipes. *Science of The Total Environment* 2014;475:8-15.
19. Guo Y, Kannan K. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. *Environmental science & technology* 2013;47(24):14442-14449.
20. Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. Parabens as urinary biomarkers of exposure in humans. *Environ Health Perspect* 2006;114(12):1843-6.
21. Darbre PD, Harvey PW. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *Journal of applied toxicology* 2008;28(5):561-578.
22. Halden RU, Paull DH. Co-occurrence of triclocarban and triclosan in US water resources. *Environmental Science & Technology* 2005;39(6):1420-1426.
23. Fang J-L, Stingley RL, Beland FA, Harrouk W, Lumpkins DL, Howard P. Occurrence, efficacy, metabolism, and toxicity of triclosan. *Journal of Environmental Science and Health, Part C* 2010;28(3):147-171.
24. Dann AB, Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action. *J Appl Toxicol* 2011;31(4):285-311.
25. United States Food and Drug Administration. Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record; Proposed Rule. In: Services DoHaH, ed. Vol. 78 Federal Register, 2013;76443-76478.
26. Latch DE, Packer JL, Stender BL, VanOverbeke J, Arnold WA, McNeill K. Aqueous photochemistry of triclosan: Formation of 2, 4-dichlorophenol, 2, 8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environmental Toxicology and Chemistry* 2005;24(3):517-525.
27. Bukowska B. Effects of 2, 4-D and its metabolite 2, 4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 2003;135(4):435-441.
28. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Quantification of urinary conjugates of bisphenol A, 2, 5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction–high performance liquid chromatography–tandem mass spectrometry. *Analytical and bioanalytical chemistry* 2005;383(4):638-644.
29. Wei Y, Zhu J, Nguyen A. Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the US National Health and Nutrition Examination Survey (NHANES) 2005–2008. *International journal of hygiene and environmental health* 2014;217(2):294-299.
30. Kadry AM, Okereke CS, Abdel-Rahman MS, Friedman MA, Davis RA. Pharmacokinetics of benzophenone-3 after oral exposure in male rats. *Journal of Applied toxicology* 1995;15(2):97-102.
31. Han C, Lim Y-H, Hong Y-C. Ten-year trends in urinary concentrations of triclosan and benzophenone-3 in the general US population from 2003 to 2012. *Environmental Pollution* 2016;208:803-810.
32. Fierens T, Servaes K, Van Holderbeke M, Geerts L, De Henauw S, Sioen I, Vanermen G. Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology* 2012;50(7):2575-2583.

33. Kawakami T, Isama K, Matsuoka A. Analysis of phthalic acid diesters, monoester, and other plasticizers in polyvinyl chloride household products in Japan. *Journal of Environmental Science and Health, Part A* 2011;46(8):855-864.
34. Mikroyannidis J. Polycarbonates of bis (4-hydroxyphenyl) phenylmethane and bis (4-hydroxyphenyl) diphenylmethane. *European polymer journal* 1985;21(10):895-898.
35. Perez P, Pulgar R, Olea-Serrano F, Villalobos M, Rivas A, Metzler M, Pedraza V, Olea N. The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups. *Environmental Health Perspectives* 1998;106(3):167.
36. Kang J-H, Katayama Y, Kondo F. Biodegradation or metabolism of bisphenol A: from microorganisms to mammals. *Toxicology* 2006;217(2):81-90.
37. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reprod Toxicol* 2007;24(2):139-77.
38. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* 2008;112(5):1557-1569.
39. Abbas AK, Lichtman AH, Pillai S. *Basic immunology: functions and disorders of the immune system* Elsevier Health Sciences, 2014.
40. Chaplin DD. 1. Overview of the human immune response. *Journal of Allergy and Clinical Immunology* 2006;117(2):S430-S435.
41. Owyang AM, Zaph C, Wilson EH, Guild KJ, McClanahan T, Miller HR, Cua DJ, Goldschmidt M, Hunter CA, Kastelein RA. Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *The Journal of experimental medicine* 2006;203(4):843-849.
42. Whittaker L, Niu N, Temann U-A, Stoddard A, Flavell RA, Ray A, Homer RJ, Cohn L. Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and interleukin-9. *American journal of respiratory cell and molecular biology* 2002;27(5):593-602.
43. Nakae S, Komiyama Y, Yokoyama H, Nambu A, Umeda M, Iwase M, Homma I, Sudo K, Horai R, Asano M. IL-1 is required for allergen-specific Th2 cell activation and the development of airway hypersensitivity response. *International immunology* 2003;15(4):483-490.
44. Helmby H, Grencis RK. Interleukin 1 plays a major role in the development of Th2-mediated immunity. *European journal of immunology* 2004;34(12):3674-3681.
45. Louten J, Rankin AL, Li Y, Murphy EE, Beaumont M, Moon C, Bourne P, McClanahan TK, Pflanz S, de Waal Malefyt R. Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. *International immunology* 2011:dxr006.
46. Hsieh C-S, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12. *Science* 1993;260:547.
47. Bradley J. TNF-mediated inflammatory disease. *The Journal of pathology* 2008;214(2):149-160.
48. Soumelis V, Liu Y-J. Human thymic stromal lymphopoietin: a novel epithelial cell-derived cytokine and a potential key player in the induction of allergic inflammation. Springer seminars in immunopathology: Springer, 2004;325-333.
49. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, Gilliet M, Ho S, Antonenko S, Lauerma A. Human epithelial cells trigger dendritic cell–mediated allergic inflammation by producing TSLP. *Nature immunology* 2002;3(7):673-680.
50. Corrigan C. Mechanisms of asthma. *Medicine* 2012;40(5):223-227.

51. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res* 2001;2(2):64-5.
52. Hart PH. Regulation of the inflammatory response in asthma by mast cell products. *Immunology and Cell Biology* 2001;79(2):149.
53. Deo SS, Mistry KJ, Kakade AM, Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. *Lung India: Official Organ of Indian Chest Society* 2010;27(2):66.
54. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma The role of interleukin-5 in allergic eosinophilic disease. *Respiratory Research* 2001;2(2):71.
55. Amin K. The role of mast cells in allergic inflammation. *Respiratory medicine* 2012;106(1):9-14.
56. Fuller R, Morris P, Richmond R, Sykes D, Varndell I, Kemeny D, Cole P, Dollery C, MacDermot J. Immunoglobulin E-dependent stimulation of human alveolar macrophages: significance in type 1 hypersensitivity. *Clinical and experimental immunology* 1986;65(2):416.
57. Kalish RS, Askenase PW. Molecular mechanisms of CD8+ T cell–mediated delayed hypersensitivity: Implications for allergies, asthma, and autoimmunity. *Journal of allergy and clinical immunology* 1999;103(2):192-199.
58. Maruyama T, Shiba T, Iizuka H, Matsuda T, Kurohane K, Imai Y. Effects of Phthalate Esters on Dendritic Cell Subsets and Interleukin-4 Production in Fluorescein Isothiocyanate-Induced Contact Hypersensitivity. *Microbiology and immunology* 2007;51(3):321-326.
59. Xu H, Shao X, Zhang Z, Zou Y, Wu X, Yang L. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. *Ecotoxicology and environmental safety* 2013;93:39-44.
60. Herberth G, Pierzchalski A, Feltens R, Bauer M, Röder S, Olek S, Hinz D, Borte M, von Bergen M, Lehmann I. Prenatal phthalate exposure associates with low regulatory T-cell numbers and atopic dermatitis in early childhood: Results from the LINA mother-child study. *Journal of Allergy and Clinical Immunology* 2016.
61. Kato T, Tada-Oikawa S, Takahashi K, Saito K, Wang L, Nishio A, Hakamada-Taguchi R, Kawanishi S, Kuribayashi K. Endocrine disruptors that deplete glutathione levels in APC promote Th2 polarization in mice leading to the exacerbation of airway inflammation. *European journal of immunology* 2006;36(5):1199-1209.
62. Jannesson L, Birkhed D, Scherl D, Gaffar A, Renvert S. Effect of oxybenzone on PGE2-production in vitro and on plaque and gingivitis in vivo. *Journal of clinical periodontology* 2004;31(2):91-94.
63. Ahn KC, Zhao B, Chen J, Cherednichenko G, Sanmarti E, Denison MS, Lasley B, Pessah IN, Kultz D, Chang D. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. *Environ Health Perspect* 2008;116(9):1203-1210.
64. Bracci L, Vukcevic M, Spagnoli G, Ducreux S, Zorzato F, Treves S. Ca2+ signaling through ryanodine receptor 1 enhances maturation and activation of human dendritic cells. *Journal of cell science* 2007;120(13):2232-2240.
65. Vukcevic M, Spagnoli GC, Iezzi G, Zorzato F, Treves S. Ryanodine receptor activation by Cav1. 2 is involved in dendritic cell major histocompatibility complex class II surface expression. *Journal of biological chemistry* 2008;283(50):34913-34922.
66. Shigeno T, Katakuse M, Fujita T, Mukoyama Y, Watanabe H. Phthalate ester-induced thymic stromal lymphopoietin mediates allergic dermatitis in mice. *Immunology* 2009;128(1pt2):e849-e857.

67. Marshall NB, Lukomska E, Long CM, Kashon ML, Sharpnack DD, Nayak AP, Anderson KL, Meade BJ, Anderson SE. Triclosan induces thymic stromal lymphopoietin in skin promoting Th2 allergic responses. *Toxicological Sciences* 2015;147(1):127-139.
68. Nagata Y, Kamijuku H, Taniguchi M, Ziegler S, Seino K-i. Differential role of thymic stromal lymphopoietin in the induction of airway hyperreactivity and Th2 immune response in antigen-induced asthma with respect to natural killer T cell function. *International archives of allergy and immunology* 2007;144(4):305-314.
69. Liu Y-J, Soumelis V, Watanabe N, Ito T, Wang Y-H, de Waal Malefyt R, Omori M, Zhou B, Ziegler SF. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu. Rev. Immunol.* 2007;25:193-219.
70. Haddad JJ, Fahlman CS. Redox-and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? *Biochemical and biophysical research communications* 2002;297(2):163-176.
71. Kim MY, Song KS, Park GH, Chang SH, Kim HW, Park JH, Jin H, Eu KJ, Cho HS, Kang G. B6C3F1 mice exposed to ozone with 4-(N-methyl-Nnitrosamino)-1-(3-pyridyl)-1-butanone and/or dibutyl phthalate showed toxicities through alterations of NF-κB, AP-1, Nrf2, and osteopontin. *Journal of veterinary science* 2004;5(2):131-137.
72. Monaco C, Andreakos E, Kiriakidis S, Mauri C, Bicknell C, Foxwell B, Cheshire N, Paleolog E, Feldmann M. Canonical pathway of nuclear factor κB activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101(15):5634-5639.
73. Yang M, Qiu W, Chen B, Chen J, Liu S, Wu M, Wang K-J. The in vitro immune modulatory effect of bisphenol A on fish macrophages via estrogen receptor α and nuclear factor-κB signaling. *Environmental science & technology* 2015;49(3):1888-1895.
74. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol Appl Pharmacol* 1998;153(1):12-9.
75. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. *Environ Health Perspect* 1997;105(8):802-11.
76. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. *Current opinion in allergy and clinical immunology* 2013;13(1):92.
77. Nakamura R, Teshima R, Sawada J-i. Effect of dialkyl phthalates on the degranulation and Ca 2+ response of RBL-2H3 mast cells. *Immunology letters* 2002;80(2):119-124.
78. Nishioka J, Iwahara C, Kawasaki M, Yoshizaki F, Nakayama H, Takamori K, Ogawa H, Iwabuchi K. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. *Inflammation Research* 2012;61(1):69-78.
79. Marshall NB, Lukomska E, Nayak AP, Long CM, Hettick JM, Anderson SE. Topical application of the anti-microbial chemical triclosan induces immunomodulatory responses through the S100A8/A9-TLR4 pathway. *Journal of Immunotoxicology* 2017;14(1):50-59.
80. Hegazy HG, Ali EH, Elgoly AHM. Interplay between pro-inflammatory cytokines and brain oxidative stress biomarkers: evidence of parallels between butyl paraben intoxication and the valproic acid brain physiopathology in autism rat model. *Cytokine* 2015;71(2):173-180.
81. Larsen ST, Lund RM, Nielsen GD, Thygesen P, Poulsen OM. Adjuvant Effect of di-n-Butyl-, di-n-Octyl-, di-iso-Nonyl-and di-iso-Decyl Phthalate in a Subcutaneous Injection Model Using BALB/c Mice. *Pharmacology & toxicology* 2002;91(5):264-272.
82. Li J, Li L, Zuo H, Ke C, Yan B, Wen H, Zhang Y, Yang X. T-helper type-2 contact hypersensitivity of Balb/c mice aggravated by dibutyl phthalate via long-term dermal exposure. *PloS one* 2014;9(2):e87887.

83. Shiue I. Association of urinary arsenic, heavy metal, and phthalate concentrations with food allergy in adults: National Health and Nutrition Examination Survey, 2005-2006. *Annals of allergy, asthma & immunology: official publication of the American College of Allergy, Asthma, & Immunology* 2013;111(5):421.
84. Watkins DJ, Ferguson KK, Del Toro LVA, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. *International journal of hygiene and environmental health* 2015;218(2):212-219.
85. Jerschow E, Parikh P, McGinn AP, De Vos G, Jariwala S, Hudes G, Rosenstreich D. Relationship between urine dichlorophenol levels and asthma morbidity. *Annals of Allergy, Asthma & Immunology* 2014;112(6):511-518. e1.
86. Jerschow E, McGinn AP, De Vos G, Vernon N, Jariwala S, Hudes G, Rosenstreich D. Dichlorophenol-containing pesticides and allergies: results from the US National Health and Nutrition Examination Survey 2005-2006. *Annals of Allergy, Asthma & Immunology* 2012;109(6):420-425.
87. Dinse GE, Jusko TA, Whitt IZ, Co CA, Parks CG, Satoh M, Chan E, Rose KM, Walker NJ, Birnbaum LS. Associations Between Selected Xenobiotics and Antinuclear Antibodies in the National Health and Nutrition Examination Survey, 1999-2004. *Environmental health perspectives* 2016;124(4):426-436.
88. Holland N, Huen K, Tran V, Street K, Nguyen B, Bradman A, Eskenazi B. Urinary phthalate metabolites and biomarkers of oxidative stress in a Mexican-American cohort: Variability in early and late pregnancy. . *Toxics* 2016;4(1):7.
89. Ferguson KK, Cantonwine DE, Rivera-González LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, Jiménez-Vélez B, Calafat AM, Ye X, Alshawabkeh AN. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. *Environmental science & technology* 2014;48(12):7018-7025.
90. Ferguson KK, Loch-Caruso R, Meeker JD. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999–2006. *Environmental science & technology* 2011;46(1):477-485.
91. Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999–2006. *Environmental research* 2011;111(5):718-726.
92. Matsuda T, Kurohane K, Imai Y. Di-(2-ethylhexyl) phthalate enhances skin sensitization to isocyanate haptens in mice. *Toxicology letters* 2010;192(2):97-100.
93. Kwon J-T, Yang Y-S, Kang M-S, Seo G-B, Lee DH, Yang M-J, Shim I, Kim H-M, Kim P, Choi K. Pulmonary toxicity screening of triclosan in rats after intratracheal instillation. *The Journal of toxicological sciences* 2013;38(3):471-475.
94. Kuo P-L, Hsu Y-L, Huang M-S, Tsai M-J, Ko Y-C. Ginger suppresses phthalate ester-induced airway remodeling. *Journal of agricultural and food chemistry* 2011;59(7):3429-3438.
95. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, Divjan A, Rosa MJ, Zhang H, Perera FP. Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort. *American journal of respiratory and critical care medicine* 2012;186(9):830-837.
96. Hurst CH, Waxman DJ. Activation of PPARα and PPARγ by environmental phthalate monoesters. *Toxicological Sciences* 2003;74(2):297-308.

97. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret M-C, Mechighel P, Megret J, Leseche G, Aubier M, Pretolani M. Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *American journal of respiratory and critical care medicine* 2001;164(8):1487-1494.
98. Lourenço A, Galbiati V, Corti D, Papale A, Martino-Andrade A, Corsini E. The plasticizer dibutyl phthalate (DBP) potentiates chemical allergen-induced THP-1 activation. *Toxicology in Vitro* 2015;29(8):2001-2008.
99. Gao L, Yuan T, Zhou C, Cheng P, Bai Q, Ao J, Wang W, Zhang H. Effects of four commonly used UV filters on the growth, cell viability and oxidative stress responses of the Tetrahymena thermophila. *Chemosphere* 2013;93(10):2507-2513.
100. Cho YS, Moon H-B. The role of oxidative stress in the pathogenesis of asthma. *Allergy, asthma & immunology research* 2010;2(3):183-187.
101. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, Wang C-R, Schumacker PT, Licht JD, Perlman H. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* 2013;38(2):225-236.
102. Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, Holmdahl R. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. *The Journal of clinical investigation* 2007;117(10):3020-3028.
103. Chen S, You H, Mao L, Yang X. Dibutyl phthalate induced oxidative stress does not lead to a significant adjuvant effect on a mouse asthma model. *Toxicology Research* 2015;4(2):260-269.
104. Anderson SE, Franko J, Kashon ML, Anderson KL, Hubbs AF, Lukomska E, Meade BJ. Exposure to triclosan augments the allergic response to ovalbumin in a mouse model of asthma. *toxicological sciences* 2012:kfs328.
105. Hwang Y-H, Paik M-J, Yee S-T. Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium. *Toxicology letters* 2017;272:49-59.
106. Wang Y, Zhao M, Liu J, Ni J, Jiao Y, Bai C. Up regulation of IL-6 is involved in di (2-ethylhexyl) phthalate (DEHP) induced migration and invasion of non small cell lung cancer (NSCLC) cells. *Biomedicine & Pharmacotherapy* 2017;89:1037-1044.
107. Stermer AR, Murphy CJ, Ghaffari R, Di Bona KR, Voss JJ, Richburg JH. Mono-(2-ethylhexyl) phthalate-induced Sertoli cell injury stimulates the production of pro-inflammatory cytokines in Fischer 344 rats. *Reproductive Toxicology* 2017;69:150-158.
108. Lee MH, Chung SW, Kang BY, Park J, Lee CH, Hwang SY, Kim TS. Enhanced interleukin-4 production in CD4+ T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+. *Immunology* 2003;109(1):76-86.
109. Lee J, Lim K-T. Plant-originated glycoprotein (36 kDa) suppresses interleukin-4 and-10 in bisphenol A–stimulated primary cultured mouse lymphocytes. *Drug and chemical toxicology* 2010;33(4):421-429.
110. Guo H, Liu T, Uemura Y, Jiao S, Wang D, Lin Z, Narita Y, Suzuki M, Hirosawa N, Ichihara Y. Bisphenol A in combination with TNF-α selectively induces Th2 cell-promoting dendritic cells in vitro with an estrogen-like activity. *Cellular & molecular immunology* 2010;7(3):227-234.
111. Shim J-U, Lim K-T. Inhibitory effect of glycoprotein isolated from Cudrania tricuspidata bureau on expression of inflammation-related cytokine in bisphenol A-treated HMC-1 cells. *Inflammation* 2009;32(4):211-217.

112. Tian X, Takamoto M, Sugane K. Bisphenol A promotes IL-4 production by Th2 cells. *International archives of allergy and immunology* 2003;132(3):240-247.
113. Yamashita U, Sugiura T, Kuroda E. Effect of endocrine disrupters on immune responses in vitro. *Journal of UOEH* 2002;24(1):1-10.
114. Lee J, Lee S-J, Lim K-T. CTB glycoprotein (75kDa) inhibits IgE releasing, TNF-α and IL-6 expressed by bisphenol A in vivo and in vitro. *Food and chemical toxicology* 2012;50(6):2109-2117.
115. Valentino R, D'Esposito V, Passaretti F, Liotti A, Cabaro S, Longo M, Perruolo G, Beguinot F, Formisano P. Bisphenol-A impairs insulin action and up-regulates inflammatory pathways in human subcutaneous adipocytes and 3T3-L1 cells. *PLoS One* 2013;8(12):e82099.
116. Lee J, Lim K-T. Expression of TNF-α and IL-6 in HMC-1 cells treated with bisphenol A is attenuated by plant-originating glycoprotein (75 kDa) by blocking p38 MAPK. *Naunyn-Schmiedeberg's archives of pharmacology* 2010;382(1):51-61.
117. Zhu J, Jiang L, Liu Y, Qian W, Liu J, Zhou J, Gao R, Xiao H, Wang J. MAPK and NF-κB pathways are involved in bisphenol A-induced TNF-α and IL-6 production in BV2 microglial cells. *Inflammation* 2015;38(2):637-648.
118. Liu Y, Mei C, Liu H, Wang H, Zeng G, Lin J, Xu M. Modulation of cytokine expression in human macrophages by endocrine-disrupting chemical Bisphenol-A. *Biochemical and biophysical research communications* 2014;451(4):592-598.
119. Xiong S, Wang Y, Li H, Zhang X. Low Dose of Bisphenol A Activates NF-κB/IL-6 Signals to Increase Malignancy of Neuroblastoma Cells. *Cellular and molecular neurobiology* 2017;37(6):1095-1103.
120. Li S, Wang B, Tang Q, Liu J, Yang X. Bisphenol A triggers proliferation and migration of laryngeal squamous cell carcinoma via GPER mediated upregulation of IL-6. *Cell biochemistry and function* 2017;35(4):209-216.
121. Xu H, Yang M, Qiu W, Pan C, Wu M. The impact of endocrine-disrupting chemicals on oxidative stress and innate immune response in zebrafish embryos. *Environmental toxicology and chemistry* 2013;32(8):1793-1799.
122. Hughes P, McLellan H, Lowes D, Kahn SZ, Bilmen J, Tovey S, Godfrey R, Michell R, Kirk C, Michelangeli F. Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca2+ pumps. *Biochemical and biophysical research communications* 2000;277(3):568-574.
123. Yan H, Takamoto M, Sugane K. Exposure to Bisphenol A Prenatally or in Adulthood Promotes T^ sub H^ 2 Cytokine Production Associated with Reduction of CD4^ sup+^ CD25^ sup+^ Regulatory T Cells. *Environmental health perspectives* 2008;116(4):514.
124. Ménard S, Guzylack-Piriou L, Lencina C, Leveque M, Naturel M, Sekkal S, Harkat C, Gaultier E, Olier M, Garcia-Villar R. Perinatal exposure to a low dose of bisphenol A impaired systemic cellular immune response and predisposes young rats to intestinal parasitic infection. *PloS one* 2014;9(11):e112752.
125. Kang J, Song J, Shen S, Li B, Yang X, Chen M. Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB. *Oncotarget* 2016;7(51):85472-85482.
126. Shen S, Li J, You H, Wu Z, Wu Y, Zhao Y, Zhu Y, Guo Q, Li X, Li R. Oral exposure to diisodecyl phthalate aggravates allergic dermatitis by oxidative stress and enhancement of thymic stromal lymphopoietin. *Food and Chemical Toxicology* 2017;99:60-69.

127. Rogers JA, Metz L, Yong VW. Review: endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. *Molecular immunology* 2013;53(4):421-430.
128. Yang YJ, Hong Y-C, Oh S-Y, Park M-S, Kim H, Leem J-H, Ha E-H. Bisphenol A exposure is associated with oxidative stress and inflammation in postmenopausal women. *Environmental research* 2009;109(6):797-801.
129. Ezz HSA, Khadrawy YA, Mourad IM. The effect of bisphenol A on some oxidative stress parameters and acetylcholinesterase activity in the heart of male albino rats. *Cytotechnology* 2015;67(1):145-155.
130. Manteiga S, Lee K. Monoethylhexyl Phthalate Elicits an Inflammatory Response in Adipocytes Characterized by Alterations in Lipid and Cytokine Pathways. *Environ Health Perspect* 2016.
131. Dearman RJ, Betts CJ, Beresford L, Bailey L, Caddick HT, Kimber I. Butyl benzyl phthalate: effects on immune responses to ovalbumin in mice. *Journal of Applied Toxicology* 2009;29(2):118-125.
132. Hansen JS, Larsen ST, Poulsen LK, Nielsen GD. Adjuvant effects of inhaled mono-2-ethylhexyl phthalate in BALB/cJ mice. *Toxicology* 2007;232(1):79-88.
133. He M, Inoue K-I, Yoshida S, Tanaka M, Takano H, Sun G, Ichinose T. Effects of airway exposure to di-(2-ethylhexyl) phthalate on allergic rhinitis. *Immunopharmacology and immunotoxicology* 2013;35(3):390-395.
134. Larsen ST, Lund RM, Nielsen GD, Thygesen P, Poulsen OM. Di-(2-ethylhexyl) phthalate possesses an adjuvant effect in a subcutaneous injection model with BALB/c mice. *Toxicology letters* 2001;125(1):11-18.
135. Larsen S, Lund R, Thygesen P, Poulsen O, Nielsen G. Investigation of the adjuvant and immuno-suppressive effects of benzyl butyl phthalate, phthalic acid and benzyl alcohol in a murine injection model. *Food and chemical toxicology* 2003;41(3):439-446.
136. Larsen ST, Hansen JS, Hansen EW, Clausen PA, Nielsen GD. Airway inflammation and adjuvant effect after repeated airborne exposures to di-(2-ethylhexyl) phthalate and ovalbumin in BALB/c mice. *Toxicology* 2007;235(1):119-129.
137. Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice. *Environmental health perspectives* 2003;111(16):1883.
138. Goto M, Takano-Ishikawa Y, Ono H, Yoshida M, Yamaki K, Shinmoto H. Orally administered bisphenol A disturbed antigen specific immunoresponses in the naive condition. *Bioscience, biotechnology, and biochemistry* 2007;71(9):2136-2143.
139. Wang I-J, Lin C-C, Lin Y-J, Hsieh W-S, Chen P-C. Early life phthalate exposure and atopic disorders in children: a prospective birth cohort study. *Environment international* 2014;62:48-54.
140. Ku HY, Su PH, Wen HJ, Sun HL, Wang CJ, Chen HY, Jaakkola JJ, Wang S-L, Group T. Prenatal and postnatal exposure to phthalate esters and asthma: a 9-year follow-up study of a taiwanese birth cohort. *PloS one* 2015;10(4):e0123309.
141. Guo J, Han B, Qin L, Li B, You H, Yang J, Liu D, Wei C, Nanberg E, Bornehag C-G. Pulmonary toxicity and adjuvant effect of di-(2-exylhexyl) phthalate in ovalbumin-immunized BALB/c mice. *PloS one* 2012;7(6):e39008.
142. Chen L, Chen J, Xie CM, Zhao Y, Wang X, Zhang YH. Maternal Disononyl Phthalate Exposure Activates Allergic Airway Inflammation via Stimulating the Phosphoinositide 3-

kinase/Akt Pathway in Rat Pups. *Biomedical and Environmental Sciences* 2015;28(3):190-198.
143. Koike E, Yanagisawa R, Sadakane K, Inoue K-i, Ichinose T, Takano H. Effects of diisononyl phthalate on atopic dermatitis in vivo and immunologic responses in vitro. *Environmental health perspectives* 2010;118(4):472.
144. Badr MZ, Shnyra A, Zoubine M, Norkin M, Herndon B, Quinn T, Miranda RN, Cunningham ML, Molteni A. Phthalate-induced liver protection against deleterious effects of the Th1 response: a potentially serious health hazard. *PPAR research* 2008;2007.
145. Qin W, Deng T, Cui H, Zhang Q, Liu X, Yang X, Chen M. Exposure to diisodecyl phthalate exacerbated Th2 and Th17-mediated asthma through aggravating oxidative stress and the activation of p38 MAPK. *Food and Chemical Toxicology* 2018;114:78-87.
146. Alfardan AS, Nadeem A, Ahmad SF, Al-Harbi NO, Al-Harbi MM, AlSharari SD. Plasticizer, di (2-ethylhexyl) phthalate (DEHP) enhances cockroach allergen extract-driven airway inflammation by enhancing pulmonary Th2 as well as Th17 immune responses in mice. *Environmental research* 2018;164:327-339.
147. Wang B, Liu F, Dong J, You M, Fu Y, Li C, Lu Y, Chen J. Maternal exposure to environmental DEHP exacerbated OVA-induced asthmatic responses in rat offspring. *Science of The Total Environment* 2018;615:253-261.
148. He M, Ichinose T, Yoshida S, Takano H, Nishikawa M, Shibamoto T, Sun G. Exposure to bisphenol A enhanced lung eosinophilia in adult male mice. *Allergy, Asthma & Clinical Immunology* 2016;12(1):16.
149. Petzold S, Averbeck M, Simon JC, Lehmann I, Polte T. Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. *PloS one* 2014;9(6):e100468.
150. O'Brien E, Dolinoy DC, Mancuso P. Perinatal bisphenol A exposures increase production of pro-inflammatory mediators in bone marrow-derived mast cells of adult mice. *Journal of immunotoxicology* 2014;11(3):205-212.
151. Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunology* 2004;112(3):489-495.
152. Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y. Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. *British journal of pharmacology* 2003;138(7):1271-1276.
153. 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(6):e38448.
154. Holladay SD, Xiao S, Diao H, Barber J, Nagy T, Ye X, Gogal RM. Perinatal bisphenol A exposure in C57B6/129svj male mice: potential altered cytokine/chemokine production in adulthood. *International journal of environmental research and public health* 2010;7(7):2845-2852.
155. Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal bisphenol a exposure promotes the development of experimental asthma in mouse pups. *Environmental health perspectives* 2010;118(2):273.
156. Bauer SM, Roy A, Emo J, Chapman TJ, Georas SN, Lawrence BP. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. *Toxicological Sciences* 2012;130(1):82-93.

157. Byun J-A, Heo Y, Kim Y-O, Pyo M-Y. Bisphenol A-induced downregulation of murine macrophage activities in vitro and ex vivo. *Environmental toxicology and pharmacology* 2005;19(1):19-24.
158. Youn J-Y, Park H-Y, Lee J-W, Jung I-O, Choi K-H, Kim K, Cho K-H. Evaluation of the immune response following exposure of mice to bisphenol A: induction of Th1 cytokine and prolactin by BPA exposure in the mouse spleen cells. *Archives of pharmacal research* 2002;25(6):946-953.
159. Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, Canfield S, Resnick D, Calafat AM, Perera FP. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. *Journal of Allergy and Clinical Immunology* 2013;131(3):736-742. e6.
160. Riu A, Grimaldi M, le Maire A, Bey G, Phillips K, Boulahtouf A, Perdu E, Zalko D, Bourguet W, Balaguer P. Peroxisome Proliferator-Activated Receptor [gamma] Is a Target for Halogenated Analogs of Bisphenol A. *Environmental health perspectives* 2011;119(9):1227.
161. 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. *Environmental Health Perspectives (Online)* 2013;121(8):912.
162. Yang G, Qiao Y, Li B, Yang J, Liu D, Yao H, Xu D, Yang X. Adjuvant effect of di-(2-ethylhexyl) phthalate on asthma-like pathological changes in ovalbumin-immunised rats. *Food and agricultural immunology* 2008;19(4):351-362.
163. Nakajima Y, Goldblum RM, Midoro-Horiuti T. Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. *Environmental Health* 2012;11(1):8.
164. Nygaard UC, Vinje NE, Samuelsen M, Andreassen M, Groeng E-C, Bølling AK, Becher R, Lovik M, Bodin J. Early life exposure to bisphenol A investigated in mouse models of airway allergy, food allergy and oral tolerance. *Food and Chemical Toxicology* 2015;83:17-25.
165. Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. *Environmental research* 2015;140:360-368.
166. Ashley-Martin J, Dodds L, Arbuckle TE, Marshall J. Prenatal triclosan exposure and cord blood immune system biomarkers. *International journal of hygiene and environmental health* 2016;219(4):454-457.
167. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, Hoepner LA, Perera FP, Miller RL. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. 2014.
168. Cakmak S, Dales RE, Hebbern C, Saravanabhavan G. The association between urinary phthalates and lung function. *Journal of occupational and environmental medicine* 2014;56(4):376.
169. Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, Martínez D. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. *Journal of Allergy and Clinical Immunology* 2015;135(2):370-378. e7.
170. Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polańska K, Ligocka D, Hanke W. The effect of prenatal exposure to phthalates on food allergy and early eczema

in inner-city children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2015;e72-e78.
171. Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat A, Ye X, Annesi-Maesano I, Siroux V. In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study. *Environmental Health Perspectives* 2017;125(9):097006.
172. Lee-Sarwar K, Hauser R, Calafat AM, Ye X, O'Connor GT, Sandel M, Bacharier LB, Zeiger RS, Laranjo N, Gold DR. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. *Journal of Allergy and Clinical Immunology* 2017.
173. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environment international* 2018;115:79-88.
174. Savage JH, Johns CB, Hauser R, Litonjua AA. Urinary triclosan levels and recent asthma exacerbations. *Annals of Allergy, Asthma & Immunology* 2014;112(2):179-181. e2.
175. Bertelsen RJ, Longnecker MP, Løvik M, Calafat AM, Carlsen KH, London SJ, Lødrup Carlsen K. Triclosan exposure and allergic sensitization in Norwegian children. *Allergy* 2013;68(1):84-91.
176. Spanier AJ, Fausnight T, Camacho TF, Braun JM. The associations of triclosan and paraben exposure with allergen sensitization and wheeze in children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2014;475-481.
177. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *Journal of Allergy and Clinical Immunology* 2012;130(2):453-460. e7.
178. Spanier AJ, Fiorino EK, Trasande L. Bisphenol A exposure is associated with decreased lung function. *The Journal of pediatrics* 2014;164(6):1403-1408. e1.
179. Kim E-H, Jeon B-H, Kim J, Kim Y-M, Han Y, Ahn K, Cheong H-K. Exposure to phthalates and bisphenol A are associated with atopic dermatitis symptoms in children: a time-series analysis. *Environmental Health* 2017;16(1):24.
180. Soomro MH, Baiz N, Philippat C, Vernet C, Siroux V, Maesano CN, Sanyal S, Slama R, Bornehag C-G, Annesi-Maesano I. Prenatal Exposure to Phthalates and the Development of Eczema Phenotypes in Male Children: Results from the EDEN Mother–Child Cohort Study. *Environmental health perspectives* 2018;126(2):027002.
181. Clayton EM, 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(3):390-6.
182. Hong S, Kwon H-J, Choi W-J, Lim WR, Kim J, Kim K. Association between exposure to antimicrobial household products and allergic symptoms. *Environmental health and toxicology* 2014;29.
183. Shiue I. IgE antibodies and urinary trimethylarsine oxide accounted for 1–7% population attributable risks for eczema in adults: USA NHANES 2005–2006. *Environmental Science and Pollution Research* 2015;22(23):18404-18409.
184. Overgaard LE, Main KM, Frederiksen H, Stender S, Szecsi PB, Williams HC, Thyssen JP. Children with atopic dermatitis and frequent emollient use have increased urinary levels of low-molecular-weight phthalate metabolites and parabens. *Allergy* 2017;72(11):1768-1777.
185. Deutschle T, Reiter R, Butte W, Heinzow B, Keck T, Riechelmann H. A controlled challenge study on di (2-ethylhexyl) phthalate (DEHP) in house dust and the immune

response in human nasal mucosa of allergic subjects. *Environmental health perspectives* 2008;116(11):1487.
186. Liao S-L, Tsai M-H, Lai S-H, Yao T-C, Hua M-C, Yeh K-W, Chiang C-H, Huang S-Y, Huang J-L. Prenatal exposure to bisphenol-A is associated with Toll-like receptor-induced cytokine suppression in neonates. *Pediatric research* 2016;79(3):438.
187. Savastano S, Tarantino G, D'Esposito V, Passaretti F, Cabaro S, Liotti A, Liguoro D, Perruolo G, Ariemma F, Finelli C. Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: a cross-sectional study on adult male population. *Journal of translational medicine* 2015;13(1):169.
188. Hauser R, Calafat A. Phthalates and human health. *Occupational and environmental medicine* 2005;62(11):806-818.
189. Larsson M, Hägerhed-Engman L, Kolarik B, James P, Lundin F, Janson S, Sundell J, Bornehag C-G. PVC–as flooring material–and its association with incident asthma in a Swedish child cohort study. *Indoor Air* 2010;20(6):494-501.
190. Smit LA, Lenters V, Høyer BB, Lindh CH, Pedersen HS, Liermontova I, Jönsson BA, Piersma AH, Bonde JP, Toft G. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. *Allergy* 2015;70(6):653-660.
191. Whyatt RM, Rundle AG, Perzanowski MS, Just AC, Donohue KM, Calafat AM, Hoepner L, Perera FP, Miller RL. Prenatal phthalate and early childhood bisphenol A exposures increase asthma risk in inner-city children. *The Journal of allergy and clinical immunology* 2014;134(5):1195.
192. Spanier AJ, Kahn RS, Kunselman AR, Schaefer EW, Hornung R, Xu Y, Calafat AM, Lanphear BP. Bisphenol a exposure and the development of wheeze and lung function in children through age 5 years. *JAMA pediatrics* 2014;168(12):1131-1137.
193. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, Lanphear BP. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. *Environmental health perspectives* 2012;120(6):916.
194. Kim K-N, Kim JH, Kwon H-J, Hong S-J, Kim B-J, Lee S-Y, Hong Y-C, Bae S. Bisphenol A exposure and asthma development in school-age children: a longitudinal study. *PloS one* 2014;9(10):e111383.
195. Vaidya SV, Kulkarni H. Association of urinary bisphenol A concentration with allergic asthma: results from the National Health and Nutrition Examination Survey 2005–2006. *Journal of Asthma* 2012;49(8):800-806.
196. Zhou A, Chang H, Huo W, Zhang B, Hu J, Xia W, Chen Z, Xiong C, Zhang Y, Wang Y. Prenatal exposure to bisphenol A and risk of allergic diseases in early life. *Pediatric Research* 2017;81(6):851-856.
197. Wang I-J, Chen C-Y, Bornehag C-G. Bisphenol A exposure may increase the risk of development of atopic disorders in children. *International journal of hygiene and environmental health* 2016;219(3):311-316.
198. Holladay SD, Smialowicz RJ. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. *Environmental health perspectives* 2000;108(Suppl 3):463.

Chapter 2: Measurement of respiratory and allergic outcomes in the CHAMACOS study

Respiratory health has been a major focus of the CHAMACOS study since it began, and the study now has a wealth of information tracking respiratory and allergic outcomes from birth through childhood. Mothers have been asked questions about their child's respiratory health at every visit point, and lung function tests were conducted on almost all children at ages five and seven. (Table 1)

Table 1. Respiratory and immune data collected at each visit point in the CHAMACOS study

	Age in years at visit points										
Outcome	0.5	1	2	3.5	5	7	9	10.5	12	14	16
Maternal report of:											
Respiratory symptoms	X	X	X	X	X	X	X	X	X	X	X
Asthma medication use	--	X	X	X	X	X	X	X	X	X	X
Doctor diagnosis of asthma	--	X	X	X	X	X	X	X	X	X	X
Symptoms or doctor diagnosis of inhalant allergy	--	--	--	X	X	X	X	X	X	X	X
Doctor diagnosis of eczema	X	X	X	X	X	X	X	X	X	--	--
Spirometry	--	--	--	--	X	X	--	--	--	--	--
Cytokines in blood	--	X	X	--	X	X	--	--	--	--	--

X: was assessed
--: was not assessed

For this analysis, we chose to examine respiratory and immune health at age seven because this age has the most comprehensive relevant data, including spirometry measurements (which are considered more valid than those at age five, a young age when spirometry is difficult for children), cytokine measurements, and respiratory and immune questionnaire data. We chose also to include cytokine data at ages two and five as previous studies have shown cytokine profiles change throughout childhood[199]. Because the respiratory outcome variables that we used differed from some previous analyses, we describe the available data and how we characterized these respiratory outcomes below. We also examine how these outcomes have tracked over time.

Data collection

Respiratory symptoms:

Mothers were asked at every visit if their child had any respiratory symptoms, and many questions were based on the ISAAC questionnaire[200]. (Table 2) While the questions were similar, they were not identical at each visit. Questions evolved with the children's age. For example, questions in younger visits included whether or not the child had had a cold recently in addition to questions on wheeze and wheeze-related doctor's visits, and later visits included additional questions on how respiratory symptoms affected children's ability to play or talk.

Table 2. Respiratory symptom questions in CHAMACOS questionnaires by visit

	Age in years at visit points					
Questionnaire item	0.5	1	2	3.5	5	7
Cold in last 12 months[1,2]	X	X	X	X		
Wheezing or whistling in the chest in last 12 months					X	X
Wheezing or difficulty breathing because of a cold in last 12 months[1]	X	X	X	X		
Wheezing or whistling in the chest without a cold in last 12 months	X	X	X	X	X	X
Age at first wheeze		X	X	X		
Saw a doctor or nurse because of wheezing or difficulty breathing in last 12 months[1]	X	X	X	X		
Emergency room visit for a breathing problem, and if the problem was due to asthma in last 12 months				X		
Tightness in the chest and difficulty breathing in last 12 months					X	X
Trouble going to sleep or being awakened from sleep because of wheezing, whistling, shortness of breath in last 12 months					X	X
Trouble going to sleep or being awakened from coughing without a cold in last 12 months					X	X
Wheezing, whistling, or shortness of breath so severe that the child couldn't finish saying a sentence in last 12 months					X	X
The child had to stop running or playing or had to sit out of active games because of wheezing, whistling, shortness of breath in last 12 months					X	X
The child had to stop running or playing or had to sit out of active games because of coughing without a cold in last 12 months					X	X
An asthma attack in last 12 months[3]				X	X	X
How often asthma attacks occurred[3]				X		

[1]Questions specified "since the child was born" instead of "in last 12 months" at the six month visit.

[2]At the one year visit, this question was asked without a specified date range

[3]Asked only of mothers who reported their child had ever been diagnosed with asthma

Asthma medication:

Mothers were asked about their child's use of asthma medication at ages one, two, three and a half, five, and seven. (Table 3) At age two, mothers were mainly asked about albuterol and prelone because these are the two most common asthma medications given to young children. At several visit points, mothers were shown a comprehensive photo of all known asthma medications known to be currently available to help her identify which medication her child was given.

Table 3. Asthma medication questions in CHAMACOS questionnaires by visit

	Age in years at visit points					
Questionnaire item	0.5	1	2	3.5	5	7
Asked if a doctor or nurse has given their child any medication for asthma or wheezing or whistling or tightness in the chest in last 12 months?			X	X	X	X
Mothers were shown photographs of all rescue, controller, and other asthma medications known to be currently on the market and were asked to point to the medications that their child currently uses.					X	X
Asked if their child uses a nebulizer machine for asthma or wheezing or whistling and shown photographs of machines to help with identification.			X	X	X	X
Asked if their child uses an inhaler for asthma or wheezing or whistling and shown photographs of inhalers to help with identification.				X	X	X
If mothers reported a doctor told her that her child had asthma, they was asked if the doctor gave her baby medicine for asthma		X				
Did a doctor or nurse gave her child albuterol or ventolin in last 12 months			X	X		
Did a doctor or nurse gave her child prelone or pediapred in last 12 months			X	X		
When her child last took any of the reported medications			X			
Asked if the asthma medication was a syrup, an inhaler, a nebulizer solution, or a pill				X		

Doctor diagnosis of asthma: Mothers were asked if their child had been diagnosed with asthma by a doctor at ages one, two, three and a half, five, and seven, but the wording on the questionnaires was not consistent at each visit. At one and two years, mothers were asked if their child had been diagnosed with asthma by a doctor since the last visit point. At all other visit points, mothers were asked if their child had ever been diagnosed with asthma by a doctor. We also have medical record information for each age, although asthma diagnoses have only been abstracted for the period between birth and age two.

Aeroallergy

At the five and seven year visits, mothers were asked "In the last 12 months, has a doctor or nurse told you that your child had hay fever or allergic rhinitis?" and "In the last 12 months has your child had runny or itchy eyes apart from colds?" or "sneezing or a runny nose apart from colds?". The

same questions were also asked at the three and a half year visit, but the questions were asked about "ever" rather than the past 12 months.

We have also collected medical record information for each age, but inhalant allergy diagnoses have only been abstracted at age two.

Eczema

At the six month, one, two, three and a half, five, and seven year visits, mothers were asked if their child had been diagnosed with eczema or an allergic skin rash. At the three and a half, five, and seven year visits, mothers were specifically asked about diagnoses in the past 12 months, while earlier visits asked about ever diagnoses. At the six month and one year visits, mothers were also asked if their baby had been given any medications that were prescribed by a doctor or that were bought without a prescription for skin allergies or eczema.

We have also collected medical record information for each age, but eczema diagnoses have only been abstracted for age two.

Spirometry

Spirometry was conducted as part of the clinical exam at ages five and seven using EasyOne spirometers programmed with software developed for the Fresno Asthmatic Children's Environment Study (FACES). Two bilingual and bicultural technicians specifically trained for the study administered the spirometry exams, and 92% of the exams were performed by the same technician. Routine calibration of three identical EasyOne (new diagnostic design) spirometers was performed every morning. Every child performed a maximum of eight expiratory maneuvers, and the EasyOne software (new diagnostic design EasyWare) kept up to three best acceptable tests. Acceptability criteria were as follows: no hesitation at the start of the test (back extrapolation volume <150ml); rapid onset of flow (time to peak expiratory flow <120 milliseconds, although otherwise acceptable tests with time to peak expiratory flow up to 200 milliseconds were accepted conditional on physician review; no evidence of air leak (seen as a decline in the time volume curve after the start of the volume plateau); no coughing or other evidence of stopping/starting of flow; expiratory time of at least three seconds; and technician assessment of subject test performance. In all attempts, children were coached to blow until the EasyOne signaled that the test had ended. Technicians verified that children did not lean forward, took enough air in and took a deep breath right from the beginning, blew enough air out, did not stop blowing too soon, took a big smooth breath, and that air did not leak out of the side. Two physicians with experience in pediatric spirometry, Dr. Ira Tager and Dr. John Balmes, individually reviewed and verified printouts of each child's maneuvers and excluded invalid data by hand. Invalid maneuvers were defined as those that did not meet criteria set by the American Thoracic Society, which required maneuvers to be smooth (no inhalation, no coughs) and to start quickly (children exhale right away). Criteria also required the best FEV_1 and FVC measurements to be within 100ml of each other. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and forced expiratory flow from 25–75% of FVC (FEF25%-75%) were measured from each maneuver.

Bronchodilator test: At age five, all children were given albuterol with a spacer and repeated the spirometry after 20 minutes to determine whether their lung function improved with the administration of a bronchodilator (an indication of asthma). At age seven, the bronchodilator test was offered only to children whose mothers answered yes to any of the asthma symptom questions because of time constraints.

Cytokines

Blood was collected from the children at ages one, two, five, and seven and mailed to the Holland laboratory for aliquoting and analysis. Unfrozen blood was analyzed for cytokines levels within 48 hours of collection. Whole blood was activated by phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO) and ionomycin (Sigma) to display CD4+ surface antigens. Monoclonal antibodies for IFN-y and IL-4 were detected using flow cytometry[201]. Cells that stained for IFN-y only were coded as Th1 cells and cells that stained for IL-4 only were coded as Th2 cells. The percentage of Th1 cells (Th1%) is the number of Th1 cells divided by the total number of CD4+ cells, and the percentage of Th2 cells (Th2%) was defined as the number of Th2 cells divided by the total number of CD4+ cells. Th1:Th2 cell ratio was defined as Th1% divided by Th2%.

This method was developed by Paurene Duramad as part of her dissertation and differs from the ELISA assay more commonly used by other researchers. The traditional ELISA assay identifies concentrations of cytokines produced by Th1 and Th2 cells, whereas our method counts actual Th1 and Th2 cells by detecting monoclonal antibodies for two major cytokines presented by the cells, which allows us to more accurately identify the relative dominance of Th1 and Th2 immunity. Our method is more appropriate for banked samples because the traditional ELISA assay requires samples to be processed at specific times, but flow cytometry does not. Our method also uses <500uL of whole blood which is more appropriate for studies like ours that have limited supplies of blood.

Definition of variables

Probable asthma variables

This analysis differs from previously published papers on CHAMACOS respiratory symptom data because we have generated a variable indicating whether a child had "probable asthma" at age seven by combining data on: 1) current respiratory symptoms, 2) current medication use, 3) ever having a doctor diagnosis of asthma, and 4) the child's response to the bronchodilator test. Details on each of these components of the probable asthma variable are detailed below:

1) *Respiratory symptoms:* We classified children as having respiratory symptoms at age seven if their mothers reported any of the symptoms listed in Table 4 in the last 12 months. There were 54 children (15%) who met this criterion. It was most common for a mother to report only one symptom, and for that symptom to be "trouble going to sleep or being awakened from coughing without a cold". Only one child reported having all nine symptoms. (Table 4)

Table 4. Respiratory symptoms in the last year, asked at age seven

	N (%)	
	Yes	Total
Tightness in the chest and difficulty breathing	11 (3%)	353
Wheezing or whistling in the chest	24 (7%)	353
Wheezing or whistling in the chest without a cold	8 (2%)	353

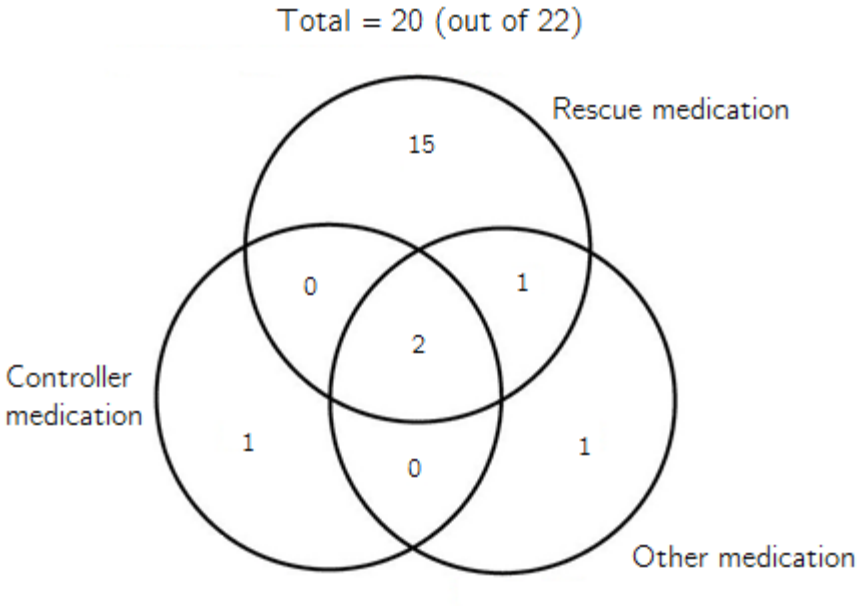
Trouble going to sleep or being awakened from sleep because of wheezing, whistling, shortness of breath	6 (2%)	353
Trouble going to sleep or being awakened from coughing without a cold	35 (10%)	353
Wheezing, whistling, or shortness of breath so severe that the child couldn't finish saying a sentence	3 (1%)	353
The child had to stop running or playing or had to sit out of active games because of wheezing, whistling, shortness of breath	12 (3%)	353
The child had to stop running or playing or had to sit out of active games because of coughing without a cold	13 (4%)	353
An asthma attack[1]	4 (11%)	35

[1]Asked only of mothers who reported their child had ever been diagnosed with asthma

There was a lack of consistency among maternal reports of respiratory symptoms at different visit points. For example, of the 89 children reported to have a symptom on the five year questionnaire, only 20% were also reported to have that symptom on the seven year questionnaire. Conversely, on average, only 19% of children reported to have a symptom on the seven year questionnaire were also reported to have that symptom on the five year questionnaire. This could reflect either the transient nature of asthma in children or inaccurate reporting by mothers. Children may have had asthmatic symptoms at earlier childhood ages and subsequently grown out of them, or developed asthma at age seven, but mothers may also have under or overreported symptoms at any age. See Appendix 1 at the end of this chapter for a comparison of maternal report of wheeze without a cold across ages, the only variable that was asked at all age points.

2) *Asthma medication:* At age seven, 22 mothers (6%) reported that their children were taking asthma medication, although two mothers did not report whether their child was using rescue, controller, or other asthma medication. (Figure 1)

Figure 1. Reports of asthma medication at age seven



At age five, 32 mothers (9%) reported their children had taken asthma medication in the last 12 months. Of those, 34% were also reported to use asthma medication at age seven. Of the 22 children who were reported to use asthma medication at age seven, 10 (45%) were reported to use it at age five. Similar to issues with respiratory symptoms, the inconsistencies in reported asthma medication use could reflect that a child truly had asthma only at certain ages, or that mothers underreported use. See Appendix 2 at the end of this chapter for a comparison of reported asthma medication use across ages.

3) *Doctor diagnosis:* Answers to questions on doctor diagnoses of asthma were not always consistent over visits. (Table 5) For example, a mother may have reported her child was ever diagnosed with asthma on the age five questionnaire, but not on the age seven questionnaire. We therefore created a variable that included children whose mothers answered yes to an asthma diagnosis question at any visit point.

Table 5. Maternal report of doctor diagnosis by age

	N (%)			
	Yes	No	Don't Know	Total
Age 6 months	--	--	--	--
Age 1	19 (4%)	405 (95%)	1 (0%)	425
Age 2	21 (5%)	392 (95%)	--	413
Age 3.5	30 (8%)	334 (92%)	1 (0%)	365
Age 5	44 (13%)	305 (87%)	1 (0%)	350
Age 7	35 (10%)	315 (90%)	--	350

On at least one questionnaire, 62 mothers (18%) reported their children were diagnosed with asthma. It was most common for mothers to respond positively to a doctor diagnosis question

only once, and age five was the most reported age. Only three mothers responded positively for every age. Of the 35 children whose mothers reported they were diagnosed with asthma at multiple ages including age seven, 27 (77%) of them consistently said their child had been diagnosed with asthma at every visit after the first positive response. Additionally, medical record data show that 35 children (7%) were diagnosed with asthma by age two (though this age is generally considered too early for a diagnosis of asthma), and only ten of these children (29%) were reported to "ever have a diagnosis" at all subsequent visits, suggesting that mothers may forget about early life diagnoses when asked on later questionnaires. An additional ten children (29%) with medical record diagnoses at age two were never reported to have a diagnosis by their mothers on subsequent visits. Unlike the respiratory symptom and asthma medication questions, these questions were asked about an "ever" diagnosis and inconsistent answers therefore should not reflect transient childhood asthma. However, mothers may have forgotten an early diagnosis or misunderstood the question. See Appendix 3 at the end of this chapter for a table comparing responses across ages.

4) *Positive bronchodilator test:* A bronchodilator test was administered to 42 children whose mothers reported any respiratory symptom at age seven. A positive bronchodilator test was defined as a 12% or more improvement from children's best measurement of FEV_1 or FVC before the bronchodilator to their best measurement of FEV_1 or FVC, respectively, after. Only ten children had a positive test, indicating that reports of current asthma symptoms may not manifest as albuterol-relieved bronchoconstriction. At age five, 278 children took the same test, regardless of reporting current respiratory symptoms, and 35 children (13%) had a positive result, although age five is a less reliable age for spirometry. Twenty-six children performed the test at both ages. Of the eight children who had a positive test result at either age, only one child had a positive bronchodilator test at both. Inconsistencies here may reflect asthma resolving or developing between ages five and seven, but could also incorporate measurement error in younger children as it is more difficult for them to perform the spirometry. Six children who had a positive result at age seven did not perform the test at age five, thereby potentially masking some consistent test results.

Probable asthma definition

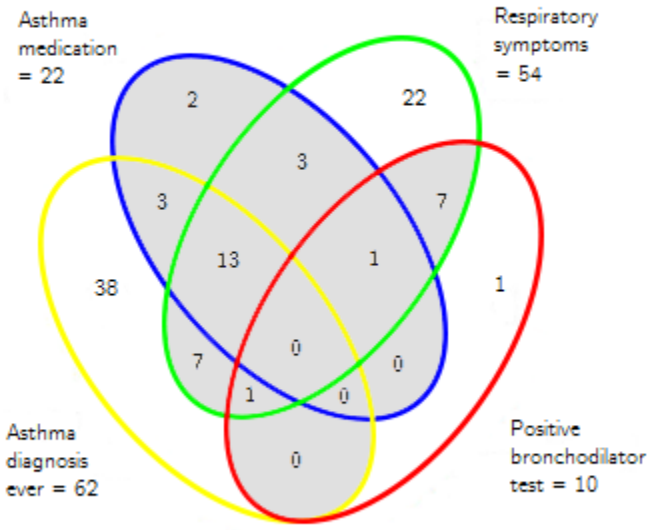
For this dissertation, we defined children as having "probable asthma" at age seven if they were currently taking asthma medication or if they had any two of the following criteria: current respiratory symptoms, maternal report of asthma diagnosis at any age, or a current positive bronchodilator test (Table 6 and Figure 2). We chose this method to increase specificity by not including children with passing symptoms or symptoms of a non-asthmatic origin, and to include children whose respiratory illness may not be captured by reportable symptoms. Out of 98 children with at least one of these criteria at age seven, 36 children (10%) met the definition of having probable asthma. In comparison, the parents of 6% of Mexican-American children age seven in 2013-2014 NHANES reported both that they "still had asthma" and that "a doctor or other health professional had ever told them that their child had asthma"[3].

Table 6. Probable asthma variables at age seven

	N (%)		
	Yes	No	Total
Probable Asthma	36 (10%)	316 (90%)	353
Any current respiratory symptoms	54 (15%)	299 (85%)	353
Any current asthma medication use	22 (6%)	331 (94%)	353
Doctor diagnosis of asthma at any age	62 (18%)	291 (82%)	353
Positive bronchodilator test	10 (24%)	32 (76%)	42

Figure 2. Criteria for classification as having probable asthma

Total with at least one criterion = 98



Shaded areas indicate children classified as having probable asthma

Previous analysis of respiratory data

Two previously published papers also analyzed respiratory symptoms in CHAMACOS and classified them slightly differently. These papers examined associations of respiratory symptoms at age seven with nearby use of organophosphate and elemental sulfur pesticides[202,203]. In these papers, Ranaan et al did not employ all the respiratory symptom questions asked at age seven. (Ranaan et al 2017 did not include "tightness in the chest and difficulty breathing" or asthma attacks in the definition of respiratory symptoms[203] and Ranaan et al 2015 did not include asthma attacks.) Additionally, in Ranaan et al 2015, the odds of a child "having to stop running or playing active games due to coughing that was not associated with a cold" was analyzed as a separate dependent variable in addition to being part of the total respiratory symptoms category[202]. In both papers, children were also classified as having "respiratory symptoms" if their mothers reported them using asthma rescue or controller medication (other asthma medication was not included), and in Ranaan et al 2017 the odds of using asthma rescue or controller medication was analyzed separately. (Table 7)

For this analysis, we chose to aggregate several respiratory data points (taking asthma medication, having respiratory symptoms, ever being diagnosed with asthma by a doctor, and performing a positive bronchodilator test) into a single classification of probable asthma. Since asthma medication is unlikely to be prescribed to children without asthma, children were also classified as having probable asthma if they had recently used asthma medication in the absence of other criteria. We chose not to analyze odds of using asthma medication separately because we did not think the question would be different enough from analyzing odds of probable asthma, and because analyzing only medication use would exclude children who had not seen a doctor or not been prescribed medication for their asthma. Additionally, to identify respiratory symptoms, we chose to include all respiratory symptom questions, including "tightness in the chest and difficulty breathing" and asthma attacks, to catch children with any symptoms that could indicate asthma. The comprehensive inclusion of respiratory data is a strength of this classification method.

Table 7. Respiratory data analyzed in CHAMACOS: current analysis compared to previously published papers

	Current analysis "Probable asthma"	Ranaan 2015 "Respiratory symptoms"	Ranaan 2017 "Respiratory symptoms"
Tightness in the chest and difficulty breathing	X	X	--
Wheezing or whistling in the chest	X	X	X
Wheezing or whistling in the chest without a cold	X	X	X
Trouble going to sleep or being awakened from sleep because of wheezing, whistling, shortness of breath	X	X	X
Trouble going to sleep or being awakened from coughing without a cold	X	X	X
Wheezing, whistling, or shortness of breath so severe that the child couldn't finish saying a sentence	X	X	X
The child had to stop running or playing or had to sit out of active games because of wheezing, whistling, shortness of breath	X	X	X
The child had to stop running or playing or had to sit out of active games because of coughing without a cold	X	X	X
An asthma attack	X	--	--
Rescue or controller asthma medication	X	X	X
Other asthma medication	X	--	--
Doctor diagnosis of asthma	X	--	--
Bronchodilator test	X	--	--

X: included in classification
--: not included in classification

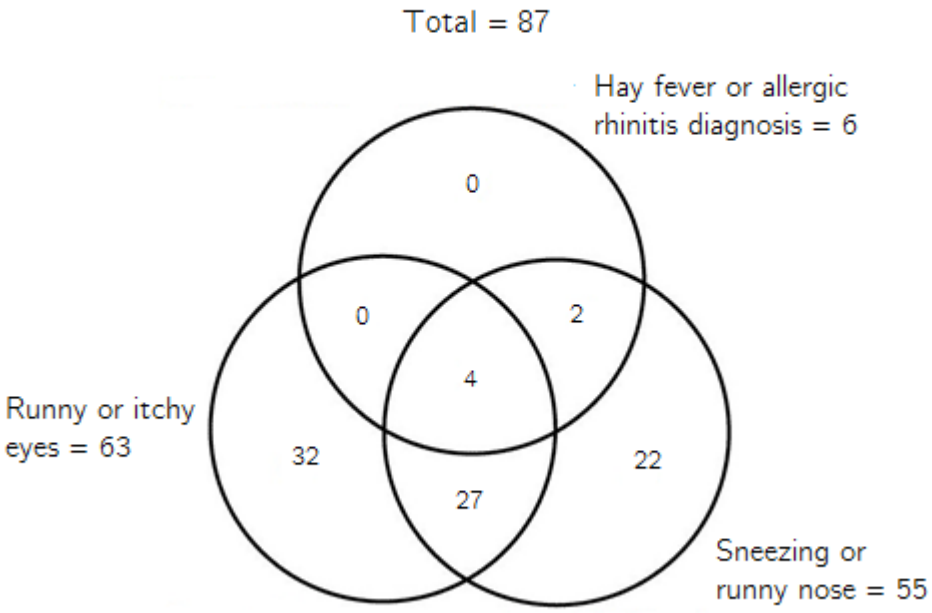
Aeroallergy

A yes to any of the three questions on aeroallergy (doctor's diagnosis of hay fever or allergic rhinitis, runny or itchy eyes without a cold, sneezing or runny nose without a cold) defined a case of aeroallergy. (Table 8 and Figure 3) We classified 87 children (26%) as having aeroallergies at age seven. Six children (2%) had been diagnosed with hay fever/allergic rhinitis and all six also experienced an aeroallergy symptom in the last 12 months. In comparison, 6% of Mexican-American children age seven in 2005-2006 NHANES reported they had ever been diagnosed with allergies, and 62% reported aeroallergy symptoms in the last year, although allergy symptoms were not specifically defined and may have been more widely interpreted by respondents[5].

Table 8. Inhalant allergy cases at age seven

	N (%)			
	Yes	No	Don't Know	Total
Total inhalant allergy cases	87 (26%)	252 (74%)	--	339
Hay fever or allergic rhinitis diagnosis	6 (2%)	332 (98%)	1 (0%)	339
Runny or itchy eyes	63 (19%)	276 (81%)	0 (0%)	339
Sneezing or runny nose	55 (16%)	284 (84%)	0 (0%)	339

Figure 3. Inhalant allergy criteria at age seven



At age five, 129 children (37%) were classified with the disease. Fifty-three children (61%) were classified as having aeroallergies at both ages five and seven, showing higher consistency than respiratory variables. Allergy symptoms may be easier for mothers to identify and thus report, or

allergy cases may not be as transient as cases of probable asthma. The decrease in classified cases from age five to age seven may reflect resolution of aeroallergy with time or use of allergy medication, which was not asked about.

Eczema

Mothers of 23 children (7%) reported that their child had been diagnosed with eczema at age seven. In comparison, 2% of parents of Mexican-American children age seven in 2005-2006 NHANES reported that their children had ever been diagnosed with eczema[5]. At age five, mothers of 28 children (8%) reported a diagnosis with the disease in the last 12 months. Mothers of nine children (39%) reported a diagnosis at both ages. Though the prevalence of eczema was similar at the two ages, individual cases were not highly consistent, although it is difficult to know whether this is due to resolution/development of individual cases between ages five and seven or maternal misreporting.

Spirometry

We administered spirometry to 328 children at age seven; 300 (91.5%) had acceptable maneuvers. In order to capture children's maximum lung function, we are using the highest measure for each of the measurement types, which may be from different maneuvers. (Table 9) In sensitivity analyses, we also used the best test result at age seven for each measurement type among children who had two or more verified maneuvers, and mean measurements were comparable. We also have these data for 287 children at age five, although spirometry is less reliable at this younger age and we have acceptable tests for only 187 (65.2%) children. Though FEV_1/FVC remained stable, all other measurements increased from age five to age seven, reflecting expected growth of lung function.

Table 9. Spirometry variables at ages five and seven

		Mean (SD)			
	N	FEV_1	FVC	FEV_1/FVC	FEF_{25-75}
Age five	104-187	1.0 (0.2) liters	1.1 (0.3) liters	0.9 (0.1)	1.3 (0.5) liters/second
Age seven	270-300	1.8 (0.5) liters	2.1 (0.6) liters	0.9 (0.1)	2.5 (0.9) liters/second

Cytokines

Cytokines measures followed expected trends across ages. (Table 10) Th1:Th2 cell ratio increased with age as the percentage of Th1 cells increased and the percentage of Th2 cells remained stable. The percentage of CD4 cells also expectedly remained stable with age.

Table 10. Descriptive statistics of T-helper cell counts in CHAMACOS participants at ages two, five, and seven

	N	Th1:Th2 cell ratio	% CD4 cells	% Th1 cells	% Th2 cells
Age 2	239	6.1 (5.6)	32.9 (7.5)	4.0 (3.0)	0.9 (0.7)
Age 5	231	14.4 (44.5)	29.9 (7.3)	6.8 (3.2)	1.1 (0.8)
Age 7	254	22.5 (36.3)	33.8 (10.2)	10.0 (5.9)	0.9 (0.7)

Relationship between cytokines and asthma, aeroallergy, and eczema

We hypothesized that cytokine measures at ages two, five, and seven would be associated with increased odds of being classified as having probable asthma, allergies, or eczema at age seven. (Table 11) We conducted logistic regression models adjusted for maternal age, parity, poverty at baseline, and family history of asthma, as determined *a priori* via DAG. Most associations were null, but a higher Th2% at age seven was associated with higher odds of probable asthma at age seven. Similarly, a higher Th1:Th2 cell ratio (i.e. lower Th2% relative to Th1%) at age seven was associated with lower odds of probable asthma at age seven, which is consistent with the hypothesis that Th2 dominance is associated with greater asthma risk. This implies that exposures associated with altered cytokine measures later in childhood may influence the child's risk for atopic diseases more than exposures associated with altered cytokine measures earlier in childhood, although it could also be due to reverse causality: if a child has asthma at age seven, this could affect their Th1:Th2 cell ratio.

Table 11. Association of Th cell percentages and asthma outcomes at age seven (N=174-254)

	Probable asthma		Allergy		Eczema	
	Crude	Adjusted[1]	Crude	Adjusted[1]	Crude	Adjusted[1]
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age 2						
Th1:Th2 cell ratio	1.26 (0.41, 3.90)	1.56 (0.45, 5.39)	0.71 (0.30, 1.67)	0.73 (0.30, 1.78)	0.62 (0.15, 2.53)	0.59 (0.14, 2.44)
Th1 %	1.82 (0.50, 6.65)	2.29 (0.56, 9.39)	0.71 (0.30, 1.67)	0.64 (0.26, 1.57)	0.41 (0.12, 1.39)	0.45 (0.13, 1.58)
Th2 %	1.43 (0.40, 5.18)	1.36 (0.36, 5.20)	1.01 (0.37, 2.79)	0.87 (0.30, 2.49)	0.48 (0.09, 2.53)	0.59 (0.11, 3.20)
	n=197	n=195	n=194	n=184	n=194	n=192
Age 5						
Th1:Th2 cell ratio	1.31 (0.47, 3.60)	1.16 (0.41, 3.30)	1.07 (0.50, 2.27)	1.02 (0.47, 2.19)	2.46 (0.79, 7.66)	2.67 (0.80, 8.86)
Th1 %	0.80 (0.18, 3.62)	0.71 (0.15, 3.38)	0.70 (0.23, 2.09)	0.71 (0.24, 2.17)	1.37 (0.16, 11.40)	1.37 (0.16, 11.49)
Th2 %	0.72 (0.28, 1.85)	0.76 (0.28, 2.05)	0.81 (0.40, 1.66)	0.86 (0.42, 1.76)	0.49 (0.17, 1.42)	0.47 (0.16, 1.41)
	n=224	n=221	n=221	n=218	n=221	n=218

Age 7						
Th1:Th2 cell ratio	0.34 (0.10, 1.12)#	0.33 (0.10, 1.13)#	0.73 (0.36, 1.49)	0.69 (0.33, 1.43)	1.39 (0.40, 4.79)	1.49 (0.43, 5.09)
Th1 %	1.10 (0.20, 5.91)	0.89 (0.15, 5.16)	1.27 (0.42, 3.79)	1.24 (0.42, 3.66)	0.66 (0.10, 4.20)	0.68 (0.10, 4.45)
Th2 %	3.33 (0.93, 11.96)#	3.08 (0.84, 11.28)#	1.53 (0.74, 3.18)	1.60 (0.76, 3.35)	0.61 (0.18, 2.03)	0.59 (0.18, 1.92)
	n=254	n=254	n=254	n=254	n=254	n=246

# p<0.1; * p<0.05; ** p<0.01
[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

Data conclusions

Many of the outcomes analyzed in this dissertation did not track well over time and it was difficult to pull the atopic story from the data points. Mothers may have forgotten about past diagnoses or symptoms, or may have misunderstood questions, and children may have had transient cases of asthma. The spirometry and cytokine data are more robust. Poorly reproducible spirometric maneuvers were eliminated by machine, and each maneuver was reviewed for validity by two pulmonary experts. Finally, although the method used in these papers for identifying cytokines is different from those of most other papers, it has been validated for use in epidemiologic studies[201].

Given the sizable amount of data available on respiratory health in this population at age seven, and the inconsistency of these data across childhood, we chose to develop a new definition for age seven probable asthma. Our definition took into account several ways asthma can manifest in children, from symptoms to medical interventions to changes in lung function, without giving too much weight to any one variable, hopefully limiting influence of misclassified data. However, as there is undoubtedly misclassification in each of the criteria that went into the definition, the overall probable asthma variable will carry misclassification as well. It is important to keep this in mind when interpreting results.

The CHAMACOS study has collected an abundance of data on respiratory and allergic outcomes throughout childhood compared to other studies. These data show inconsistencies over time and reveal the difficulty in obtaining information about diagnoses and symptoms from parents. They also indicate possible weakness in classifying these outcomes using fewer data points, as some other studies have done. The data also reveal a more nuanced picture of how these illnesses may change throughout childhood and provide an opportunity to develop more intricate and sensitive measures of these outcomes as we have done.

References

1. Smart JM, Kemp AS. Ontogeny of T-helper 1 and T-helper 2 cytokine production in childhood. *Pediatric allergy and immunology* 2001;12(4):181-187.
2. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8(3):483-91.
3. Duramad P, McMahon CW, Hubbard A, Eskenazi B, Holland NT. Flow cytometric detection of intracellular TH1/TH2 cytokines using whole blood: validation of immunologic biomarker for use in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2004;13(9):1452-8.
4. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2013-2014.
5. Raanan R, Harley KG, Balmes JR, Bradman A, Lipsett M, Eskenazi B. Early-life exposure to organophosphate pesticides and pediatric respiratory symptoms in the CHAMACOS cohort. *Environ Health Perspect* 2015;123(2):179-85.
6. Raanan R, Gunier RB, Balmes JR, Beltran AJ, Harley KG, Bradman A, Eskenazi B. Elemental Sulfur Use and Associations with Pediatric Lung Function and Respiratory Symptoms in an Agricultural Community (California, USA). *Environmental Health Perspectives* 2017;87007:1.
7. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2005-2006.

Appendix 1. Comparison of maternal report of wheeze without a cold by age

Age 6mo	Age 1	Age 2	Age 3.5	Age 5	Age 7
Yes	No	No	No	No	No
Yes	No	No	No	No	No
Yes	No	No	No	No	No
Yes	No	No	No	No	No
Yes	No	No	No	No	No
Yes	No	No	No	No	No
Yes	No	No	No	No	No
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No	No	Yes	No	No	No
No	No	Yes	No	No	No
No	No	Yes	No	No	No
No	No	Yes	No	No	No
No	No	Yes	No	No	No
No	No	Yes	No	No	No
No	No	Yes	No	No	No

Yes	No	Yes	No	No	No
No	Yes	Yes	No	No	No
Yes	Yes	Yes	No	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
No	No	No	Yes	No	No
Yes	No	No	Yes	No	No
Yes	Yes	Yes	Missing	No	No
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No	No	No	No	Yes	No
No	No	No	No	Yes	No
No	No	No	No	Yes	No
No	No	No	No	Yes	No
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No	Yes	No	Missing	Missing	Missing

No	Yes	No	Missing	Missing	Missing
Missing	Yes	No	Missing	Missing	Missing
Yes	No	Missing	Missing	Missing	Missing
No	Yes	Missing	Missing	Missing	Missing
Yes	Missing	Missing	Missing	Missing	Missing

Appendix 2. Comparison across ages of maternal report of child using asthma medication across ages

Age 1	Age 2	Age 3.5	Age 5	Age 7
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
Yes	No	No	No	No
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Yes	Yes	No	No	No

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No	No	No	Yes	No
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Yes	Missing	Missing	Missing	Missing

Appendix 3. Comparison of reports on doctor diagnosis of asthma across ages

Age 1 maternal report	Age 2 maternal report	Age 2 medical records	Age 3.5 maternal report	Age 5 maternal report	Age 7 maternal report
Yes	No	No	No	No	No
Yes	No	No	No	No	No
No	Yes	No	No	No	No
No	No	Yes	No	No	No
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No	No	Yes	No	No	No
Yes	No	Yes	No	No	No
Don't Know	No	Yes	No	No	No
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Chapter 3: Associations between prenatal maternal urinary concentrations of high molecular weight phthalates, BPA, and childhood respiratory and allergic outcomes in the CHAMACOS study

Introduction

Rates of asthma, aeroallergy, and eczema in children have been increasing for several decades[1-3]. Currently, prevalence of these diseases in the U.S. are at an all-time high, with 15.9% children estimated to have asthma[4], 24.6% estimated to have aeroallergies[5], and 14.3% estimated to have eczema[5].

Exposure to high molecular weight phthalates and bisphenol A (BPA), chemicals commonly found in plastics, may be one factor behind the rising prevalence of these atopic diseases. Benzylbutyl phthalate (BBzP), di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), and di(2-ethylhexyl) phthalate (DEHP), are widely used as plasticizers in food packages, building materials, children's toys, medical devices, vinyl flooring, and paints[6,7]. Most of their metabolites were detected in 90% or more of 2013-2014 National Health and Nutrition Examination Survey (NHANES) participants[4]. Bisphenol A (BPA) is used in some plastics manufacturing and is found in food and beverage packaging, dental sealants, and certain receipts[8]. BPA was detected in 96% of 2013-2014 NHANES participants[4].

T helper immune cells are a type of lymphocyte that release cytokines, proteins that act as chemical messengers to direct the action of other immune cells[9] and play a role in asthma and allergy development[10,11]. There are two main types of T helper cells: T helper 1 (Th1) cells release cytokines such as interferon gamma (IFN-y), interleukin-2 (IL-2), and IL-3 and act through cell-mediated immunity to attack intracellular viruses and bacteria. T helper 2 (Th2) cells release cytokines such as IL-4, IL-5, and IL-6 and are involved in allergic and inflammatory immunity[12]. A shift in the ratio of Th1:Th2 cytokines towards a higher concentration of Th2 cytokines has been associated with increased risk for asthma and aeroallergies[10,11].

Animal studies on high molecular weight phthalates suggest an association with decreased lung function and an increased Th2 immune response. For example, oral exposure in adult mice to an allergen and 30 to 3,000 ug/kg of DEHP led to increases in concentrations of the Th2 cytokine IL-4 and increases in asthma-like lung inflammation, airway hyperresponsiveness (AHR), and lung eosinophils compared to mice given the allergen only, suggesting DEHP may act as an adjuvant[13]. Several other murine studies have shown that exposure to DiNP, DiDP, or DEHP leads to Th2-dominated immunity, evidenced by increases in Th2 cytokines and decreases in Th1 cytokines[14-25], and to asthma-like changes in the lungs[18,20,22-26]. In addition, two mouse studies have looked at high molecular weight phthalates and allergic dermatitis, and found adult oral exposure to 2, 20, or 200 mg/kg DiNP[14] and DiDP[27] led to increased dermatitis symptoms. Animal studies on BPA also suggest an association with decreased lung function[28-33], eosinophil infiltration into the lungs[34], and Th2 dominant immunity[30,33,35-43], though some studies show Th1 cytokine increases[35,37,39,42,44,45].

Several longitudinal epidemiologic studies have found associations between high molecular weight phthalates[46-50] and BPA[48,51-54] with asthma or respiratory symptoms. For example, the Dampness in Buildings and Health follow-up study of 4,779 children in Sweden found that having polyvinyl chloride (PVC) flooring (which may contain BBzP, DiNP, DiDP, or DEHP[55]) in the home at ages 1-3 was associated with increased risk of asthma at ages 6-8[46]. Other longitudinal studies have also found positive associations between prenatal and early childhood concentrations of high molecular

weight phthalate metabolites and asthma[48-50], but the Polish Mother and Child Cohort study found no association in 144 children with wheeze at age two and a French birth cohort study of 587 children found no associations with asthma or wheeze until age five. Among 727 children in the Columbia Center for Children's Environmental Health cohort, BPA urinary concentrations at early childhood ages, but not concentrations *in utero*, were associated with asthma and wheeze throughout childhood up to age 12[51]. Several other longitudinal epidemiological studies on exposures to BPA have also shown an association with asthma or respiratory symptoms[48,52-54,56], but studies have also found some null results[48,53,57].

Investigations of aeroallergy and eczema have shown less consistent associations. Most studies have not found associations of exposures to high molecular weight phthalates with aeroallergy or eczema[46-48,56,58-61]. However, a French birth cohort found an association between prenatal urinary concentrations of a DEHP metabolite and eczema at age five[62], and the Dampness in Buildings and Health study did find an association between PVC flooring in early childhood and rhinitis later in childhood[46]. Only two human studies have looked at cytokines in relation to exposures to high molecular weight phthalates, and results are contrasting. An experimental study that exposed 16 people to DEHP-containing dust for three hours found increased concentrations of Th2 cytokines IL-6 and IL-5 in nasal fluid compared to 16 unexposed controls[63]. However, a study of 1,121 infants found maternal urinary concentrations of high molecular weight phthalates during pregnancy were not associated with concentrations of Th2 cytokine IL-33 in cord blood[64].

Human studies suggest there is no relationship between BPA and aeroallergies in NHANES 2003-2006[65], in NHANES 2005-2006[66], in the Infancia y Medio Ambiente birth cohort[48], or in the Childhood Environment and Allergic Diseases Study[67]. There was also no relationship between BPA and eczema in the Mount Sinai Children's Environmental Health birth cohort of 164 children ages six and seven, or in NHANES 2005-2006[68]. However two studies found a positive association: *In utero* BPA concentrations were associated with increased odds of combined eczema and wheeze at 6 months in 412 Chinese infants[69], and pooled urinary BPA concentrations taken twice daily for 230 days were associated with symptoms of atopic dermatitis in 18 Korean children ages 3-7 diagnosed with atopic dermatitis[59]. The few human studies of urinary BPA concentrations and Th2 cytokines are conflicting, with studies showing positive[70], negative[71] and no associations[64].

Overall, the human literature suggests exposures to high molecular weight phthalates and BPA may be associated with asthma, but associations are lacking for aeroallergy or eczema. The literature is conflicting on a relationship between these chemicals and Th2 cytokines. The prenatal period is crucial for immune system development[72], and exposures during this period may have greater impact on immune system functioning than exposures later in life. There is also evidence that these plasticizing chemicals can cross the placental barrier, making prenatal exposure biologically plausible[73,74].

To further examine the relationship between these chemicals and childhood immune dysfunction, we measured urinary concentrations of eight phthalate metabolites and BPA at two time points during pregnancy and analyzed associations with asthma, aeroallergies, eczema, and lung function at age seven, and with Th1 and Th2 cell percentages in whole blood at ages two, five, and seven. This is the first study to analyze associations between prenatal high molecular weight phthalates and BPA and childhood cytokine measures and the second to examine prenatal concentrations of MCOP, MCNP, or MCPP in relation to asthma, aeroallergies, or eczema in childhood.

Methods

CHAMACOS Study. The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) Study is a longitudinal birth cohort study that has followed children living in the Salinas Valley, California from birth to age 16. The study investigates effects of early environmental exposures on child development in this largely Latino and agricultural community. Enrollment in the CHAMACOS Study occurred between October 1999 and 2000. All women attending first prenatal care visits at a network of five community clinics or one hospital clinic serving low-income farmworker populations were screened for eligibility. Women were invited to participate if they were spoke Spanish or English, were ≤20 weeks' pregnant, were ≥18 years old, qualified for Medicaid (low income health insurance), and planned to deliver at the county hospital. CHAMACOS mothers were primarily Mexican-born and Spanish-speaking. They were also mostly under 30 years of age, married, and low-income. Almost all were farmworkers or lived with farmworkers. Of the 601 women enrolled, 531 were followed through a live birth, and 517 children had at least one prenatal high molecular weight phthalate or BPA measurement. Of those, 392 children had data on respiratory or allergy symptoms or spirometry at age seven, or had cytokine measurements at two, five, or seven years of age. Our final sample did not differ substantially, compared to children who did not have outcome data, in demographics or in maternal chemical concentrations. The University of California, Berkeley Committee for the Protection of Human Subjects approved research protocols, written informed consent was obtained from the mothers, and children gave verbal assent at age seven.

Questionnaire data collection. Mothers completed interviewer-administered, structured questionnaires twice during pregnancy (mean 13 and 26 weeks gestation), at delivery, and when their children were six months, one year, two years, three and a half years, five years, and seven years of age. Questionnaires were in English or Spanish. During the first pregnancy interview, questionnaires asked about maternal age, parity, family income, and family history of asthma.

Mothers were asked at the seven year visit if their child had been diagnosed with "eczema or an allergic skin rash", or "hay fever or allergic rhinitis" in the last year. Mothers were also asked if their child had "runny or itchy eyes apart from colds" or "sneezing or a runny nose apart from colds", which were considered aeroallergy symptoms, in the last year.

At each visit point, mothers were asked if their child had ever been diagnosed with asthma by a doctor or nurse. A child was considered to have been diagnosed with asthma if the mother had responded "yes" at any age.

At the seven year visit, mothers also answered questions about their child's respiratory symptoms in the past year based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[75]. Mothers were asked if their child experienced any of the following during the previous year: a) tightness in the chest and difficulty breathing; b) wheezing or whistling in the chest; c) trouble going to sleep or being awakened from sleep because of wheezing, whistling, shortness of breath, or coughing without a cold; d) wheezing, whistling, or shortness of breath so severe that the child couldn't finish saying a sentence; e) having to stop running or playing or having to sit out of active games because of wheezing, whistling, shortness of breath, or coughing without a cold; f) an asthma attack. A positive response to any of these questions was coded as the child having current respiratory symptoms. Mothers were also asked if their child was currently taking asthma medication.

Spirometry methods. Spirometry was administered as part of the seven year clinical examination. Routine calibration of three identical EasyOne (new diagnostic design) spirometers was performed every morning. Every child performed up to eight expiratory maneuvers, and the best three acceptable tests were kept by the spirometric software. Each acceptable maneuver lasted at least three seconds. Two bilingual and bicultural technicians specifically trained for the study administered the exams, and the same technician performed 92% of the exams. Technicians confirmed that children did not lean forward, that they inhaled enough air deeply and smoothly from the start of the maneuver, that they exhaled enough air and blew until the EasyOne signaled the end of the test, and that air did not leak from the side of the spirometer. Two physicians with experience in pediatric spirometry reviewed and verified all maneuvers. Forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and forced expiratory flow from 25–75% of FVC ($FEF_{25\%-75\%}$) were measured from each maneuver. FEV_1 measurements were obtained for 300 children, and the other spirometry measurements were available for 270 children.

All children were offered spirometry; children whose mothers reported they had respiratory symptoms on the age seven questionnaire were additionally administered an inhaled bronchodilator (albuterol) and repeated spirometry 20 minutes later. A positive bronchodilator test was defined as a 12% or more improvement from their best measurement of FEV_1 or FVC before the bronchodilator to their best measurement after.

Definition of outcome variables. We defined having eczema as reporting a diagnosis of eczema or an allergic skin rash in the last year. Having aeroallergies was defined 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. We defined probable asthma at age seven as currently taking asthma medication or having two or more of the following criteria: any current respiratory symptom, doctor diagnosis of asthma at any age, or a positive bronchodilator test.

Cytokine detection. Flow cytometry was used in the detection of intracellular Th1 and Th2 cells in unfrozen pediatric whole blood and has been previously described and validated for use in epidemiologic studies[76]. Briefly, phorbol 12-myristate 13-acetate (PMA) (Sigma, St. Louis, MO) and ionomycin (Sigma) activated 500 uL whole blood for 4 hours for the Th surface antigen CD4+. Monoclonal antibodies specific for IFN-y (Becton Dickinson) or IL-4 (Becton Dickinson) cytokines were used to detect Th1 and Th2 cells, respectively. Cells that stained positive for IFN-y only were classified as Th1 cells, and cells that stained positive for IL-4 only were classified as Th2 cells. The number of Th1 positive cells divided by the total number of CD4+ cells yielded the percentage of Th1 cells (Th1%). The number of Th2 positive cells divided by the total number of CD4+ cells yielded the percentage of Th2 cells (Th2%). Th1:Th2 cell ratio was defined as Th1% divided by Th2%. Cytokine data were available for 101 children at all three time points. At age two, 239 children had cytokine data, along with 231 children at age five and 254 children at age seven.

Exposure assessment. Mothers provided urine samples at the two interviews during pregnancy. Phthalate- and BPA-free polypropylene urine cups were used to collect samples, which were aliquoted into glass vials and stored at -80ºC until shipment to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia for analysis.

We used solid phase extraction coupled with isotope dilution high performance liquid chromatography-electrospray ionization-tandem mass spectrometry to quantify concentrations of the following eight phthalate metabolites of four parent compounds,: monobenzyl phthalate

[MBzP, a metabolite of benzylbutyl phthalate (BBzP)]; four metabolites of di(2-ethylhexyl) phthalate (DEHP) [mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECCP)]; mono(carboxyoctyl) phthalate [MCOP; a metabolite of di-isononyl phthalate (DiNP)]; mono(carboxynonyl) phthalate [MCNP; a metabolite of di-isodecyl phthalate (DiDP)]; and mono(3-carboxypropyl) phthalate [MCPP; a metabolite of several high molecular weight phthalates and a minor metabolite of dibutyl phthalate]. To determine concentrations of bisphenol A (BPA), we used isotope dilution on-line solid-phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometry. Analytic methods have been published previously for phthalates[77] and BPA[78]. Phthalate metabolite and BPA concentrations were reported in ng/mL of urine and limits of detection (LOD) ranged from 0.2 ng/mL – 0.5 ng/mL. Concentrations below the LOD were assigned the machine read values, if available, or an imputed value below the LOD selected randomly from the log-normal distribution using maximum likelihood estimation[79]. We combined MEHP, MEHHP, MEOHP, and MECPP to create a molar sum of DEHP metabolites, which is expressed as μmol/ml.

A hand-held refractometer (National Instrument Company Inc., Baltimore, MD) was used to measure urinary specific gravity. We used the formula (analyte concentration * 0.24)/(sample specific gravity – 1) to correct for urinary dilution[80]. We imputed urinary specific gravity based on urinary creatinine concentrations for 77 women missing specific gravity measurements.

Statistical analysis. We averaged the two samples given during pregnancy. Chemical concentrations were log-normally distributed and were thus $\log_2$-transformed and examined as continuous variables. Separate models were constructed for each chemical, although additional chemicals were included in sensitivity models. Probable asthma, aeroallergies, and eczema were analyzed as binary variables using multivariate logistic regression to assess changes associated with *in utero* urinary chemical concentrations.

We conducted multivariable linear regression to assess the change in lung function associated with chemical concentrations. FEV_1 and FVC were assessed as continuous variables and were normally distributed. FEV_1/FVC and $FEF_{25-75\%}$ were assessed as continuous variables and were $\log_{10}$-transformed, as they were log-normally distributed. Thus, for each two-fold increase in chemical concentration, regression coefficients represent mean (FEV_1 and FVC) or percent (FEV_1/FVC and $FEF_{25-75\%}$) change in outcomes. We performed analyses on all children who had at least one valid maneuver. In sensitivity analyses, we excluded children who were currently taking any asthma medication and also limited the analyses to children who had at least two valid maneuvers.

We analyzed Th1%, Th2%, and Th1:Th2 cell ratio at each age continuously using multivariate linear regression and $\log_{10}$-transformed the variables, as they were all log-normally distributed. To determine the longitudinal association of cytokine variables at two, five, and seven years of age with chemical concentrations, we used generalized estimating equations (GEEs) with Gaussian specification. We also conducted GEE models with interaction terms for child age to obtain p-values for interaction by age.

We included maternal age at birth, parity, poverty index at baseline, and family history of asthma (mother, father, or siblings) as confounders in demographically adjusted models, as determined *a priori* via directed acyclic graph. Because people are not exposed to one chemical at a time, but rather are exposed to a mixture of chemicals from different materials, we wanted to control for some of the other chemicals measured in this population, including other phthalates (monoethyl

phthalate, mono-n-butyl phthalate, mono-isobutyl phthalate) and phenols (methyl paraben, propyl paraben, triclosan, 2,4-dichlorophenol, 2,5-dichlorophenol, benzophenone-3). Controlling for this number of chemicals in a regression model, however, could lead to overfitting. To avoid over fitted models and to identify the most important variables to include in the fully adjusted models, we used Bayesian Model Averaging (BMA). BMA estimates models for all possible combinations of the specified chemicals with an outcome, and weights different models by their posterior model probability while adjusting for specified demographic covariates to determine how influential given variables are on an outcome[81]. BMA produces Posterior Inclusion Probabilities (PIPs), which are measurements of each variable's influence on the outcome relative to other variables in the BMA model. We conducted BMA to obtain PIPs for each outcome and then chose the three most influential variables (those with the highest PIPs) to include in fully adjusted regression models for that outcome.

In sensitivity analyses for probable asthma and spirometry, we included as covariates the sum of urinary concentrations of dialkyl phosphate metabolites (DAPs), which reflect exposure to organophosphate pesticides, and measures of elemental sulfur sprayed near participants' homes. We chose to include these chemicals because we have previously found associations in this cohort between them and respiratory symptoms and spirometry[82,83].

Results

Mothers were mostly aged 18-29 (76%), below 100% of the federal poverty index (61%), multiparous (68%), and did not have a history of asthma in their child's family (90%). (Table 1) All chemicals were detected in over 90% of participants in both measurements. (Table 2) Correlations between chemicals are shown in Figure 1. Our study population had lower concentrations of all chemicals, except for MEHP[84], compared with NHANES data at the closest years to CHAMACOS data collection.

At age seven, 22 children (6%) were taking medication for asthma and 54 (15%) currently had any respiratory symptoms. Seventy children (16%) had received an asthma diagnosis from a doctor at any age, as reported by the mother. Ten children (24%) had a positive result on the bronchodilator test of the 42 children with respiratory symptoms who took it. We categorized 37 children (11%) as having probable asthma, 87 children (25%) with aeroallergies, and 23 children (7%) with eczema. (Table 3)

Children had a mean FEV_1 volume of 1.8 liters (SD: 0.5) and a mean FVC volume of 2.1 liters (SD: 0.6). FEV_1/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). Means and standard deviations were similar when restricted to children with 2 or more valid maneuvers. (Table 3)

Th1% increased as the children aged, with a mean of 4.0 (SD: 3.0) at age two, 6.8 (SD: 3.2) at age five, and 10.0 (SD: 5.9) at age seven. Th2% stayed stable with age: ages two and seven had a mean of 0.9% (SD: 0.7) and age five had a mean of 1.1% (SD: 0.8). Correspondingly, Th1:Th2 cell ratios increased with age. The mean ratio at age two was 6.13 (SD: 5.58), which increased to 14.40 (SD: 44.53) at age five and to 22.51 (SD: 36.29) at age seven. (Table 4)

The PIPs for each outcome are shown in Table 5. Chemicals with the highest three PIPs were chosen for inclusion as covariates in models found in Tables 6, 7, 8, 9, and 10 and can be found in

footnotes of those tables. For probable asthma, aeroallergies, FEV_1, FVC, and $FEF_{25-75\%}$, the chemical with the highest PIP also exhibited the strongest association with the outcome.

Table 6 shows that prenatal maternal urinary MCOP concentrations were associated with increased odds for having probable asthma (OR: 1.54, 95% CI: 1.12, 2.12) in fully adjusted models and was also associated with increased odds for having aeroallergies, but in demographically adjusted models only (OR: 1.28, 95% CI: 1.02, 1.62). Prenatal urinary BPA (OR: 1.35, 95% CI: 1.06, 1.73) and MCPP concentrations (OR: 1.36, 95% CI: 1.07, 1.73) were also associated with increased odds for having aeroallergies in demographically adjusted models. Prenatal urinary MCNP concentrations was associated with borderline significance with increased odds for probable asthma and aeroallergies in demographically adjusted models. There were no associations with eczema.

As shown in Table 7, each two-fold increase in MCOP concentration was associated with lower FEV_1 (RR: -0.09, 95% CI: -0.15, -0.03) and $FEF_{25-75\%}$ (RR: -6.98, 95% CI: -10.95, -2.84) in fully adjusted models, as well as lower FVC (RR: -0.07, 95% CI: -0.13, 0.00) in demographically adjusted models. MCNP concentrations was associated with lower FEV_1 (RR: -0.05, 95% CI: -0.11, 0.01) with borderline significance and with lower $FEF_{25-75\%}$ (RR: -5.03, 95% CI: -9.45, -0.40) in demographically adjusted models. MBzP concentrations was also associated with lower FEV_1 (RR: -0.04, 95% CI: -0.09, 0.00) with borderline significance and with lower $FEF_{25-75\%}$ (RR: -4.11, 95% CI: -7.59, -0.50) in demographically adjusted models. Results were similar in children with 2+ verified maneuvers, and when excluding children who were currently using asthma medication (Table 8).

Few associations were seen with cytokines at any age. Table 9 shows associations between maternal urinary chemical concentrations and cytokine levels at individual ages two, five, and seven. MCNP concentrations were associated with lower Th1% at age 5 (RR: -10.03, 95% CI: -18.18, -1.06) in fully adjusted models. MBzP concentrations were associated with higher Th2% at age 2 in demographically adjusted models (RR: 7.77, 95% CI: -0.33, 16.54), and the sum of DEHP metabolite concentrations were associated with lower Th1:Th2 cell ratio at age two in demographically adjusted models (RR: -10.11, 95% CI: 19.85, 0.80). Table 10 shows longitudinal associations across the three ages and p-values for interaction by age. No associations were seen in the GEE models, though MBzP concentrations showed possible interaction by age for Th2%.

Results were similar for probable asthma and spirometry in sensitivity analyses when controlling for the sum of dialkyl phosphate metabolites and elemental sulfur (data not shown).

Discussion

We found that prenatal urinary concentrations of MCOP (a metabolite of a phthalate used commonly in PVC, sealants, and paints) were associated with lower lung function performance measured by FEV_1 and $FEF_{25-75\%}$, and with increased odds for having probable asthma at age seven, after controlling for other, related chemical exposures, and were associated with increased odds for aeroallergies in demographically adjusted models only. Prenatal urinary MCNP concentrations (a metabolite of a phthalate commonly used in PVC, particularly PVC flooring and car interiors) was associated with lower FEV_1 and $FEF_{25-75\%}$, and with increased odds for probable asthma and for aeroallergies, but not after adjusting for other phthalates and phenols measured in the same urine samples. Prenatal urinary MBzP concentrations (a metabolite of a phthalate commonly used in PVC, particularly in flooring) was associated in some models with lower FEV_1 and $FEF_{25-75\%}$, and prenatal urinary MCPP (a metabolite of several phthalates used in PVC) and

BPA concentrations (commonly used in water bottles, sports equipment, medical devices, and receipts) were associated in some models with increased odds for aeroallergies.

Previous studies of exposures to high molecular weight phthalates and BPA with respiratory and allergic responses have tended examine one chemical at a time, failing to account for the fact that individuals experience exposure to multiple chemicals once. One strength of our study is that this is the first study to use BMA variable selection to control for other, potentially confounding chemicals in addition to those of specific interest. This data-driven method allowed us to further identify independent associations of each chemical. Results from these fully adjusted models showed markedly fewer significant associations than models adjusted for demographic confounders only. Results from demographically adjusted models resemble those from other studies to a greater extent that do our fully adjusted models, suggesting that other studies' findings may be partially due to confounding by exposure to other chemicals found in similar materials as those studied.

Our strongest findings were with MCOP, a phthalate metabolite that has been little studied in the past. This is consistent with the Dampness in Buildings and Health study that found an association between living with PVC flooring, which may contain the parent compounds of MCOP and MCPP, at ages 1-3 and risk for asthma and aeroallergies at ages 6-8 in 4,779 children[46]. Like our study, this study found no association of early life exposure with eczema risk. Our results also agree with the cross-sectional associations found between MCOP, MCNP, and MCPP and asthma in 1091 participants aged 18 and over in the 2005-2006 NHANES study[85]. However, a French birth cohort study found no association between prenatal urinary concentrations of these chemicals and asthma or FEV_1 at age five[57].

Unlike other studies[48-50,53,54,85,86], we did not find associations of *in utero* exposures to DEHP, MBzP, or BPA and atopy risk. However some previous studies also found no associations[48,49,56,67]. Interestingly, a study of 727 children from Columbia Center for Children's Environmental Health found that while early childhood urinary concentrations of BPA were associated with childhood asthma, *in utero* exposures were not associated[51]. The Infancia y Medio Ambiente study also found *in utero* exposures to BPA were not associated with childhood asthma in 655 children[48]. Together, these results may suggest that early childhood exposure to BPA may be more impactful on childhood lung health than *in utero* exposure and may explain our null results.

Our study found little evidence of associations of prenatal maternal urinary concentrations of metabolites of high molecular weight phthalates or BPA with Th1 and Th2 profiles in childhood. No other studies have examined the effect of these plasticizing chemicals on Th1 and Th2 function in childhood, and only a small number have examined prenatal exposure and cytokine function in cord blood. One study found no association between prenatal maternal urinary concentrations of high molecular weight phthalates or BPA and cord blood Th2 cytokine concentrations in 1,121 infants[64], while another found an association between prenatal maternal blood concentrations of BPA and higher Th2 cytokines in cord blood[71]. However, T helper cell-related immunity changes drastically from birth to age two[87], and these previous studies may not be appropriate comparisons. Additionally, our study classified cells according to their production of two cytokines; future studies should examine associations between these chemicals and childhood levels of a wider array of cytokines.

Our study differs from previous human studies in several substantive ways. Our population is from a largely Mexican-American farm working community of low income, and may not be comparable to other study populations. For example, compared to NHANES, our population has lower

geometric means of urinary chemical concentrations for all high molecular weight phthalates and BPA[84]. A strength of our study is that we measured urinary concentrations prenatally, and that we measured them twice. Measuring chemical exposures *in utero* is important because gestational exposure to environmental chemicals may influence lung development and function more substantially than later exposure, as much of the immune and respiratory systems develop *in utero*[88-90]. Intraclass correlation coefficients (ICCs) for these chemicals are generally low in our population (high molecular phthalates range from 0.14 to 0.37 and the ICC for BPA is 0.16), indicating high variability between samples. However, unlike many previous studies, our exposure estimates are based on two measures of urinary chemical concentrations during pregnancy rather than one.

In accordance with animal studies, our study found *in utero* exposure to MCOP, a metabolite of DiNP, was associated with lower lung performance[18]. In contrast to animal studies, however, we did not find that exposure to MCOP was associated with higher Th2 cytokines[14,16,18,22], although most of the experimental groups in these studies were exposed to 10 to 37 times the DiNP that humans are exposed to[91]. Also in contrast to animal studies, our study did not find associations between DEHP metabolite urinary concentrations and lung performance[13,17,19,20,26], though most of these studies found effects with doses that were 23 to 75,000 times higher than expected in humans[92]. Our study also did not find that *in utero* exposure to BPA was associated with lung function or Th2 dominated immunity, unlike several animal studies[28-31,33]. While some of the BPA studies produced organ concentrations similar to those found in humans[28-30], they exposed animals to ten to over 20,000 times the amounts of BPA in comparable human exposures[93,94].

Another strength of our study is we have focused on a comprehensive evaluation of atopic diseases in childhood. We measured cytokine levels at several ages, spanning important changes in the juvenile immune system. We used an integrated classification of probable asthma based on a number of asthma-related variables, including a bronchodilator test. Most previous studies looked at only one indicator of asthma (such as respiratory symptoms or medication use or doctor diagnosis), which may exclude children whose symptoms are not apparent or who had not visited a doctor. Including several criteria for probable asthma captured children who may have been difficult to identify with a more limited case definition. Requiring more than one of these criteria, as opposed to including children with only respiratory symptoms or only a diagnosis, excluded children with passing or non-asthmatic symptoms, and children whose past respiratory conditions may have resolved. However, one limitation of our study is that although the focus of this paper was atopic illnesses, some of our probable asthma cases may be non-atopic. In other populations, 10% of asthma cases have been reported as non-atopic[95], and these cases may be related to a variety of outside factors, including air pollution and tobacco smoke exposure[96]. Our inability to differentiate atopic versus non-atopic cases obscures any differentiation by etiology. Another limitation is that while our probable asthma and aeroallergy case definitions were based on both symptoms and medical management, we were unable to apply the same rigor to our eczema cases, which were based only on maternal report of a doctor diagnosis of eczema in their children. Mothers may not have taken their children to a doctor for apparent eczema due to access barriers, or may not have identified existing eczema. We therefore may not have identified every case.

Our findings of an association between prenatal urinary MCOP concentrations and probable asthma concurrent with our null findings on cytokine levels suggest that MCOP may be acting along a pathway not directly involved with IL-4 or IFN-y. Phthalates have been shown to activate nuclear peroxisome proliferator–activated receptors (PPAR)[97], which are involved in a wide range

of physiological actions, including airway inflammation and airway remodeling[98]. High molecular weight phthalates are also xenoestogens[99,100], which have been shown to lower FEV_1 and FVC and increase bronchial hyperactivity[101]. High molecular weight phthalates are associated with increased biomarkers of inflammation[20,35,40,102-108], and of oxidative stress[109-112] which can play a crucial role in asthma origins[113] by increasing inflammation and promoting bronchial hyperresponsiveness and bronchospasm[114].

We present evidence that prenatal maternal urinary concentrations of high molecular weight phthalates, particularly MCOP, a metabolite of DiNP, are associated with increased odds of having probable asthma and aeroallergies at age seven, and with lower lung function at age seven. We found limited evidence that prenatal urinary concentrations of BPA may be associated with increased odds of having aeroallergies at age seven but did not confirm findings of other studies that showed associations between BPA and respiratory symptoms. This is the first study to examine the relationship between prenatal maternal urinary concentrations of any high molecular weight phthalate or BPA and cytokine measurements in childhood. It is also the second study to evaluate associations between *in utero* exposures to MCOP, MCNP, or MCPP and childhood asthma, aeroallergies, or eczema.

References

1. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the United States, 1980–2007. *Pediatrics* 2009;123(Supplement 3):S131-S145.
2. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H, Group IPTS. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet* 2006;368(9537):733-743.
3. Jackson KD, Howie LD, Akinbami OJ. Trends in allergic conditions among children: United States, 1997-2011. 2013.
4. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2013-2014.
5. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2005-2006.
6. Fierens T, Servaes K, Van Holderbeke M, Geerts L, De Henauw S, Sioen I, Vanermen G. Analysis of phthalates in food products and packaging materials sold on the Belgian market. *Food and Chemical Toxicology* 2012;50(7):2575-2583.
7. Kawakami T, Isama K, Matsuoka A. Analysis of phthalic acid diesters, monoester, and other plasticizers in polyvinyl chloride household products in Japan. *Journal of Environmental Science and Health, Part A* 2011;46(8):855-864.
8. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). *Reprod Toxicol* 2007;24(2):139-77.
9. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* 2008;112(5):1557-1569.
10. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res* 2001;2(2):64-5.
11. Deo SS, Mistry KJ, Kakade AM, Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. *Lung India: Official Organ of Indian Chest Society* 2010;27(2):66.
12. Volcheck GW. Overview of the Human Immune Response. *Clinical Allergy* Springer, 2008;1-39.
13. Guo J, Han B, Qin L, Li B, You H, Yang J, Liu D, Wei C, Nanberg E, Bornehag C-G. Pulmonary toxicity and adjuvant effect of di-(2-exylhexyl) phthalate in ovalbumin-immunized BALB/c mice. *PloS one* 2012;7(6):e39008.
14. Kang J, Song J, Shen S, Li B, Yang X, Chen M. Diisononyl phthalate aggravates allergic dermatitis by activation of NF-kB. *Oncotarget* 2016;7(51):85472-85482.
15. Li J, Li L, Zuo H, Ke C, Yan B, Wen H, Zhang Y, Yang X. T-helper type-2 contact hypersensitivity of Balb/c mice aggravated by dibutyl phthalate via long-term dermal exposure. *PloS one* 2014;9(2):e87887.
16. Koike E, Yanagisawa R, Sadakane K, Inoue K-i, Ichinose T, Takano H. Effects of diisononyl phthalate on atopic dermatitis in vivo and immunologic responses in vitro. *Environmental health perspectives* 2010;118(4):472.
17. Hansen JS, Larsen ST, Poulsen LK, Nielsen GD. Adjuvant effects of inhaled mono-2-ethylhexyl phthalate in BALB/cJ mice. *Toxicology* 2007;232(1):79-88.
18. Chen L, Chen J, Xie CM, Zhao Y, Wang X, Zhang YH. Maternal Disononyl Phthalate Exposure Activates Allergic Airway Inflammation via Stimulating the Phosphoinositide 3-kinase/Akt Pathway in Rat Pups. *Biomedical and Environmental Sciences* 2015;28(3):190-198.

19. Badr MZ, Shnyra A, Zoubine M, Norkin M, Herndon B, Quinn T, Miranda RN, Cunningham ML, Molteni A. Phthalate-induced liver protection against deleterious effects of the Th1 response: a potentially serious health hazard. *PPAR research* 2008;2007.
20. Larsen ST, Hansen JS, Hansen EW, Clausen PA, Nielsen GD. Airway inflammation and adjuvant effect after repeated airborne exposures to di-(2-ethylhexyl) phthalate and ovalbumin in BALB/c mice. *Toxicology* 2007;235(1):119-129.
21. Stermer AR, Murphy CJ, Ghaffari R, Di Bona KR, Voss JJ, Richburg JH. Mono-(2-ethylhexyl) phthalate-induced Sertoli cell injury stimulates the production of pro-inflammatory cytokines in Fischer 344 rats. *Reproductive Toxicology* 2017;69:150-158.
22. Hwang Y-H, Paik M-J, Yee S-T. Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium. *Toxicology letters* 2017;272:49-59.
23. Wang B, Liu F, Dong J, You M, Fu Y, Li C, Lu Y, Chen J. Maternal exposure to environmental DEHP exacerbated OVA-induced asthmatic responses in rat offspring. *Science of The Total Environment* 2018;615:253-261.
24. Qin W, Deng T, Cui H, Zhang Q, Liu X, Yang X, Chen M. Exposure to diisodecyl phthalate exacerbated Th2 and Th17-mediated asthma through aggravating oxidative stress and the activation of p38 MAPK. *Food and Chemical Toxicology* 2018;114:78-87.
25. Alfardan AS, Nadeem A, Ahmad SF, Al-Harbi NO, Al-Harbi MM, AlSharari SD. Plasticizer, di (2-ethylhexyl) phthalate (DEHP) enhances cockroach allergen extract-driven airway inflammation by enhancing pulmonary Th2 as well as Th17 immune responses in mice. *Environmental research* 2018;164:327-339.
26. Yang G, Qiao Y, Li B, Yang J, Liu D, Yao H, Xu D, Yang X. Adjuvant effect of di-(2-ethylhexyl) phthalate on asthma-like pathological changes in ovalbumin-immunised rats. *Food and agricultural immunology* 2008;19(4):351-362.
27. Shen S, Li J, You H, Wu Z, Wu Y, Zhao Y, Zhu Y, Guo Q, Li X, Li R. Oral exposure to diisodecyl phthalate aggravates allergic dermatitis by oxidative stress and enhancement of thymic stromal lymphopoietin. *Food and Chemical Toxicology* 2017;99:60-69.
28. Nakajima Y, Goldblum RM, Midoro-Horiuti T. Fetal exposure to bisphenol A as a risk factor for the development of childhood asthma: an animal model study. *Environmental Health* 2012;11(1):8.
29. Midoro-Horiuti T, Tiwari R, Watson CS, Goldblum RM. Maternal bisphenol a exposure promotes the development of experimental asthma in mouse pups. *Environmental health perspectives* 2010;118(2):273.
30. Petzold S, Averbeck M, Simon JC, Lehmann I, Polte T. Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. *PloS one* 2014;9(6):e100468.
31. Bauer SM, Roy A, Emo J, Chapman TJ, Georas SN, Lawrence BP. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. *Toxicological Sciences* 2012;130(1):82-93.
32. 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. *Environmental Health Perspectives (Online)* 2013;121(8):912.
33. He M, Ichinose T, Yoshida S, Takano H, Nishikawa M, Shibamoto T, Sun G. Exposure to bisphenol A enhanced lung eosinophilia in adult male mice. *Allergy, Asthma & Clinical Immunology* 2016;12(1):16.

34. Nygaard UC, Vinje NE, Samuelsen M, Andreassen M, Groeng E-C, Bølling AK, Becher R, Lovik M, Bodin J. Early life exposure to bisphenol A investigated in mouse models of airway allergy, food allergy and oral tolerance. *Food and Chemical Toxicology* 2015;83:17-25.
35. Goto M, Takano-Ishikawa Y, Ono H, Yoshida M, Yamaki K, Shinmoto H. Orally administered bisphenol A disturbed antigen specific immunoresponses in the naive condition. *Bioscience, biotechnology, and biochemistry* 2007;71(9):2136-2143.
36. Ménard S, Guzylack-Piriou L, Lencina C, Leveque M, Naturel M, Sekkal S, Harkat C, Gaultier E, Olier M, Garcia-Villar R. Perinatal exposure to a low dose of bisphenol A impaired systemic cellular immune response and predisposes young rats to intestinal parasitic infection. *PloS one* 2014;9(11):e112752.
37. Yan H, Takamoto M, Sugane K. Exposure to Bisphenol A Prenatally or in Adulthood Promotes T^ sub H^ 2 Cytokine Production Associated with Reduction of CD4^ sup+^ CD25^ sup+^ Regulatory T Cells. *Environmental health perspectives* 2008;116(4):514.
38. O'Brien E, Dolinoy DC, Mancuso P. Perinatal bisphenol A exposures increase production of pro-inflammatory mediators in bone marrow-derived mast cells of adult mice. *Journal of immunotoxicology* 2014;11(3):205-212.
39. Yoshino S, Yamaki K, Li X, Sai T, Yanagisawa R, Takano H, Taneda S, Hayashi H, Mori Y. Prenatal exposure to bisphenol A up-regulates immune responses, including T helper 1 and T helper 2 responses, in mice. *Immunology* 2004;112(3):489-495.
40. Lee MH, Chung SW, Kang BY, Park J, Lee CH, Hwang SY, Kim TS. Enhanced interleukin-4 production in CD4+ T cells and elevated immunoglobulin E levels in antigen-primed mice by bisphenol A and nonylphenol, endocrine disruptors: involvement of nuclear factor-AT and Ca2+. *Immunology* 2003;109(1):76-86.
41. Yoshino S, Yamaki K, Yanagisawa R, Takano H, Hayashi H, Mori Y. Effects of bisphenol A on antigen-specific antibody production, proliferative responses of lymphoid cells, and TH1 and TH2 immune responses in mice. *British journal of pharmacology* 2003;138(7):1271-1276.
42. 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(6):e38448.
43. Holladay SD, Xiao S, Diao H, Barber J, Nagy T, Ye X, Gogal RM. Perinatal bisphenol A exposure in C57B6/129svj male mice: potential altered cytokine/chemokine production in adulthood. *International journal of environmental research and public health* 2010;7(7):2845-2852.
44. Byun J-A, Heo Y, Kim Y-O, Pyo M-Y. Bisphenol A-induced downregulation of murine macrophage activities in vitro and ex vivo. *Environmental toxicology and pharmacology* 2005;19(1):19-24.
45. Youn J-Y, Park H-Y, Lee J-W, Jung I-O, Choi K-H, Kim K, Cho K-H. Evaluation of the immune response following exposure of mice to bisphenol A: induction of Th1 cytokine and prolactin by BPA exposure in the mouse spleen cells. *Archives of pharmacal research* 2002;25(6):946-953.
46. Larsson M, Hägerhed-Engman L, Kolarik B, James P, Lundin F, Janson S, Sundell J, Bornehag C-G. PVC–as flooring material–and its association with incident asthma in a Swedish child cohort study. *Indoor Air* 2010;20(6):494-501.
47. Smit LA, Lenters V, Høyer BB, Lindh CH, Pedersen HS, Liermontova I, Jönsson BA, Piersma AH, Bonde JP, Toft G. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. *Allergy* 2015;70(6):653-660.

48. Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, Martínez D. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. *Journal of Allergy and Clinical Immunology* 2015;135(2):370-378. e7.
49. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, Hoepner LA, Perera FP, Miller RL. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. 2014.
50. Ku HY, Su PH, Wen HJ, Sun HL, Wang CJ, Chen HY, Jaakkola JJ, Wang S-L, Group T. Prenatal and postnatal exposure to phthalate esters and asthma: a 9-year follow-up study of a taiwanese birth cohort. *PloS one* 2015;10(4):e0123309.
51. Donohue KM, Miller RL, Perzanowski MS, Just AC, Hoepner LA, Arunajadai S, Canfield S, Resnick D, Calafat AM, Perera FP. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. *Journal of Allergy and Clinical Immunology* 2013;131(3):736-742. e6.
52. Whyatt RM, Rundle AG, Perzanowski MS, Just AC, Donohue KM, Calafat AM, Hoepner L, Perera FP, Miller RL. Prenatal phthalate and early childhood bisphenol A exposures increase asthma risk in inner-city children. *The Journal of allergy and clinical immunology* 2014;134(5):1195.
53. Spanier AJ, Kahn RS, Kunselman AR, Schaefer EW, Hornung R, Xu Y, Calafat AM, Lanphear BP. Bisphenol a exposure and the development of wheeze and lung function in children through age 5 years. *JAMA pediatrics* 2014;168(12):1131-1137.
54. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, Lanphear BP. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. *Environmental health perspectives* 2012;120(6):916.
55. Hauser R, Calafat A. Phthalates and human health. *Occupational and environmental medicine* 2005;62(11):806-818.
56. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environment international* 2018;115:79-88.
57. Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat A, Ye X, Annesi-Maesano I, Siroux V. In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study. *Environmental Health Perspectives* 2017;125(9):097006.
58. Herberth G, Pierzchalski A, Feltens R, Bauer M, Röder S, Olek S, Hinz D, Borte M, von Bergen M, Lehmann I. Prenatal phthalate exposure associates with low regulatory T-cell numbers and atopic dermatitis in early childhood: Results from the LINA mother-child study. *Journal of Allergy and Clinical Immunology* 2016.
59. Kim E-H, Jeon B-H, Kim J, Kim Y-M, Han Y, Ahn K, Cheong H-K. Exposure to phthalates and bisphenol A are associated with atopic dermatitis symptoms in children: a time-series analysis. *Environmental Health* 2017;16(1):24.
60. Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polańska K, Ligocka D, Hanke W. The effect of prenatal exposure to phthalates on food allergy and early eczema in inner-city children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2015;e72-e78.

61. Overgaard LE, Main KM, Frederiksen H, Stender S, Szecsi PB, Williams HC, Thyssen JP. Children with atopic dermatitis and frequent emollient use have increased urinary levels of low-molecular-weight phthalate metabolites and parabens. *Allergy* 2017;72(11):1768-1777.
62. Soomro MH, Baiz N, Philippat C, Vernet C, Siroux V, Maesano CN, Sanyal S, Slama R, Bornehag C-G, Annesi-Maesano I. Prenatal Exposure to Phthalates and the Development of Eczema Phenotypes in Male Children: Results from the EDEN Mother–Child Cohort Study. *Environmental health perspectives* 2018;126(2):027002.
63. Deutschle T, Reiter R, Butte W, Heinzow B, Keck T, Riechelmann H. A controlled challenge study on di (2-ethylhexyl) phthalate (DEHP) in house dust and the immune response in human nasal mucosa of allergic subjects. *Environmental health perspectives* 2008;116(11):1487.
64. Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. *Environmental research* 2015;140:360-368.
65. Clayton EM, 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(3):390-6.
66. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012;130(2):453-60 e7.
67. Wang I-J, Chen C-Y, Bornehag C-G. Bisphenol A exposure may increase the risk of development of atopic disorders in children. *International journal of hygiene and environmental health* 2016;219(3):311-316.
68. Shiue I. IgE antibodies and urinary trimethylarsine oxide accounted for 1–7% population attributable risks for eczema in adults: USA NHANES 2005–2006. *Environmental Science and Pollution Research* 2015;22(23):18404-18409.
69. Zhou A, Chang H, Huo W, Zhang B, Hu J, Xia W, Chen Z, Xiong C, Zhang Y, Wang Y. Prenatal exposure to bisphenol A and risk of allergic diseases in early life. *Pediatric Research* 2017;81(6):851-856.
70. Savastano S, Tarantino G, D'Esposito V, Passaretti F, Cabaro S, Liotti A, Liguoro D, Perruolo G, Ariemma F, Finelli C. Bisphenol-A plasma levels are related to inflammatory markers, visceral obesity and insulin-resistance: a cross-sectional study on adult male population. *Journal of translational medicine* 2015;13(1):169.
71. Liao S-L, Tsai M-H, Lai S-H, Yao T-C, Hua M-C, Yeh K-W, Chiang C-H, Huang S-Y, Huang J-L. Prenatal exposure to bisphenol-A is associated with Toll-like receptor-induced cytokine suppression in neonates. *Pediatric research* 2016;79(3):438.
72. Holladay SD, Smialowicz RJ. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. *Environmental health perspectives* 2000;108(Suppl 3):463.
73. Balakrishnan B, Henare K, Thorstensen EB, Ponnampalam AP, Mitchell MD. Transfer of bisphenol A across the human placenta. *American Journal of Obstetrics & Gynecology* 2010;202(4):393. e1-393. e7.
74. Mose T, Mortensen GK, Hedegaard M, Knudsen LE. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. *Reproductive Toxicology* 2007;23(1):83-91.

75. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8(3):483-91.
76. Duramad P, McMahon CW, Hubbard A, Eskenazi B, Holland NT. Flow cytometric detection of intracellular TH1/TH2 cytokines using whole blood: validation of immunologic biomarker for use in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2004;13(9):1452-8.
77. Silva MJ SE, Preau JL Jr, Reidy JA, Needham LL, Calafat AM. Quantification of 22 phthalate metabolites in human urine. *Journal of Chromatography* 2007(860):106–112.
78. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 2005;77(16):5407-13.
79. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 2004;112(17):1691-6.
80. Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL. Normalization of urinary drug concentrations with specific gravity and creatinine. *Journal of Analytical Toxicology* 2009;33(1):1-7.
81. Madigan D, Raftery AE, Volinsky C, Hoeting J. Bayesian model averaging. Proceedings of the AAAI Workshop on Integrating Multiple Learned Models, Portland, OR, 1996;77-83.
82. Raanan R, Gunier RB, Balmes JR, Beltran AJ, Harley KG, Bradman A, Eskenazi B. Elemental Sulfur Use and Associations with Pediatric Lung Function and Respiratory Symptoms in an Agricultural Community (California, USA). *Environmental Health Perspectives* 2017;87007:1.
83. Raanan R, Balmes JR, Harley KG, Gunier RB, Magzamen S, Bradman A, Eskenazi B. Decreased lung function in 7-year-old children with early-life organophosphate exposure. *Thorax* 2016;71(2):148-53.
84. Centers for Disease Control and Prevention. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2015. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. http://www.cdc.gov/exposurereport/
85. Strassle PD, Smit LA, Hoppin JA. Endotoxin enhances respiratory effects of phthalates in adults: Results from NHANES 2005-6. *Environmental research* 2018;162:280-286.
86. Kim K-N, Kim JH, Kwon H-J, Hong S-J, Kim B-J, Lee S-Y, Hong Y-C, Bae S. Bisphenol A exposure and asthma development in school-age children: a longitudinal study. *PloS one* 2014;9(10):e111383.
87. Smart JM, Kemp AS. Ontogeny of T-helper 1 and T-helper 2 cytokine production in childhood. *Pediatric allergy and immunology* 2001;12(4):181-187.
88. Duijts L. Fetal and infant origins of asthma. *European journal of epidemiology* 2012;27(1):5-14.
89. Pinkerton KE, Joad JP. The mammalian respiratory system and critical windows of exposure for children's health. *Environmental health perspectives* 2000;108(Suppl 3):457.
90. Warner J. The early life origins of asthma and related allergic disorders. *Archives of disease in childhood* 2004;89(2):97-102.
91. Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. *International journal of hygiene and environmental health* 2007;210(5):623-634.

92. Barr DB, Breysse PN, Chapin R, Marcus M. NTP-CERHR Expert Panel Update on the Reproductive and Developmental Toxicity of di (2-ethylhexyl) phthalate. *Reproductive toxicology* 2006;22:291-399.
93. Lakind JS, Naiman DQ. Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003-2004 NHANES urinary BPA data. *Journal of Exposure Science and Environmental Epidemiology* 2008;18(6):608.
94. Arnold SM, Clark KE, Staples CA, Klecka GM, Dimond SS, Caspers N, Hentges SG. Relevance of drinking water as a source of human exposure to bisphenol A. *Journal of exposure science & environmental epidemiology* 2013;23(2):137.
95. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D. Allergic vs nonallergic asthma: what makes the difference? *Allergy* 2002;57(7):607-613.
96. Noutsios GT, Floros J. Childhood asthma: causes, risks, and protective factors; a role of innate immunity. *Swiss Med Wkly* 2014;144:w14036.
97. Hurst CH, Waxman DJ. Activation of PPARα and PPARγ by environmental phthalate monoesters. *Toxicological Sciences* 2003;74(2):297-308.
98. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret M-C, Mechighel P, Megret J, Leseche G, Aubier M, Pretolani M. Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *American journal of respiratory and critical care medicine* 2001;164(8):1487-1494.
99. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. *Environ Health Perspect* 1997;105(8):802-11.
100. Rogers JA, Metz L, Yong VW. Review: endocrine disrupting chemicals and immune responses: a focus on bisphenol-A and its potential mechanisms. *Molecular immunology* 2013;53(4):421-430.
101. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. *Current opinion in allergy and clinical immunology* 2013;13(1):92.
102. Nakamura R, Teshima R, Sawada J-i. Effect of dialkyl phthalates on the degranulation and Ca 2+ response of RBL-2H3 mast cells. *Immunology letters* 2002;80(2):119-124.
103. Nishioka J, Iwahara C, Kawasaki M, Yoshizaki F, Nakayama H, Takamori K, Ogawa H, Iwabuchi K. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. *Inflammation Research* 2012;61(1):69-78.
104. Manteiga S, Lee K. Monoethylhexyl Phthalate Elicits an Inflammatory Response in Adipocytes Characterized by Alterations in Lipid and Cytokine Pathways. *Environ Health Perspect* 2016.
105. Guo H, Liu T, Uemura Y, Jiao S, Wang D, Lin Z, Narita Y, Suzuki M, Hirosawa N, Ichihara Y. Bisphenol A in combination with TNF-α selectively induces Th2 cell-promoting dendritic cells in vitro with an estrogen-like activity. *Cellular & molecular immunology* 2010;7(3):227-234.
106. Dearman RJ, Betts CJ, Beresford L, Bailey L, Caddick HT, Kimber I. Butyl benzyl phthalate: effects on immune responses to ovalbumin in mice. *Journal of Applied Toxicology* 2009;29(2):118-125.
107. Larsen ST, Lund RM, Nielsen GD, Thygesen P, Poulsen OM. Adjuvant Effect of di-n-Butyl-, di-n-Octyl-, di-iso-Nonyl-and di-iso-Decyl Phthalate in a Subcutaneous Injection Model Using BALB/c Mice. *Pharmacology & toxicology* 2002;91(5):264-272.

108. Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice. *Environmental health perspectives* 2003;111(16):1883.
109. Ferguson KK, Loch-Caruso R, Meeker JD. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999–2006. *Environmental science & technology* 2011;46(1):477-485.
110. Ferguson KK, Cantonwine DE, Rivera-González LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, Jiménez-Vélez B, Calafat AM, Ye X, Alshawabkeh AN. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. *Environmental science & technology* 2014;48(12):7018-7025.
111. Holland N, Huen K, Tran V, Street K, Nguyen B, Bradman A, Eskenazi B. Urinary phthalate metabolites and biomarkers of oxidative stress in a Mexican-American cohort: Variability in early and late pregnancy. . *Toxics* 2016;4(1):7.
112. Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999–2006. *Environmental research* 2011;111(5):718-726.
113. Kato T, Tada-Oikawa S, Takahashi K, Saito K, Wang L, Nishio A, Hakamada-Taguchi R, Kawanishi S, Kuribayashi K. Endocrine disruptors that deplete glutathione levels in APC promote Th2 polarization in mice leading to the exacerbation of airway inflammation. *European journal of immunology* 2006;36(5):1199-1209.
114. Cho YS, Moon H-B. The role of oxidative stress in the pathogenesis of asthma. *Allergy, asthma & immunology research* 2010;2(3):183-187.

Table 1. Demographic characteristics of the study population, CHAMACOS Study, Salinas, CA

Characteristics at time of pregnancy	N	(%)
Age		
18-24	174	(44.7)
25-29	123	(31.6)
30-34	62	(15.9)
35+	30	(7.7)
Poverty		
<100%	240	(61.2)
100-200%	138	(35.2)
>200%	14	(3.6)
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)

Table 2. Distribution of specific gravity corrected phthalate monoester metabolites in maternal prenatal urine collected at two time points in pregnancy

	% > LOD		Average of 2 Measurements						
Phthalate	Early Pregnancy[1]	Late Pregnancy[2]	Geo. Mean	10th%	25th%	50th%	75th%	90th%	95th%
MBzP (ng/mL)	98.3%	98.9%	8.9	2.5	4.9	9.2	17.7	28.3	40.1
MCNP (ng/mL)	97.5%	97.4%	2.3	1.0	1.5	2.3	3.4	5.2	7.6
MCOP (ng/mL)	97.0%	96.8%	3.8	1.5	2.4	3.9	5.5	8.7	11.6
MCPP (ng/mL)	90.3%	95.1%	2.2	0.8	1.4	2.4	3.8	5.1	7.1
∑DEHP (umol/mL)	96.95%[3]	96.54%[3]	0.2	0.1	0.1	0.2	0.4	0.6	0.8
BPA (ug/mL)	97.4%	97.8%	1.5	0.6	0.9	1.3	2.2	3.7	5.8

[1]Mean ± SD = 14.0 ± 5.0 weeks gestation

[2]Mean ± SD = 26.9 ± 2.5 weeks gestation

[3]MEHP used as proxy for ∑DEHP in % > LOD

Abbreviations: LOD = Limit of detection

Table 3. Respiratory symptom and spirometry data in CHAMACOS participants at age seven

Maternal report	N (%)	Spirometry	N	Mean (SD)
Any current symptoms	54 (15%)	Best maneuver (all children)		
Any current asthma medication use	22 (6%)	FEV_1	300	1.8 (0.5) liters
Past diagnosis of asthma	62 (18%)	FVC	270	2.1 (0.6) liters
Positive bronchodilator test[1]	10 (3%)	FEV_1/FVC	270	0.9 (0.1)
Probable Asthma[2]	37 (11%)	FEF_{25-75}	270	2.5 (0.9) liters/second
Aeroallergy symptoms or diagnosis	87 (25%)	Best maneuver (those with 2+ maneuvers)		
Eczema diagnosis	23 (7%)	FEV_1	276	1.8 (0.5) liters
		FVC	234	2.1 (0.5) liters
		FEV_1/FVC	234	0.9 (0.1)
		FEF_{25-75}	234	2.6 (0.9) liters/second

[1] Completed by children with respiratory symptoms (N=42)

[2] Defined as: taking asthma medications or any two of current respiratory symptoms, diagnosis of asthma, positive bronchodilator test

Table 4. Descriptive statistics of T-helper cell counts in CHAMACOS participants at ages 2, 5, and 7

Variable	N	Mean (SD)
Age 2		
Th1:Th2 cell ratio	239	6.13 (5.58)
% CD4 cells	239	32.9 (7.5)
% Th1 cells	239	4.0 (3.0)
% Th2 cells	239	0.9 (0.7)
Age 5		
Th1:Th2 cell ratio	231	14.40 (44.53)
% CD4 cells	231	29.9 (7.3)
% of Th1 cells	231	6.8 (3.2)
% of Th2 cells	231	1.1 (0.8)
Age 7		
Th1:Th2 cell ratio	254	22.51 (36.29)
% CD4 cells	254	33.8 (10.2)
% of Th1 cells	254	10.0 (5.9)
% of Th2 cells	254	0.9 (0.7)

Table 5. Posterior Inclusion Probabilities (PIP) for all phthalate metabolites, parabens, other phenols, and dialkyl phosphate metabolites included in Bayesian Model Averaging

Probable asthma		Aeroallergy		Eczema	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MCOP	0.5072	MCPP	0.39754	MCPP	0.15586
PP	0.32714	BP	0.26668	MBP	0.115
2,4-DCP	0.24914	BPA	0.19336	MiBP	0.11436
MCNP	0.23314	MCOP	0.18742	TCS	0.09724
2,5-DCP	0.22578	MCNP	0.12052	MP	0.068
TCS	0.14808	DAPs	0.09588	MCNP	0.0673
MEP	0.10582	MBzP	0.07654	2,5-DCP	0.0661
DEHP	0.08882	BP3	0.06858	BPA	0.0635
BP	0.08844	MEP	0.06592	MEP	0.06096
MCPP	0.08466	2,5-DCP	0.06576	DAPs	0.0555
MP	0.0776	2,4-DCP	0.06462	DEHP	0.0554
MBzP	0.0768	MiBP	0.06158	MBzP	0.05456
MBP	0.06452	PP	0.05946	2,4-DCP	0.05418
DAPs	0.06166	MP	0.0572	PP	0.05334
BPA	0.0593	DEHP	0.05676	MCOP	0.0525
BP3	0.0586	MBP	0.05508	BP	0.05224
MiBP	0.0541	TCS	0.05292	BP3	0.0503

FEV_1		FVC		FEV_1/FVC		$FEF_{25-75\%}$	
Chemical	PIP	Chemical	PIP	Chemical	PIP	Chemical	PIP
MCOP	0.64582	MCOP	0.36054	DAPs	0.1423	MCOP	0.8658
MBP	0.26968	DAPs	0.18786	MBzP	0.11322	MEP	0.508
MEP	0.20392	BP	0.1511	MEP	0.10604	TCS	0.2652
MBzP	0.1683	MCNP	0.13188	MCOP	0.10162	MBP	0.21876
TCS	0.16306	MiBP	0.13144	MBP	0.10084	BP	0.1341
MiBP	0.13012	MEP	0.13	BPA	0.09898	MiBP	0.13254
DEHP	0.1199	2,4-DCP	0.11852	2,4-DCP	0.08358	MBzP	0.11116
MCNP	0.10164	MBP	0.09842	PP	0.06794	DEHP	0.09706
MCPP	0.0999	MBzP	0.08204	BP3	0.06734	MCNP	0.09106
BP	0.09472	2,5-DCP	0.08046	2,5-DCP	0.06686	MP	0.08538
2,5-DCP	0.08226	MCPP	0.07828	MP	0.06438	PP	0.07494
DAPs	0.08134	TCS	0.07486	TCS	0.06436	MCPP	0.07456
2,4-DCP	0.07718	BP3	0.07228	MCNP	0.06404	BPA	0.07404
BP3	0.06144	BPA	0.0642	BP	0.0637	BP3	0.07236
PP	0.06064	MP	0.06392	DEHP	0.05966	2,4-DCP	0.0708
MP	0.05648	PP	0.06016	MiBP	0.05908	2,5-DCP	0.06996
BPA	0.0541	DEHP	0.0551	MCPP	0.0585	DAPs	0.05576

Th1:Th2 cell ratio, age 2		Th1 %, age 2		Th2 %, age 2	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MBP	0.90552	2,5-DCP	0.35408	MBP	0.57386
MEP	0.76292	2,4-DCP	0.2366	MP	0.28364

PP	0.295	PP	0.23254	2,5-DCP	0.2258
MCOP	0.12096	MEP	0.2082	2,4-DCP	0.20322
DEHP	0.12014	DEHP	0.17696	MBzP	0.19724
MCNP	0.0979	MP	0.1515	BP3	0.19178
MiBP	0.0964	MBzP	0.10768	MEP	0.1269
MCPP	0.08616	MBP	0.10274	MiBP	0.09624
TCS	0.08614	BP3	0.09516	BP	0.0961
MP	0.07832	MCOP	0.09176	PP	0.07786
DAPs	0.07684	DAPs	0.08812	DEHP	0.07492
BP	0.07598	MiBP	0.08154	MCPP	0.07436
BPA	0.0699	BPA	0.07886	DAPs	0.07218
2,5-DCP	0.06924	MCNP	0.07386	MCNP	0.07156
2,4-DCP	0.05948	MCPP	0.06766	TCS	0.07118
BP3	0.0568	BP	0.0645	MCOP	0.07074
MBzP	0.05638	TCS	0.06166	BPA	0.05964

Th1:Th2 cell ratio, age 5		Th1 %, age 5		Th2 %, age 5	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MiBP	0.22998	MBP	0.35058	MBP	0.6689
TCS	0.1997	MCNP	0.2633	MEP	0.15788
MBP	0.1435	MCPP	0.13324	TCS	0.15366
BP	0.11892	MEP	0.12886	DAPs	0.14222
2,5-DCP	0.10386	MCOP	0.09936	MCPP	0.1057
DAPs	0.09814	MiBP	0.095	MiBP	0.10328
BPA	0.09238	DAPs	0.09376	MCOP	0.09626
2,4-DCP	0.09148	MBzP	0.09354	BP	0.09018
MBzP	0.08868	DEHP	0.09056	MCNP	0.08976
MCNP	0.0881	2,5-DCP	0.0832	BPA	0.08728
DEHP	0.0876	BPA	0.07642	MP	0.0851
MCPP	0.08292	BP	0.07544	MBzP	0.07924
MCOP	0.07552	PP	0.07416	BP3	0.07596
MEP	0.07538	2,4-DCP	0.07178	DEHP	0.07492
MP	0.07362	MP	0.06852	2,4-DCP	0.07206
PP	0.07124	BP3	0.06714	2,5-DCP	0.06666
BP3	0.06704	TCS	0.06448	PP	0.0538

Th1:Th2 cell ratio, age 7		Th1 %, age 7		Th2 %, age 7	
Chemical	PIP	Chemical	PIP	Chemical	PIP
BP	0.14764	BP	0.28148	MP	0.24174
TCS	0.10314	MP	0.2132	DEHP	0.1231
DEHP	0.09426	MCPP	0.16302	MBzP	0.09164
MBP	0.083	2,4-DCP	0.1368	MEP	0.09132
MBzP	0.08152	DAPs	0.13384	2,5-DCP	0.09028
MCOP	0.07676	2,5-DCP	0.1244	MCPP	0.0805
2,5-DCP	0.07562	PP	0.12406	PP	0.07378
MP	0.07464	MBP	0.0919	BPA	0.073

2,4-DCP	0.07354	MEP	0.09094	TCS	0.07122
MiBP	0.06596	BPA	0.0812	2,4-DCP	0.07094
MCNP	0.0648	MCOP	0.07918	BP	0.06978
DAPs	0.06374	MBzP	0.07452	BP3	0.06936
BPA	0.06356	TCS	0.07236	MCOP	0.06658
MEP	0.06186	DEHP	0.06646	DAPs	0.06396
BP3	0.0618	MCNP	0.06578	MBP	0.0604
MCPP	0.06098	BP3	0.06484	MiBP	0.0595
PP	0.05862	MiBP	0.06354	MCNP	0.05782

Th1:Th2 cell ratio, longitudinal		Th1 %, longitudinal		Th2 %, longitudinal	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MiBP	0.5219	MP	0.25002	MBP	0.50824
MBP	0.1206	2,4-DCP	0.1823	MP	0.449
TCS	0.10798	2,5-DCP	0.1292	MEP	0.16016
MEP	0.09542	PP	0.12382	DAPs	0.11294
MBzP	0.06374	MiBP	0.08824	TCS	0.1035
2,4-DCP	0.0618	BP3	0.07436	MiBP	0.08742
PP	0.05982	DAPs	0.07312	DEHP	0.0852
BP3	0.05108	DEHP	0.06332	2,5-DCP	0.08326
MP	0.04842	MBzP	0.05634	PP	0.05696
BP	0.04678	BP	0.0536	2,4-DCP	0.04638
2,5-DCP	0.04554	MCOP	0.0469	BP	0.04586
MCPP	0.04446	MBP	0.0453	MCOP	0.0443
MCNP	0.04104	MCPP	0.045	MCPP	0.04402
BPA	0.0391	BPA	0.04376	BPA	0.04138
MCOP	0.03894	MCNP	0.04154	MBzP	0.04024
DAPs	0.03706	MEP	0.03724	BP3	0.0377
DEHP	0.03602	TCS	0.03462	MCNP	0.03138

Table 6. Association of log 2 prenatal chemical concentrations and asthma outcomes

	Probable asthma			Aeroallergies			Eczema		
Chemical	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[2] OR (95% CI)	Crude OR (95% CI)	Demographically adjusted[1] OR (95% CI)	Fully adjusted[2] OR (95% CI)
	n=336-346	n=333-343	n=327	n=327-334	n=324-331	n=321	n=326-331	n=323-330	n=320-323
MBzP	0.99 (0.77, 1.29)	1.04 (0.79, 1.36)	0.76 (0.54, 1.08)	1.01 (0.84, 1.21)	1.05 (0.86, 1.27)	0.85 (0.67, 1.07)	0.98 (0.71, 1.36)	1.00 (0.71, 1.39)	1.33 (0.86, 2.06)
MCNP	1.34 (0.98, 1.84)#	1.36 (0.95, 1.94)#	1.11 (0.65, 1.90)	1.24 (0.98, 1.56)#	1.27 (0.99, 1.63)#	1.08 (0.81, 1.45)	0.91 (0.60, 1.39)	0.93 (0.61, 1.44)	1.17 (0.70, 1.94)
MCOP	1.37 (1.04, 1.80)*	1.42 (1.03, 1.95)*	1.53 (1.11, 2.13)*	1.22 (0.99, 1.52)#	1.28 (1.02, 1.62)*	1.10 (0.83, 1.46)	0.99 (0.68, 1.46)	1.03 (0.69, 1.55)	1.39 (0.87, 2.20)
MCPP	1.24 (0.91, 1.70)	1.29 (0.91, 1.82)	1.06 (0.70, 1.59)	1.30 (1.04, 1.64)*	1.36 (1.07, 1.73)*	1.26 (0.97, 1.63)#	0.79 (0.58, 1.07)	0.77 (0.56, 1.07)	0.83 (0.57, 1.19)
∑DEHP	1.05 (0.78, 1.42)	1.11 (0.81, 1.52)	0.90 (0.61, 1.33)	1.03 (0.83, 1.29)	1.07 (0.86, 1.35)	0.87 (0.66, 1.16)	0.88 (0.59, 1.32)	0.88 (0.58, 1.33)	1.09 (0.70, 1.69)
BPA	1.04 (0.75, 1.45)	1.19 (0.83, 1.71)	0.98 (0.65, 1.49)	1.24 (0.99, 1.56)#	1.35 (1.06, 1.73)*	1.21 (0.92, 1.59)	0.86 (0.56, 1.31)	0.89 (0.57, 1.38)	0.99 (0.61, 1.63)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, 2,4-dichlorophenol, 2,5-dichlorophenol

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monocarboxyisononyl phthalate, monobenzyl phthalate

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, 2,4-dichlorophenol, 2,5-diclorophenol, mono(3-carboxypropyl) phthalate

Table 7. Association of log 2 prenatal chemical concentrations and spirometry outcomes

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=290-296	n=287-293	n=285-287	n=260-266	n=257-263	n=255
MBzP	-0.04 (-0.08, 0.00)#	-0.04 (-0.09, 0.00)#	-0.03 (-0.09, 0.03)	-0.02 (-0.07, 0.03)	-0.02 (-0.07, 0.03)	0.00 (-0.06, 0.06)
MCNP	-0.05 (-0.11, 0.00)#	-0.05 (-0.11, 0.01)#	0.00 (-0.07, 0.08)	-0.05 (-0.12, 0.01)	-0.05 (-0.12, 0.02)	-0.01 (-0.10, 0.08)
MCOP	-0.07 (-0.12, -0.02)**	-0.07 (-0.12, -0.02)**	-0.09 (-0.15, -0.03)**	-0.07 (-0.12, -0.01)*	-0.07 (-0.13, 0.00)*	-0.07 (-0.14, -0.01)*
MCPP	-0.04 (-0.08, 0.01)	-0.04 (-0.08, 0.01)	-0.02 (-0.07, 0.04)	-0.02 (-0.08, 0.03)	-0.03 (-0.08, 0.03)	0.00 (-0.06, 0.06)
∑DEHP	0.00 (-0.05, 0.05)	0.00 (-0.05, 0.05)	0.03 (-0.04, 0.09)	-0.01 (-0.07, 0.05)	-0.01 (-0.07, 0.05)	0.03 (-0.04, 0.09)
BPA	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	0.00 (-0.06, 0.06)	-0.03 (-0.09, 0.03)	-0.04 (-0.11, 0.02)	-0.02 (-0.09, 0.05)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=260-266	n=257-263	n=255	n=260-266	n=257-263	n=255
MBzP	-0.42 (-1.05, 0.22)	-0.36 (-1.01, 0.29)	-0.51 (-1.18, 0.17)	-3.89 (-7.29, -0.36)*	-4.11 (-7.59, -0.50)*	-0.26 (-4.50, 4.16)
MCNP	-0.35 (-1.16, 0.46)	-0.20 (-1.04, 0.65)	-0.19 (-1.12, 0.74)	-5.40 (-9.63, -0.97)*	-5.03 (-9.45, -0.40)*	-0.40 (-6.47, 6.07)
MCOP	-0.48 (-1.21, 0.25)	-0.36 (-1.14, 0.42)	-0.29 (-1.20, 0.64)	-6.84 (-10.57, -2.95)**	-6.81 (-10.82, -2.63)**	-6.98 (-10.95, -2.84)**
MCPP	-0.16 (-0.83, 0.51)	-0.08 (-0.76, 0.61)	0.01 (-0.77, 0.80)	-3.49 (-7.10, 0.26)#	-3.43 (-7.10, 0.37)#	0.44 (-3.90, 4.97)
∑DEHP	0.17 (-0.57, 0.92)	0.17 (-0.58, 0.93)	0.16 (-0.69, 1.02)	-0.67 (-4.80, 3.63)	-0.69 (-4.86, 3.67)	3.30 (-1.34, 8.16)
BPA	0.45 (-0.32, 1.23)	0.46 (-0.34, 1.26)	0.69 (-0.16, 1.55)	-0.38 (-4.65, 4.08)	-0.95 (-5.32, 3.62)	2.74 (-1.99, 7.69)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, sum of dialkyl phosphate metabolites, butyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, sum of dialkyl phosphate metabolites, monobenzyl phthalate, bisphenol A

[5]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monoethyl phthalate, triclosan

[6]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, triclosan, monoethyl phthalate

[7]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, 2,4-dichlorophenol, mono-isobutyl phthalate

[8]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[9]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, monocarboxyisooctyl phthalate, triclosan

Table 8. Sensitivity analyses for associations of log2 prenatal chemical concentrations and spirometry outcomes

For children with two or more verified maneuvers

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=267-272	n=264-269	n=262	n=227-231	n=224-228	n=223
MBzP	-0.04 (-0.08, 0.00)#	-0.04 (-0.09, 0.00)#	-0.01 (-0.07, 0.05)	-0.02 (-0.07, 0.03)	-0.02 (-0.07, 0.03)	0.02 (-0.05, 0.09)
MCNP	-0.05 (-0.11, 0.00)#	-0.05 (-0.11, 0.01)#	-0.02 (-0.10, 0.06)	-0.05 (-0.12, 0.01)	-0.05 (-0.12, 0.02)	0.00 (0.00, 0.00)
MCOP	-0.07 (-0.12, -0.02)**	-0.07 (-0.12, -0.02)**	-0.09 (-0.15, -0.04)**	-0.07 (-0.12, -0.01)*	-0.07 (-0.13, 0.00)*	-0.04 (-0.13, 0.05)
MCPP	-0.04 (-0.08, 0.01)	-0.04 (-0.08, 0.01)	-0.01 (-0.07, 0.04)	-0.02 (-0.08, 0.03)	-0.03 (-0.08, 0.03)	0.01 (-0.06, 0.08)
∑DEHP	0.00 (-0.05, 0.05)	0.00 (-0.05, 0.05)	0.01 (-0.05, 0.07)	-0.01 (-0.07, 0.05)	-0.01 (-0.07, 0.05)	0.01 (-0.07, 0.08)
BPA	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	-0.01 (-0.07, 0.05)	-0.03 (-0.09, 0.03)	-0.04 (-0.11, 0.02)	-0.02 (-0.09, 0.05)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=227-231	n=224-228	n=223-224	n=227-231	n=224-228	n=223
MBzP	-0.42 (-1.05, 0.22)	-0.36 (-1.01, 0.29)	-0.56 (-1.34, 0.22)	-3.89 (-7.29, -0.36)*	-4.11 (-7.59, -0.50)*	0.95 (-3.38, 5.48)
MCNP	-0.35 (-1.16, 0.46)	-0.20 (-1.04, 0.65)	0.03 (-0.86, 0.92)	-5.40 (-9.63, -0.97)*	-5.03 (-9.45, -0.40)*	-0.94 (-7.09, 5.62)
MCOP	-0.48 (-1.21, 0.25)	-0.36 (-1.14, 0.42)	-0.53 (-1.36, 0.31)	-6.84 (-10.57, -2.95)**	-6.81 (-10.82, -2.63)**	-5.63 (-9.59, -1.50)**
MCPP	-0.16 (-0.83, 0.51)	-0.08 (-0.76, 0.61)	-0.24 (-0.98, 0.50)	-3.49 (-7.10, 0.26)#	-3.43 (-7.10, 0.37)#	0.34 (-3.83, 4.70)
∑DEHP	0.17 (-0.57, 0.92)	0.17 (-0.58, 0.93)	-0.08 (-0.92, 0.78)	-0.67 (-4.80, 3.63)	-0.69 (-4.86, 3.67)	2.58 (-2.00, 7.37)
BPA	0.45 (-0.32, 1.23)	0.46 (-0.34, 1.26)	0.34 (-0.52, 1.21)	-0.38 (-4.65, 4.08)	-0.95 (-5.32, 3.62)	1.26 (-3.40, 6.14)

For children who have not taken asthma medication in the last year

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=274-290	n=271-277	n=269-271	n=248-254	n=245-251	n=243
MBzP	-0.04 (-0.08, 0.00)#	-0.04 (-0.09, 0.00)#	-0.02 (-0.08, 0.04)	-0.02 (-0.07, 0.03)	-0.02 (-0.08, 0.03)	0.01 (-0.05, 0.07)
MCNP	-0.05 (-0.11, 0.00)#	-0.05 (-0.11, 0.01)#	0.02 (-0.06, 0.10)	-0.06 (-0.12, 0.01)#	-0.06 (-0.12, 0.01)	0.00 (-0.09, 0.09)

MCOP	-0.08 (-0.13, -0.03)**	-0.08 (-0.13, -0.02)**	-0.10 (-0.15, -0.04)**	-0.08 (-0.14, -0.02)**	-0.08 (-0.14, -0.02)*	-0.09 (-0.15, -0.02)**
MCPP	-0.03 (-0.08, 0.02)	-0.03 (-0.08, 0.01)	-0.01 (-0.06, 0.05)	-0.02 (-0.08, 0.03)	-0.03 (-0.08, 0.03)	0.01 (-0.05, 0.07)
∑DEHP	0.00 (-0.05, 0.05)	0.00 (-0.05, 0.05)	0.03 (-0.03, 0.09)	-0.01 (-0.08, 0.05)	-0.01 (-0.07, 0.05)	0.03 (-0.04, 0.10)
BPA	-0.02 (-0.08, 0.03)	-0.03 (-0.08, 0.03)	0.00 (-0.06, 0.06)	-0.04 (-0.11, 0.02)	-0.05 (-0.12, 0.01)	-0.03 (-0.10, 0.04)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=248-254	n=245-251	n=243	n=248-254	n=245-251	n=243
MBzP	-0.41 (-1.05, 0.24)	-0.35 (-1.02, 0.31)	-0.52 (-1.20, 0.17)	-3.91 (-7.34, -0.34)*	-4.13 (-7.67, -0.46)*	0.39 (-3.92, 4.89)
MCNP	-0.30 (-1.11, 0.52)	-0.15 (-1.00, 0.71)	-0.12 (-1.06, 0.82)	-5.43 (-9.68, -0.98)*	-5.08 (-9.55, -0.40)*	0.23 (-5.88, 6.73)
MCOP	-0.45 (-1.19, 0.30)	-0.34 (-1.14, 0.47)	-0.24 (-1.17, 0.70)	-7.48 (-11.23, -3.57)**	-7.43 (-11.48, -3.20)**	-7.63 (-11.60, -3.47)**
MCPP	-0.12 (-0.80, 0.57)	-0.03 (-0.73, 0.67)	0.07 (-0.72, 0.87)	-3.19 (-6.87, 0.64)	-3.21 (-6.96, 0.69)	1.43 (-3.01, 6.07)
∑DEHP	0.16 (-0.60, 0.92)	0.15 (-0.62, 0.93)	0.13 (-0.74, 0.99)	-0.95 (-5.14, 3.43)	-0.93 (-5.18, 3.52)	3.26 (-1.39, 8.14)
BPA	0.41 (-0.39, 1.21)	0.49 (-0.34, 1.32)	0.73 (-0.15, 1.62)	-1.01 (-5.38, 3.57)	-1.41 (-5.93, 3.33)	2.64 (-2.21, 7.73)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, sum of dialkyl phosphate metabolites, butyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, sum of dialkyl phosphate metabolites, monobenzyl phthalate, bisphenol A

[5]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monoethyl phthalate, triclosan

[6]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, triclosan, monoethyl phthalate

[7]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, 2,4-dichlorophenol, mono-isobutyl phthalate

[8]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[9]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, monocarboxyisooctyl phthalate, triclosan

Table 9. Association of log 2 prenatal chemical concentrations and TH cell parameters

	Th1:Th2 cell ratio, age 2			Th1 %, age 2			Th2 %, age 2		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=219-234	n=217-232	n=215	n=219-234	n=217-232	n=215-232	n=219-234	n=217-232	n=215
MBzP	-4.78 (-13.14, 4.38)	-4.61 (-13.31, 4.96)	0.96 (-9.38, 12.48)	3.23 (-5.60, 12.90)	2.80 (-6.33, 12.82)	4.89 (-4.74, 15.50)	8.42 (0.44, 17.03)*	7.77 (-0.33, 16.54)#	4.58 (-4.20, 14.15)
MCNP	0.45 (-10.39, 12.60)	2.21 (-9.45, 15.37)	6.98 (-5.54, 21.15)	2.52 (-8.26, 14.56)	3.03 (-8.40, 15.89)	4.74 (-7.08, 18.06)	2.06 (-7.26, 12.31)	0.80 (-8.76, 11.37)	-1.26 (-10.99, 9.54)
MCOP	-1.04 (-10.90, 9.92)	0.40 (-10.44, 12.56)	8.01 (-4.34, 21.96)	4.35 (-5.77, 15.56)	3.60 (-7.28, 15.76)	4.23 (-6.79, 16.55)	5.44 (-3.42, 15.12)	3.19 (-6.06, 13.35)	-2.08 (-11.50, 8.34)
MCPP	-2.05 (-11.85, 8.83)	-1.73 (-11.74, 9.42)	5.76 (-6.13, 19.16)	0.75 (-9.07, 11.63)	0.70 (-9.28, 11.79)	1.03 (-9.07, 12.27)	2.86 (-5.83, 12.36)	2.48 (-6.19, 11.94)	-3.12 (-12.27, 6.98)
∑DEHP	-7.40 (-17.19, 3.54)	-6.80 (-16.95, 4.58)	-2.53 (-14.12, 10.62)	-2.14 (-12.25, 9.15)	-3.64 (-13.87, 7.80)	-3.80 (-14.15, 7.80)	5.69 (-3.77, 16.07)	3.39 (-5.98, 13.70)	-2.36 (-12.12, 8.48)
BPA	-7.77 (-18.07, 3.83)	-6.72 (-17.60, 5.61)	-4.03 (-15.63, 9.17)	-3.74 (-14.37, 8.21)	-4.73 (-15.75, 7.74)	-5.31 (-16.18, 6.98)	4.37 (-5.62, 15.41)	2.13 (-7.91, 13.27)	-1.75 (-11.67, 9.29)

	Th1:Th2 cell ratio, age 5			Th1 %, age 5			Th2 %, age 5		
	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=221-229	n=218-226	n=218	n=221-229	n=218-226	n=218	n=221-229	n=218-226	n=218
MBzP	0.01 (-8.99, 9.90)	1.16 (-8.37, 11.69)	8.82 (-3.26, 22.41)	3.38 (-2.84, 10.01)	3.26 (-3.29, 10.25)	1.53 (-6.59, 10.36)	3.37 (-6.04, 13.73)	2.07 (-7.69, 12.86)	-4.06 (-14.52, 7.67)
MCNP	-6.10 (-16.57, 5.69)	-4.98 (-16.18, 7.73)	-3.73 (-15.61, 9.81)	-4.96 (-12.09, 2.74)	-5.35 (-12.89, 2.84)	-10.03 (-18.18, -4.59 (-6.78, 17.34)	1.21 (-10.24, 14.13)	-0.39 (-12.33, 13.17)	-3.51 (-15.41, 10.06)
MCOP	-1.83 (-11.64, 9.06)	0.32 (-10.57, 12.54)	4.81 (-7.96, 19.35)	1.14 (-5.65, 8.42)	1.39 (-6.04, 9.42)	3.03 (-7.39, 14.63)	1.07 (-10.07, 13.58)	-5.52 (-16.96, 7.50)	
MCPP	-4.95 (-14.64, 5.85)	-4.33 (-14.30, 6.80)	-0.42 (-12.79, 13.70)	3.79 (-3.32, 11.43)	4.01 (-3.30, 11.87)	5.29 (-4.10, 15.59)	9.20 (-2.04, 21.72)	8.72 (-2.74, 21.53)	4.06 (-8.64, 18.52)
∑DEHP	-2.21 (-11.99, 8.65)	-1.92 (-11.93, 9.23)	2.69 (-8.82, 15.64)	-2.20 (-8.76, 4.84)	-2.58 (-9.29, 4.62)	-5.93 (-13.26, 2.02)	0.01 (-10.12, 11.28)	-0.68 (-10.97, 10.80)	-6.63 (-16.95, 4.97)
BPA	-5.67 (-16.04, 5.99)	-5.76 (-16.52, 6.39)	-4.46 (-15.83, 8.44)	-0.85 (-8.28, 7.19)	-1.12 (-8.86, 7.27)	-2.51 (-10.94, 6.72)	5.11 (-6.72, 18.44)	4.92 (-7.36, 18.84)	2.11 (-10.12, 16.01)

	Th1:Th2 cell ratio, age 7			Th1 %, age 7			Th2 %, age 7		
	Crude	Demographically adjusted[1]	Fully adjusted[2]	Crude	Demographically adjusted[1]	Fully adjusted[2]	Crude	Demographically adjusted[1]	Fully adjusted[2]

	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
	n=244-250	n=244-250	n=242	n=244-250	n=244-250	n=242-250	n=244-250	n=244-250	n=242
MBzP	3.58 (-4.96, 12.88)	4.61 (-4.16, 14.19)	3.36 (-6.66, 14.44)	0.13 (-5.26, 5.82)	0.13 (-5.38, 5.95)	0.15 (-5.60, 6.26)	-3.33 (-11.15, 5.18)	-4.29 (-12.17, 4.30)	-2.22 (-11.16, 7.62)
MCNP	-3.63 (-13.48, 7.34)	-2.59 (-12.70, 8.69)	-5.90 (-16.41, 5.92)	-3.15 (-9.63, 3.78)	-2.77 (-9.39, 4.33)	-3.24 (-10.24, 4.30)	0.50 (-9.60, 11.72)	-0.19 (-10.36, 11.14)	4.44 (-7.11, 17.43)
MCOP	-3.03 (-12.56, 7.52)	-2.50 (-12.33, 8.43)	-7.09 (-17.61, 4.79)	-2.49 (-8.75, 4.20)	-2.19 (-8.66, 4.73)	-3.53 (-10.32, 3.77)	0.56 (-9.14, 11.30)	0.31 (-9.61, 11.33)	4.60 (-7.10, 17.77)
MCPP	-0.63 (-9.46, 9.05)	0.31 (-8.68, 10.19)	-2.39 (-12.27, 8.59)	-4.35 (-9.87, 1.51)	-4.24 (-9.84, 1.70)	-4.79 (-10.80, 1.63)	-3.73 (-12.12, 5.45)	-4.54 (-12.93, 4.65)	-1.45 (-11.04, 9.17)
∑DEHP	5.29 (-4.97, 16.67)	5.16 (-5.25, 16.71)	2.93 (-8.59, 15.90)	-0.27 (-6.64, 6.53)	-0.16 (-6.65, 6.78)	-1.18 (-8.03, 6.18)	-5.28 (-14.35, 4.74)	-5.06 (-14.28, 5.15)	-4.70 (-14.04, 5.65)
BPA	2.41 (-8.07, 14.09)	2.49 (-8.31, 14.57)	1.13 (-10.21, 13.92)	2.94 (-4.02, 10.40)	3.19 (-4.02, 10.93)	3.69 (-3.51, 11.43)	0.51 (-9.66, 11.83)	0.67 (-9.83, 12.40)	2.57 (-8.89, 15.47)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, monoethyl phthalate, propyl paraben

[3]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, 2,5-dichlorophenol, 2,4-dichlorophenol, propyl paraben

[4]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, methyl paraben, 2,5-dichlorophenol

[5]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, triclosan, mono-n-butyl phthalate

[6]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, monocarboxyisononyl phthalate, mono(3-carboxypropyl) phthalate

[7]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, triclosan, monoethyl phthalate

[8]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, butyl paraben, triclosan, mono-n-butyl phthalate

[9]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, butyl paraben, bisphenol A, methyl paraben

[10]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, methyl paraben, triclosan, sum of di(2-ethylhexyl) phthalate metabolites

Table 10. Longitudinal association of log 2 prenatal chemical concentrations and Th cell parameters at various ages, with P-values for age interaction

	Th1:Th2 cell ratio				Th1%				Th2%			
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int
	n=341-362	n=338-359	n=335		n=341-362	n=338-359	n=335-359		n=341-362	n=338-359	n=335	
MBzP	-1.21 (-7.14, 5.09)	-0.24 (-6.49, 6.42)	5.18 (-1.83, 12.69)	0.25	1.40 (-2.87, 5.87)	1.48 (-2.91, 6.07)	1.85 (-2.49, 6.39)	0.73	2.76 (-2.77, 8.60)	1.82 (-3.98, 7.97)	-0.06 (-5.99, 6.25)	0.10
MCNP	-2.52 (-8.43, 3.77)	-0.90 (-7.20, 5.82)	0.57 (-6.23, 7.86)	0.72	-1.37 (-6.10, 3.59)	-0.93 (-5.92, 4.33)	-0.32 (-5.41, 5.05)	0.29	1.19 (-4.84, 7.61)	-0.03 (-6.27, 6.63)	-0.64 (-7.15, 6.32)	0.90
MCOP	-2.31 (-7.70, 3.40)	-0.38 (-6.26, 5.87)	2.88 (-3.93, 10.17)	0.85	0.64 (-3.34, 4.78)	1.09 (-3.30, 5.67)	1.00 (-3.40, 5.60)	0.46	2.81 (-2.87, 8.82)	1.24 (-4.87, 7.75)	-1.46 (-7.60, 5.08)	0.83
MCPP	-3.45 (-9.56, 3.07)	-2.80 (-9.00, 3.83)	0.27 (-6.72, 7.78)	0.89	-1.44 (-5.90, 3.23)	-1.21 (-5.77, 3.56)	-0.91 (-5.50, 3.91)	0.16	1.94 (-4.69, 9.02)	1.48 (-5.15, 8.58)	-0.39 (-7.08, 6.78)	0.20
∑DEHP	-1.88 (-7.44, 4.00)	-1.44 (-7.24, 4.72)	2.88 (-4.06, 10.31)	0.22	-2.14 (-7.01, 3.00)	-2.60 (-7.61, 2.69)	-3.20 (-8.22, 2.08)	0.81	-0.42 (-6.44, 5.98)	-1.33 (-7.45, 5.20)	-4.66 (-11.69, 2.92)	0.42
BPA	-3.46 (-10.28, 3.88)	-2.57 (-9.65, 5.07)	-0.94 (-8.40, 7.12)	0.42	-0.36 (-5.76, 5.36)	-0.38 (-5.91, 5.47)	-0.80 (-6.27, 4.99)	0.44	3.30 (-3.42, 10.50)	2.30 (-4.60, 9.70)	0.76 (-6.34, 8.38)	0.75

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

Separate models created for each chemical

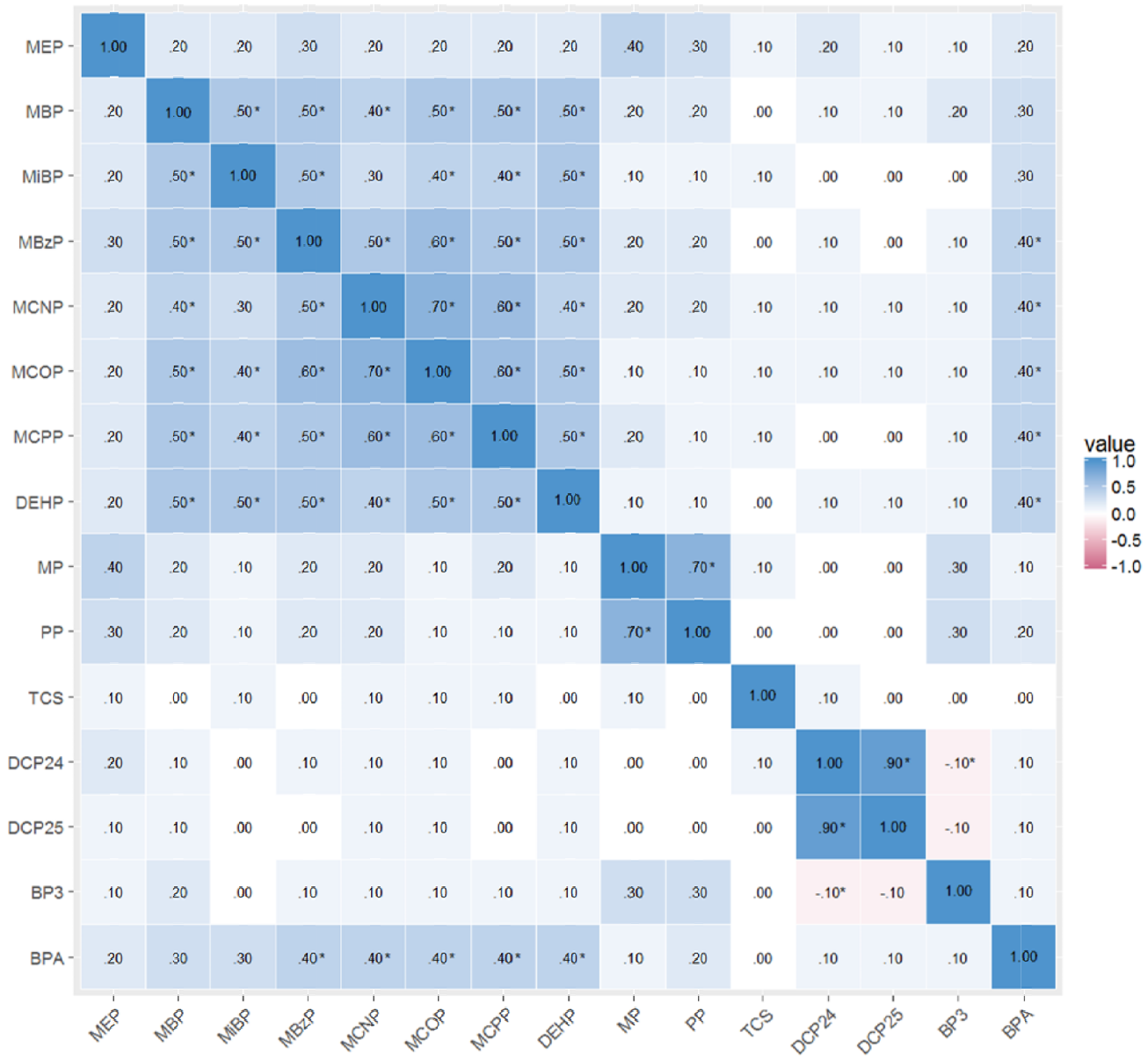
[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, triclosan

[3]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, methyl paraben, 2,4-dichlorophenol, propyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, mono-n-butyl phthalate, methyl paraben, monoethyl phthalate

Figure 1. Log2 specific gravity corrected phthalate, paraben, and phenol correlations in CHAMACOS



Chapter 4: Associations between prenatal maternal urinary concentrations of personal care product chemicals and childhood respiratory and allergic outcomes in the CHAMACOS study

Introduction

Chemicals in personal care products may impact children's immune development and risk for asthma. Chemicals commonly found in fragrance, cosmetics, and other personal care products include low molecular weight phthalates, parabens, and other phenols. Low molecular weight phthalates (diethyl phthalate (DEP), dibutyl phthalate (DBP), and diisobutyl phthalate (DiBP)) are used in fragrances, cosmetics, and medications[1,2]. Parabens (methyl paraben (MP), propyl paraben (PP), and butyl paraben (BP)) are used as preservatives in cosmetics, pharmaceuticals, and paper products because of their bactericidal and fungicidal properties[3,4]. Triclosan is an antibacterial found in some toothpastes, deodorants, and antimicrobial fabrics[5]. Triclosan was recently banned from antibacterial soaps for not being generally recognized as safe and effective[6]. 2,4-dichlorophenol (2,4-DCP) is an intermediate in pesticide manufacturing, but is also a photo-degradation product of triclosan[7]. 2,5-dichlorophenol (2,5-DCP) is used in moth balls and room and toilet deodorizers[8]. Benzophenone-3 (BP-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[9]. Exposure to these chemicals 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[10].

Rates of childhood asthma, aeroallergy, and eczema are high in the U.S. and have been increasing for several decades[11-13]. In the 2013-2014 NHANES, 15.9% of children had ever been told they had asthma[10], and in the 2005-2006 NHANES self-reported doctor diagnosis of aeroallergy and eczema prevalence were 24.6% and 14.3%, respectively[14]. Activity of T helper cells, a type of lymphocyte, may be on the causal pathway to asthma, inhalant allergies, and other allergic diseases[15,16]. T helper cells release cytokines that send chemical messages to either attract other immune cells, or signal a direction for other immune cells to go in[17]. Of the two main types of T helper cells, T helper 1 (Th1) cells are designed to combat intracellular viruses and bacteria through cell-mediated immunity and they secrete cytokines such as interferon gamma (IFN-y), 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[18]. A higher ratio of Th2 cytokines to Th1 cytokines has been associated with increased risks of aeroallergy and asthma[15,16].

Previous studies have shown that exposure to personal care product chemicals may be related to immune dysfunction[19,20]. Animal studies suggest exposure to some low molecular weight phthalates, parabens, and triclosan leads to higher Th2 cytokine concentrations[21-26], although other studies found no association with low molecular weight phthalates[27,28]. Animal studies also suggest that exposure to triclosan leads to reduced lung function[24]. Epidemiologic studies suggest no association between exposures to low molecular weight phthalates, parabens, and other phenols with cytokine activity[29-32]. However, *in vitro* research suggests there is biological evidence that personal care product chemicals may be modifying different immune system pathways. *In vitro* studies show DEP and DBP act as adjuvants to increase concentrations of IL-4, a Th2 cytokine, in animal lymph node cells[33]. Phthalates may also influence cytokine activity through regulatory T cells, which control the activity of T helper cells[34]. In addition, benzophenones have been shown to

inhibit Th1 cytokine production and promote Th2 cytokine production[35,36], and triclosan may bind to the ryanodine receptor[37] which can cause T-cell stimulation[38,39]. Several of these pathways may involve activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)[40,41], which induces pro-inflammatory cytokine production and has been associated with autoimmune diseases[42]. Some phthalates[21] and triclosan[25] also promote thymic stromal lymphopoietin (TSLP) protein expression, which activates inflammatory and Th2-related immune responses[43,44].

Some epidemiologic studies on prenatal exposure to these chemicals corroborate *in vitro* and animal evidence. In 610 children, prenatal maternal urinary concentrations of MiBP were associated with dermatitis at age three[34], and a French birth cohort of 604 children found prenatal MiBP and MEP were associated with eczema in early childhood[45]. However, other studies have found no association between low molecular weight phthalates and eczema[46-48] or aeroallergies[34,46,47,49]. There have only been two prenatal studies of parabens or phenols: the Mount Sinai Children's Environmental Health study found that 2,5-DCP was associated with eczema in 164 six and seven year olds[48], and the Vitamin D Antenatal Asthma Reduction Trial study found no association between triclosan or parabens and aeroallergies in 463 three and four year olds[50]. Cross-sectional studies suggest that exposures to parabens[20,51] and triclosan[20,51-54], but not to BP-3[20], are associated with aeroallergy, and that none of these chemicals is associated with eczema[51,54-56].

These chemicals may also act on several mechanisms involved in the biology of asthma. Phthalates, including DEP and DBP, have been found to propagate release of inflammatory cytokines *in vitro* directly from airway epithelial cells which then lead to proliferation and migration of bronchial smooth muscle cells[57]. These are key steps in the airway remodeling that characterizes asthma[58]. DEP is also associated with increased fractional exhaled nitric oxide (FE_{NO}), a biomarker for eosinophilic airway inflammation that is associated with increased risk for and exacerbation of asthma symptoms[59]. Phthalates have also been shown to activate nuclear peroxisome proliferator–activated receptors (PPAR)[60], which are involved in a wide range of physiological actions, including airway inflammation and airway remodeling[61]. Phthalates and parabens are also xenoestogens[62,63], and estrogens have been shown to lower FEV_1 and FVC and increase bronchial hyperactivity[64]. Estrogens have also been shown to encourage Th2 differentiation and other allergic inflammatory responses[64]. Exposures to low molecular phthalates, parabens, and phenols are also associated with increased biomarkers of oxidative stress in humans[30,31,65,66] and *in vitro*[67-70] which can play a crucial role in asthma development[35] by increasing inflammation and promoting bronchial hyperresponsiveness[71].

Asthma has been examined in epidemiologic studies in relation to *in utero* exposures to low molecular weight phthalates. A study of 154 children found that prenatal maternal urinary concentrations of MBP, but not MEP, were associated with increased risk of asthma from ages 5-11[72], and a study of 171 eight-year-old children found prenatal maternal and early childhood urinary concentrations of MEP, but not MBP, were associated with increased risk for wheeze and asthma in boys only[73]. However, additional longitudinal studies that examined *in utero* low molecular weight phthalates found null results[47-49,74]. Although two longitudinal studies on parabens and other phenols also found null results with asthma[48,50], a French birth cohort study of 587 children found that prenatal urinary concentrations of parabens and 2,5-DCP were associated with wheeze and asthma in early childhood[74]. Several cross-sectional studies have also looked at urinary concentrations of parabens and other phenols in relation to asthma. Results suggest that exposures to triclosan[51,53,75-77] and the dichlorophenols[78] may be associated with risk of asthma and atopy, but parabens[51,76] and BP-3[51,76,77] may not.

Phthalates, parabens, and some other phenols have been detected in human placentas, furthering the case for studying these exposures prenatally[79,80]. Pregnancy also represents an essential 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.

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 that is mostly Latino. English or Spanish-speaking mothers who were eligible for low-income health insurance (Medicaid), 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 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 chemical concentrations compared to children who did not have outcome data. Research protocols were approved by the University of California, Berkeley Committee for the Protection of Human Subjects. Mothers provided written informed consent and children provided verbal assent at age seven.

Questionnaire data collection. Interviews were conducted with the mothers in English or Spanish using structured questionnaires two times during pregnancy (mean 13 and 26 weeks gestation), when they were asked their age, family income, parity, and if they had a family history of asthma. 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.

When the child was seven, mothers were asked whether her child was 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[81]: a) tightness in the chest and difficulty breathing; b) wheezing or whistling in the chest; c) trouble going to sleep or being awakened from sleep because of wheezing, whistling, shortness of breath, or coughing without a cold; d) wheezing, whistling, or shortness of breath so severe that the child couldn't finish saying a sentence; e) having to stop running or playing or having to sit out of active games because of wheezing, whistling, shortness of breath, or coughing without a cold; f) an asthma attack. We defined respiratory symptoms as a binary outcome based on a positive response to any of the symptom questions.

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.

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. Ninety-two percent of the tests were performed by the same technician. Technicians verified that children did not lean forward, that they inhaled enough air deeply and smoothly from the start of the maneuver, that they exhaled enough air, and that air did not leak from the side of the spirometer. Three identical EasyOne (new diagnostic design) spirometers were used in the study and routine calibration was performed every morning. Every child performed maneuvers lasting at least 3 seconds with a maximum of eight expiratory maneuvers each. Children were coached in all attempts to blow until the EasyOne signaled that the test had ended. The spirometric software kept up to best three acceptable tests. All maneuvers were reviewed and verified by two physicians with experience in pediatric spirometry. The following were measured from each maneuver: forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), FEV_1/FVC, and forced expiratory flow from 25–75% of FVC ($FEF_{25\%-75\%}$). FEV_1 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 FEV_1 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 using flow cytometry that has been described previously and validated for epidemiologic studies[82]. Briefly, 500 uL 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-y (Becton Dickinson) or IL-4 (Becton Dickinson) cytokines. Cells that stained positive for IFN-y 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 defined having eczema as a positive response to having 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.

Exposure assessment. Urine samples were collected from mothers at the two interviews during pregnancy. Samples were collected in phthalate-free polypropylene urine cups, aliquoted into glass

vials, and stored at -80ºC until shipment to the Centers for Disease Control and Prevention (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: (analyte concentration * 0.24)/(sample specific gravity – 1)[83]. Urinary specific gravity was imputed based on urinary creatinine concentrations for 77 women who were missing specific gravity measurements.

Solid phase extraction coupled with isotope dilution high performance liquid chromatography-electrospray ionization-tandem mass spectrometry was used to quantify concentrations of the following three phthalate metabolites: monoethyl phthalate [MEP, a metabolite of diethyl phthalate (DEP)]; mono-n-butyl phthalate [MBP, a metabolite of dibutyl phthalate (DBP)]; and mono-isobutyl phthalate [MiBP, a metabolite of di-isobutyl phthalate (DiBP)]. Isotope dilution on-line solid-phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometry was used for the determination of eight phenols: methyl paraben (MP), propyl paraben (PP), butyl paraben (BP), triclosan, 2,4-dichlorphenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), and benzophenone-3 (BP-3). BP 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[84] and phenols[85]. 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. If concentrations were below the LOD, we assigned the machine read values, if available, or we used maximum likelihood estimation to imputed a randomly selected value below the LOD from the log-normal distribution[86]. 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]). These were replaced with the highest calibrator concentration used (100 ng/mL for 2,4-dichlorophenol, 1000 ng/mL for all other parabens and phenols).

Statistical analysis. We conducted multivariate logistic regression models to assess the odds of probable asthma, aeroallergy, and eczema, and we conducted linear regression models to assess the risk of altered lung function and cytokine levels associated with prenatal maternal urinary chemical concentrations. Chemical 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. Case variables for probable asthma, aeroallergies, and eczema were analyzed as binary.

FEV_1 and FVC were normally distributed and analyzed as continuous variables. FEV_1/FVC and $FEF_{25-75\%}$ were $\log_{10}$-transformed because they were log-normally distributed, and were analyzed as continuous variables. Regression coefficients thus represent mean (FEV_1 and FVC) or percent change (FEV_1/FVC and $FEF_{25-75\%}$) in outcomes for each two-fold increase in chemical concentration. We first conducted analyses on children who had at least one valid 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 valid maneuvers in sensitivity analyses, and also excluded children who were currently taking any asthma medication.

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. For each two-fold increase in

chemical 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 chemical concentrations across ages. Additionally, we obtained p-values for interaction by age by conducting GEE models with interaction terms for child age.

We controlled for maternal age at birth, parity, poverty index at baseline, and family history of asthma (mother, father, or siblings) as confounders. These were determined via directed acyclic graphs *a priori*. Modeling exposure to one chemical at a time unrealistically represents exposure to these chemicals, which are often found in products together. To reduce dimensions while modeling joint 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 chemicals with an outcome and weights different models by their posterior model probability, adjusted for specified demographic covariates, to determine the influence of different chemicals on a health outcome relative to other chemicals[87], measured as Posterior Inclusion Probabilities (PIPs). We included the following chemicals in the BMA models, in addition to the chemicals focused on in this chapter: other phthalates (monobenzyl phthalate, monocarboxy-isononyl phthalate, monocarboxy-octyl phthalate, mono-(3-carboxypropyl) phthalate) and phenols (bisphenol A). We chose the three most influential chemicals (i.e. those with the highest PIPs in the BMA model) to add to fully adjusted regression models for that outcome.

Because we previously found associations in this cohort between respiratory symptoms, spirometry, and the sum of urinary concentrations of dialkyl phosphate metabolites (DAPs)[88], which reflect exposure to organophosphate pesticides, and measures of elemental sulfur sprayed near participants' homes[89], we chose to include these two additional covariates in sensitivity analyses for probable asthma and spirometry.

Results

As seen in Table 1, mothers were mostly under 30 (76%), living below 100% of the federal poverty index (61%), and multiparous (68%). Most children did not have a family history of asthma (90%).

All chemicals were detected in over 95% of participants in both measurements, with the exception of TCS, which was detected in 84% of measurements at the first pregnancy visit and 88% of measurements at the second pregnancy visit. (Table 2) Figure 1 shows correlations between chemicals. Compared with NHANES data at the closest years to CHAMACOS data collection, our study population had higher concentrations of all low molecular weight phthalates, parabens, and other phenols, notably MEP, MP, PP, and 2,5-DCP[90].

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. (Table 3)

The mean FVC volume was 2.1 liters (SD: 0.6), FEV_1/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. (Table 3)

Th1:Th2 cell ratios increased with age. The mean ratio at age two was 6.13 (SD: 5.58), which increased to 14.40 (SD: 44.53) at age five and to 22.51 (SD: 36.29) at age seven. This corresponded with Th1% increasing from the age two mean of 4.0 (SD: 3.0) to the age five mean of 6.8 (SD: 3.2) to the age seven mean of 10.0 (SD: 5.9). Th2% stayed stable with age: ages two and seven had a mean of 0.9% (SD: 0.7) and age five had a mean of 1.1% (SD: 0.8). (Table 4)

Table 5 shows the PIPs for each phthalate metabolite, paraben, other phenol, and DAP metabolite included in the BMA analysis. The top three ranked chemicals for each outcome were included as covariates in regression analyses for those outcomes and can also be found in the footnotes of Tables 6, 7, 8, 9, and 10. The top ranked chemical for age two Th1:Th2 cell ratio; age two Th2%; age five Th1:Th2 cell ratio, Th1%, and Th2%; age seven Th2%, Th1:Th2 cell ratio longitudinally, and Th2% longitudinally also showed the strongest association with those outcomes.

Tables 6, 7, 8, 9, and 10 show crude models, demographically adjusted models, and fully adjusted models. As shown in Table 6 in fully adjusted models, prenatal urinary concentrations of 2,5-DCP (OR: 0.63, 95% CI: 0.47, 0.86) and PP (OR: 0.84, 95% CI: 0.73, 0.97) were associated with decreased odds of probable asthma. There were no associations with aeroallergies or eczema.

Table 7 shows that each 2-fold increase in MEP urinary concentration was associated in fully adjusted models with lower FEV_1 (RR: -0.03, 95% CI: -0.07, 0.00) at borderline significance, but MBP concentrations were associated with higher FEV_1 (RR: 0.06, 95% CI: 0.01, 0.11). MEP concentrations were also associated with lower $FEF_{25-75\%}$ (RR: -3.23, 95% CI: -5.87, -0.52), but MBP concentrations were associated with higher $FEF_{25-75\%}$ (RR: 4.29, 95% CI: 0.04, 8.71), as were triclosan concentrations (RR: 1.76, 95% CI: 0.07, 3.47). Results were similar in children with 2+ verified maneuvers, and when excluding children who were currently using asthma medication (Table 8).

Associations between maternal urinary chemical concentrations and cytokine levels at individual ages two, five, and seven are shown in Table 9. In fully adjusted models, MBP concentrations were associated with lower Th1:Th2 cell ratio (more Th2-dominant) at age two (RR: -17.32, 95% CI: -25.68, -8.03), as were PP concentrations with borderline significance (RR: -4.70, 95% CI: -9.24, 0.08), but MEP concentrations were associated with higher (more Th1-dominant) Th1:Th2 cell ratio at age two (RR: 13.60, 95% CI: 5.06, 22.84). Also in age two fully adjusted models, MEP concentrations were associated with higher Th1% at borderline significance (RR: 7.11, 95% CI: -0.81, 15.66). 2,4-DCP and 2,5-DCP concentrations were also associated with higher Th1% in demographically adjusted models only, although 2,5-DCP was associated with higher Th2% at age two with borderline significance (RR: 3.38, 95% CI: -0.56, 7.47) in fully adjusted models. MBP concentrations were associated with higher Th2% at ages two (RR: 13.02, 95% CI: 3.58, 23.33) and five (RR: 18.20, 95% CI: 6.07, 31.73), and were borderline associated with higher Th1% at age seven (RR: 5.86, 95% CI: -0.94, 13.13). MP concentrations were associated at borderline significance with lower Th2% at age seven (RR: -6.22, 95% CI: -12.41, -0.42) in fully adjusted models and were also associated with lower Th1% at age seven, but in demographically adjusted models.

GEE results modeling cytokine associations across ages are shown in Table 10, as are p-values for interaction by age. In fully adjusted models, MiBP concentrations were associated with lower Th1:Th2 cell ratio (RR: -6.19, 95% CI: -11.70, -0.32) and MBP concentrations were associated with higher Th2% (RR: 8.40, 95% CI: 1.95, 15.26). MP concentrations were associated with lower Th1% in demographically adjusted models, and was associated with lower Th2%, but with borderline significance (RR: -4.36, 95% CI: -8.59, 0.06) in fully adjusted models. P-values for age interaction in GEE models indicate that results differed across ages for the associations between MBP concentrations and Th1:Th2 cell ratio and Th2%.

Sensitivity analyses for probable asthma and spriometry including the sum of dialkyl phosphate metabolites and elemental sulfur yielded similar results (data not shown).

Discussion

We found that prenatal maternal urinary MBP concentrations were associated with higher Th2% and lower Th1:Th2 cell ratio at ages two and five, but were also associated with higher lung function at age seven. We also found that MiBP concentrations were associated with lower Th1:Th2 cell ratio in longitudinal analyses, which is consistent with an atopic phenotype. We found that MEP concentrations were associated with lower FEV_1 and $FEF_{25-75\%}$ at age seven, however, no low molecular weight phthalates were associated with increased odds of asthma, aeroallergy, or eczema. Of the phenols, we found that PP and 2,5-DCP concentrations were associated with lowered odds of probable asthma, triclosan concentrations were associated with higher lung function, and MP was associated with lower age seven Th2%.

In contrast to previous studies, we did not find an association between phthalates and atopic diseases, although we did show an association with an atopic cytokine profile. Two previous longitudinal studies found *in utero* exposures to phthalates were associated with childhood asthma[72,73]. One study found that prenatal urinary concentrations of MBP were associated with asthma at ages 5-11, but MEP concentrations were not[72]. Similarly, a study of 171 children that found prenatal urinary MEP concentrations were related to increased asthma risk at age eight, but prenatal MBP concentrations were not[73]. 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[47-49,74]. One previous longitudinal study of 610 children found prenatal MiBP exposure was associated with dermatitis at age three[34], and another found MiBP and MEP were associated with eczema in early childhood[45], however several other longitudinal studies, like ours, did not find an association between prenatal exposures to low molecular phthalates and aeroallergies or eczema[34,46-49]. Additionally, in contrast to our findings, two previous studies of exposures to low molecular weight phthalates found no associations with cytokine concentrations. One study measured phthalates and cytokines in the cord blood of 1,121 infants[29], and the other measured phthalates and cytokines in the plasma of 120 pregnant women[30]. 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[21,22], but did not affect lung function[28]. Overall, our findings confirm those of many previous longitudinal studies that show exposures to low molecular weight phthalates are not associated with atopic diseases, but our findings suggest there may still be an increased risk for immune dysregulation.

There were several unexpected protective findings of exposures to parabens and phenols in our study, in addition to expected negative associations. Our unexpected finding that 2,5-DCP concentrations were associated with lowered odds of probable asthma is in contrast with a study that found a positive association between prenatal 2,5-DCP and wheeze in early childhood[74], and with a study that found no association between prenatal 2,5-DCP and asthma at ages six and seven[48]. Our unexpected finding that PP concentrations were associated with lowered odds of probable asthma is in contrast with a study that found prenatal paraben concentrations were associated with asthma and wheeze in early childhood[74], and with a study that found null results[50]. Additionally, a study of 106 pregnant women found no association between urinary concentrations of parabens and blood cytokine concentrations[31], which is in contrast to our finding that MP concentrations are associated with lower levels of Th1 and Th2 cytokines. This study, however, did not look at cytokine levels in childhood, and therefore differs greatly from our study as cytokine profiles change throughout childhood[91]. Finally, our unexpected finding that triclosan was associated with improved lung function is in contrast with previous birth cohort studies that found null results[48,50,74]. Though difficult to confirm with other studies, our results suggest that *in utero* exposures to various parabens and phenols have inconsistent associations with components of atopic diseases in childhood.

Many of the chemicals analyzed in this study are present as co-exposures in personal care products. For example, a single product may have artificial scents, preservatives, and sunblock, containing phthalates, parabens, and BP-3 at once. Exploring associations of one of these chemicals at a time does not accurately model how people are exposed. One strength of our study is we implemented BMA to identify additional chemicals measured in this population that may confound relationships between other chemicals and atopic outcomes. This has allowed us to include the most relevant potentially confounding chemicals in the model and isolate the associations that one chemical may have with an atopic outcome to a greater extent than previous studies. Nevertheless, the chemicals studied here all have relatively low ICCs (low molecular weight phthalates range from 0.19 to 0.38, parabens range from 0.41 to 0.46, and the other phenols range from 0.46 to 0.56), which demonstrates high variability among samples. While we do have two urinary chemical concentration measurements during pregnancy, it is more difficult to capture representative exposure to chemicals with high temporal variability.

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[92-94]. At age seven, when we were able to obtain reliable spirometry, we performed a thorough assessment of atopic diseases. 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. 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. Non-atopic childhood asthma can be related to exposure to air pollution, exercise-induced stress, obesity, tobacco smoke, or respiratory infections[95]. Around 10% of asthmatic patients are usually non-atopic[96].

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.

Our study found that prenatal maternal urinary concentrations of low molecular weight phthalates found in personal care products are associated with atopic cytokine profiles, that parabens and dichlorophenols are associated with increased odds of probable asthma, and that *in utero* exposures to several phenols are unexpectedly associated with decreased odds of probable asthma or higher lung function. This is the first study to evaluate the association between *in utero* exposure to prenatal personal care product chemicals and childhood cytokine expression.

References

1. Kelley KE, Hernández-Díaz S, Chaplin EL, Hauser RB, Mitchell AA. Identification of phthalates in medications and dietary supplement formulations in the United States and Canada. 2011.
2. Koniecki D, Wang R, Moody RP, Zhu J. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. *Environmental research* 2011;111(3):329-336.
3. Liao C, Kannan K. Concentrations and composition profiles of parabens in currency bills and paper products including sanitary wipes. *Science of The Total Environment* 2014;475:8-15.
4. Guo Y, Kannan K. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. *Environmental science & technology* 2013;47(24):14442-14449.
5. Dann AB, Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action. *J Appl Toxicol* 2011;31(4):285-311.
6. United States Food and Drug Administration. Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record; Proposed Rule. In: Services DoHaH, ed. Vol. 78 Federal Register, 2013;76443-76478.
7. Latch DE, Packer JL, Stender BL, VanOverbeke J, Arnold WA, McNeill K. Aqueous photochemistry of triclosan: Formation of 2, 4-dichlorophenol, 2, 8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environmental Toxicology and Chemistry* 2005;24(3):517-525.
8. Wei Y, Zhu J, Nguyen A. Urinary concentrations of dichlorophenol pesticides and obesity among adult participants in the US National Health and Nutrition Examination Survey (NHANES) 2005–2008. *International journal of hygiene and environmental health* 2014;217(2):294-299.
9. Han C, Lim Y-H, Hong Y-C. Ten-year trends in urinary concentrations of triclosan and benzophenone-3 in the general US population from 2003 to 2012. *Environmental Pollution* 2016;208:803-810.
10. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2013-2014.
11. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the United States, 1980–2007. *Pediatrics* 2009;123(Supplement 3):S131-S145.
12. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H, Group IPTS. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet* 2006;368(9537):733-743.
13. Jackson KD, Howie LD, Akinbami OJ. Trends in allergic conditions among children: United States, 1997-2011. 2013.
14. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2005-2006.
15. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res* 2001;2(2):64-5.

16. Deo SS, Mistry KJ, Kakade AM, Niphadkar PV. Role played by Th2 type cytokines in IgE mediated allergy and asthma. *Lung India: Official Organ of Indian Chest Society* 2010;27(2):66.
17. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* 2008;112(5):1557-1569.
18. Volcheck GW. Overview of the Human Immune Response. *Clinical Allergy* Springer, 2008;1-39.
19. Robinson L, Miller R. The impact of bisphenol A and phthalates on allergy, asthma, and immune function: a review of latest findings. *Current environmental health reports* 2015;2(4):379-387.
20. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012;130(2):453-60 e7.
21. Shigeno T, Katakuse M, Fujita T, Mukoyama Y, Watanabe H. Phthalate ester-induced thymic stromal lymphopoietin mediates allergic dermatitis in mice. *Immunology* 2009;128(1pt2):e849-e857.
22. Li J, Li L, Zuo H, Ke C, Yan B, Wen H, Zhang Y, Yang X. T-helper type-2 contact hypersensitivity of Balb/c mice aggravated by dibutyl phthalate via long-term dermal exposure. *PloS one* 2014;9(2):e87887.
23. Hegazy HG, Ali EH, Elgoly AHM. Interplay between pro-inflammatory cytokines and brain oxidative stress biomarkers: evidence of parallels between butyl paraben intoxication and the valproic acid brain physiopathology in autism rat model. *Cytokine* 2015;71(2):173-180.
24. Anderson SE, Franko J, Kashon ML, Anderson KL, Hubbs AF, Lukomska E, Meade BJ. Exposure to triclosan augments the allergic response to ovalbumin in a mouse model of asthma. *toxicological sciences* 2012:kfs328.
25. Marshall NB, Lukomska E, Long CM, Kashon ML, Sharpnack DD, Nayak AP, Anderson KL, Meade BJ, Anderson SE. Triclosan induces thymic stromal lymphopoietin in skin promoting Th2 allergic responses. *Toxicological Sciences* 2015;147(1):127-139.
26. Kwon J-T, Yang Y-S, Kang M-S, Seo G-B, Lee DH, Yang M-J, Shim I, Kim H-M, Kim P, Choi K. Pulmonary toxicity screening of triclosan in rats after intratracheal instillation. *The Journal of toxicological sciences* 2013;38(3):471-475.
27. Matsuda T, Kurohane K, Imai Y. Di-(2-ethylhexyl) phthalate enhances skin sensitization to isocyanate haptens in mice. *Toxicology letters* 2010;192(2):97-100.
28. Chen S, You H, Mao L, Yang X. Dibutyl phthalate induced oxidative stress does not lead to a significant adjuvant effect on a mouse asthma model. *Toxicology Research* 2015;4(2):260-269.
29. Ashley-Martin J, Dodds L, Levy AR, Platt RW, Marshall JS, Arbuckle TE. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. *Environmental research* 2015;140:360-368.
30. Ferguson KK, Cantonwine DE, Rivera-González LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, Jiménez-Vélez B, Calafat AM, Ye X, Alshawabkeh AN. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. *Environmental science & technology* 2014;48(12):7018-7025.
31. Watkins DJ, Ferguson KK, Del Toro LVA, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. *International journal of hygiene and environmental health* 2015;218(2):212-219.

32. Ashley-Martin J, Dodds L, Arbuckle TE, Marshall J. Prenatal triclosan exposure and cord blood immune system biomarkers. *International journal of hygiene and environmental health* 2016;219(4):454-457.
33. Maruyama T, Shiba T, Iizuka H, Matsuda T, Kurohane K, Imai Y. Effects of Phthalate Esters on Dendritic Cell Subsets and Interleukin-4 Production in Fluorescein Isothiocyanate-Induced Contact Hypersensitivity. *Microbiology and immunology* 2007;51(3):321-326.
34. Herberth G, Pierzchalski A, Feltens R, Bauer M, Röder S, Olek S, Hinz D, Borte M, von Bergen M, Lehmann I. Prenatal phthalate exposure associates with low regulatory T-cell numbers and atopic dermatitis in early childhood: Results from the LINA mother-child study. *Journal of Allergy and Clinical Immunology* 2016.
35. Kato T, Tada-Oikawa S, Takahashi K, Saito K, Wang L, Nishio A, Hakamada-Taguchi R, Kawanishi S, Kuribayashi K. Endocrine disruptors that deplete glutathione levels in APC promote Th2 polarization in mice leading to the exacerbation of airway inflammation. *European journal of immunology* 2006;36(5):1199-1209.
36. Jannesson L, Birkhed D, Scherl D, Gaffar A, Renvert S. Effect of oxybenzone on PGE2-production in vitro and on plaque and gingivitis in vivo. *Journal of clinical periodontology* 2004;31(2):91-94.
37. Ahn KC, Zhao B, Chen J, Cherednichenko G, Sanmarti E, Denison MS, Lasley B, Pessah IN, Kultz D, Chang D. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: receptor-based bioassay screens. *Environ Health Perspect* 2008;116(9):1203-1210.
38. Bracci L, Vukcevic M, Spagnoli G, Ducreux S, Zorzato F, Treves S. Ca2+ signaling through ryanodine receptor 1 enhances maturation and activation of human dendritic cells. *Journal of cell science* 2007;120(13):2232-2240.
39. Vukcevic M, Spagnoli GC, Iezzi G, Zorzato F, Treves S. Ryanodine receptor activation by Cav1. 2 is involved in dendritic cell major histocompatibility complex class II surface expression. *Journal of biological chemistry* 2008;283(50):34913-34922.
40. Haddad JJ, Fahlman CS. Redox-and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? *Biochemical and biophysical research communications* 2002;297(2):163-176.
41. Kim MY, Song KS, Park GH, Chang SH, Kim HW, Park JH, Jin H, Eu KJ, Cho HS, Kang G. B6C3F1 mice exposed to ozone with 4-(N-methyl-Nnitrosamino)-1-(3-pyridyl)-1-butanone and/or dibutyl phthalate showed toxicities through alterations of NF-κB, AP-1, Nrf2, and osteopontin. *Journal of veterinary science* 2004;5(2):131-137.
42. Monaco C, Andreakos E, Kiriakidis S, Mauri C, Bicknell C, Foxwell B, Cheshire N, Paleolog E, Feldmann M. Canonical pathway of nuclear factor κB activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101(15):5634-5639.
43. Nagata Y, Kamijuku H, Taniguchi M, Ziegler S, Seino K-i. Differential role of thymic stromal lymphopoietin in the induction of airway hyperreactivity and Th2 immune response in antigen-induced asthma with respect to natural killer T cell function. *International archives of allergy and immunology* 2007;144(4):305-314.
44. Liu Y-J, Soumelis V, Watanabe N, Ito T, Wang Y-H, de Waal Malefyt R, Omori M, Zhou B, Ziegler SF. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. *Annu. Rev. Immunol.* 2007;25:193-219.
45. Soomro MH, Baiz N, Philippat C, Vernet C, Siroux V, Maesano CN, Sanyal S, Slama R, Bornehag C-G, Annesi-Maesano I. Prenatal Exposure to Phthalates and the Development

of Eczema Phenotypes in Male Children: Results from the EDEN Mother–Child Cohort Study. *Environmental health perspectives* 2018;126(2):027002.
46. Wang I-J, Lin C-C, Lin Y-J, Hsieh W-S, Chen P-C. Early life phthalate exposure and atopic disorders in children: a prospective birth cohort study. *Environment international* 2014;62:48-54.
47. Stelmach I, Majak P, Jerzynska J, Podlecka D, Stelmach W, Polańska K, Ligocka D, Hanke W. The effect of prenatal exposure to phthalates on food allergy and early eczema in inner-city children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2015;e72-e78.
48. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environment international* 2018;115:79-88.
49. Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, Martínez D. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. *Journal of Allergy and Clinical Immunology* 2015;135(2):370-378. e7.
50. Lee-Sarwar K, Hauser R, Calafat AM, Ye X, O'Connor GT, Sandel M, Bacharier LB, Zeiger RS, Laranjo N, Gold DR. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. *Journal of Allergy and Clinical Immunology* 2017.
51. Spanier AJ, Fausnight T, Camacho TF, Braun JM. The associations of triclosan and paraben exposure with allergen sensitization and wheeze in children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2014;475-481.
52. Clayton EM, 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(3):390-6.
53. Bertelsen RJ, Longnecker MP, Løvik M, Calafat AM, Carlsen KH, London SJ, Lødrup Carlsen K. Triclosan exposure and allergic sensitization in Norwegian children. *Allergy* 2013;68(1):84-91.
54. Hong S, Kwon H-J, Choi W-J, Lim WR, Kim J, Kim K. Association between exposure to antimicrobial household products and allergic symptoms. *Environmental health and toxicology* 2014;29.
55. Shiue I. IgE antibodies and urinary trimethylarsine oxide accounted for 1–7% population attributable risks for eczema in adults: USA NHANES 2005–2006. *Environmental Science and Pollution Research* 2015;22(23):18404-18409.
56. Overgaard LE, Main KM, Frederiksen H, Stender S, Szecsi PB, Williams HC, Thyssen JP. Children with atopic dermatitis and frequent emollient use have increased urinary levels of low-molecular-weight phthalate metabolites and parabens. *Allergy* 2017;72(11):1768-1777.
57. Kuo P-L, Hsu Y-L, Huang M-S, Tsai M-J, Ko Y-C. Ginger suppresses phthalate ester-induced airway remodeling. *Journal of agricultural and food chemistry* 2011;59(7):3429-3438.
58. Glue C, Platzer MH, Larsen ST, Nielsen GD, Skov PS, Poulsen LK. Phthalates potentiate the response of allergic effector cells. *Basic & clinical pharmacology & toxicology* 2005;96(2):140-142.
59. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, Divjan A, Rosa MJ, Zhang H, Perera FP. Children's urinary phthalate metabolites and fractional exhaled nitric

oxide in an urban cohort. *American journal of respiratory and critical care medicine* 2012;186(9):830-837.
60. Hurst CH, Waxman DJ. Activation of PPARα and PPARγ by environmental phthalate monoesters. *Toxicological Sciences* 2003;74(2):297-308.
61. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret M-C, Mechighel P, Megret J, Leseche G, Aubier M, Pretolani M. Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *American journal of respiratory and critical care medicine* 2001;164(8):1487-1494.
62. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol Appl Pharmacol* 1998;153(1):12-9.
63. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. *Environ Health Perspect* 1997;105(8):802-11.
64. Bonds RS, Midoro-Horiuti T. Estrogen effects in allergy and asthma. *Current opinion in allergy and clinical immunology* 2013;13(1):92.
65. Ferguson KK, Loch-Caruso R, Meeker JD. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999–2006. *Environmental science & technology* 2011;46(1):477-485.
66. Holland N, Huen K, Tran V, Street K, Nguyen B, Bradman A, Eskenazi B. Urinary phthalate metabolites and biomarkers of oxidative stress in a Mexican-American cohort: Variability in early and late pregnancy. . *Toxics* 2016;4(1):7.
67. Lourenço A, Galbiati V, Corti D, Papale A, Martino-Andrade A, Corsini E. The plasticizer dibutyl phthalate (DBP) potentiates chemical allergen-induced THP-1 activation. *Toxicology in Vitro* 2015;29(8):2001-2008.
68. Xu H, Shao X, Zhang Z, Zou Y, Wu X, Yang L. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. *Ecotoxicology and environmental safety* 2013;93:39-44.
69. Gao L, Yuan T, Zhou C, Cheng P, Bai Q, Ao J, Wang W, Zhang H. Effects of four commonly used UV filters on the growth, cell viability and oxidative stress responses of the Tetrahymena thermophila. *Chemosphere* 2013;93(10):2507-2513.
70. Bukowska B. Effects of 2, 4-D and its metabolite 2, 4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 2003;135(4):435-441.
71. Cho YS, Moon H-B. The role of oxidative stress in the pathogenesis of asthma. *Allergy, asthma & immunology research* 2010;2(3):183-187.
72. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, Hoepner LA, Perera FP, Miller RL. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. 2014.
73. Ku HY, Su PH, Wen HJ, Sun HL, Wang CJ, Chen HY, Jaakkola JJ, Wang S-L, Group T. Prenatal and postnatal exposure to phthalate esters and asthma: a 9-year follow-up study of a taiwanese birth cohort. *PloS one* 2015;10(4):e0123309.
74. Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat A, Ye X, Annesi-Maesano I, Siroux V. In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study. *Environmental Health Perspectives* 2017;125(9):097006.

75. Savage JH, Johns CB, Hauser R, Litonjua AA. Urinary triclosan levels and recent asthma exacerbations. *Annals of Allergy, Asthma & Immunology* 2014;112(2):179-181. e2.
76. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *Journal of Allergy and Clinical Immunology* 2012;130(2):453-460. e7.
77. Spanier AJ, Fiorino EK, Trasande L. Bisphenol A exposure is associated with decreased lung function. *The Journal of pediatrics* 2014;164(6):1403-1408. e1.
78. Jerschow E, Parikh P, McGinn AP, De Vos G, Jariwala S, Hudes G, Rosenstreich D. Relationship between urine dichlorophenol levels and asthma morbidity. *Annals of Allergy, Asthma & Immunology* 2014;112(6):511-518. e1.
79. Mose T, Mortensen GK, Hedegaard M, Knudsen LE. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. *Reproductive Toxicology* 2007;23(1):83-91.
80. Valle-Sistac J, Molins-Delgado D, Díaz M, Ibáñez L, Barceló D, Díaz-Cruz MS. Determination of parabens and benzophenone-type UV filters in human placenta. First description of the existence of benzyl paraben and benzophenone-4. *Environment international* 2016;88:243-249.
81. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8(3):483-91.
82. Duramad P, McMahon CW, Hubbard A, Eskenazi B, Holland NT. Flow cytometric detection of intracellular TH1/TH2 cytokines using whole blood: validation of immunologic biomarker for use in epidemiologic studies. *Cancer Epidemiol Biomarkers Prev* 2004;13(9):1452-8.
83. Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL. Normalization of urinary drug concentrations with specific gravity and creatinine. *Journal of Analytical Toxicology* 2009;33(1):1-7.
84. Silva MJ SE, Preau JL Jr, Reidy JA, Needham LL, Calafat AM. Quantification of 22 phthalate metabolites in human urine. *Journal of Chromatography* 2007(860):106–112.
85. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 2005;77(16):5407-13.
86. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 2004;112(17):1691-6.
87. Madigan D, Raftery AE, Volinsky C, Hoeting J. Bayesian model averaging. Proceedings of the AAAI Workshop on Integrating Multiple Learned Models, Portland, OR, 1996;77-83.
88. Raanan R, Balmes JR, Harley KG, Gunier RB, Magzamen S, Bradman A, Eskenazi B. Decreased lung function in 7-year-old children with early-life organophosphate exposure. *Thorax* 2016;71(2):148-53.
89. Raanan R, Gunier RB, Balmes JR, Beltran AJ, Harley KG, Bradman A, Eskenazi B. Elemental Sulfur Use and Associations with Pediatric Lung Function and Respiratory Symptoms in an Agricultural Community (California, USA). *Environ Health Perspect* 2017;125(8):087007.
90. Centers for Disease Control and Prevention. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, February 2015. Atlanta, GA: U.S. Department

of Health and Human Services, Centers for Disease Control and Prevention. http://www.cdc.gov/exposurereport/
91. Smart JM, Kemp AS. Ontogeny of T-helper 1 and T-helper 2 cytokine production in childhood. *Pediatric allergy and immunology* 2001;12(4):181-187.
92. Duijts L. Fetal and infant origins of asthma. *European journal of epidemiology* 2012;27(1):5-14.
93. Pinkerton KE, Joad JP. The mammalian respiratory system and critical windows of exposure for children's health. *Environmental health perspectives* 2000;108(Suppl 3):457.
94. Warner J. The early life origins of asthma and related allergic disorders. *Archives of disease in childhood* 2004;89(2):97-102.
95. Noutsios GT, Floros J. Childhood asthma: causes, risks, and protective factors; a role of innate immunity. *Swiss Med Wkly* 2014;144:w14036.
96. Romanet-Manent S, Charpin D, Magnan A, Lanteaume A, Vervloet D. Allergic vs nonallergic asthma: what makes the difference? *Allergy* 2002;57(7):607-613.

Table 1. Demographic characteristics of the study population, CHAMACOS Study, Salinas, CA

Characteristics at time of pregnancy	N	(%)
Age		
18-24	174	(44.7)
25-29	123	(31.6)
30-34	62	(15.9)
35+	30	(7.7)
Poverty		
<100%	240	(61.2)
100-200%	138	(35.2)
>200%	14	(3.6)
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)

Table 2. Distribution of specific gravity corrected phthalate monoester metabolites in maternal prenatal urine collected at two time points in pregnancy

	% > LOD		Average of 2 Measurements						
Phthalate	Early Pregnancy[1]	Late Pregnancy[2]	Geo. Mean	10th%	25th%	50th%	75th%	90th%	95th%
MEP (ng/mL)	100.0%	99.7%	233.3	46.5	108.5	223.6	490.2	934.7	1574.2
MBP (ng/mL)	99.4%	100.0%	27.8	9.5	15.9	26.4	47.5	91.3	119.5
MiBP (ng/mL)	95.2%	97.5%	3.4	1.1	1.9	3.3	6.1	10.9	16.3
MP (ug/mL)	100.0%	100.0%	152.8	29.5	75.5	168.9	365.0	631.3	767.1
PP (ug/mL)	98.5%	98.7%	39.6	2.6	11.6	48.0	166.4	446.5	636.6
BP (ug/mL)	66.5%	71.0%	0.5	0.1	0.1	0.3	1.7	12.6	23.5
TCS (ug/mL)	83.5%	88.1%	22.4	1.6	6.0	20.8	112.1	363.3	593.2
2,4-DCP (ug/mL)	100.0%	100.0%	5.8	1.5	2.3	3.8	14.6	47.8	69.3
2,5-DCP (ug/mL)	99.7%	100.0%	74.5	6.4	16.3	68.4	451.3	859.9	1109.1
BP3 (ug/mL)	99.7%	99.5%	27.1	2.5	5.2	18.3	152.1	564.8	944.4

[1]Mean ± SD = 14.0 ± 5.0 weeks gestation

[2]Mean ± SD = 26.9 ± 2.5 weeks gestation

Abbreviations: LOD = Limit of detection

Table 3. Respiratory symptom and spirometry data in CHAMACOS participants at age seven

Maternal report	N (%)	Spirometry	N	Mean (SD)
Any current symptoms	54 (15%)	Best maneuver (all children)		
Any current asthma medication use	22 (6%)	FEV_1	300	1.8 (0.5) liters
Past diagnosis of asthma	62 (18%)	FVC	270	2.1 (0.6)liters
Positive bronchodilator test[1]	10 (3%)	FEV_1/FVC	270	0.9 (0.1)
Probable Asthma[2]	37 (11%)	FEF_{25-75}	270	2.5 (0.9) liters/second
Aeroallergy symptoms or diagnosis	87 (25%)	Best maneuver (those with 2+ maneuvers)		
Eczema diagnosis	23 (7%)	FEV_1	276	1.8 (0.5) liters
		FVC	234	2.1 (0.5) liters
		FEV_1/FVC	234	0.9 (0.1)
		FEF_{25-75}	234	2.6 (0.9) liters/second

[1] Completed by children with respiratory symptoms (N=42)

[2] Defined as: taking asthma medications or any two of current respiratory symptoms, diagnosis of asthma, positive bronchodilator test

Table 4. Descriptive statistics of T-helper cell counts in CHAMACOS participants at ages 2, 5, and 7

Variable	N	Mean (SD)
Age 2		
Th1:Th2 cell ratio	239	6.13 (5.58)
% CD4 cells	239	32.9 (7.5)
% Th1 cells	239	4.0 (3.0)
% Th2 cells	239	0.9 (0.7)
Age 5		
Th1:Th2 cell ratio	231	14.40 (44.53)
% CD4 cells	231	29.9 (7.3)
% of Th1 cells	231	6.8 (3.2)
% of Th2 cells	231	1.1 (0.8)
Age 7		
Th1:Th2 cell ratio	254	22.51 (36.29)
% CD4 cells	254	33.8 (10.2)
% of Th1 cells	254	10.0 (5.9)
% of Th2 cells	254	0.9 (0.7)

Table 5. Posterior Inclusion Probabilities (PIP) for all phthalate metabolites, parabens, other phenols, and dialkl phosphate metabolites included in Bayesian Model Averaging

Probable asthma		Aeroallergy		Eczema	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MCOP	0.5072	MCPP	0.39754	MCPP	0.15586
PP	0.32714	BP	0.26668	MBP	0.115
2,4-DCP	0.24914	BPA	0.19336	MiBP	0.11436
MCNP	0.23314	MCOP	0.18742	TCS	0.09724
2,5-DCP	0.22578	MCNP	0.12052	MP	0.068
TCS	0.14808	DAPs	0.09588	MCNP	0.0673
MEP	0.10582	MBzP	0.07654	2,5-DCP	0.0661
DEHP	0.08882	BP3	0.06858	BPA	0.0635
BP	0.08844	MEP	0.06592	MEP	0.06096
MCPP	0.08466	2,5-DCP	0.06576	DAPs	0.0555
MP	0.0776	2,4-DCP	0.06462	DEHP	0.0554
MBzP	0.0768	MiBP	0.06158	MBzP	0.05456
MBP	0.06452	PP	0.05946	2,4-DCP	0.05418
DAPs	0.06166	MP	0.0572	PP	0.05334
BPA	0.0593	DEHP	0.05676	MCOP	0.0525
BP3	0.0586	MBP	0.05508	BP	0.05224
MiBP	0.0541	TCS	0.05292	BP3	0.0503

FEV_1		FVC		FEV_1/FVC		$FEF_{25-75\%}$	
Chemical	PIP	Chemical	PIP	Chemical	PIP	Chemical	PIP
MCOP	0.64582	MCOP	0.36054	DAPs	0.1423	MCOP	0.8658
MBP	0.26968	DAPs	0.18786	MBzP	0.11322	MEP	0.508
MEP	0.20392	BP	0.1511	MEP	0.10604	TCS	0.2652
MBzP	0.1683	MCNP	0.13188	MCOP	0.10162	MBP	0.21876
TCS	0.16306	MiBP	0.13144	MBP	0.10084	BP	0.1341
MiBP	0.13012	MEP	0.13	BPA	0.09898	MiBP	0.13254
DEHP	0.1199	2,4-DCP	0.11852	2,4-DCP	0.08358	MBzP	0.11116
MCNP	0.10164	MBP	0.09842	PP	0.06794	DEHP	0.09706
MCPP	0.0999	MBzP	0.08204	BP3	0.06734	MCNP	0.09106
BP	0.09472	2,5-DCP	0.08046	2,5-DCP	0.06686	MP	0.08538
2,5-DCP	0.08226	MCPP	0.07828	MP	0.06438	PP	0.07494
DAPs	0.08134	TCS	0.07486	TCS	0.06436	MCPP	0.07456
2,4-DCP	0.07718	BP3	0.07228	MCNP	0.06404	BPA	0.07404
BP3	0.06144	BPA	0.0642	BP	0.0637	BP3	0.07236
PP	0.06064	MP	0.06392	DEHP	0.05966	2,4-DCP	0.0708
MP	0.05648	PP	0.06016	MiBP	0.05908	2,5-DCP	0.06996
BPA	0.0541	DEHP	0.0551	MCPP	0.0585	DAPs	0.05576

Th1:Th2 cell ratio, age 2		Th1 %, age 2		Th2 %, age 2	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MBP	0.90552	2,5-DCP	0.35408	MBP	0.57386
MEP	0.76292	2,4-DCP	0.2366	MP	0.28364

PP	0.295	PP	0.23254	2,5-DCP	0.2258
MCOP	0.12096	MEP	0.2082	2,4-DCP	0.20322
DEHP	0.12014	DEHP	0.17696	MBzP	0.19724
MCNP	0.0979	MP	0.1515	BP3	0.19178
MiBP	0.0964	MBzP	0.10768	MEP	0.1269
MCPP	0.08616	MBP	0.10274	MiBP	0.09624
TCS	0.08614	BP3	0.09516	BP	0.0961
MP	0.07832	MCOP	0.09176	PP	0.07786
DAPs	0.07684	DAPs	0.08812	DEHP	0.07492
BP	0.07598	MiBP	0.08154	MCPP	0.07436
BPA	0.0699	BPA	0.07886	DAPs	0.07218
2,5-DCP	0.06924	MCNP	0.07386	MCNP	0.07156
2,4-DCP	0.05948	MCPP	0.06766	TCS	0.07118
BP3	0.0568	BP	0.0645	MCOP	0.07074
MBzP	0.05638	TCS	0.06166	BPA	0.05964

Th1:Th2 cell ratio, age 5

Chemical	PIP
MiBP	0.22998
TCS	0.1997
MBP	0.1435
BP	0.11892
2,5-DCP	0.10386
DAPs	0.09814
BPA	0.09238
2,4-DCP	0.09148
MBzP	0.08868
MCNP	0.0881
DEHP	0.0876
MCPP	0.08292
MCOP	0.07552
MEP	0.07538
MP	0.07362
PP	0.07124
BP3	0.06704

Th1 %, age 5

Chemical	PIP
MBP	0.35058
MCNP	0.2633
MCPP	0.13324
MEP	0.12886
MCOP	0.09936
MiBP	0.095
DAPs	0.09376
MBzP	0.09354
DEHP	0.09056
2,5-DCP	0.0832
BPA	0.07642
BP	0.07544
PP	0.07416
2,4-DCP	0.07178
MP	0.06852
BP3	0.06714
TCS	0.06448

Th2 %, age 5

Chemical	PIP
MBP	0.6689
MEP	0.15788
TCS	0.15366
DAPs	0.14222
MCPP	0.1057
MiBP	0.10328
MCOP	0.09626
BP	0.09018
MCNP	0.08976
BPA	0.08728
MP	0.0851
MBzP	0.07924
BP3	0.07596
DEHP	0.07492
2,4-DCP	0.07206
2,5-DCP	0.06666
PP	0.0538

Th1:Th2 cell ratio, age 7

Chemical	PIP
BP	0.14764
TCS	0.10314
DEHP	0.09426
MBP	0.083
MBzP	0.08152
MCOP	0.07676
2,5-DCP	0.07562
MP	0.07464

Th1 %, age 7

Chemical	PIP
BP	0.28148
MP	0.2132
MCPP	0.16302
2,4-DCP	0.1368
DAPs	0.13384
2,5-DCP	0.1244
PP	0.12406
MBP	0.0919

Th2 %, age 7

Chemical	PIP
MP	0.24174
DEHP	0.1231
MBzP	0.09164
MEP	0.09132
2,5-DCP	0.09028
MCPP	0.0805
PP	0.07378
BPA	0.073

2,4-DCP	0.07354	MEP	0.09094	TCS	0.07122
MiBP	0.06596	BPA	0.0812	2,4-DCP	0.07094
MCNP	0.0648	MCOP	0.07918	BP	0.06978
DAPs	0.06374	MBzP	0.07452	BP3	0.06936
BPA	0.06356	TCS	0.07236	MCOP	0.06658
MEP	0.06186	DEHP	0.06646	DAPs	0.06396
BP3	0.0618	MCNP	0.06578	MBP	0.0604
MCPP	0.06098	BP3	0.06484	MiBP	0.0595
PP	0.05862	MiBP	0.06354	MCNP	0.05782

Th1:Th2 cell ratio, longitudinal		Th1 %, longitudinal		Th2 %, longitudinal	
Chemical	PIP	Chemical	PIP	Chemical	PIP
MiBP	0.5219	MP	0.25002	MBP	0.50824
MBP	0.1206	2,4-DCP	0.1823	MP	0.449
TCS	0.10798	2,5-DCP	0.1292	MEP	0.16016
MEP	0.09542	PP	0.12382	DAPs	0.11294
MBzP	0.06374	MiBP	0.08824	TCS	0.1035
2,4-DCP	0.0618	BP3	0.07436	MiBP	0.08742
PP	0.05982	DAPs	0.07312	DEHP	0.0852
BP3	0.05108	DEHP	0.06332	2,5-DCP	0.08326
MP	0.04842	MBzP	0.05634	PP	0.05696
BP	0.04678	BP	0.0536	2,4-DCP	0.04638
2,5-DCP	0.04554	MCOP	0.0469	BP	0.04586
MCPP	0.04446	MBP	0.0453	MCOP	0.0443
MCNP	0.04104	MCPP	0.045	MCPP	0.04402
BPA	0.0391	BPA	0.04376	BPA	0.04138
MCOP	0.03894	MCNP	0.04154	MBzP	0.04024
DAPs	0.03706	MEP	0.03724	BP3	0.0377
DEHP	0.03602	TCS	0.03462	MCNP	0.03138

Table 6. Association of log 2 prenatal chemical concentrations and asthma outcomes

	Probable asthma			Aeroallergies			Eczema		
Chemical	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[2] OR (95% CI)	Crude OR (95% CI)	Demographically adjusted[1] OR (95% CI)	Fully adjusted[2] OR (95% CI)
	n=336-346	n=333-343	n=327	n=327-334	n=324-331	n=319-321	n=326-331	n=323-330	n=318-323
MEP	0.89 (0.73, 1.10)	0.86 (0.69, 1.07)	0.81 (0.64, 1.03)#	1.10 (0.95, 1.27)	1.09 (0.94, 1.27)	1.02 (0.87, 1.19)	1.01 (0.78, 1.31)	1.00 (0.77, 1.30)	1.06 (0.81, 1.40)
MBP	1.01 (0.76, 1.33)	1.05 (0.78, 1.40)	0.90 (0.64, 1.28)	1.08 (0.88, 1.32)	1.10 (0.89, 1.35)	0.90 (0.70, 1.15)	0.79 (0.55, 1.13)	0.80 (0.55, 1.15)	0.95 (0.60, 1.48)
MiBP	1.06 (0.83, 1.35)	1.08 (0.85, 1.38)	0.99 (0.76, 1.28)	1.03 (0.87, 1.23)	1.04 (0.87, 1.24)	0.91 (0.75, 1.11)	0.81 (0.60, 1.10)	0.81 (0.60, 1.10)	0.88 (0.61, 1.27)
MP	0.94 (0.77, 1.15)	0.95 (0.77, 1.17)	0.88 (0.70, 1.11)	1.06 (0.91, 1.23)	1.07 (0.92, 1.24)	0.99 (0.84, 1.17)	1.04 (0.80, 1.35)	1.04 (0.80, 1.35)	1.11 (0.83, 1.47)
PP	0.89 (0.79, 1.01)#	0.89 (0.78, 1.01)#	0.84 (0.73, 0.97)*	1.02 (0.93, 1.11)	1.02 (0.93, 1.12)	0.97 (0.88, 1.07)	1.01 (0.86, 1.18)	1.01 (0.86, 1.18)	1.00 (0.85, 1.18)
BP	0.91 (0.79, 1.05)	0.91 (0.79, 1.06)	0.91 (0.78, 1.07)	1.08 (0.99, 1.18)#	1.09 (0.99, 1.19)#	1.09 (0.99, 1.19)#	0.98 (0.83, 1.15)	0.98 (0.83, 1.15)	1.00 (0.84, 1.18)
TCS	1.14 (1.01, 1.29)*	1.11 (0.98, 1.27)#	1.04 (0.91, 1.19)	1.00 (0.92, 1.08)	0.99 (0.91, 1.08)	0.99 (0.90, 1.08)	1.10 (0.95, 1.28)	1.09 (0.94, 1.27)	1.11 (0.95, 1.30)
2,4-DCP	1.10 (0.92, 1.32)	1.12 (0.93, 1.36)	1.94 (1.24, 3.05)**	1.01 (0.88, 1.15)	1.02 (0.89, 1.17)	0.97 (0.84, 1.12)	0.98 (0.77, 1.25)	0.99 (0.78, 1.25)	1.04 (0.81, 1.32)
2,5-DCP	0.98 (0.86, 1.12)	1.00 (0.87, 1.14)	0.64 (0.47, 0.89)**	0.99 (0.90, 1.09)	1.00 (0.91, 1.10)	0.96 (0.87, 1.07)	0.94 (0.80, 1.11)	0.95 (0.81, 1.12)	0.98 (0.83, 1.16)
BP3	1.02 (0.91, 1.15)	0.99 (0.88, 1.12)	0.97 (0.85, 1.11)	1.04 (0.95, 1.13)	1.05 (0.96, 1.14)	1.02 (0.93, 1.12)	1.02 (0.88, 1.18)	1.01 (0.87, 1.17)	1.03 (0.89, 1.20)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, 2,4-dichlorophenol, 2,5-dichlorophenol

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monocarboxyisononyl phthalate, monobenzyl phthalate

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, 2,4-dichlorophenol, 2,5-diclorophenol, mono(3-carboxypropyl) phthalate

Table 7. Association of log 2 prenatal chemical concentrations and spirometry outcomes

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=294-296	n=287-293	n=283-287	n=260-266	n=257-263	n=253-255
MEP	-0.03 (-0.06, 0.00)#	-0.03 (-0.06, 0.00)#	-0.03 (-0.07, 0.00)#	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)
MBP	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.06)	0.06 (0.01, 0.11)*	0.01 (-0.04, 0.07)	0.02 (-0.04, 0.07)	0.04 (-0.02, 0.10)
MiBP	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.06)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.07)	0.03 (-0.02, 0.08)
MP	0.00 (-0.03, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.02, 0.05)	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)	0.00 (-0.04, 0.04)
PP	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.00 (-0.03, 0.02)
BP	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.02 (-0.01, 0.04)
TCS	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.02 (0.00, 0.04)#	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.04)
2,4-DCP	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.02)	-0.03 (-0.07, 0.00)#	-0.03 (-0.07, 0.01)	-0.02 (-0.06, 0.02)
2,5-DCP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	-0.01 (-0.04, 0.01)	-0.01 (-0.04, 0.02)	-0.01 (-0.03, 0.02)
BP3	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.01)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.03)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=260-266	n=257-263	n=253-255	n=260-266	n=257-263	n=253-255
MEP	-0.29 (-0.78, 0.21)	-0.29 (-0.79, 0.21)	-0.26 (-0.77, 0.25)	-3.68 (-6.32, -0.96)**	-3.48 (-6.16, -0.72)*	-3.23 (-5.87, -0.52)*
MBP	0.23 (-0.44, 0.90)	0.28 (-0.40, 0.97)	0.35 (-0.43, 1.14)	0.02 (-3.74, 3.92)	0.22 (-3.61, 4.20)	4.29 (0.04, 8.71)*
MiBP	0.16 (-0.42, 0.75)	0.19 (-0.39, 0.79)	0.33 (-0.30, 0.97)	0.68 (-2.62, 4.08)	0.76 (-2.56, 4.20)	2.22 (-1.13, 5.68)
MP	-0.09 (-0.57, 0.40)	-0.09 (-0.57, 0.40)	-0.08 (-0.57, 0.42)	-0.13 (-2.83, 2.64)	-0.37 (-3.08, 2.41)	1.92 (-1.15, 5.09)
PP	-0.06 (-0.36, 0.24)	-0.06 (-0.36, 0.25)	-0.01 (-0.33, 0.31)	-0.20 (-1.89, 1.52)	-0.28 (-1.99, 1.46)	0.86 (-1.02, 2.79)
BP	-0.07 (-0.37, 0.22)	-0.05 (-0.35, 0.25)	-0.12 (-0.43, 0.18)	0.62 (-1.05, 2.31)	0.59 (-1.10, 2.31)	1.19 (-0.52, 2.94)
TCS	0.04 (-0.25, 0.33)	0.07 (-0.23, 0.37)	0.10 (-0.20, 0.40)	0.99 (-0.65, 2.66)	1.29 (-0.40, 3.00)	1.76 (0.07, 3.47)*
2,4-DCP	0.15 (-0.30, 0.61)	0.16 (-0.29, 0.62)	0.01 (-0.46, 0.48)	-0.93 (-3.44, 1.64)	-0.70 (-3.24, 1.90)	-0.66 (-3.20, 1.94)
2,5-DCP	0.00 (-0.32, 0.31)	0.00 (-0.32, 0.32)	-0.09 (-0.41, 0.23)	-0.61 (-2.35, 1.17)	-0.55 (-2.31, 1.25)	-0.40 (-2.16, 1.39)
BP3	-0.03 (-0.31, 0.25)	0.00 (-0.29, 0.28)	-0.01 (-0.30, 0.28)	-0.49 (-2.05, 1.09)	-0.44 (-2.02, 1.17)	0.41 (-1.22, 2.06)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, sum of dialkyl phosphate metabolites, butyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, sum of dialkyl phosphate metabolites, monobenzyl phthalate, bisphenol A

[5]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monoethyl phthalate, triclosan

[6]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, triclosan, monoethyl phthalate

[7]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, 2,4-dichlorophenol, mono-isobutyl phthalate
[8]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, monoethyl phthalate
[9]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, monocarboxyisooctyl phthalate, triclosan

Table 8. Sensitivity analyses for associations of log2 prenatal chemical concentrations and spirometry outcomes

For children with two or more verified maneuvers

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=267-272	n=264-269	n=262	n=227-231	n=224-228	n=223
MEP	-0.03 (-0.06, 0.00)#	-0.03 (-0.06, 0.00)#	-0.04 (-0.08, -0.01)*	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)	-0.03 (-0.08, 0.01)
MBP	0.02 (-0.03, 0.06)	0.02 (-0.03, 0.06)	0.06 (0.00, 0.11)#	0.01 (-0.04, 0.07)	0.02 (-0.04, 0.07)	0.04 (-0.03, 0.11)
MiBP	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.02 (-0.03, 0.06)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.07)	0.05 (0.00, 0.10)#
MP	0.00 (-0.03, 0.03)	0.00 (-0.04, 0.03)	0.01 (-0.03, 0.05)	0.01 (-0.03, 0.05)	0.00 (-0.04, 0.04)	0.04 (-0.01, 0.08)
PP	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.04)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.03 (0.00, 0.06)#
BP	0.01 (-0.01, 0.03)	0.01 (-0.02, 0.03)	0.00 (-0.02, 0.02)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)	0.01 (-0.01, 0.04)
TCS	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.02 (0.00, 0.04)*	0.01 (-0.02, 0.03)	0.01 (-0.02, 0.03)	0.02 (-0.01, 0.04)
2,4-DCP	-0.02 (-0.05, 0.01)	-0.01 (-0.04, 0.02)	-0.02 (-0.05, 0.01)	-0.03 (-0.07, 0.00)#	-0.03 (-0.07, 0.01)	-0.03 (-0.07, 0.01)
2,5-DCP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	-0.01 (-0.04, 0.01)	-0.01 (-0.04, 0.02)	0.04 (-0.02, 0.11)
BP3	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.01)	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	0.01 (-0.02, 0.03)
BPA	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	-0.01 (-0.07, 0.05)	-0.03 (-0.09, 0.03)	-0.04 (-0.11, 0.02)	-0.02 (-0.09, 0.05)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=227-231	n=224-228	n=223-224	n=227-231	n=224-228	n=223
MEP	-0.29 (-0.78, 0.21)	-0.29 (-0.79, 0.21)	-0.50 (-1.04, 0.04)	-3.68 (-6.32, -0.96)**	-3.48 (-6.16, -0.72)*	-4.18 (-6.87, -1.40)**
MBP	0.23 (-0.44, 0.90)	0.28 (-0.40, 0.97)	0.27 (-0.55, 1.09)	0.02 (-3.74, 3.92)	0.22 (-3.61, 4.20)	5.27 (1.09, 9.62)*
MiBP	0.16 (-0.42, 0.75)	0.19 (-0.39, 0.79)	0.38 (-0.30, 1.07)	0.68 (-2.62, 4.08)	0.76 (-2.56, 4.20)	3.85 (0.53, 7.27)*
MP	-0.09 (-0.57, 0.40)	-0.09 (-0.57, 0.40)	-0.03 (-0.58, 0.53)	-0.13 (-2.83, 2.64)	-0.37 (-3.08, 2.41)	1.89 (-1.14, 5.02)
PP	-0.06 (-0.36, 0.24)	-0.06 (-0.36, 0.25)	0.09 (-0.27, 0.44)	-0.20 (-1.89, 1.52)	-0.28 (-1.99, 1.46)	1.81 (-0.12, 3.77)#
BP	-0.07 (-0.37, 0.22)	-0.05 (-0.35, 0.25)	-0.11 (-0.43, 0.21)	0.62 (-1.05, 2.31)	0.59 (-1.10, 2.31)	0.85 (-0.87, 2.60)
TCS	0.04 (-0.25, 0.33)	0.07 (-0.23, 0.37)	0.17 (-0.14, 0.49)	0.99 (-0.65, 2.66)	1.29 (-0.40, 3.00)	2.35 (0.62, 4.10)**
2,4-DCP	0.15 (-0.30, 0.61)	0.16 (-0.29, 0.62)	0.17 (-0.31, 0.65)	-0.93 (-3.44, 1.64)	-0.70 (-3.24, 1.90)	-1.13 (-3.69, 1.49)
2,5-DCP	0.00 (-0.32, 0.31)	0.00 (-0.32, 0.32)	-0.01 (-0.34, 0.32)	-0.61 (-2.35, 1.17)	-0.55 (-2.31, 1.25)	-0.59 (-2.34, 1.20)
BP3	-0.03 (-0.31, 0.25)	0.00 (-0.29, 0.28)	-0.10 (-0.41, 0.21)	-0.49 (-2.05, 1.09)	-0.44 (-2.02, 1.17)	0.30 (-1.35, 1.97)
BPA	0.45 (-0.32, 1.23)	0.46 (-0.34, 1.26)	0.34 (-0.52, 1.21)	-0.38 (-4.65, 4.08)	-0.95 (-5.32, 3.62)	1.26 (-3.40, 6.14)

For children who have not taken asthma medication in the last year

	FEV_1			FVC		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=274-290	n=271-277	n=269-271	n=248-254	n=245-251	n=243
MEP	-0.04 (-0.07, 0.00)*	-0.04 (-0.07, 0.00)*	-0.04 (-0.07, 0.00)*	-0.03 (-0.07, 0.01)#	-0.03 (-0.07, 0.01)	-0.04 (-0.08, 0.00)#
MBP	0.01 (-0.03, 0.06)	0.01 (-0.03, 0.06)	0.06 (0.01, 0.11)*	0.01 (-0.05, 0.06)	0.01 (-0.05, 0.06)	0.03 (-0.03, 0.09)
MiBP	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.07)	0.03 (-0.02, 0.08)	0.03 (-0.02, 0.07)	0.04 (-0.01, 0.09)
MP	-0.01 (-0.04, 0.03)	-0.01 (-0.05, 0.02)	0.01 (-0.03, 0.05)	-0.01 (-0.05, 0.04)	-0.01 (-0.05, 0.03)	-0.01 (-0.05, 0.03)
PP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.01 (-0.02, 0.03)	0.00 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	-0.01 (-0.04, 0.02)
BP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.02)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.04)	0.01 (-0.02, 0.03)	0.02 (-0.01, 0.04)
TCS	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.04)	0.02 (0.00, 0.04)*	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.04)	0.02 (-0.01, 0.04)
2,4-DCP	-0.01 (-0.04, 0.02)	-0.01 (-0.04, 0.03)	0.00 (-0.04, 0.03)	-0.02 (-0.06, 0.01)	-0.02 (-0.06, 0.02)	-0.01 (-0.05, 0.03)
2,5-DCP	0.00 (-0.02, 0.02)	0.00 (-0.02, 0.03)	0.00 (-0.02, 0.03)	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	0.00 (-0.03, 0.03)
BP3	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.01)	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	0.00 (-0.03, 0.02)
BPA	-0.02 (-0.08, 0.03)	-0.03 (-0.08, 0.03)	0.00 (-0.06, 0.06)	-0.04 (-0.11, 0.02)	-0.05 (-0.12, 0.01)	-0.03 (-0.10, 0.04)

	FEV_1/FVC			$FEF_{25-75\%}$		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=248-254	n=245-251	n=243	n=248-254	n=245-251	n=243
MEP	-0.23 (-0.73, 0.27)	-0.24 (-0.75, 0.27)	-0.22 (-0.74, 0.31)	-3.81 (-6.49, -1.05)**	-3.70 (-6.44, -0.88)*	-3.52 (-6.18, -0.78)*
MBP	0.32 (-0.36, 1.02)	0.40 (-0.30, 1.11)	0.51 (-0.29, 1.32)	-0.06 (-3.90, 3.93)	0.13 (-3.80, 4.23)	4.61 (0.29, 9.11)*
MiBP	0.19 (-0.40, 0.78)	0.21 (-0.39, 0.81)	0.35 (-0.29, 0.99)	1.05 (-2.28, 4.49)	1.06 (-2.31, 4.55)	2.80 (-0.57, 6.29)
MP	-0.11 (-0.61, 0.39)	-0.10 (-0.60, 0.41)	-0.08 (-0.59, 0.43)	-0.75 (-3.50, 2.07)	-0.98 (-3.76, 1.88)	1.31 (-1.86, 4.58)
PP	-0.07 (-0.38, 0.24)	-0.07 (-0.38, 0.25)	-0.01 (-0.34, 0.31)	-0.53 (-2.25, 1.21)	-0.64 (-2.38, 1.14)	0.45 (-1.46, 2.40)
BP	-0.11 (-0.40, 0.19)	-0.08 (-0.38, 0.23)	-0.15 (-0.45, 0.16)	0.42 (-1.27, 2.13)	0.39 (-1.33, 2.14)	1.01 (-0.72, 2.77)
TCS	0.05 (-0.25, 0.34)	0.06 (-0.25, 0.36)	0.09 (-0.22, 0.40)	1.36 (-0.33, 3.08)	1.60 (-0.14, 3.38)#	2.07 (0.34, 3.83)*
2,4-DCP	0.13 (-0.34, 0.61)	0.13 (-0.35, 0.61)	-0.04 (-0.54, 0.46)	-0.46 (-3.11, 2.26)	-0.24 (-2.93, 2.53)	-0.16 (-2.84, 2.60)
2,5-DCP	-0.03 (-0.35, 0.30)	-0.03 (-0.36, 0.30)	-0.13 (-0.46, 0.21)	-0.46 (-2.26, 1.38)	-0.37 (-2.21, 1.51)	-0.20 (-2.02, 1.66)
BP3	-0.07 (-0.36, 0.21)	-0.04 (-0.33, 0.25)	-0.04 (-0.34, 0.25)	-0.99 (-2.57, 0.62)	-0.95 (-2.57, 0.69)	-0.08 (-1.74, 1.60)
BPA	0.41 (-0.39, 1.21)	0.49 (-0.34, 1.32)	0.73 (-0.15, 1.62)	-1.01 (-5.38, 3.57)	-1.41 (-5.93, 3.33)	2.64 (-2.21, 7.73)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[3]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, sum of dialkyl phosphate metabolites, butyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, sum of dialkyl phosphate metabolites, monobenzyl phthalate, bisphenol A

[5]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, monoethyl phthalate, triclosan

[6]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monocarboxyisooctyl phthalate, triclosan, monoethyl phthalate

[7]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, 2,4-dichlorophenol, mono-isobutyl phthalate

[8]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, monoethyl phthalate

[9]Models control for maternal age, parity, poverty index at baseline, child's family history of asthma, monoethyl phthalate, monocarboxyisooctyl phthalate, triclosan

Table 9. Association of log 2 prenatal chemical concentrations and TH cell parameters

	Th1:Th2 cell ratio, age 2			Th1 %, age 2			Th2 %, age 2		
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=219-234	n=217-232	n=215	n=219-234	n=217-232	n=215-232	n=219-234	n=217-232	n=215
MEP	7.10 (-0.52, 15.31)#	7.67 (-0.12, 16.07)#	13.60 (5.06, 22.84)**	4.93 (-2.37, 12.78)	4.41 (-2.99, 12.36)	7.11 (-0.81, 15.66)#	-2.03 (-7.95, 4.27)	-3.03 (-8.88, 3.20)	-2.91 (-9.33, 3.96)
MBP	-14.02 (-22.43, -4.70)**	-13.96 (-22.51, -4.46)**	-17.32 (-25.68, -8.03)**	-4.02 (-13.32, 6.29)	-5.45 (-14.73, 4.85)	-4.17 (-13.78, 6.50)	11.64 (2.36, 21.75)*	9.89 (0.75, 19.86)*	13.02 (3.58, 23.33)**
MiBP	-7.55 (-15.02, 0.56)#	-7.55 (-15.18, 0.76)#	-2.01 (-11.17, 8.09)	-3.17 (-10.83, 5.15)	-2.93 (-10.77, 5.59)	-2.03 (-9.98, 6.61)	4.74 (-2.42, 12.43)	4.99 (-2.21, 12.73)	1.26 (-6.70, 9.89)
MP	0.29 (-6.94, 8.09)	-0.02 (-7.36, 7.90)	2.77 (-7.27, 13.90)	-5.69 (-12.36, 1.48)	-5.08 (-11.95, 2.33)	-1.60 (-10.28, 7.92)	-5.97 (-11.70, 0.13)#	-5.06 (-10.87, 1.12)	-6.09 (-11.90, 0.10)#
PP	-2.41 (-6.71, 2.08)	-2.64 (-6.97, 1.89)	-4.70 (-9.24, 0.08)#	-4.21 (-8.34, 0.11)#	-3.90 (-8.12, 0.51)#	-3.30 (-7.56, 1.16)	-1.84 (-5.51, 1.98)	-1.30 (-4.97, 2.52)	2.43 (-2.61, 7.74)
BP	1.14 (-3.13, 5.60)	1.16 (-3.16, 5.67)	2.07 (-2.52, 6.87)	-1.13 (-5.24, 3.16)	-0.86 (-5.06, 3.51)	-0.34 (-4.67, 4.19)	-2.24 (-5.74, 1.38)	-2.00 (-5.49, 1.62)	-1.48 (-5.14, 2.33)
TCS	1.41 (-2.82, 5.83)	0.92 (-3.38, 5.41)	1.49 (-2.99, 6.17)	0.58 (-3.56, 4.90)	0.92 (-3.34, 5.36)	1.07 (-3.24, 5.57)	-0.81 (-4.33, 2.84)	0.00 (-3.56, 3.69)	-1.26 (-4.91, 2.53)
2,4-DCP	1.52 (-4.86, 8.32)	1.64 (-4.85, 8.56)	0.54 (-5.97, 7.51)	7.04 (0.47, 14.04)*	6.75 (0.06, 13.88)*	-1.52 (-15.55, 14.85)	5.44 (-0.16, 11.35)#	5.03 (-0.54, 10.91)#	1.56 (-11.37, 16.36)
2,5-DCP	1.25 (-3.19, 5.88)	1.50 (-3.03, 6.23)	0.84 (-3.78, 5.68)	5.65 (1.15, 10.35)*	5.37 (0.76, 10.18)*	5.93 (-4.80, 17.86)	4.35 (0.51, 8.34)*	3.81 (-0.03, 7.80)#	3.38 (-0.56, 7.47)#
BP3	0.04 (-3.96, 4.20)	0.07 (-3.99, 4.29)	0.96 (-3.30, 5.41)	-2.96 (-6.76, 1.00)	-3.09 (-6.95, 0.94)	-1.88 (-5.89, 2.30)	-2.99 (-6.26, 0.39)#	-3.15 (-6.40, 0.21)#	-2.18 (-5.75, 1.52)

	Th1:Th2 cell ratio, age 5			Th1 %, age 5			Th2 %, age 5		
	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=221-229	n=218-226	n=218	n=221-229	n=218-226	n=218	n=221-229	n=218-226	n=218
MEP	1.26 (-5.92, 8.98)	0.99 (-6.33, 8.88)	2.52 (-5.15, 10.82)	-2.19 (-6.82, 2.66)	-2.33 (-7.08, 2.65)	-3.95 (-8.76, 1.11)	-3.41 (-10.33, 4.05)	-3.29 (-10.40, 4.37)	-6.10 (-13.12, 1.50)
MBP	-7.32 (-16.26, 2.57)	-7.15 (-16.35, 3.06)	-4.22 (-15.38, 8.41)	6.74 (-0.15, 14.11)#	6.64 (-0.46, 14.25)#	6.57 (-1.61, 15.43)	15.17 (4.05, 27.49)**	14.85 (3.42, 27.54)**	18.20 (6.07, 31.73)**
MiBP	-6.83 (-14.23, 1.22)#	-6.95 (-14.49, 1.26)#	-5.28 (-14.33, 4.72)	-0.33 (-5.67, 5.31)	-0.57 (-6.03, 5.20)	-5.22 (-11.42, 1.42)	6.97 (-1.65, 16.35)	6.85 (-1.95, 16.44)	0.88 (-8.80, 11.58)
MP	2.53 (-5.04, 10.71)	2.43 (-5.27, 10.77)	3.16 (-4.78, 11.76)	-0.28 (-5.26, 4.97)	-0.39 (-5.48, 4.98)	-0.51 (-5.69, 4.95)	-2.74 (-10.09, 5.21)	-2.75 (-10.25, 5.37)	-2.09 (-10.12, 6.64)
PP	-0.86 (-5.17, 3.64)	-0.97 (-5.33, 3.60)	-0.18 (-4.73, 4.59)	0.14 (-2.79, 3.15)	0.13 (-2.85, 3.20)	-0.45 (-3.46, 2.66)	1.01 (-3.48, 5.71)	1.11 (-3.46, 5.90)	0.45 (-4.28, 5.41)
BP	2.54 (-1.82, 7.08)	2.58 (-1.87, 7.23)	3.17 (-1.38, 7.93)	0.21 (-2.66, 3.16)	0.20 (-2.75, 3.23)	0.23 (-2.70, 3.26)	-2.27 (-6.52, 2.17)	-2.32 (-6.67, 2.22)	-1.60 (-6.00, 3.00)

TCS	2.87 (-1.24, 7.14)	2.58 (-1.64, 6.99)	3.41 (-0.91, 7.92)	0.24 (-2.46, 3.02)	0.26 (-2.54, 3.13)	0.35 (-2.47, 3.26)	-2.55 (-6.54, 1.61)	-2.27 (-6.40, 2.05)	-3.09 (-7.15, 1.15)
2,4-DCP	1.55 (-4.82, 8.35)	1.55 (-4.95, 8.50)	1.33 (-5.38, 8.51)	-0.02 (-4.26, 4.40)	-0.16 (-4.50, 4.37)	0.30 (-4.13, 4.93)	-1.55 (-7.88, 5.21)	-1.69 (-8.14, 5.23)	-0.85 (-7.46, 6.23)
2,5-DCP	-1.45 (-5.68, 2.96)	-1.27 (-5.60, 3.25)	-1.02 (-5.45, 3.62)	-1.00 (-3.85, 1.94)	-1.07 (-4.00, 1.94)	-0.91 (-3.87, 2.14)	0.47 (-3.95, 5.09)	0.20 (-4.31, 4.92)	0.37 (-4.15, 5.10)
BP3	0.58 (-3.35, 4.68)	0.67 (-3.36, 4.88)	0.26 (-3.94, 4.66)	0.92 (-1.73, 3.64)	0.89 (-1.84, 3.70)	0.43 (-2.35, 3.29)	0.33 (-3.68, 4.52)	0.21 (-3.91, 4.52)	0.05 (-4.14, 4.43)

	Th1:Th2 cell ratio, age 7			Th1 %, age 7			Th2 %, age 7		
	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)
	n=244-250	n=244-250	n=240-242	n=244-250	n=244-250	n=242-250	n=244-250	n=244-250	n=240-242
MEP	2.40 (-4.31, 9.59)	1.93 (-4.79, 9.12)	1.76 (-5.50, 9.57)	-1.61 (-5.81, 2.76)	-1.83 (-6.05, 2.57)	0.10 (-4.69, 5.14)	-3.92 (-10.09, 2.67)	-3.69 (-9.91, 2.96)	-0.53 (-7.73, 7.23)
MBP	4.42 (-4.58, 14.26)	4.13 (-4.90, 14.02)	4.91 (-4.38, 15.11)	2.44 (-3.32, 8.55)	2.53 (-3.29, 8.69)	2.85 (-3.32, 9.41)	-1.89 (-10.20, 7.19)	-1.54 (-9.93, 7.63)	1.12 (-8.57, 11.84)
MiBP	-0.89 (-8.82, 7.73)	-0.16 (-8.19, 8.59)	-2.09 (-11.33, 8.11)	-0.37 (-5.57, 5.10)	-0.31 (-5.56, 5.22)	0.24 (-5.21, 6.00)	0.52 (-7.38, 9.08)	-0.16 (-8.04, 8.40)	2.32 (-6.47, 11.93)
MP	2.51 (-4.21, 9.70)	2.73 (-4.04, 9.98)	3.75 (-3.52, 11.56)	-4.40 (-8.49, -0.14)*	-4.34 (-8.47, -0.03)*	-3.71 (-8.01, 0.79)	-6.74 (-12.74, -0.33)*	-6.89 (-12.91, -0.45)*	-6.59 (-12.73, -0.02)*
PP	0.05 (-3.88, 4.15)	0.11 (-3.85, 4.24)	0.81 (-3.50, 5.32)	-1.43 (-3.95, 1.16)	-1.44 (-3.99, 1.18)	0.55 (-2.83, 4.04)	-1.48 (-5.30, 2.50)	-1.55 (-5.40, 2.45)	1.03 (-4.23, 6.59)
BP	-2.59 (-6.59, 1.59)	-2.31 (-6.38, 1.93)	-2.41 (-6.57, 1.94)	-2.48 (-5.09, 0.21)#	-2.30 (-4.96, 0.43)#	-1.76 (-4.52, 1.08)	0.11 (-3.97, 4.37)	0.01 (-4.12, 4.31)	1.03 (-3.28, 5.54)
TCS	1.34 (-2.60, 5.44)	1.12 (-2.87, 5.27)	1.20 (-2.88, 5.44)	0.41 (-2.14, 3.03)	0.53 (-2.07, 3.20)	0.53 (-2.05, 3.18)	-0.92 (-4.73, 3.04)	-0.58 (-4.46, 3.45)	-0.50 (-4.42, 3.58)
2,4-DCP	1.47 (-4.64, 7.97)	1.66 (-4.49, 8.21)	1.44 (-5.07, 8.40)	2.13 (-1.89, 6.32)	2.20 (-1.86, 6.43)	2.49 (-1.66, 6.81)	0.65 (-5.34, 7.02)	0.53 (-5.49, 6.93)	1.72 (-4.63, 8.49)
2,5-DCP	0.38 (-3.88, 4.84)	0.66 (-3.66, 5.17)	0.50 (-4.02, 5.24)	1.38 (-1.43, 4.27)	1.41 (-1.44, 4.35)	1.54 (-1.37, 4.53)	0.99 (-3.26, 5.43)	0.75 (-3.53, 5.22)	1.69 (-2.80, 6.39)
BP3	-1.25 (-4.99, 2.63)	-1.54 (-5.30, 2.36)	-1.41 (-5.36, 2.70)	-0.81 (-3.26, 1.70)	-1.04 (-3.51, 1.49)	-0.42 (-3.00, 2.22)	0.45 (-3.31, 4.36)	0.51 (-3.28, 4.45)	1.77 (-2.22, 5.91)

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

Separate models created for each chemical

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, monoethyl phthalate, propyl paraben

[3]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, 2,5-dichlorophenol, 2,4-dichlorophenol, propyl paraben

[4]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, methyl paraben, 2,5-dichlorophenol

[5]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-isobutyl phthalate, triclosan, mono-n-butyl phthalate

[6]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, monocarboxyisononyl phthalate, mono(3-carboxypropyl) phthalate

[7]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, mono-n-butyl phthalate, triclosan, monoethyl phthalate
[8]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, butyl paraben, triclosan, mono-n-butyl phthalate
[9]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, butyl paraben, bisphenol A, methyl paraben
[10]Model controls for maternal age, parity, poverty index at baseline, child's family history of asthma, methyl paraben, triclosan, sum of di(2-ethylhexyl) phthalate metabolites

Table 10. Longitudinal association of log 2 prenatal chemical concentrations and Th cell parameters at various ages, with P-values for age interaction

	Th1:Th2 cell ratio				Th1%				Th2%			
Chemical	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int	Crude β (95% CI)	Demographically adjusted[1] β (95% CI)	Fully adjusted[2] β (95% CI)	P-int
	n=341-362	n=338-359	n=333-335		n=341-362	n=338-359	n=335-359		n=341-362	n=338-359	n=333-335	
MEP	3.18 (-1.59, 8.17)	3.25 (-1.49, 8.21)	4.58 (-0.35, 9.75)#	0.39	-0.07 (-3.53, 3.52)	-0.23 (-3.76, 3.43)	1.26 (-2.56, 5.22)	0.16	-3.21 (-7.25, 1.00)	-3.43 (-7.39, 0.70)	-3.27 (-7.75, 1.43)	0.95
MBP	-5.58 (-11.40, 0.63)#	-5.16 (-11.13, 1.21)	-1.96 (-8.98, 5.59)	0.01	0.83 (-4.70, 6.69)	0.71 (-4.98, 6.75)	1.21 (-4.44, 7.20)	0.29	6.54 (0.53, 12.91)*	5.98 (-0.08, 12.40)#	8.40 (1.95, 15.26)*	0.02
MiBP	-6.70 (-11.58, -1.54)*	-6.65 (-11.60, -1.43)*	-6.19 (-11.70, -0.32)*	0.31	-2.82 (-7.01, 1.55)	-3.02 (-7.15, 1.30)	-2.89 (-7.07, 1.47)	0.89	4.14 (-0.94, 9.49)	3.88 (-1.16, 9.17)	1.80 (-3.43, 7.31)	0.50
MP	1.07 (-3.17, 5.50)	0.94 (-3.26, 5.33)	1.43 (-2.64, 5.67)	0.77	-4.13 (-7.32, -0.82)*	-4.11 (-7.26, -0.85)*	-3.74 (-8.06, 0.77)	0.33	-5.17 (-8.96, -1.23)*	-5.04 (-8.82, -1.10)*	-4.36 (-8.59, 0.06)#	0.59
PP	-1.01 (-3.73, 1.79)	-1.16 (-3.89, 1.64)	-0.97 (-3.83, 1.98)	0.47	-1.76 (-3.83, 0.36)	-1.77 (-3.84, 0.35)	-0.38 (-3.21, 2.54)	0.39	-0.76 (-3.14, 1.67)	-0.62 (-3.01, 1.83)	1.75 (-1.59, 5.21)	0.63
BP	0.44 (-2.41, 3.38)	0.46 (-2.44, 3.45)	1.28 (-1.77, 4.42)	0.31	-1.00 (-3.24, 1.29)	-0.94 (-3.20, 1.37)	-0.56 (-2.99, 1.93)	0.43	-1.73 (-4.10, 0.70)	-1.69 (-4.10, 0.77)	-0.77 (-3.26, 1.77)	0.88
TCS	1.51 (-1.24, 4.33)	1.44 (-1.31, 4.26)	2.18 (-0.63, 5.07)	0.78	0.16 (-1.97, 2.33)	0.29 (-1.83, 2.46)	0.14 (-2.01, 2.34)	0.96	-1.45 (-3.88, 1.05)	-1.25 (-3.67, 1.24)	-1.75 (-4.15, 0.72)	0.64
2,4-DCP	1.61 (-2.79, 6.21)	1.86 (-2.56, 6.48)	1.76 (-2.78, 6.52)	0.98	2.98 (-0.29, 6.37)#	2.92 (-0.38, 6.33)#	2.94 (-0.34, 6.33)#	0.31	1.32 (-2.56, 5.36)	1.00 (-2.84, 5.00)	1.29 (-2.59, 5.32)	0.27
2,5-DCP	0.18 (-2.91, 3.37)	0.44 (-2.66, 3.65)	0.64 (-2.56, 3.94)	0.75	1.90 (-0.42, 4.26)	1.86 (-0.46, 4.23)	-0.28 (-6.35, 6.18)	0.11	1.65 (-1.04, 4.42)	1.35 (-1.32, 4.09)	1.33 (-1.40, 4.13)	0.46
BP3	-0.79 (-3.24, 1.71)	-0.86 (-3.34, 1.68)	-1.00 (-3.58, 1.64)	0.84	-1.38 (-3.30, 0.57)	-1.48 (-3.42, 0.50)	-0.80 (-2.80, 1.23)	0.27	-0.58 (-2.88, 1.79)	-0.60 (-2.91, 1.78)	0.02 (-2.50, 2.60)	0.43

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

Separate models created for each chemical

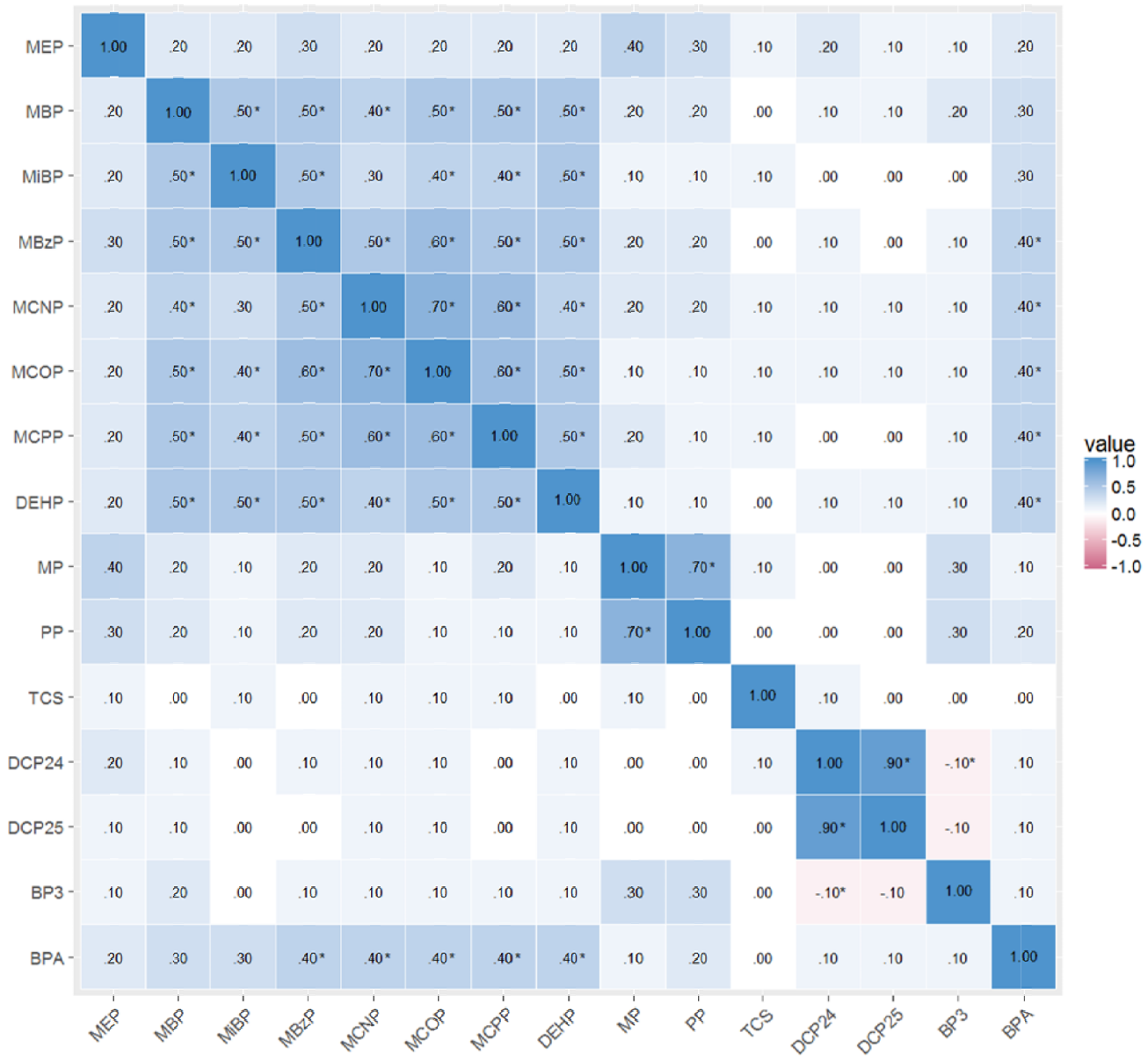
[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

[2]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, mono-isobutyl phthalate, mono-n-butyl phthalate, triclosan

[3]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, methyl paraben, 2,4-dichlorophenol, propyl paraben

[4]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma, mono-n-butyl phthalate, methyl paraben, monoethyl phthalate

Figure 1. Log2 specific gravity corrected phthalate, paraben, and phenol correlations in CHAMACOS



Chapter 5: Using Bayesian Kernel Machine Regression and Bayesian Profile Regression to evaluate relationships between prenatal exposure to mixtures of personal care product and plasticizing chemicals with select measures of childhood immune dysfunction

Introduction

Phthalates, parabens, and other phenols found in personal care products and plastics have demonstrated associations with childhood immune outcomes, including asthma[1-11], aeroallergies[3,12-14], and spirometry[6,15,16]. In chapter three of this dissertation, we found that the phthalates monocarboxyisooctyl phthalate (MCOP) and monocarboxyisononyl phthalate (MCNP) were associated with lower lung performance and increased odds of having probable asthma and aeroallergies, that monobenzyl phthalate (MBzP) was associated with lower lung performance, and that mono(3-carboxypropyl) phthalate (MCPP) and bisphenol A (BPA) were associated with increased odds for having aeroallergies. In chapter four of this dissertation, we found that monoethyl phthalate (MEP) was associated with lower lung performance and mono-n-butyl phthalate (MBP) and triclosan were associated with higher lung performance. The models used in the previous two chapters controlled for concentrations of other, potentially confounding chemicals chosen via Bayesian Model Averaging (BMA). BMA evaluated the performance of regression models with all possible combinations of the 15 personal care product and plasticizing chemicals for each outcome, ranking the chemicals based on how often each was included in high performing models. The top three chemicals from these lists were included in final regression models for each outcome to control for confounding by exposure to other chemicals.

Controlling for confounding by exposure to other chemicals, however, does not examine the effects of joint exposure to those chemicals. Methods are needed that account for joint exposure to multiple chemicals, rather than attempt to control for them as confounders[17]. In this chapter, I examine joint exposure in two ways, the first of which groups individuals into clusters based on their joint chemical exposures, and the second of which computes risk for a health outcome as a nonlinear function of joint exposure to several chemicals.

Bayesian Profile Regression (BPR) clusters participants into groups based on profiles of joint exposure to chemicals[18]. For example, clusters may reflect that some people have high concentrations of all included chemicals, some people have high concentrations of only a few chemicals, and some people have low concentrations of all chemicals. The number of clusters is determined by the model, not the researcher. We can evaluate how risk of a health outcome varies for individuals in different clusters, as one way to account for joint exposure to multiple chemicals.

Bayesian Kernel Machine Regression (BKMR) fits multiple chemical exposures into one model as joint independent variables[19]. It ranks chemicals by their impact on a chosen health outcome and visually represents each chemical's association in the context of joint exposure to all other selected chemicals in the model. The simultaneous testing of chemical associations (as opposed to sequentially testing one chemical at a time) also results in a more realistic estimation of uncertainty.

Joint exposure to several chemicals may result in non-additive risk for a health outcome, rather than the sum of the chemicals' individual risks[19]. Instead, we need to estimate associations while appropriately accounting for joint exposure to other relevant chemical exposures. BPR and BKMR, newly applied to environmental chemicals and children's health, allow us to estimate these

associations, along with uncertainties that account for variance across several steps of the modeling process.

BPR has been used in the current study population to analyze the associations between prenatal exposure to pesticides and childhood neurodevelopment[20]. Few other studies have applied BPR or BKMR methods to the field of environmental health[18,19,21-24]. This is the first study to use these methods to analyze atopic outcomes.

To assess the relationship of prenatal joint exposures to these chemicals with childhood atopy, we measured maternal urinary concentrations of eleven phthalate metabolites, three parabens, and five other phenols at two time points during pregnancy and used BPR and BKMR to analyze associations with probable asthma, aeroallergies, and lung function at age seven.

Methods

CHAMACOS Study. Participants were mothers and their children in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a longitudinal study investigating *in utero* and childhood exposures to environmental chemicals and their associations with a multitude of health outcomes across childhood. Mothers in the Salinas Valley of California, a largely Latino agricultural community, were recruited from 1999-2000 if they were eligible for MediCal, at least 18 years old, less than 20 weeks' pregnant, and planning on delivering at the county hospital. Research protocols were approved by the University of California, Berkeley Committee for the Protection of Human Subjects. Written informed consent was obtained from mothers and verbal assent was obtained from children at age seven.

Outcome definitions. Spirometry methods are described in more detail in chapters three and four. At age seven, children underwent spirometry administered by two technicians specifically trained for the study, using EasyOne dry-seal spirometers. Children performed up to eight expiratory maneuvers, and the spirometric software kept up to three best acceptable tests. All maneuvers were reviewed and verified by two physicians with experience in pediatric spirometry. Forced expiratory volume in one second (FEV_1) was measured from each maneuver. The definition of probable asthma in this population were also described in the previous two chapters of this dissertation. Briefly, when children were seven, mothers were asked if their children had respiratory symptoms via questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[25]. Mothers were also asked if their children took asthma medication or had been diagnosed with asthma ever by a doctor. Children with respiratory symptoms also underwent additional spirometry before and after taking a bronchodilator to determine a percent change in their lung function. A composite of these variables defined whether or not a child had probable asthma. The definition of aeroallergies was also described in chapters three and four of this dissertation. Briefly, children were determined to have aeroallergies based on maternal questionnaires on aeroallergy symptoms when the children were seven.

Exposure assessment. Urine samples were collected from mothers at two interviews during pregnancy (mean ± SD: 14.0 ± 5.0 and 26.9 ± 2.5 weeks gestation). Samples were collected in polypropylene urine cups, aliquoted into glass vials, and stored at -80ºC until shipment to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia for analysis.

Solid phase extraction coupled with isotope dilution high performance liquid chromatography-electrospray ionization-tandem mass spectrometry was used to quantify concentrations of the following eleven phthalate metabolites of eight parent compounds: monoethyl phthalate [MEP, a metabolite of diethyl phthalate (DEP)]; mono-n-butyl phthalate [MBP, a metabolite of dibutyl phthalate (DBP)]; and mono-isobutyl phthalate [MiBP, a metabolite of di-isobutyl phthalate (DiBP)]; monobenzyl phthalate [MBzP, a metabolite of benzylbutyl phthalate (BBzP)]; four metabolites of di(2-ethylhexyl) phthalate (DEHP) [mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECCP)]; mono(carboxyoctyl) phthalate [MCOP; a metabolite of di-isononyl phthalate (DiNP)]; mono(carboxynonyl) phthalate [MCNP; a metabolite of di-isodecyl phthalate (DiDP)]; and mono(3-carboxypropyl) phthalate [MCPP; a metabolite of several high molecular weight phthalates and a minor metabolite of dibutyl phthalate]. Isotope dilution on-line solid-phase extraction coupled to high-performance liquid chromatography-tandem mass spectrometry was used for the determination of nine phenols: methyl paraben (MP), propyl paraben (PP), butyl paraben (BP), triclosan, 2,4-dichlorphenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), and benzophenone-3 (BP-3), and bisphenol A (BPA). We decided to drop BP from the 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 for both phthalates[26] and phenols[27]. 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. Concentrations below the LOD were assigned the machine read values, if available, or an imputed value below the LOD selected randomly from the log-normal distribution using maximum likelihood estimation[28].

Urinary specific gravity was measured using a hand-held refractometer (National Instrument Company Inc., Baltimore, MD). We corrected for urinary dilution using the formula: (analyte concentration * 0.24)/(sample specific gravity – 1)[29]. We imputed urinary specific gravity based on urinary creatinine concentrations for 77 women missing specific gravity measurements.

Statistical analysis. We used the average of the two measurements during pregnancy in all analyses. We conducted BPR in order to group participants into clusters based on their patterns of log2 urinary chemical concentrations. BPR clusters individual observations using model averaging with Markov chain Monte Carlo estimation and is based on Dirichlet Process mixture modeling methods[18]. The number of clusters is allowed to vary across iterations of the model, and the final number of clusters is data-driven. BPR models can be supervised by an outcome variable, which would allow clustering to be influenced by the outcome and would run a random effects regression model of the resulting clusters on that outcome. However, we chose to run our models unsupervised so our clusters were determined independently of any health outcome. This yielded us one set of clusters based on urinary chemical concentrations which we could analyze further for relationships to each of our outcomes. We included maternal age, parity, poverty at baseline, and family history of asthma as covariates in cluster formation. We determined the mean concentrations of each chemical in each cluster and used Analysis of Variance (ANOVA) tests to assess if clusters had significantly different mean chemical concentrations. We then determined the frequency of cases of probable asthma and aeroallergy across clusters, and the mean of FEV_1 across clusters. We conducted chi squared tests and ANOVAs to evaluate if clusters differed significantly on the outcomes. We also conducted logistic regressions for probable asthma and aeroallergies and a linear regression for FEV_1 with cluster assignment as a categorical predictor and with no other variables in the models.

We used BKMR analysis to evaluate the associations of joint exposure to log2 phthalates, parabens, and other phenols with probable asthma, aeroallergies, and FEV_1 at age seven, controlling for maternal age, parity, poverty at baseline, and family history of asthma. BKMR models each health outcome as a flexible kernel function of the chemical exposure variables, adjusted for covariates[19]. It reduces dimensionality by selecting variables into the model only if they show evidence of an association with the outcome, and it penalizes the complexity of the multivariate surface. The functions estimated are nonlinear and nonadditive and they encapsulate many possible underlying functional forms. Uncertainty in the variable selection process is applied to the function estimation. We included all phthalates, parabens, and other phenols in all models, and we used BKMR's hierarchal variable selection option, which runs variable selection in two phases: first, selection takes place at the group level and each of the three groups is evaluated for its importance in the model; next, chemicals are selected within their groups (group one: phthalates, group two: parabens, group three: other phenols) based on their importance for the model. We used this option because a few of our chemicals were highly correlated within these groups. After variable selection, BKMR outputs Posterior Inclusion Probabilities (PIPs), which rank each chemical (regardless of group) by importance in the model and also each group by importance in the model.

BKMR outputs several types of plots to summarize the relationships between each chemical concentration and each outcome while accounting for the presence of all other included chemicals: univariate plots, bivariate plots, and overall risk plots. The univariate plots (see Figures 7, 10, and 13) represent predicted probability of a health outcome as a function of joint exposure to the 15 chemicals, with each subplot focusing on the association of a particular chemical with others held at their medians. The x-axis of each univariate plot represents the z-score of the chemical's concentration and the y-axis of each univariate plot represents the predicted probability of the health outcome. BKMR also outputs bivariate plots (see Figures 8, 11, and 14), which use the function of predicted probability to examine the relationship between each chemical and an outcome as the concentration of a second chemical increases in quantiles, while holding all additional chemicals at their medians. These plots can show additive or multiplicative effects of exposure to mixtures of chemicals. Chemicals listed along the right side of the bivariate figures are represented in rows as the plots of their associations with the health outcome. Chemicals listed at the top of the bivariate figures are represented in columns as five colored lines, each a different quantile of its concentration. Thus, within each bivariate plot, one can see the association between one chemical and the outcome at different concentrations of a second chemical. Lastly, the overall risk plots (see Figures 9, 12, and 16) use the same predicted probability function to compare probability for the outcome when all chemicals are in a particular quantile (for example, 0.75 or 0.95) compared to when all chemicals are at the 0.50 quantile.

For chemicals that demonstrated multiplicative interaction in BKMR bivariate plots, we conducted regressions with interaction terms for the two chemicals, controlling for the same covariates as in BKMR.

Both Bayesian analyses were conducted in R[30] (Vienna, Austria): BPR with the PReMiuM package[31] (version 3.1.4) and BKMR with the bkmr package[32] (version 0.2.0). Details on BPR[31,33,34] and BKMR[19,35] have been published previously. ANOVAs, chi squared tests, and regressions were conducted in Stata 14 (College Station, TX).

Results

There were 601 women enrolled in the study, and 531 were followed through a live birth. Of these, there were 392 children with information on both a prenatal urinary concentration of a phthalate, paraben, or other phenol, and a health outcome. However, since BPR and BKMR only analyze complete cases, including covariate data, 319 children were ultimately included in the analyses. Table 1 shows demographic information of these children and their mothers. Mothers were young (42% were <25 years old) and of low income (62% were below 100% federal poverty). Many mothers already had two or more children (39%). Only 10% of children had a family history of asthma.

Most chemicals were detected in over 90% of samples, as shown in Table 2. Figure 1 shows correlations for all chemicals included in these analyses. The highest correlated chemicals are 2,4-DCP and 2,5-DCP (0.85, P<0.0001). Remaining significantly correlated chemicals range from 0.65 (MCNP and MCOP, P<0.0001) to 0.11 (PP and MCPP, P=0.05). Most moderate and high correlations were within chemical groups, but there were some moderate intergroup correlations as well. As shown in Table 3, 33 children (10%) had probable asthma and 81 (25%) had aeroallergies at age seven. We had FEV_1 data for 282 children at age seven (Mean=1.8 liters (SD=0.5)).

BPR results

The BPR analysis yielded seven clusters, chemical concentrations for which are shown in Figures 2 (phthalates) and 3 (parabens and other phenols). Cluster three, with 68 people, was characterized by high concentrations of most phthalates, parabens, and other phenols and the absence of any low concentrations, relative to other clusters. Cluster five, with 45 people, was characterized by the inverse pattern: low concentrations of most phthalates, parabens, and other phenols with no high chemical concentrations, relative to other clusters. Clusters two (26 people) and seven (52 people) were characterized by high concentrations of personal care product chemicals and low concentrations of plasticizing chemicals, relative to other clusters, and cluster six (31 people) was characterized by the inverse pattern: high concentrations of plasticizing chemicals and low concentrations of personal care product chemicals, relative to other clusters. Cluster four (51 people) was characterized by low concentrations of all chemicals except the dichlorophenols and cluster one (46 people) was characterized by high concentrations of all chemicals except the dichlorophenols, relative to other clusters. ANOVA p-values indicated clusters differed significantly on all chemicals except for triclosan.

Table 5 shows the frequency of probably asthma and aeroallergies by cluster, as well as mean FEV_1 volume. Chi squared tests indicated that clustering was not related to odds of having probable asthma (Figure 4), to FEV_1 volume (Figure 5) or aeroallergies (Figure 6). However, cluster three, characterized by relatively high concentrations of all chemicals, exhibited the lowest average FEV_1 volume (Table 5). Regressing probable asthma, aeroallergies, and FEV_1 on cluster assignment as a categorical predictor yielded no significant associations (Table 6).

BKMR results

The BKMR program determined that all 15 chemicals were to be included in models for each outcome. PIPs from BKMR also indicated that the phthalate group was more influential for probable asthma and FEV_1, but that the other phenols group was most influential for aeroallergies (Table 4).

The univariate BKMR plots for probable asthma largely confirm regression results seen in chapters 3 and 4. MCOP, MCNP and 2,4-DCP appear to have strong positive relationships, while 2,5-DCP and PP appear to have strong negative (protective) relationships (Figure 7). The PIPs for probable asthma indicate that PP, MCOP, and TCS were ranked in order as the most influential chemicals (Table 4). Figure 8 shows that in bivariate plots for probable asthma, the slightly downward slope seen for MEP does not change with differing quantiles of any additional chemical, but the intercept for MEP increases as 2,4-DCP increases in quantiles of concentration. This demonstrates a positive additive effect between the two chemicals. 2,4-DCP appears to have a positive additive effect on associations of all other chemicals for probable asthma, and 2,5-DCP and PP appear to have negative additive effects. MCOP, MCNP, and triclosan also appear to have slight positive additive effects. The plot of overall risks for probable asthma indicates a u-shaped curve, with lower and higher concentrations of all chemicals associated with increased risk (Figure 9).

The univariate BKMR plots for FEV_1 volume show a strong negative (harmful) association with MCOP, additional negative associations with MEP, MBzP, and 2,4-DCP (Figure 10), and a positive (protective) association with MBP. These associations are consistent with regression results in chapters 3 and 4. MCOP, PP, and MP were ranked in order as most influential via PIPs (Table 4). In bivariate plots, MCOP, triclosan, and 2,4-DCP show weak additive effects on the associations of other chemicals (Figure 11). The overall risk plot shows that with higher concentrations of all chemicals, FEV_1 volume is lower, with a slight downward u-shape (Figure 12).

In the univariate plots for aeroallergy, MCPP and BPA show strong positive (harmful) associations, with BPA showing a downward u-shaped curve (Figure 13). These directions are consistent with results from chapters 3 and 4, though the logistic regressions in these chapters were not statistically significant. PIPs indicate that BPA, MP, and MCPP were the most influential chemicals, ranked respectively, for aeroallergies (Table 4). In bivariate plots, MCPP and BPA appear to have positive additive effects on the associations of other chemicals (Figure 14). The plot also shows an antagonistic interaction between MEP and BPA: each chemical shows a positive association on its own, but increasing levels of one of these chemicals appears to attenuate the association of the other. An enhanced plot of this relationship is shown in Figure 15. To further explore the antagonistic relationship seen between MEP and BPA in relation to aeroallergy, we conducted a logistic regression model with interaction terms for MEP and BPA, controlling for the same covariates as in BKMR (maternal age, parity, poverty at baseline, and family history of asthma). Odds ratios for both chemical concentrations were above 1 and significant (MEP: OR=1.21, 95% CI: 1.01, 1.47; BPA: OR=6.79, 95% CI: 1.84, 25.03), and the odds ratio for their interaction was below 1 and significant (OR=0.82, 95% CI: 0.70, 0.96), indicating the chemicals interact antagonistically. The BKMR overall risk plot for aeroallergies shows that with higher concentrations of all chemicals, aeroallergy shows a linear positive trend, with lower concentrations associated with a decreased risk and higher concentrations associated with an increased risk (Figure 16).

Discussion

We aimed to use BPR to cluster participants into groups based on joint exposure to prenatal urinary concentrations of phthalates, parabens, and other phenols found in personal care products and plastics and to evaluate associations between cluster assignment and probable asthma, aeroallergies, and FEV_1. We found that the seven clusters produced by BPR were characterized by patterns of exposure consistent with varying personal care product and plastic use, but no cluster

was significantly associated with increased risk for any of the outcomes. We also aimed to estimate associations of a flexible kernel function of joint exposure to these chemicals with the outcomes above using BKMR. The BKMR analysis showed that MCOP was associated both with increased predicted probability of having probable asthma and with increased predicted probability of having a lower FEV_1 volume, as was 2,4-DCP to a lesser extent. 2,5-DCP and PP showed an association with a decreased predicted probability of having probable asthma, but did not show an association with FEV_1 volume. We also found that MCPP and BPA were associated with increased predicted probability of having aeroallergies. Several chemicals that showed associations in the univariate plots also showed positive additive effects in bivariate plots, such as 2,4-DCP and probable asthma and MCPP and aeroallergy, and MEP and BPA showed an antagonistic multiplicative interaction in their relationship to aeroallergy.

The number of clusters produced by BPR, as well as the relative chemical concentrations seen within them, seem to represent expected patterns of joint exposure. Clusters two and seven, higher than other clusters in MEP, MP, and PP and lower than other clusters in most other chemicals, may represent women who use more makeup or artificial scents. Higher urinary concentrations of MEP, MP, and PP have been associated in previous studies with use of makeup, lotion, deodorant, and perfume[36-39]. Clusters one and three seem to represent women who have higher exposure to many chemicals compared to other clusters, in the absence of comparatively low exposure, whereas clusters four and five seem to represent women who have the inverse exposure pattern. Cluster six, higher than other clusters in high molecular weight phthalates and BPA and lower than other clusters in low molecular weight phthalates, parabens, and other phenols, may represent women with high molecular weight phthalates present in building materials in their homes or women who use more plastic products. These clusters seem to reflect patterns consistent with personal care product or plasticizer use.

The lack of significant associations in regressions with cluster assignments may reflect a loss in power to detect relationships due to reduced sample sizes within clusters. Additionally, although the ANOVA p-value indicated clusters did not differ significantly, cluster three (characterized by higher concentrations of all chemicals) had the lowest FEV_1 volume, suggesting a relationship between respiratory health and high concentrations of many chemicals.

The associations seen in BKMR plots between MCOP and probable asthma and FEV_1 appear strong and are consistent with associations seen in logistic and linear regressions in chapters 3 and 4 of this dissertation. With these data, BKMR serves as a useful tool alongside single-chemical regression analyses to confirm if individual chemical associations from traditional regression methods persist when accounting for joint exposure to other chemicals.

Although the BKMR plots point to trends in how chemical concentrations are related to atopic outcomes, the posterior probability distributions (represented by credible intervals) indicate that these trends may take another form within the credible interval. It is difficult to compare this to statistical significance in the traditional sense, but although the bounds of the credible intervals overlap on the y-axis, the parameter estimate provides a useful representation of the most probable overall trend. Credible intervals also take into account the uncertainty of the variable selection process, which contributes to their width. With 15 chemicals and three outcomes in this study, these credible intervals should also be interpreted with multiple comparisons in mind.

In bivariate BKMR plots, several chemicals showed evidence of a positive additive effect when in the presence of other chemicals. For example, at higher quantiles of MCPP, MEP was associated

with even higher probability of aeroallergies while maintaining the slope of its overall positive trend. The chemicals that showed these additive effects also generally showed an association in univariate plots, indicating that if a chemical is strongly associated with an outcome, it will exert a positive additive effect on the association of other chemicals and that outcome. Multiplicative, antagonistic interaction was seen in the plots of MEP and BPA in relation to aeroallergy, to the effect that high concentrations of each chemical appeared to diminish the associations of the other. This was supported by interaction terms in a logistic regression model. There are no other epidemiologic studies on the joint effects of these two chemicals on aeroallergies to compare to, nor are there animal or *in vitro* studies of this chemical combination to our knowledge. Existing literature on possible immunologic mechanisms of these chemicals does not suggest a biologically antagonistic relationship[40-43], but the interaction has not been specifically studied. It may also be a statistical interaction influenced by confounding present at the extreme concentrations of either chemical. Multiplicative interactions were not seen between MEP and BPA for the other outcomes.

The plots of overall risk were difficult to interpret for these data in part because of their wide credible intervals. Few people had cumulative chemical concentrations below the 25th quantile or above the 75th quantile so it was difficult to obtain estimates comparing them to people in the 50th quantile. In addition, for probable asthma, and to a lesser extent FEV_1, the overall risk plots were estimating associations of a mixture of both positively and negatively associated chemicals. For example, an association of probable asthma with combined concentrations of 2,4-DCP and 2,5-DCP, whose univariate plots essentially mirror each other in direction, would appear null although associations with each chemical individually are not null. These plots may be more useful in a scenario where data on cumulative chemical concentrations were more evenly distributed across quantiles and where each chemical's individual association with the outcome were in the same direction.

The concentrations of phthalates, parabens, and other phenols in our data are mostly moderately correlated and are thus more suited to these methods than to inclusion as confounders in traditional regression models, as both BPR and BKMR can better account for collinearity of data. However, moderate correlation between chemicals could limit power to detect the effect of one chemical in the context of joint exposure to others. For example, a subtle association between a chemical and an outcome may not be apparent in a BKMR univariate plot, and BPR clusters do not allow us to discern the effects of any one chemical within a cluster.

One limitation of these methods is they do not allow for the presence of missing data. Only complete cases can be used, which can cut down on sample size and power to detect associations, and can bias results if the participants with missing data differ significantly from those with complete data. While our study had 74 people removed due to missing data, those removed did not differ greatly on exposure, outcome, or covariate summary measures (data not shown). One limitation of our data is the relatively high variability in urinary concentrations of these chemicals over time. Although we have two urinary measurements during pregnancy, these may not fully represent a child's exposure to these chemicals throughout gestation.

Our data show that participants cluster into seven groups based on prenatal urinary concentrations of phthalates, parabens, and other phenols, and that these groups are not significantly related to having probable asthma or aeroallergies, or to FEV_1 volume. Our data also show that MCOP is associated with higher predicted probability of having probable asthma and with lower FEV_1 volume, when accounting for joint exposure to other personal care product and plasticizing chemicals. We found that results from the BKMR analysis are similar to those from traditional

regression methods, but can additionally evaluate how the presence of joint chemical exposure influences the associations of any one chemical. This is the first study to analyze chemical mixtures in relation to respiratory and allergic outcomes.

References

1. Gascon M, Casas M, Morales E, Valvi D, Ballesteros-Gómez A, Luque N, Rubio S, Monfort N, Ventura R, Martínez D. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. *Journal of Allergy and Clinical Immunology* 2015;135(2):370-378. e7.
2. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, Hoepner LA, Perera FP, Miller RL. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. 2014.
3. Larsson M, Hägerhed-Engman L, Kolarik B, James P, Lundin F, Janson S, Sundell J, Bornehag C-G. PVC–as flooring material–and its association with incident asthma in a Swedish child cohort study. *Indoor Air* 2010;20(6):494-501.
4. Jerschow E, Parikh P, McGinn AP, De Vos G, Jariwala S, Hudes G, Rosenstreich D. Relationship between urine dichlorophenol levels and asthma morbidity. *Annals of Allergy, Asthma & Immunology* 2014;112(6):511-518. e1.
5. Savage JH, Johns CB, Hauser R, Litonjua AA. Urinary triclosan levels and recent asthma exacerbations. *Annals of Allergy, Asthma & Immunology* 2014;112(2):179-181. e2.
6. Spanier AJ, Kahn RS, Kunselman AR, Schaefer EW, Hornung R, Xu Y, Calafat AM, Lanphear BP. Bisphenol a exposure and the development of wheeze and lung function in children through age 5 years. *JAMA pediatrics* 2014;168(12):1131-1137.
7. Spanier AJ, Kahn RS, Kunselman AR, Hornung R, Xu Y, Calafat AM, Lanphear BP. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. *Environmental health perspectives* 2012;120(6):916.
8. Whyatt RM, Rundle AG, Perzanowski MS, Just AC, Donohue KM, Calafat AM, Hoepner L, Perera FP, Miller RL. Prenatal phthalate and early childhood bisphenol A exposures increase asthma risk in inner-city children. *The Journal of allergy and clinical immunology* 2014;134(5):1195.
9. Zhou A, Chang H, Huo W, Zhang B, Hu J, Xia W, Chen Z, Xiong C, Zhang Y, Wang Y. Prenatal exposure to bisphenol A and risk of allergic diseases in early life. *Pediatric Research* 2017;81(6):851-856.
10. Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat A, Ye X, Annesi-Maesano I, Siroux V. In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study. *Environmental Health Perspectives* 2017;125(9):097006.
11. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environment international* 2018;115:79-88.
12. Clayton EM, 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(3):390-6.
13. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012;130(2):453-60 e7.

14. Spanier AJ, Fausnight T, Camacho TF, Braun JM. The associations of triclosan and paraben exposure with allergen sensitization and wheeze in children. Allergy and Asthma Proceedings: OceanSide Publications, Inc, 2014;475-481.
15. Spanier AJ, Fiorino EK, Trasande L. Bisphenol A exposure is associated with decreased lung function. *The Journal of pediatrics* 2014;164(6):1403-1408. e1.
16. Cakmak S, Dales RE, Hebbern C, Saravanabhavan G. The association between urinary phthalates and lung function. *Journal of occupational and environmental medicine* 2014;56(4):376.
17. Carpenter DO, Arcaro K, Spink DC. Understanding the human health effects of chemical mixtures. *Environ Health Perspect* 2002;110 Suppl 1:25-42.
18. Molitor J, Papathomas M, Jerrett M, Richardson S. Bayesian profile regression with an application to the National Survey of Children's Health. *Biostatistics* 2010;11(3):484-98.
19. Bobb JF VL, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, Godleski JJ, Coull BA. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics* 2015;16(3):493-508.
20. Coker E, Gunier R, Bradman A, Harley K, Kogut K, Molitor J, Eskenazi B. Association between Pesticide Profiles Used on Agricultural Fields near Maternal Residences during Pregnancy and IQ at Age 7 Years. *International journal of environmental research and public health* 2017;14(5):506.
21. Coker E, Liverani S, Ghosh JK, Jerrett M, Beckerman B, Li A, Ritz B, Molitor J. Multi-pollutant exposure profiles associated with term low birth weight in Los Angeles County. *Environ Int* 2016;91:1-13.
22. Pirani M, Best N, Blangiardo M, Liverani S, Atkinson RW, Fuller GW. Analysing the health effects of simultaneous exposure to physical and chemical properties of airborne particles. *Environment international* 2015;79:56-64.
23. Coker E, Chevrier J, Rauch S, Bradman A, Obida M, Crause M, Bornman R, Eskenazi B. Association between prenatal exposure to multiple insecticides and child body weight and body composition in the VHEMBE South African birth cohort. *Environment international* 2018;113:122-132.
24. Chiu Y-H, Bellavia A, James-Todd T, Correia KF, Valeri L, Messerlian C, Ford JB, Mínguez-Alarcón L, Calafat AM, Hauser R. Evaluating effects of prenatal exposure to phthalate mixtures on birth weight: A comparison of three statistical approaches. *Environment international* 2018;113:231-239.
25. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, Mitchell EA, Pearce N, Sibbald B, Stewart AW, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8(3):483-91.
26. Silva MJ SE, Preau JL Jr, Reidy JA, Needham LL, Calafat AM. Quantification of 22 phthalate metabolites in human urine. *Journal of Chromatography* 2007(860):106–112.
27. Ye X, Kuklenyik Z, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal Chem* 2005;77(16):5407-13.
28. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, Bernstein L, Hartge P. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environ Health Perspect* 2004;112(17):1691-6.
29. Cone EJ, Caplan YH, Moser F, Robert T, Shelby MK, Black DL. Normalization of urinary drug concentrations with specific gravity and creatinine. *Journal of Analytical Toxicology* 2009;33(1):1-7.

30. Team RC. R: A language and environment for statistical computing. 2013.
31. Liverani S, Hastie DI, Azizi L, Papathomas M, Richardson S. PReMiuM: an R package for profile regression mixture models using Dirichlet processes. *arXiv preprint arXiv:1303.2836* 2013.
32. Bobb JF. bkmr: Bayesian Kernel Machine Regression. *R package version 0.2.0. https://CRAN.R-project.org/package=bkmr* 2017.
33. Hastie DI, Liverani S, Azizi L, Richardson S, Stücker I. A semi-parametric approach to estimate risk functions associated with multi-dimensional exposure profiles: application to smoking and lung cancer. *BMC medical research methodology* 2013;13(1):129.
34. Papathomas M, Molitor J, Richardson S, Riboli E, Vineis P. Examining the joint effect of multiple risk factors using exposure risk profiles: lung cancer in nonsmokers. *Environmental health perspectives* 2011;119(1):84.
35. Coull B, Bobb J, Wellenius G, Kioumourtzoglou M, Mittleman M, Koutrakis P, Godleski J. Part 1. Statistical Learning Methods for the Effects of Multiple Air Pollution Constituents. *Research report (Health Effects Institute)* 2015(183 Pt 1-2):5-50.
36. Berger K, Kogut K, Bradman A, She J, Qi G, Zahedi R, Parra KL, K.G H. Personal care product use as a predictor of urinary concentrations of certain phthalates, parabens, and phenols in the HERMOSA study. *Journal of exposure science & environmental epidemiology* 2018.
37. Meeker JD, Cantonwine DE, Rivera-Gonzalez LO, Ferguson KK, Mukherjee B, Calafat AM, Ye X, Anzalota Del Toro LV, Crespo-Hernandez N, Jimenez-Velez B, Alshawabkeh AN, Cordero JF. Distribution, variability, and predictors of urinary concentrations of phenols and parabens among pregnant women in Puerto Rico. *Environ Sci Technol* 2013;47(7):3439-47.
38. Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. *Journal of Exposure Science and Environmental Epidemiology* 2014;24(5):459-466.
39. Parlett LE, Calafat AM, Swan SH. Women's exposure to phthalates in relation to use of personal care products. *Journal of Exposure Science and Environmental Epidemiology* 2013;23(2):197-206.
40. Maruyama T, Shiba T, Iizuka H, Matsuda T, Kurohane K, Imai Y. Effects of Phthalate Esters on Dendritic Cell Subsets and Interleukin-4 Production in Fluorescein Isothiocyanate-Induced Contact Hypersensitivity. *Microbiology and immunology* 2007;51(3):321-326.
41. Tian X, Takamoto M, Sugane K. Bisphenol A promotes IL-4 production by Th2 cells. *International archives of allergy and immunology* 2003;132(3):240-247.
42. Herberth G, Pierzchalski A, Feltens R, Bauer M, Röder S, Olek S, Hinz D, Borte M, von Bergen M, Lehmann I. Prenatal phthalate exposure associates with low regulatory T-cell numbers and atopic dermatitis in early childhood: Results from the LINA mother-child study. *Journal of Allergy and Clinical Immunology* 2016.
43. Yan H, Takamoto M, Sugane K. Exposure to bisphenol A prenatally or in adulthood promotes TH2 cytokine production associated with reduction of CD4+ CD25+ regulatory T cells. *Environmental health perspectives* 2008;116(4):514.

Table 1. Demographic characteristics of the study population, CHAMACOS Study, Salinas, CA

Characteristics at time of pregnancy	N (%)
Age	
18-24	135 (42)
25-29	106 (33)
30-34	51 (16)
35+	27 (8)
Poverty	
<100%	198 (62)
100-200%	109 (34)
>200%	12 (4)
Parity	
First child	102 (32)
Second child	92 (29)
Third child or greater	125 (39)
Child's mother, father, or siblings have asthma history	
No	288 (90)
Yes	31 (10)

Table 2. Distribution of specific gravity corrected phthalate monoester metabolites in maternal prenatal urine collected at two time points in pregnancy

	% > LOD		Average of 2 Measurements						
Phthalate	Early Pregnancy[1]	Late Pregnancy[2]	Geo. Mean	10th%	25th%	50th%	75th%	90th%	95th%
MEP (ng/mL)	100.0%	99.7%	237.9	46.7	106.5	223.6	490.9	992.3	1615.9
MBP (ng/mL)	98.4%	100.0%	27.9	9.6	16.2	26.6	46.6	89.9	119.5
MiBP (ng/mL)	92.4%	95.7%	3.4	1.0	1.8	3.4	6.7	11.0	16.6
MBZP (ng/mL)	97.8%	98.7%	8.9	2.5	4.9	9.1	17.7	29.4	40.6
MCNP (ng/mL)	95.0%	96.7%	2.3	0.9	1.5	2.3	3.5	5.4	7.6
MCOP (ng/mL)	96.5%	96.4%	3.8	1.5	2.4	3.9	5.6	8.9	13.1
MCPP (ng/mL)	88.6%	93.8%	2.2	0.8	1.4	2.4	3.8	5.3	7.6
∑DEHP (umol/mL)	87.7%[3]	91.8%[3]	0.2	0.1	0.1	0.2	0.4	0.6	0.8
MP (ug/mL)	99.7%	100.0%	152.8	29.2	73.9	171.5	365.8	633.7	783.7
PP (ug/mL)	96.5%	97.6%	38.9	2.6	11.6	47.9	164.9	435.7	631.4
BP (ug/mL)	44.8%	44.1%	0.5	0.1	0.1	0.3	1.8	12.0	21.0
TCS (ug/mL)	70.1%	75.1%	22.5	1.4	6.1	10.5	139.2	364.7	593.2
2,4-DCP (ug/mL)	100.0%	100.0%	5.7	1.5	2.2	3.7	13.9	48.7	61.5
2,5-DCP (ug/mL)	99.7%	100.0%	71.2	6.5	15.1	66.4	412.9	857.1	1109.1
BP3 (ug/mL)	99.7%	99.3%	26.1	2.5	5.0	17.9	145.0	559.4	1005.8
BPA (ug/mL)	82.9%	89.6%	1.5	0.6	0.9	1.4	2.3	3.7	5.8

[1]Mean ± SD = 14.0 ± 5.0 weeks gestation

[2]Mean ± SD = 26.9 ± 2.5 weeks gestation

[3]MEHP used as proxy for sumDEHP in % > LOD

Abbreviations: LOD = Limit of detection

Table 3. Descriptive statistics of immune outcomes in CHAMACOS participants

Asthma symptoms and medication variables

	N (%)	
Variable	Yes	No
Probable Asthma	33 (10%)	285 (90%)
Aeroallergy	81 (25%)	237 (75%)

Variable	N	Mean (SD)
FEV_1	282	1.8 (0.5)

Table 4. Posterior Inclusion Probabilities (PIP) for all phthalate metabolites, parabens, other phenols, included in Bayesian Kernel Machine Regression

Probable asthma		Aeroallergy		FEV1	
Chemical	PIP	Chemical	PIP	Chemical	PIP
PP	0.82213	BPA	0.69181	MCOP	0.55379
MCOP	0.47427	MP	0.56505	PP	0.54632
TCS	0.44584	MCPP	0.43896	MP	0.45368
MCNP	0.30915	PP	0.43495	DCP24	0.29411
DCP25	0.2038	MCOP	0.13803	TCS	0.27699
MP	0.17787	MCNP	0.13077	DCP25	0.25121
BP3	0.16763	MEP	0.11722	MBzP	0.13752
DCP24	0.12369	BP3	0.09915	MCNP	0.09468
MCPP	0.0871	TCS	0.08006	BPA	0.09085
BPA	0.05903	DCP25	0.06801	BP3	0.08684
MEP	0.03816	DCP24	0.06097	MEP	0.08055
MBzP	0.03624	DEHP	0.05303	MCPP	0.06068
MiBP	0.02443	MBP	0.04665	MBP	0.02826
DEHP	0.01558	MBzP	0.03859	MiBP	0.02226
MBP	0.01506	MiBP	0.03676	DEHP	0.02226
Group	PIP	Group	PIP	Group	PIP
Phthalate	0.54188	Phthalate	0.50164	Phthalate	0.45288
Paraben	0.49968	Paraben	0.25888	Paraben	0.0842
Other phenol	0.42688	Other phenol	0.5176	Other phenol	0.18932

Table 5. Frequency of probable asthma and aeroallergy cases and mean FEV_1 volume by cluster

		Frequency of cases (% of cluster)				Mean (standard deviation)
Clusters	N	Probable asthma	Allergy	Clusters	N	FEV_1
1	46	4 (9%)	12 (46%)	1	43	1.75 (0.46)
2	26	1 (4%)	3 (12%)	2	21	1.86 (0.44)
3	68	9 (13%)	22 (32%)	3	57	1.73 (0.53)
4	51	6 (12%)	11 (22%)	4	47	1.88 (0.45)
5	45	6 (13%)	9 (20%)	5	42	1.74 (0.51)
6	31	3 (10%)	10 (32%)	6	27	1.81 (0.51)
7	52	4 (8%)	14 (27%)	7	46	1.96 (0.49)
Total	319	Chi^2=2.88, P=0.82	Chi^2=6.30, P=0.39	Total	283	ANOVA P=0.19

Table 6. Association of BPR cluster assignment and probable asthma, aeroallergies, and FEV_1

	Probable asthma	Aeroallergies	FEV_1
Chemical	OR (95% CI)	OR (95% CI)	β (95% CI)
	n=319	n=319	n=283
Cluster 1	Ref	Ref	Ref
Cluster 2	0.62 (0.16, 2.36)	1.41 (0.53, 3.77)	0.00 (-0.21, 0.21)
Cluster 3	0.26 (0.03, 2.29)	0.52 (0.13, 2.13)	0.12 (-0.14, 0.38)
Cluster 4	0.99 (0.33, 3.01)	1.91 (0.79, 4.66)	-0.02 (-0.21, 0.18)
Cluster 5	0.87 (0.26, 2.91)	1.10 (0.41, 2.96)	0.14 (-0.07, 0.34)
Cluster 6	0.70 (0.16, 3.02)	1.90 (0.67, 5.44)	0.07 (-0.17, 0.31)
Cluster 7	0.54 (0.14, 2.06)	1.47 (0.57, 3.82)	0.22 (0.01, 0.42)

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

[1]Models control for maternal age, parity, poverty index at baseline, and child's family history of asthma

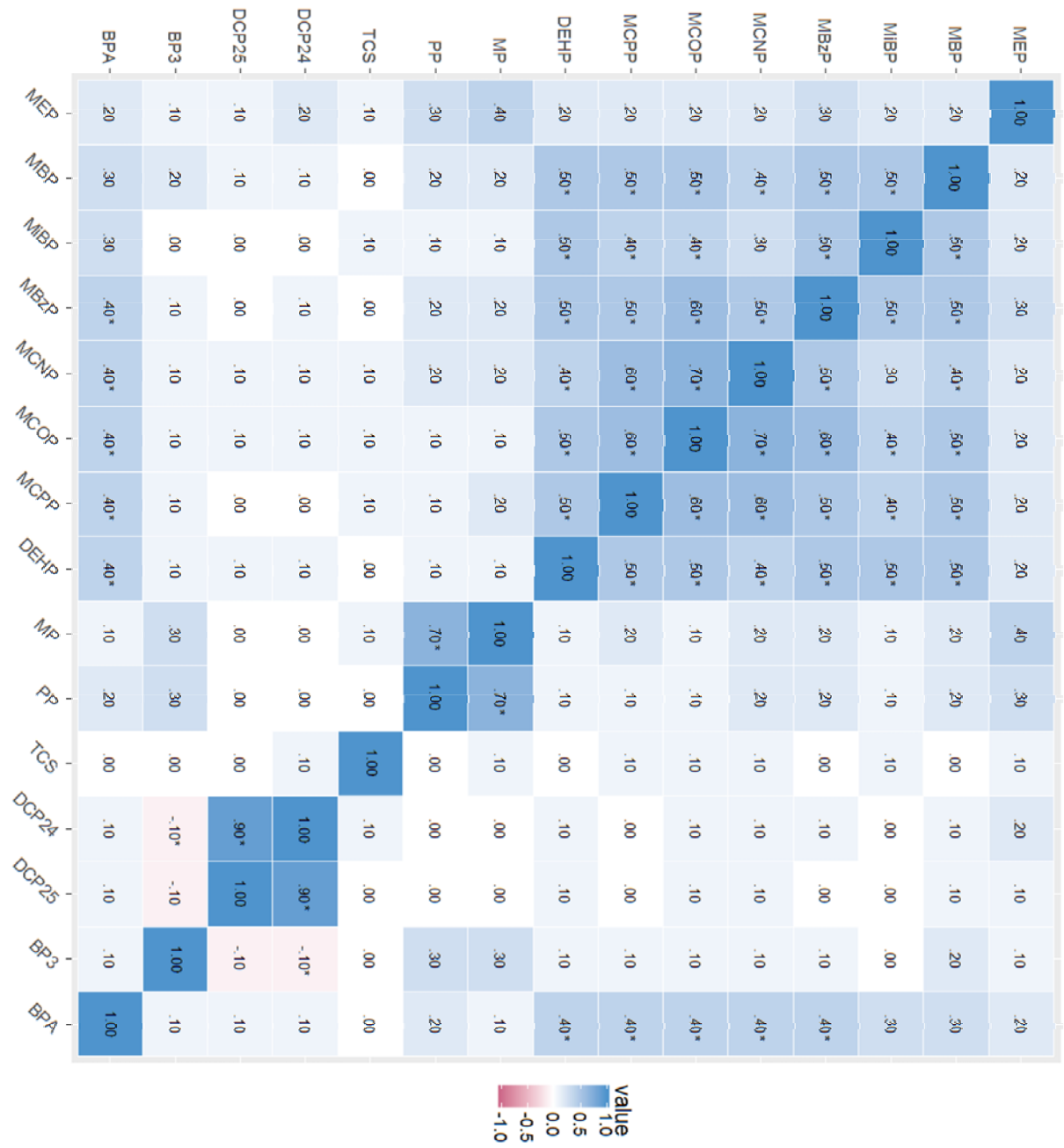


Figure 1. Log2 specific gravity corrected phthalate, paraben, and phenol correlations in CHAMACOS

Figure 2. Geographic means and geographic standard deviations of phthalate concentrations across clusters generated by Bayesian Profile Regression

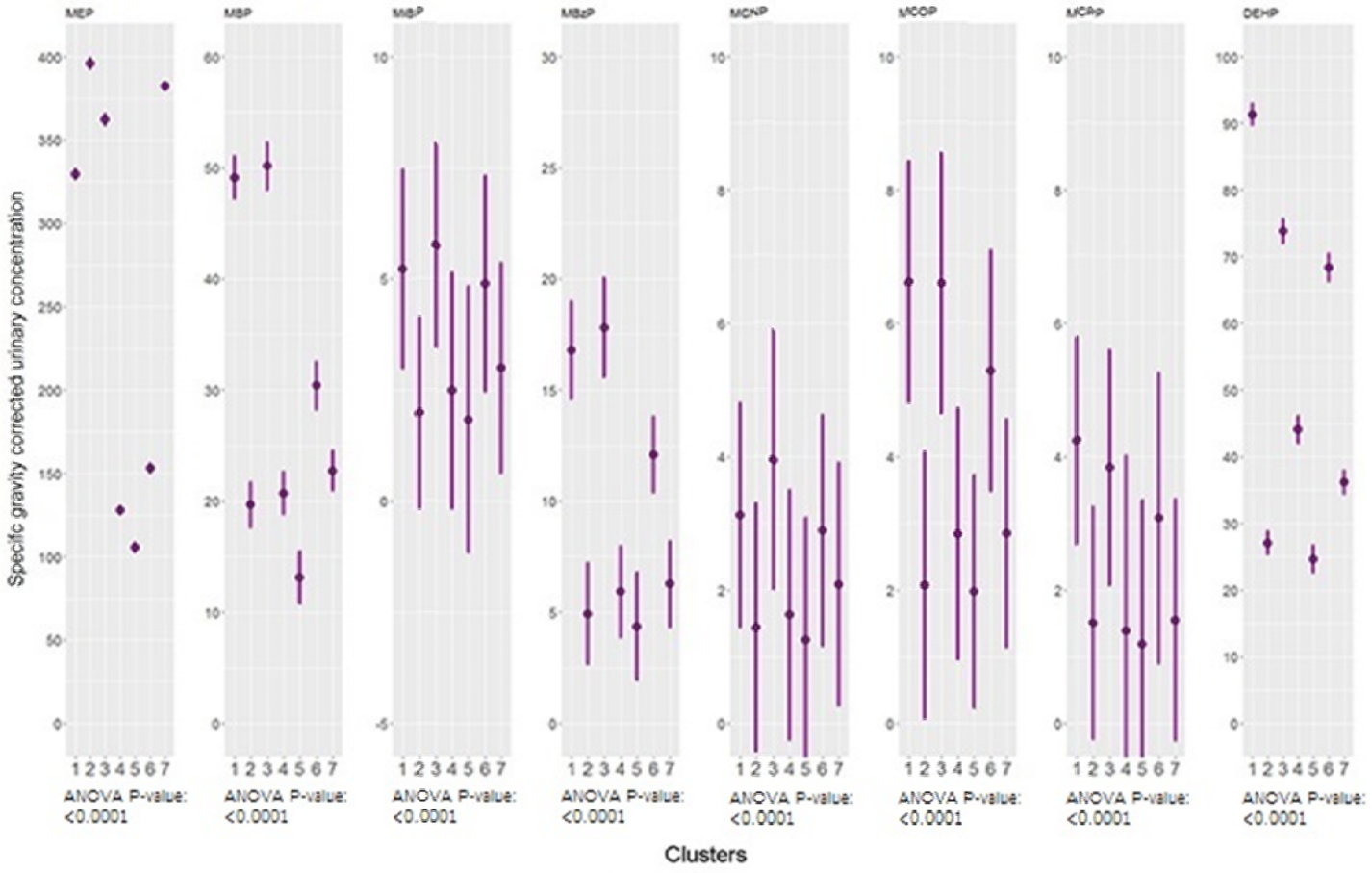


Figure 3. Geographic means and geographic standard deviations of paraben and other phenol concentrations across clusters generated by Bayesian Profile Regression

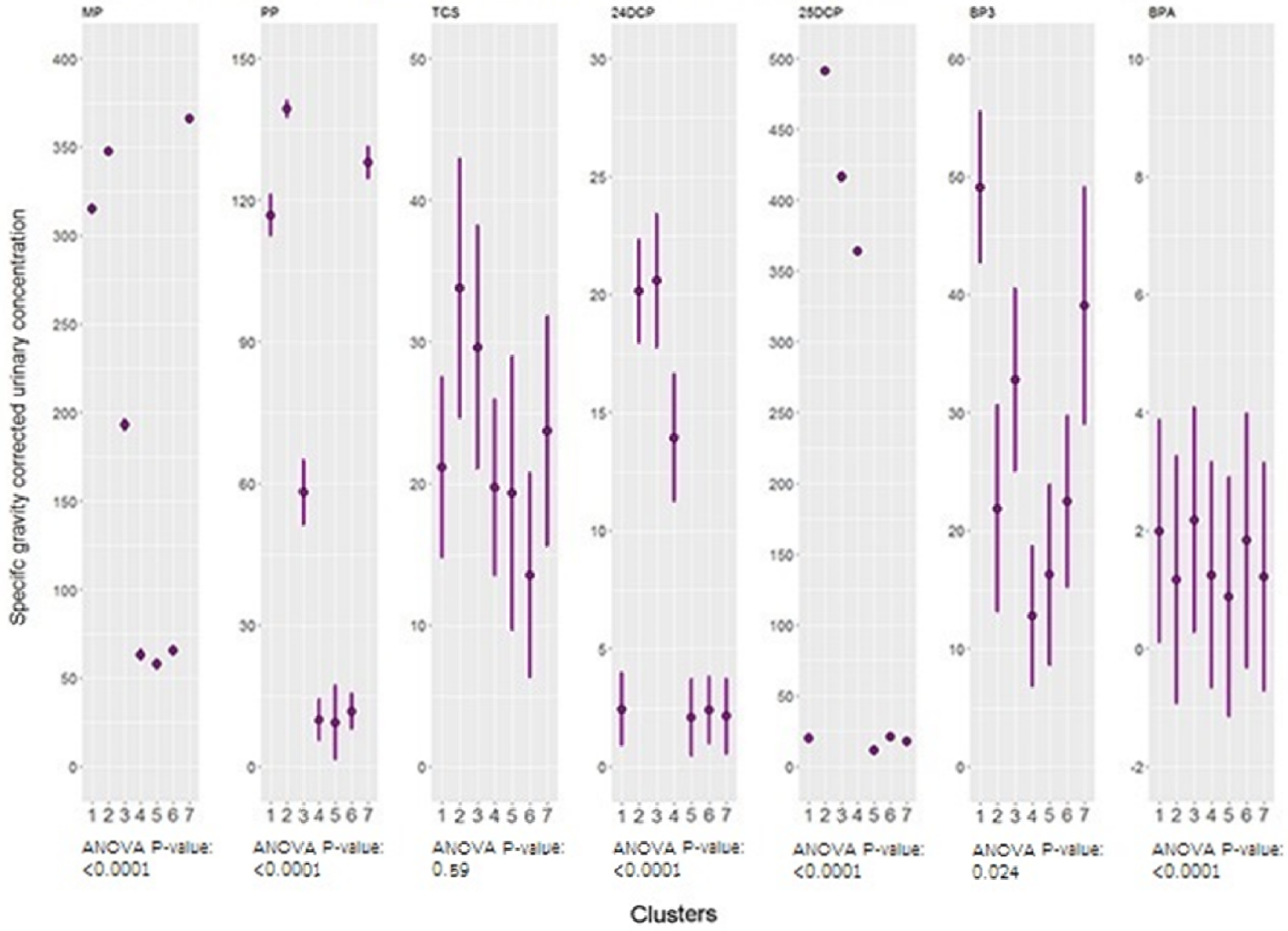


Figure 4. Percent of probable asthma cases in clusters generated by Bayesian Profile Regression

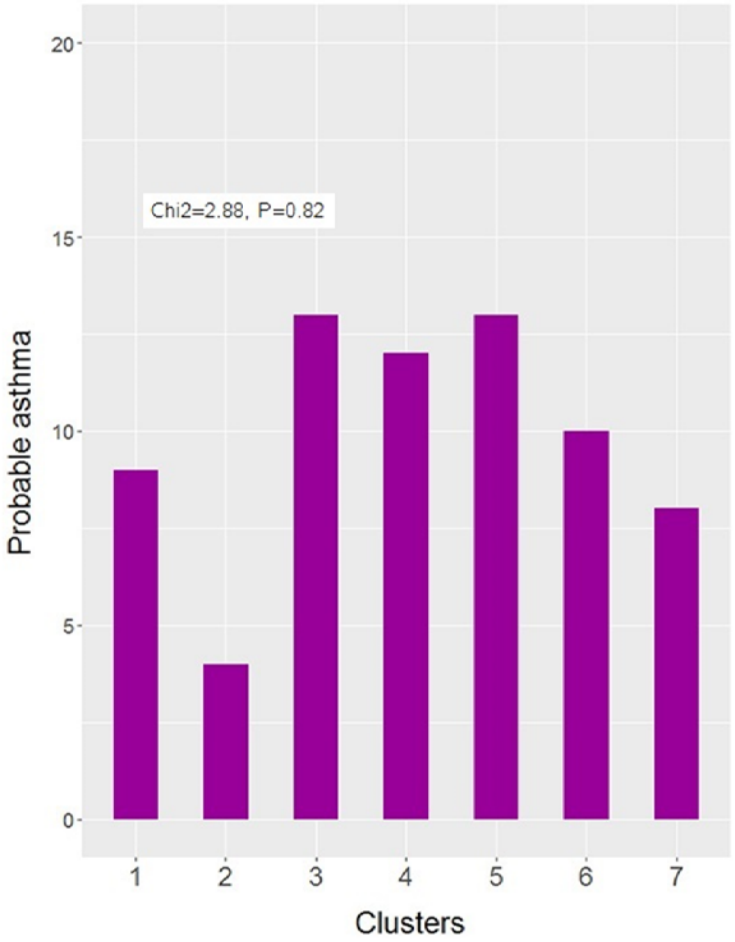


Figure 5. Mean (SD) FEV_1 volume across clusters generated by Bayesian Profile Regression

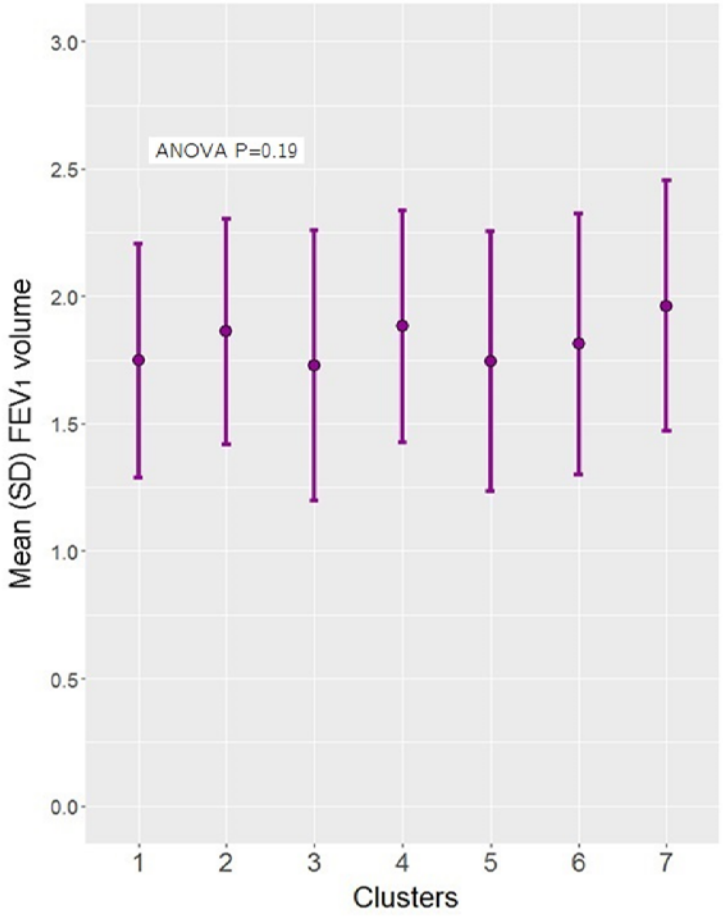


Figure 6. Percent of aeroallergy cases across clusters generated by Bayesian Profile Regression

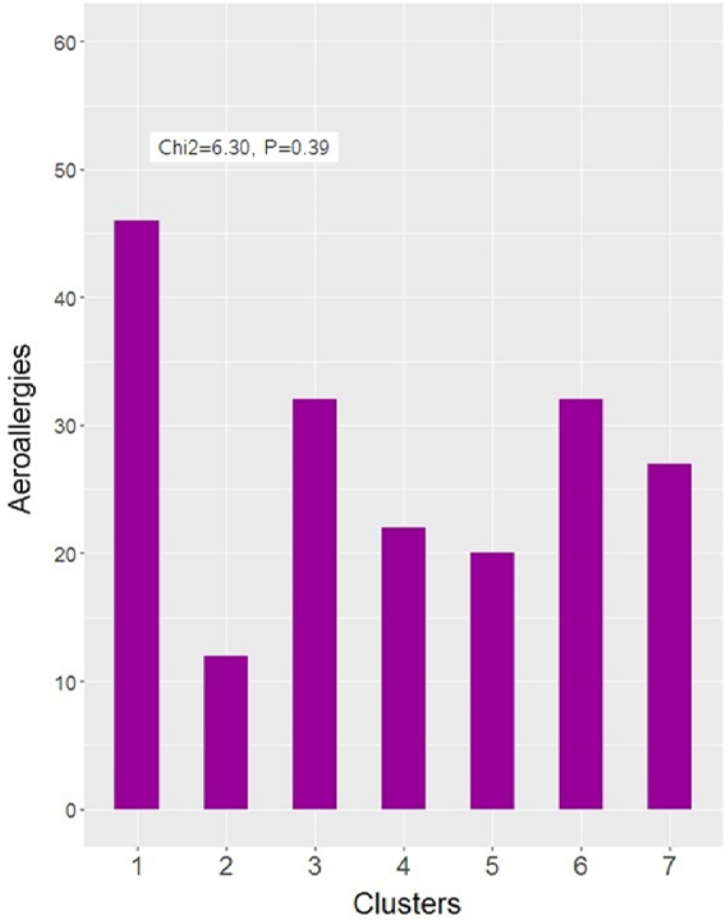


Figure 7. Predicted probability of having probable asthma by z-score of log2 chemical concentrations, holding all other chemicals at their medians

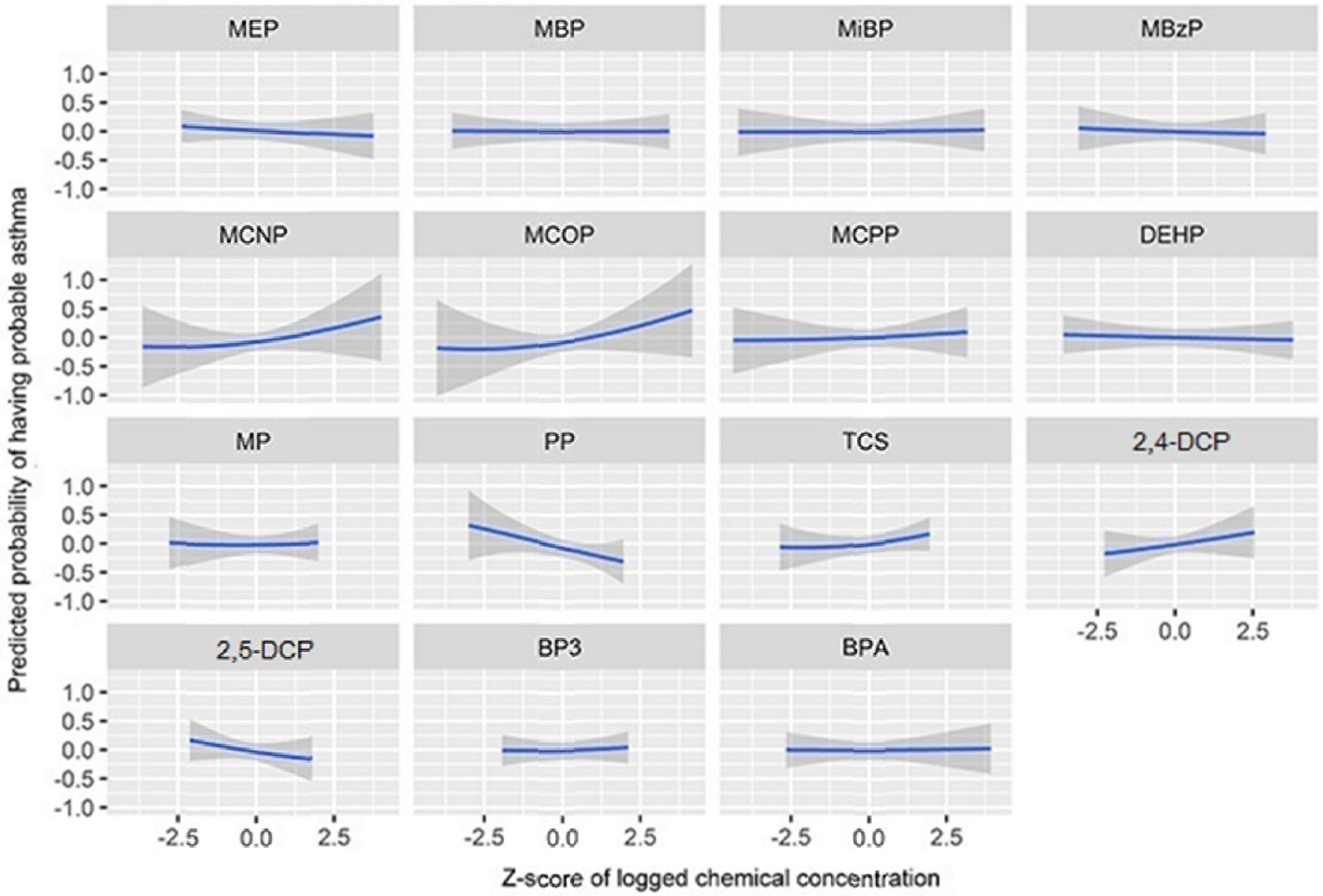


Figure 8. Predicted probability of having probable asthma by z-score of chemical concentrations for one chemical (row) by quantiles of a second chemical (columns), holding all additional chemicals at their medians

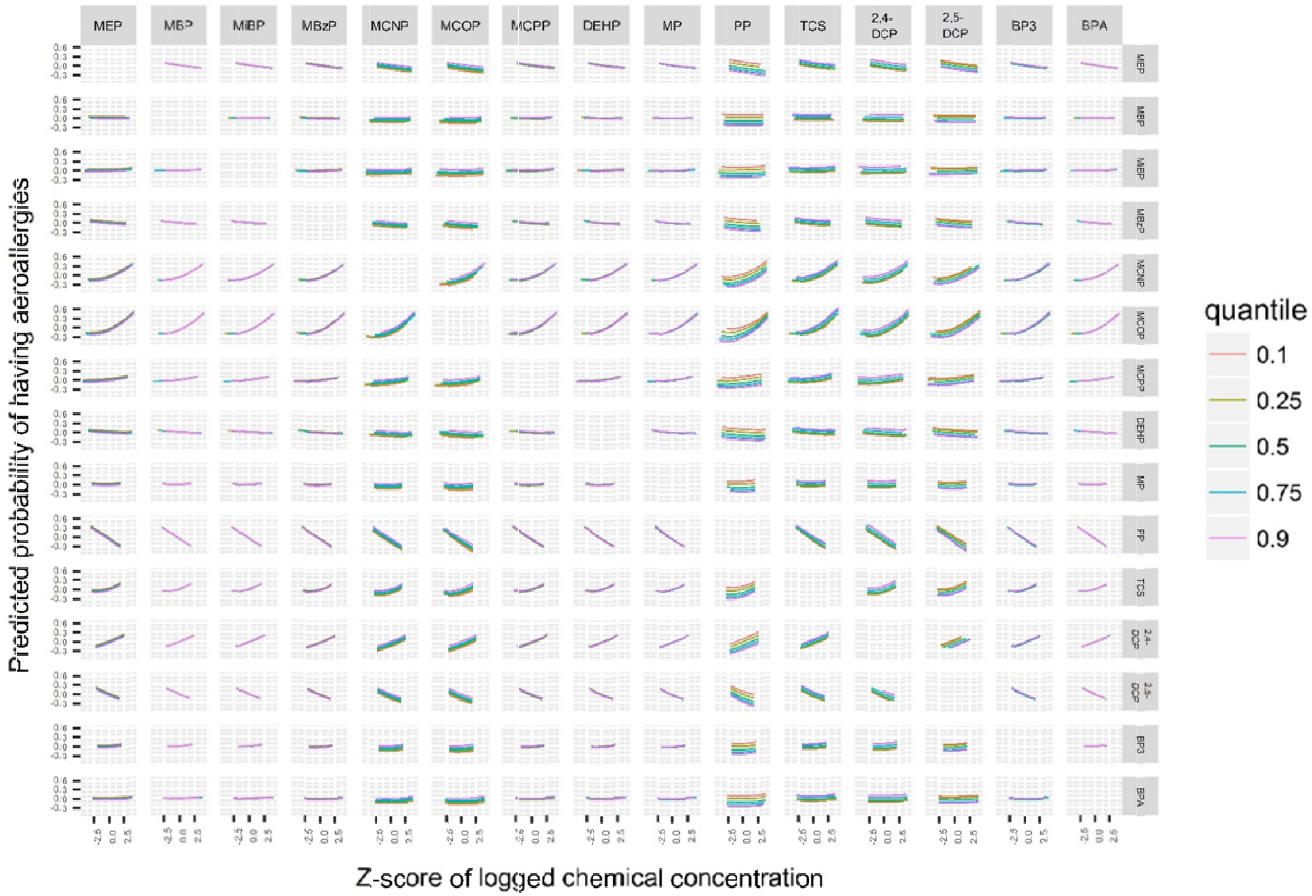


Figure 9. Predicted probability of having probable asthma with increasing quantiles of total chemical concentration

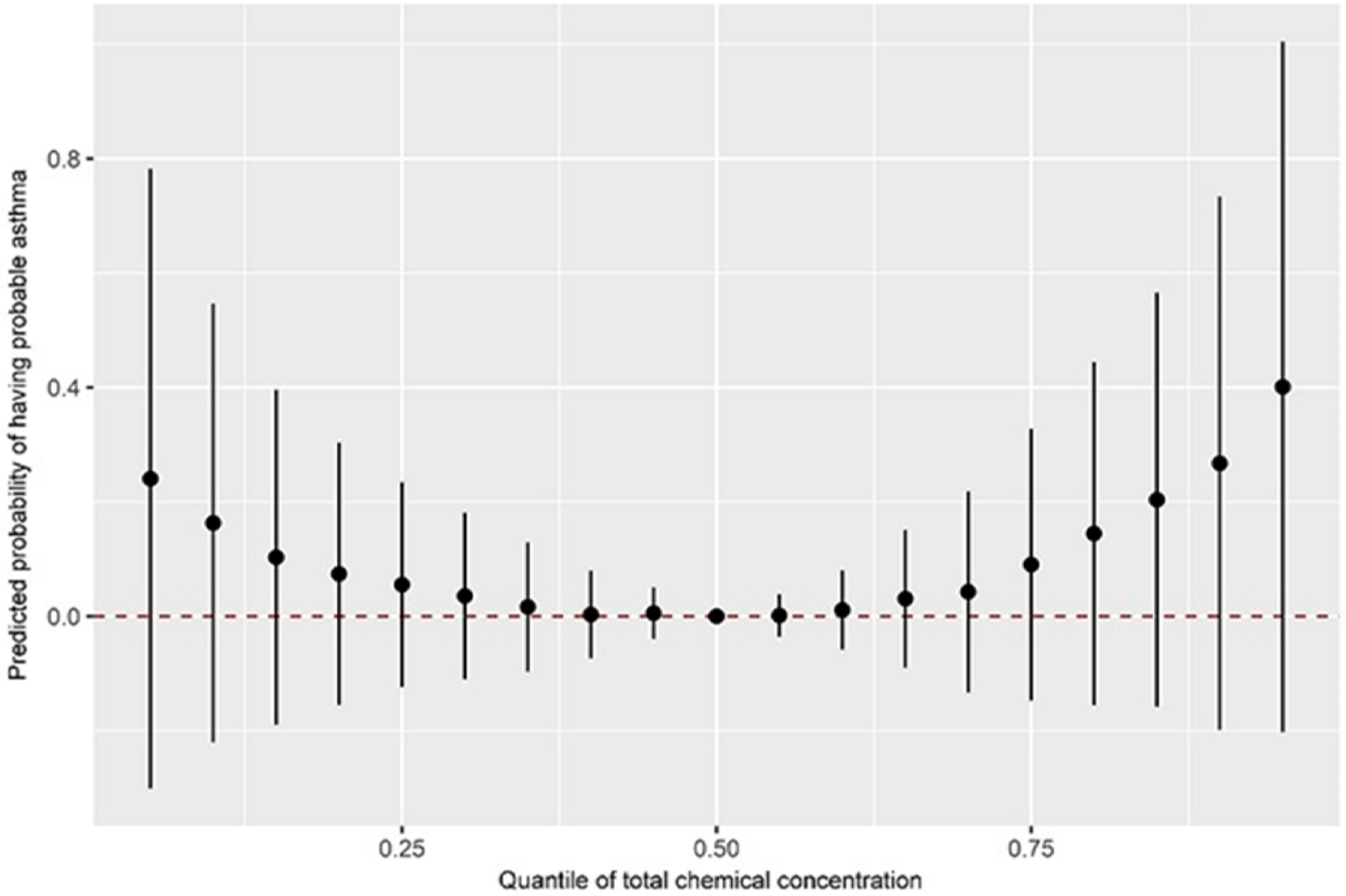


Figure 10. Predicted probability of FEV_1 volume by z-score of log2 chemical concentrations, holding all other chemicals at their medians

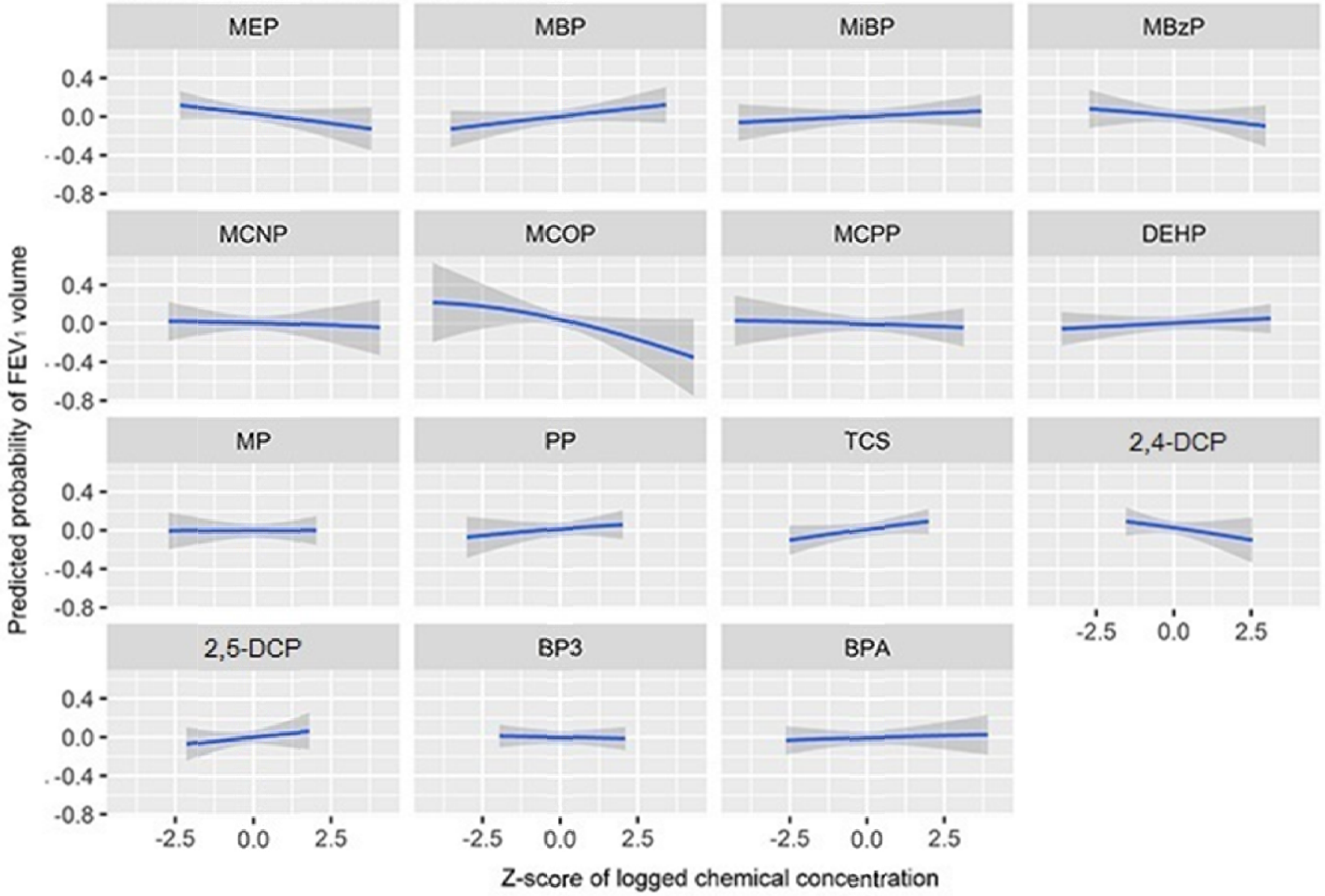


Figure 11. Predicted probability of FEV_1 volume by z-score of chemical concentrations for one chemical (row) by quantiles of a second chemical (columns), holding all additional chemicals at their medians

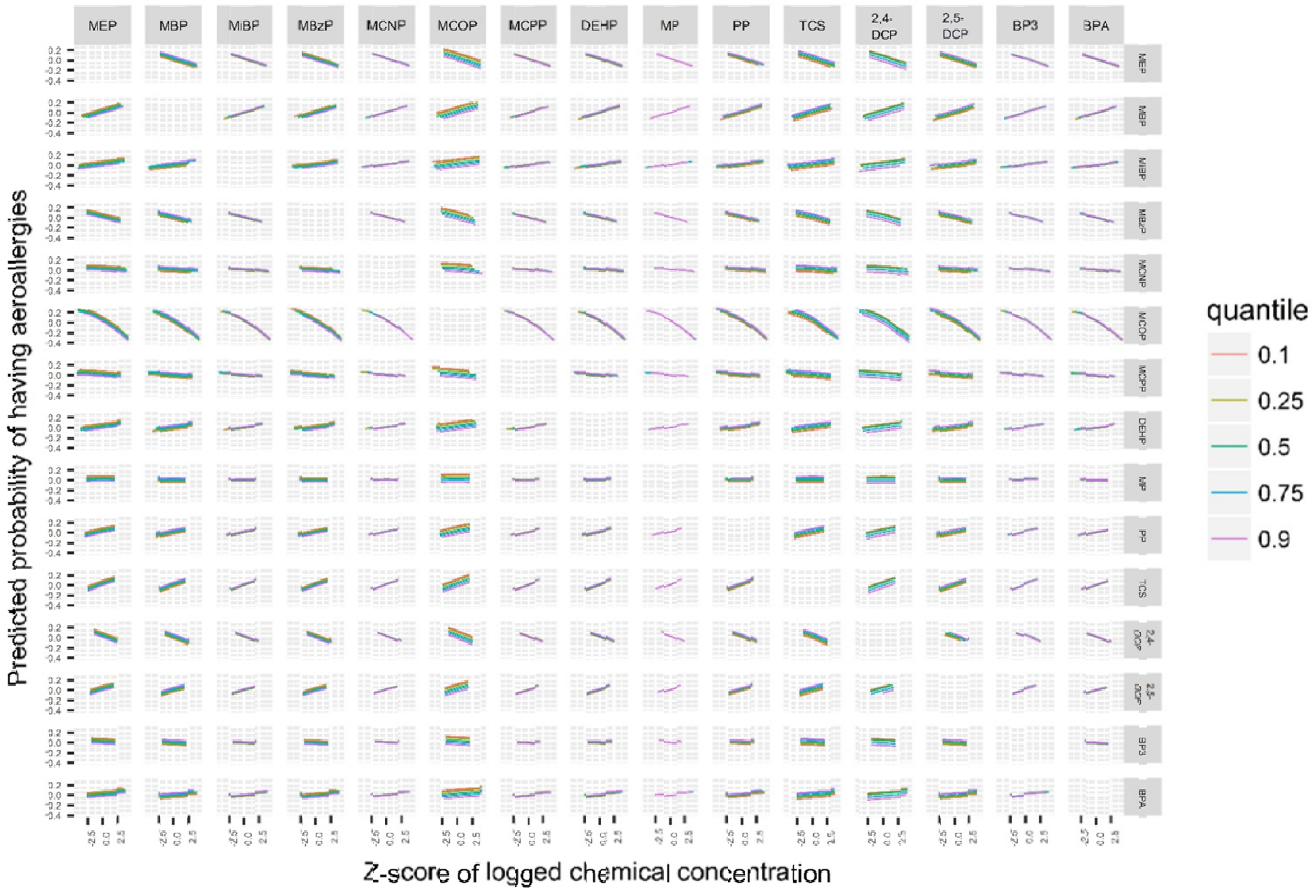


Figure 12. Predicted probability of FEV_1 volume with increasing quantiles of total chemical concentration

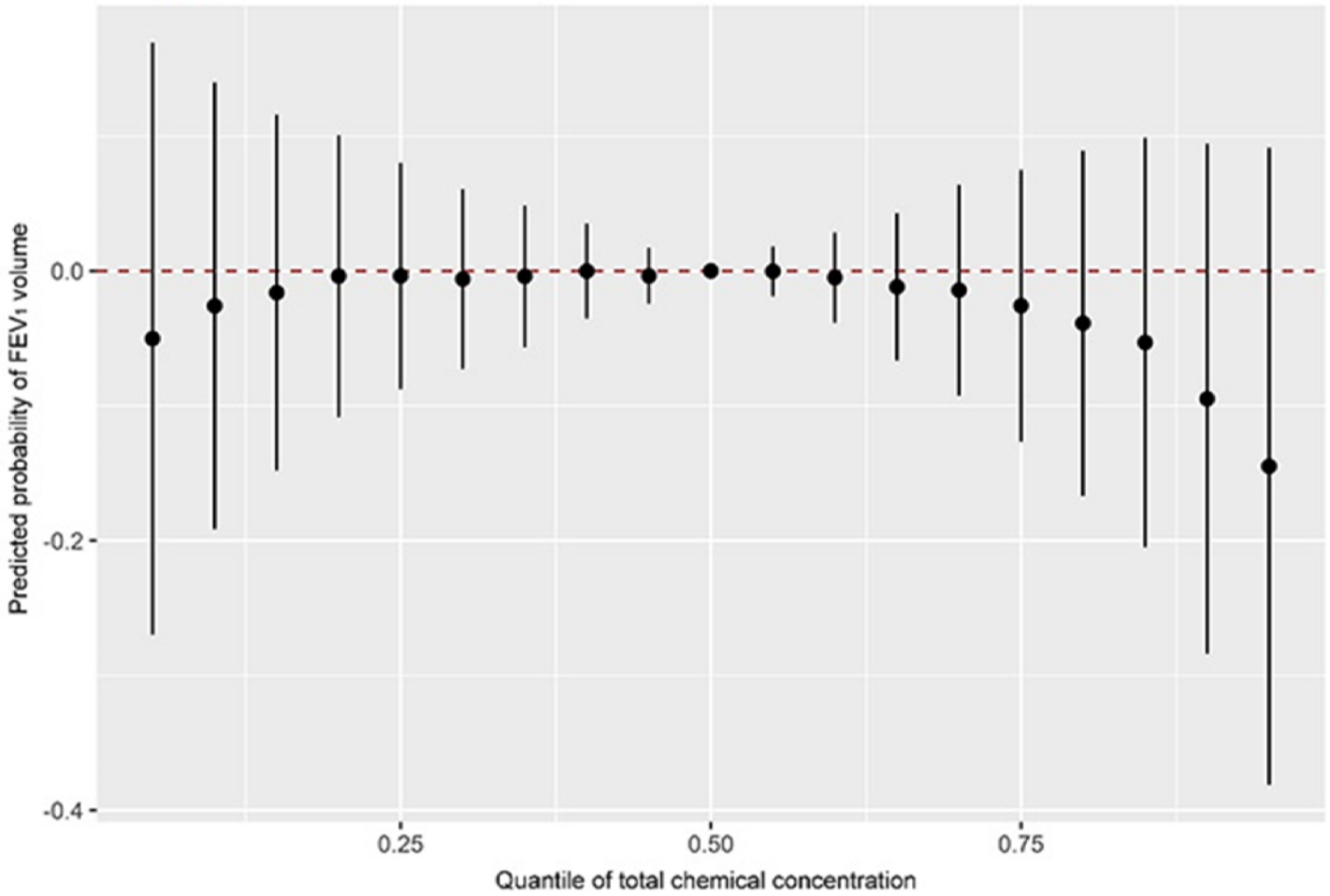


Figure 13. Predicted probability of having aeroallergies by z-score of log2 chemical concentrations, holding all other chemicals at their medians

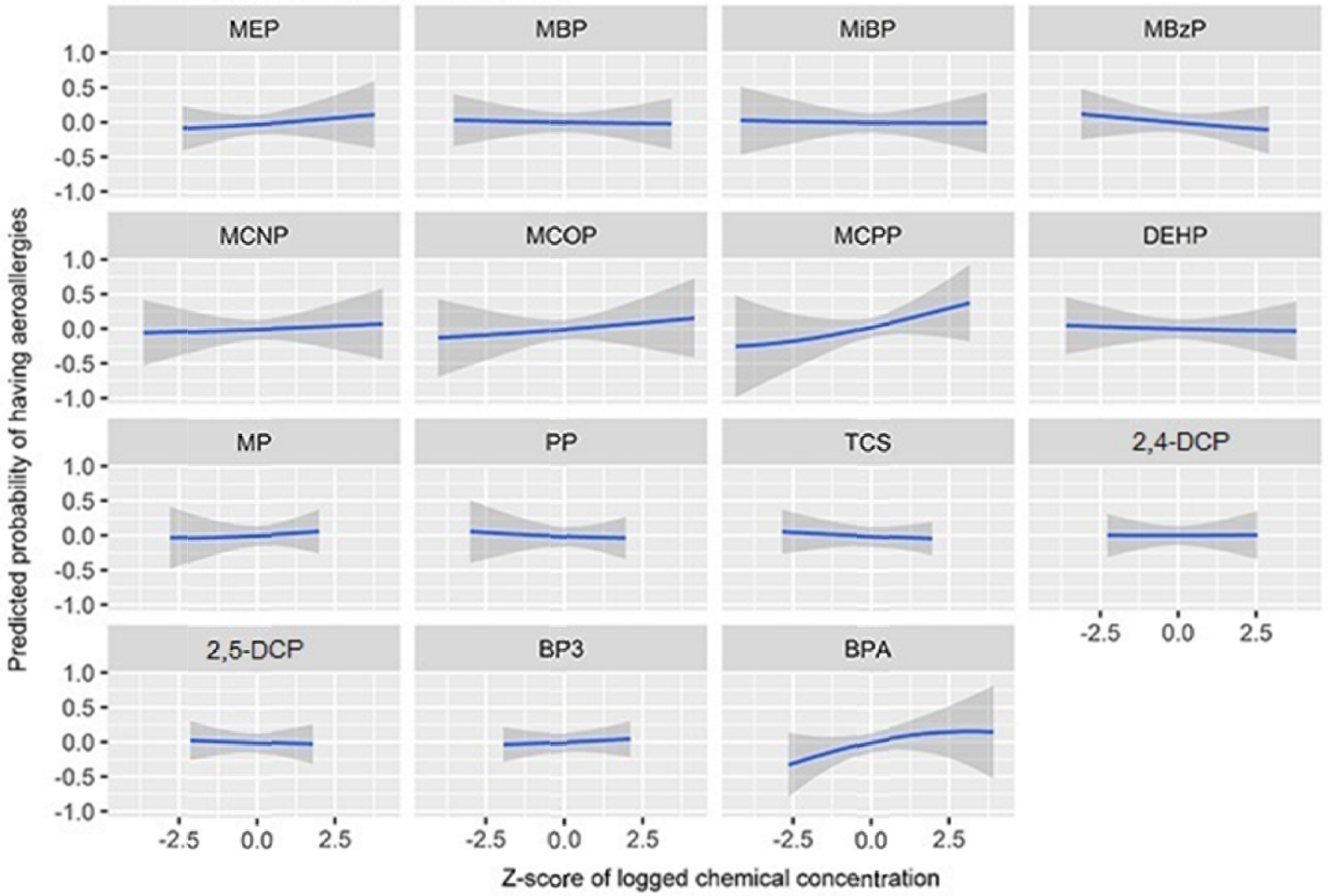


Figure 14. Predicted probability of having aeroallergies by z-score of chemical concentrations for one chemical (row) by quantiles of a second chemical (columns), holding all additional chemicals at their medians

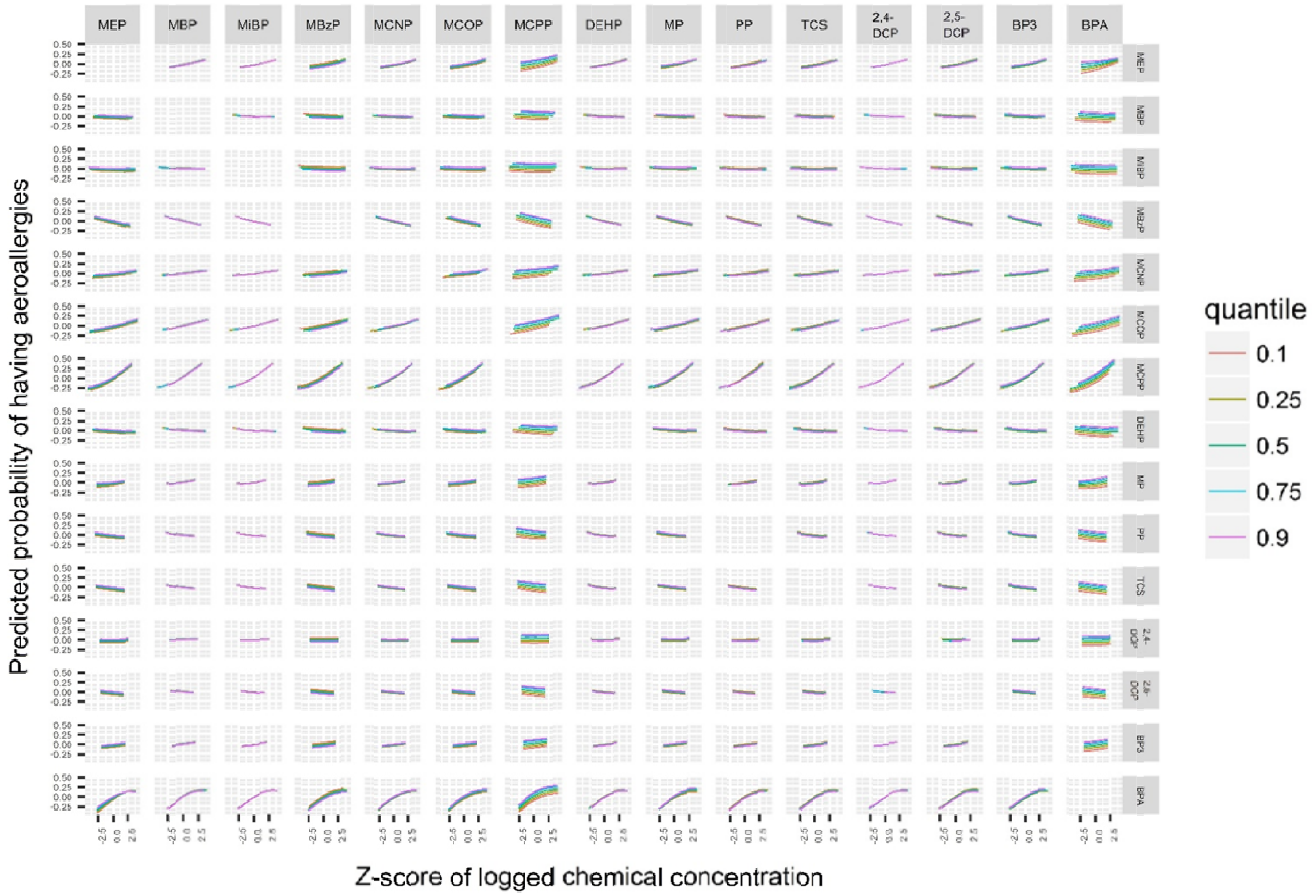


Figure 15. Predicted probability of having aeroallergies by z-score of (A) MEP by quantiles of BPA and (B) BPA by quantiles of MEP

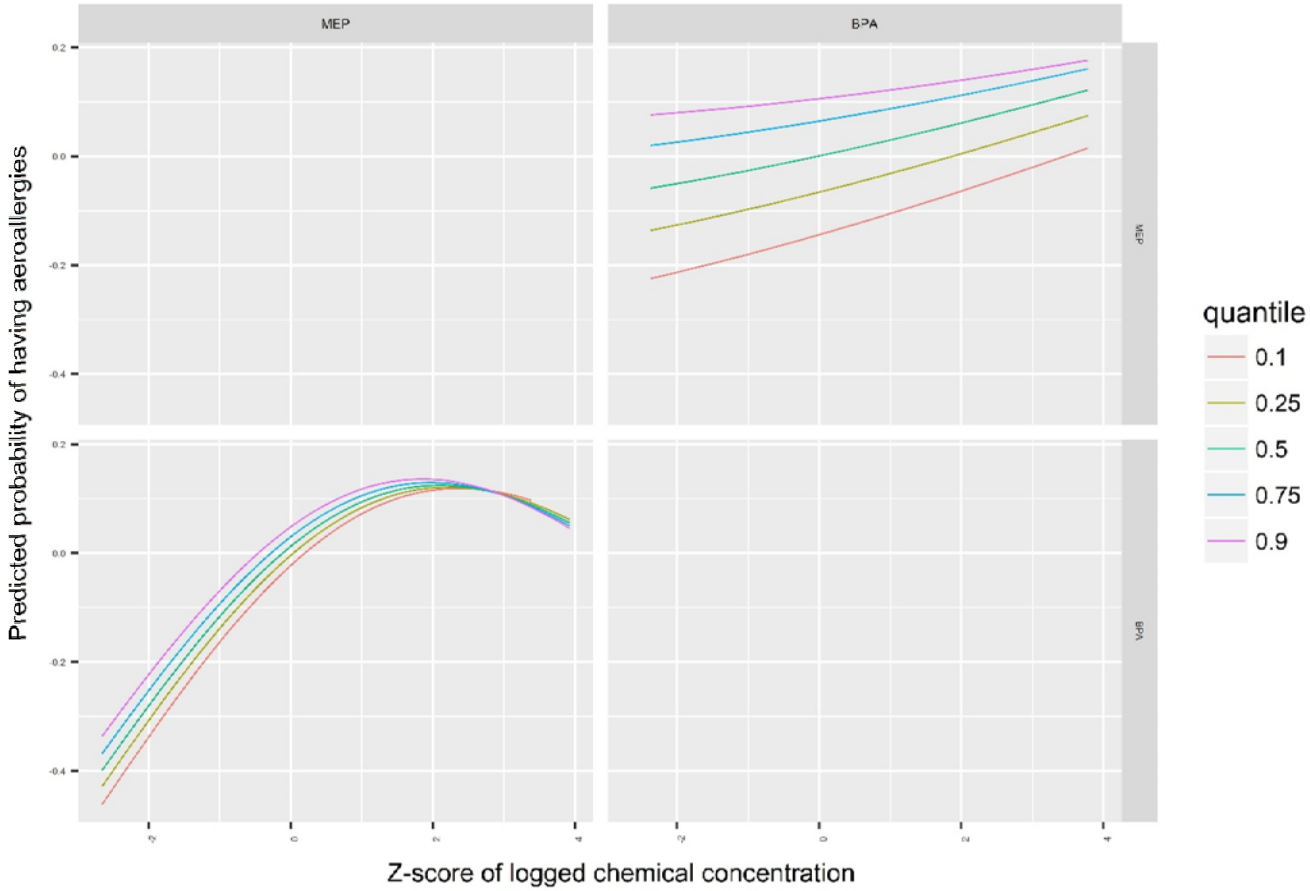
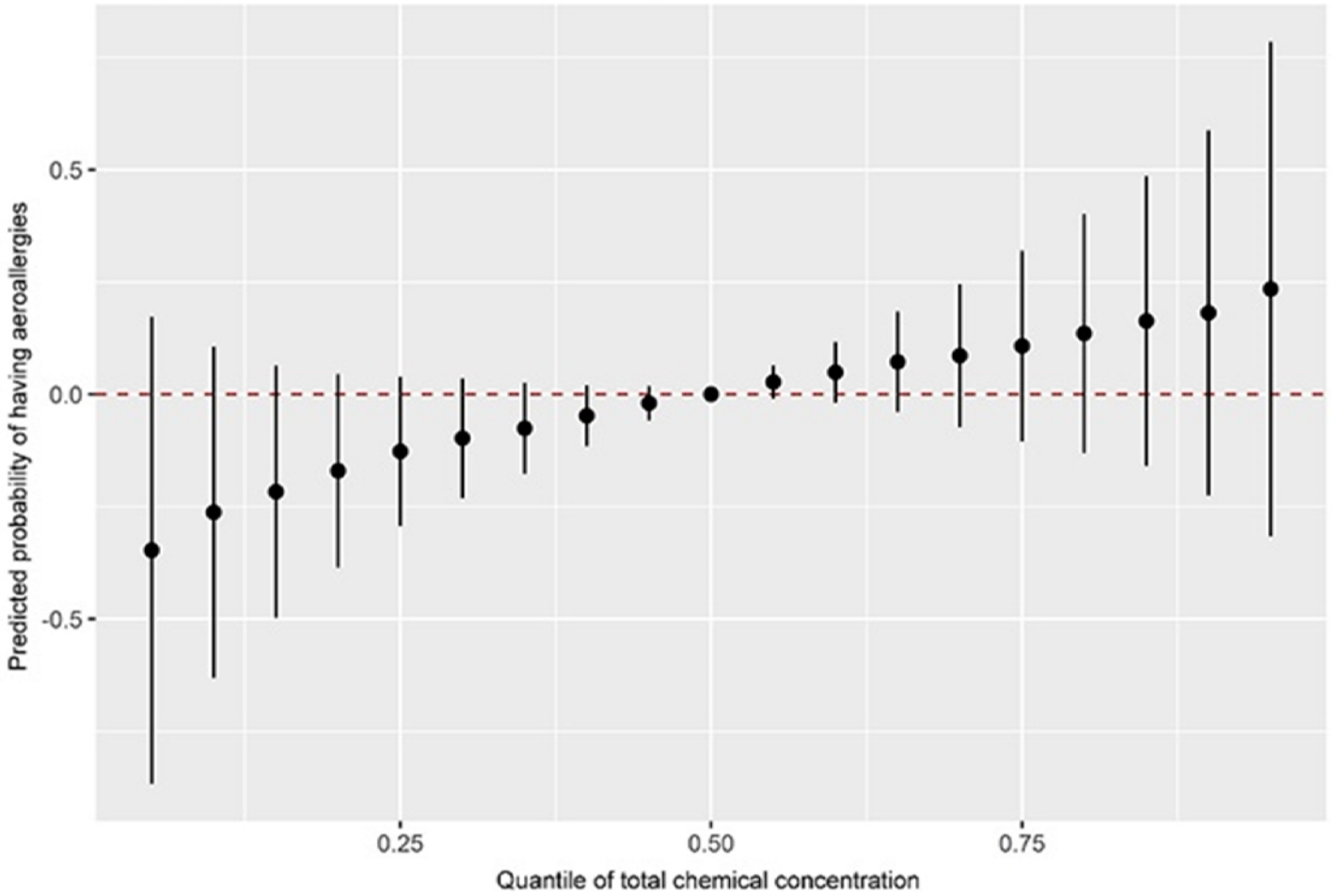


Figure 16. Predicted probability of having aeroallergies with increasing quantiles of total chemical concentration



Chapter 6: Conclusions

This dissertation aimed to quantify the relationship between *in utero* exposure to chemicals found in personal care products and plastics and several measures of childhood immune dysfunction, using multiple methods to account for joint exposure to similar chemicals. The phthalates, parabens, and other phenols analyzed in this study are used as fragrance compounds, preservatives, antimicrobials, and plasticizers in products that are widely used in the United States and globally.

One strength of this study is our classification of children as having probable asthma using comprehensive criteria based on data across childhood in order to minimize case misclassification. This is the first study to analyze the association between *in utero* exposure to these chemicals and cytokine levels in childhood, and it is the second study to examine associations between prenatal urinary concentrations of monocarboxyisooctyl phthalate (MCOP), monocarboxyisononyl phthalate (MCNP), mono(3-carboxypropyl) phthalate (MCPP) in relation to atopic diseases in childhood. While previous studies have examined associations between the other phthalates, parabens, and other phenols in this study with atopic outcomes, none have looked at atopic diseases and biomarkers of immune function together, which is a strength of our study.

All previous studies on these outcomes have examined associations with one chemical at a time; this is the first study to evaluate associations with mixtures of parabens and other phenols. Strengths of our study include using Bayesian Model Averaging (BMA) as a dimension reduction tool to select confounders from concurrent chemical exposures, and using Bayesian Kernel Machine Regression (BKMR) and Bayesian Profile Regression (BPR) to evaluate exposure to mixtures of phthalates, parabens, and other phenols.

Summary of findings and conclusions

Considering both the results of previous studies and the findings of this dissertation, *in utero* urinary concentrations of high molecular weight phthalates appear to be associated with asthma and adverse respiratory function in childhood, and possibly with aeroallergies in childhood. *In utero* urinary concentrations of low molecular weight phthalates appear to be associated with altered cytokine levels in early childhood, and concentrations of parabens and other phenols show limited or no association with respiratory or immune function in childhood. Expansion on these conclusions follows.

The association between monocarboxyisooctyl phthalate (MCOP, a metabolite of the high molecular weight di-isononyl phthalate which is used in PVC, sealants, and paints) and probable asthma and lower spirometry performance was consistent across models and was the most compelling finding of this dissertation. The sparse findings in cytokine associations suggest that MCOP may affect development of the respiratory system on a path independent of allergic/atopic biomechanisms. Although *in vitro* and animal studies provide evidence that high molecular weight phthalates may be involved with allergic/atopic immunity, there is also evidence that they may act on other mechanisms to affect respiratory function. For example, high molecular weight phthalates lead to proliferation and migration of bronchial smooth muscle cells *in vitro*[1] and are associated with increased fractional exhaled nitric oxide[2] and nuclear peroxisome proliferator–activated receptors[3] in humans (both associated with airway remodeling and asthma symptoms[2,4]). Additionally, in rats and mice, high molecular weight phthalates lead to increased airway hyperresponsiveness[5,6], increased airway wall thickness[5], decreased volume of inspired air[7], and increased respiratory rate[7]. With either mechanistic route, the ubiquitous nature of MCOP

exposure (detected in over 99% of participants in the 2013-2014 National Health and Nutrition Examination Survey[8]) could be significantly contributing to the high the prevalence of childhood asthma (15.9% of children according to the 2013-2014 National Health and Nutrition Examination Survey[9]).

The longitudinal association between mono-n-butyl phthalate (MBP, a metabolite of the low molecular weight dibutyl phthalate which is used in fragrances, cosmetics, and medications) and higher Th2% across childhood was also seen in individual time points at ages two and five, but not at age seven. The relative levels of cytokine-producing T cells are quite variable throughout childhood, and early childhood immune systems could be more susceptible to phthalate influence; this influence could become less effectual as children's immune systems continue to develop. MBP was not associated with probable asthma, aeroallergies, or eczema at age seven in this study, suggesting that early childhood changes in Th2% may not be associated with age seven atopic disease. Although associations between early childhood cytokine levels and later childhood atopy have not been studied, several previous studies report an association between cytokine levels and atopic illnesses at various ages[10-15]. Associations between *in utero* concentrations of low molecular weight phthalates and childhood cytokine levels have not been previously studied, so we cannot confirm if associations between them become null with increasing age in other cohorts.

Additionally, the unexpected protective findings between 2,5-dichlorophenol, propyl paraben, and probable asthma; and MBP, triclosan, and lung function are inconsistent with previous studies[16-20]. These unexpected findings persisted in BKMR univariate plots and are therefore not likely due to confounding by additional phthalate, paraben, or phenol exposure.

With the above findings, we created separate models for each chemical but used BMA to select five additional chemical covariates from the list of 15 phthalates, parabens, and other phenols in our study in order to control for exposure to multiple chemicals. Controlling for additional chemical exposure in regression models, while more comprehensive than approaches of previous studies, did not examine the effects of joint exposure to those chemicals. Because joint exposure to multiple chemicals may yield patterns of association different from the additive sum of single-chemical effects, we performed additional analyses to explore these joint effects.

BKMR analysis largely mimicked the results of linear and logistic regressions seen in chapters three and four, reinforcing its utility as a tool for quantifying associations between chemicals and health outcomes. Some unexpected findings from the linear and logistic regressions were attenuated in the BKMR univariate plots, suggesting that these findings may have been due to chemical confounding beyond what was controlled for using BMA. In data sets with a large number of possible chemical confounders, BKMR can be used to assess the robustness of single-chemical findings from traditional regressions when put in context of additional chemical exposures.

For the data in this study, the overall summaries plots outputted by the BKMR were not particularly useful, with the possible exception of the aeroallergy plot, because the included chemical covariates were associated with the outcomes in inconsistent directions. The overall summaries plot attempts to visualize how risk for an outcome changes with increasing total exposure to chemical covariates and would be useful in data where each chemical were associated with an outcome in the same direction (*e.g.* all associated with increased risk or null). The bivariate BKMR plots, however, provided useful information on how associations of one chemical changed in the presence of differing levels of a second chemical and functioned as an exploratory tool that lead to additional, more precise interaction analyses.

In addition to BKMR's chemical mixture analysis, BPR provided information on how participants clustered into discrete groups of joint exposure. We were able to identify a group with relatively high overall exposure, a group with relatively low overall exposure, and groups specifically with high personal care product exposure or high plasticizer exposure. We were additionally able to identify if these groups differed by any of the outcome measures which, although on a less nuanced scale than regression results from chapters three and four, is valuable information on whether or not the exposure clustering (*i.e.* real-world exposure patterns) is relevant to outcome risk. Although we did not find any significant associations between cluster assignment and disease risk, this may be due to decreased power from reduced sample sizes within each cluster. Regardless of associations with atopic diseases, the clustering itself is an interesting component of looking at participant exposure. Compared to other methods of clustering or data reduction, such as k-means clustering, principle components analysis, or factor analysis, BPR is a model averaging approach that, after thousands of iterative runs, yields higher standard errors with inconsistent clustering and smaller standard errors with consistent clustering. Compared to other clustering methods, it is also data-driven, with the analyst making few decisions that affect the modeling, and the number of clusters and which participants are in them is allowed to vary over iterations.

The CHAMACOS cohort is unique in the large number of chemicals measured from biological samples or estimated using geographical data. Most studies do not have the luxury of controlling for additional chemical covariates or evaluating exposure to chemical mixtures. However, as seen when comparing fully adjusted models to demographically adjusted models in this study, model adjustment for multiple chemical exposure can impact significance and magnitude of regression results. It is possible that results from previous studies on this subject and others are incomplete or inaccurate because they were unable to take into account additional chemical exposures.

Future research directions

Several of the associations evaluated in this study have not been sufficiently examined and should therefore be analyzed in other populations to assess reproducibility, especially the unexpected protective findings discussed above. Future research should also measure IgE in children along with asthma status in order to distinguish atopic versus non-atopic asthma and any associations with prenatal chemical exposure that are differential by this distinction. In addition, other classes of CD4+ cells such as Th17 cells and regulatory T cells should be evaluated for their associations with prenatal exposure to the chemicals in this study to further characterize the behavior of the childhood immune system in conjunction with these chemicals. Finally, future studies should take multiple chemical exposure into consideration by prioritizing measurement of multiple biomarkers and using methods like BMA, BPR, and BKMR to evaluate the effects of joint chemical exposure.

References

1. Kuo P-L, Hsu Y-L, Huang M-S, Tsai M-J, Ko Y-C. Ginger suppresses phthalate ester-induced airway remodeling. *Journal of agricultural and food chemistry* 2011;59(7):3429-3438.
2. Just AC, Whyatt RM, Miller RL, Rundle AG, Chen Q, Calafat AM, Divjan A, Rosa MJ, Zhang H, Perera FP. Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort. *American journal of respiratory and critical care medicine* 2012;186(9):830-837.
3. Hurst CH, Waxman DJ. Activation of PPARα and PPARγ by environmental phthalate monoesters. *Toxicological Sciences* 2003;74(2):297-308.
4. Benayoun L, Letuve S, Druilhe A, Boczkowski J, Dombret M-C, Mechighel P, Megret J, Leseche G, Aubier M, Pretolani M. Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling. *American journal of respiratory and critical care medicine* 2001;164(8):1487-1494.
5. Yang G, Qiao Y, Li B, Yang J, Liu D, Yao H, Xu D, Yang X. Adjuvant effect of di-(2-ethylhexyl) phthalate on asthma-like pathological changes in ovalbumin-immunised rats. *Food and agricultural immunology* 2008;19(4):351-362.
6. Chen L, Chen J, Xie CM, Zhao Y, Wang X, Zhang YH. Maternal Disononyl Phthalate Exposure Activates Allergic Airway Inflammation via Stimulating the Phosphoinositide 3-kinase/Akt Pathway in Rat Pups. *Biomedical and Environmental Sciences* 2015;28(3):190-198.
7. Larsen ST, Hansen JS, Hansen EW, Clausen PA, Nielsen GD. Airway inflammation and adjuvant effect after repeated airborne exposures to di-(2-ethylhexyl) phthalate and ovalbumin in BALB/c mice. *Toxicology* 2007;235(1):119-129.
8. CDC. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables (February, 2017). *Atlanta, GA, USA: Department of Health and Human Services, Centers for Disease Control and Prevention* 2017.
9. CDC. National Health and Nutrition Examination Survey Data. *U.S. Department of Health and Human Services* 2013-2014.
10. Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy, and asthma. *Current opinion in allergy and clinical immunology* 2005;5(2):161-166.
11. Sly PD, Boner AL, Björksten B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ. Early identification of atopy in the prediction of persistent asthma in children. *The Lancet* 2008;372(9643):1100-1106.
12. Black S, Teixeira A, Loh A, Vinall L, Holloway J, Hardy R, Swallow D. Contribution of functional variation in the IL13 gene to allergy, hay fever and asthma in the NSHD longitudinal 1946 birth cohort. *Allergy* 2009;64(8):1172-1178.
13. Robinson D, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley A, Corrigan C, Durham S, Kay A. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *NEJM* 1992;326(5):298-304.
14. Steinke JW, Borish L. Th2 cytokines and asthma—Interleukin-4: its role in the pathogenesis of asthma, and targeting it for asthma treatment with interleukin-4 receptor antagonists. *Respiratory research* 2001;2(2):66.
15. Greenfeder S, Umland SP, Cuss FM, Chapman RW, Egan RW. Th2 cytokines and asthma The role of interleukin-5 in allergic eosinophilic disease. *Respiratory Research* 2001;2(2):71.

16. Whyatt RM, Perzanowski MS, Just AC, Rundle AG, Donohue KM, Calafat AM, Hoepner LA, Perera FP, Miller RL. Asthma in inner-city children at 5–11 years of age and prenatal exposure to phthalates: the Columbia Center for Children's Environmental Health Cohort. 2014.
17. Cakmak S, Dales RE, Hebbern C, Saravanabhavan G. The association between urinary phthalates and lung function. *Journal of occupational and environmental medicine* 2014;56(4):376.
18. Buckley JP, Quirós-Alcalá L, Teitelbaum SL, Calafat AM, Wolff MS, Engel SM. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7 years. *Environment international* 2018;115:79-88.
19. Lee-Sarwar K, Hauser R, Calafat AM, Ye X, O'Connor GT, Sandel M, Bacharier LB, Zeiger RS, Laranjo N, Gold DR. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. *Journal of Allergy and Clinical Immunology* 2017.
20. Vernet C, Pin I, Giorgis-Allemand L, Philippat C, Benmerad M, Quentin J, Calafat A, Ye X, Annesi-Maesano I, Siroux V. In Utero Exposure to Select Phenols and Phthalates and Respiratory Health in Five-Year-Old Boys: A Prospective Study. *Environmental Health Perspectives* 2017;125(9):097006.