Environmental Research 162 (2018) 280–286

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Endotoxin enhances respiratory effects of phthalates in adults: Results from NHANES 2005-6

Paula D. Strassle[a], Lidwien A.M. Smit[b], Jane A. Hoppin[c,d,*]

[a] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
[b] Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands
[c] Center for Human Health and the Environment, North Carolina State University, Campus Box 7633, Raleigh 27695-7633, NC, United States
[d] Department of Biological Sciences, North Carolina State University, Raleigh, NC, United States

ARTICLE INFO

Keywords:
Allergy
Asthma
Wheeze
Dust endotoxin
Phthalates

ABSTRACT

Phthalates have been associated with respiratory symptoms in adults; they may enhance effects of inflammatory compounds. To assess the potential interactions of phthalates and endotoxin on respiratory and allergic symptoms in adults, we used cross-sectional information from the 1091 adults with complete data on urinary phthalates and house dust endotoxin from NHANES 2005–2006. We used multivariable logistic regression to assess whether endotoxin levels modified the association between nine phthalate metabolites and four current allergic symptoms (asthma, wheeze, hay fever, and rhinitis). Endotoxin was classified into tertiles (< 10, 10–25, ≥ 25 EU/mg dust). Urinary phthalate and dust endotoxin levels were not correlated ($r < |0.02|$). Under low endotoxin conditions, no associations between phthalates and respiratory outcomes were observed. Under medium or high endotoxin conditions, exposure-response relationships were observed between specific phthalates and wheeze and asthma. For wheeze, three phthalates (mono-benzyl phthalate (MBzP), mono(carboxyoctyl) phthalate (MCOP), and di-ethylhexyl phthalate (DEHP) had significant interactions with endotoxin); for asthma, two phthalates (MCOP and mono(carboxyoctyl) phthalate (MCNP)) had significant interactions. Endotoxin did not modify the associations between phthalates and hay fever or rhinitis. These results are consistent with the hypothesis that endotoxin enhances the respiratory toxicity of phthalates; however this cross-sectional study cannot address key temporal issues. The lack of an association between wheeze or asthma and phthalates when endotoxin exposure was low suggests that phthalates alone may not increase these symptoms.

1. Introduction

Phthalates are a class of chemicals commonly found in cosmetics, personal care products, plastics, and food packaging (Hauser and Calafat, 2005). Exposure to phthalates is ubiquitous and poorly characterized. While the relationship between phthalate exposure and health outcomes are not fully understood, recent studies in adults from multiple countries have shown that higher levels of phthalates are associated with increased prevalence of asthma and allergy (Hoppin et al., 2013; Jaakkola et al., 2006; Jaakkola and Knight, 2008; Kimber and Dearman, 2010; North et al., 2014). A recent review by North (North et al., 2014) concluded "Despite mounting evidence implicating phthalates, causation of allergic disease by these compounds cannot currently be established. Another review by Robinson and Miller (Robinson and Miller, 2015) concluded "Emerging science indicates that deleterious immunologic changes, including increased propensity to develop wheeze, allergy, and asthma after dietary and inhalation exposure to these chemicals, may be occurring." Understanding the mechanisms by which phthalates may contribute to allergy and asthma is an active area of research. A proposed mechanism is that phthalates may act as adjuvants and exacerbate allergic symptoms through either allergenic or inflammatory mechanisms (North et al., 2014). Exposure to diethylhexylphthalate (DEHP) enhances inflammatory responses in rats in a model of allergic airway response (Guo et al., 2012). In vitro studies have shown that phthalates (di-ethyl and di-butyl phthalates) influence human innate and adaptive immunity as measured by enhanced cytokine response to endotoxin (Hansen et al., 2015). One common inflammatory agent associated with wheeze and other asthma symptoms in human populations is endotoxin (Smit et al., 2008; Thorne et al., 2005, 2015; Mendell et al., 2011).

In previous work, we showed that specific phthalate metabolites were associated with respiratory and allergic symptoms in adults; at that time we lacked information on endotoxin to examine whether endotoxin influenced the impact of phthalates on respiratory and

* Corresponding author at: Center for Human Health and the Environment, North Carolina State University, Campus Box 7633, Raleigh 27695-7633, NC, United States.
E-mail address: jahoppin@ncsu.edu (J.A. Hoppin).

https://doi.org/10.1016/j.envres.2018.01.017
Received 8 August 2017; Received in revised form 6 October 2017; Accepted 16 January 2018
Available online 04 February 2018
0013-9351/

allergic outcomes (Hoppin et al., 2013). Since then, the National Health and Nutrition Examination Survey (NHANES) 2005 – 2006 has released the data on endotoxin and allergens in dust providing the opportunity to explore this hypothesis using the same data set. Other investigators have shown an association between endotoxin and wheeze, independent of allergic sensitization status in the whole NHANES 2005–2006 sample; no association was seen for current asthma (Thorne et al., 2015). To expand on both our earlier work and these other findings from NHANES 2005–2006, here we explore whether endotoxin levels in the home modify the association between phthalates and respiratory symptoms in adults.

2. Materials and methods

We used publicly available data from the NHANES 2005–2006 to assess the potential interaction between phthalates and endotoxin on allergic and respiratory symptoms in adults (≥18 years old) (CDC, 2015a). This dataset is the only recent NHANES dataset to include the respiratory and allergic outcome questions along with endotoxin measurement. Analysis was limited to adults because our earlier findings for phthalates were stronger in adults and the sample size for adults was larger to assess potential interactions. NHANES participants provided informed consent and were assured that data collected will be used only for stated purposes. This analysis used the publicly available, de-identified data.

Self-reported current respiratory and allergic symptoms (asthma, hay fever, rhinitis, and wheeze), defined as symptoms occurring within the last 12 months, were obtained from the self-administered questionnaire during the clinic visit. In the NHANES data set, wheeze is defined as any episode of wheezing or whistling in the chest in the past year, while current asthma is defined based on both a doctor diagnosis of asthma and experiencing symptoms in the past year. Covariate data were obtained through either the questionnaire (age, race/ethnicity, and gender) or measured (body mass index [BMI], urinary creatinine, and cotinine).

Spot urine samples were analyzed for 15 phthalate metabolites using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (CDC, 2015b). Samples below the limit of detection (LOD) were assigned a value of the LOD divided by the square root of 2. A summary DEHP variable was created by summing the concentrations (ng/mL) of the DEHP metabolites: mono-(2-ethyl)-hexyl phthalate, mono-2-ethyl-5-carboxypentyl phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, and mono-(2-ethyl-5-oxohexyl) phthalate.

Endotoxin was measured in the combined dust from the participant's bed and bedroom floor (Thorne et al., 2015). On average, dust samples were collected in participants' homes within seven days of the clinic visit. Dust endotoxin was measured using a *Limulus* amebocyte lysate assay (CDC, 2015c).

Multivariable logistic regression was used to model the potential interaction between each phthalate and dust endotoxin exposure on respiratory and allergic outcomes. Each phthalate was modeled individually. Because we were interested in whether endotoxin modified the association of phthalates, we modeled phthalate concentrations as a continuous linear variable ($\log_{10}$-transformed) and endotoxin was categorized into tertiles (low: < 10 EU/mg, medium: 10–25 EU/mg, and high: ≥25 EU/mg). All phthalates detected in ≥50% of the study population were included. Models were adjusted for the same covariates previously (Hoppin et al., 2013) included: age (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, and other), gender, creatinine ($\log_{10}$-transformed, continuous), cotinine (< LOD, 0.015–10.0 ng/mL, ≥10 ng/mL) (Pirkle et al., 1996), and BMI (< 25, 25–30, ≥30) (Hoppin et al., 2013). Participants missing information on any of these variables were excluded. We tested for interactions using an overall difference in slope test (Wald test). We also assessed interactions between phthalates and total dust weight and sieved dust weight to provide evidence that the associations observed were from the endotoxin present in the dust and not the dust itself. For comparison purposes, we also present the main effects of phthalates without endotoxin in each table, as the sample size differed from our earlier analysis. All analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). We regarded $p < 0.05$ as statistically significant and $p < 0.1$ as borderline significant for interactions.

Table 1
Demographic, medical, and allergic characteristics for the adults (≥18 years old) with complete demographic, urinary phthalate metabolite and household dust data, NHANES 2005–2006, n = 1091.

Age, in years, mean (SD)	44.5 (19.7)
Race/ethnicity, n (%)	
Non-Hispanic White	501 (45.9)
Non-Hispanic Black	278 (25.5)
Mexican American	239 (21.9)
Other	73 (6.7)
Gender, n (%)	
Female	544 (49.9)
Male	547 (50.1)
Cotinine, ng/mL, n (%)	
< LOD (< 0.015)	174 (16.0)
Low (0.015–10.0)	609 (55.8)
High (≥ 10.0)	308 (28.2)
Creatinine, mg/dL, mean (SD)	136.5 (77.6)
BMI[a], n (%)	
Underweight/normal	349 (32.0)
Overweight	356 (32.6)
Obese	386 (35.4)
Current allergic conditions[b], n (%)	
Asthma	90 (8.3)
Hay fever	55 (5.0)
Rhinitis	335 (30.7)
Wheeze	160 (14.7)
Dust endotoxin[c], EU/mg, n (%)	
Low (< 10)	364 (33.4)
Medium (10–25)	352 (32.3)
High (≥ 25)	375 (34.4)

SD: standard deviation; LOD: limit of detection.
Participants with missing sIgE information were excluded, n = 1.
[a] Adult BMI was classified using calculated BMI as underweight/normal (< 25), overweight (25–30), and obese (≥30).
[b] Self-reported symptoms for the past 12 months; asthma and hay fever were assessed only among individuals reporting ever having a doctor diagnosis.
[c] Endotoxin levels below the LOD (0.00034 EU/g dust) were recorded as LOD/√2, n = 2.

3. Results

A total of 1091 adults had complete data on phthalates, dust endotoxin levels, and potential confounders, and had not moved between the clinic visit and dust collection. Rhinitis was the most commonly reported symptom (30.7%); 14.7% of participants reported wheeze in the past year and 8.3% reported current asthma (Table 1). Nine different phthalates were detected in ≥50% of our sample (Table 2); endotoxin was detected in all but two homes. There was no correlation between urinary phthalates and dust endotoxin levels (See Supplemental Figure 1).

For both wheeze and asthma, we observed significant interactions between phthalates and endotoxin (Figs. 1 and 2). For wheeze (Table 3), three phthalates [mono-n-butyl (MnBP), mono-carboxyoctyl (MCOP), and DEHP] had significant interactions; an additional three phthalates mono-isobutyl (MiBP), monoethyl (MEP), and monobenzyl (MBzP)] had borderline significant interactions ($0.05 < p < 0.10$). The odds ratios for all six of these phthalates showed evidence of a monotonic increase with increasing phthalate concentration, with the largest association between phthalates and wheeze observed in the highest category of endotoxin. While we observed significant interactions between endotoxin and specific phthalates for wheeze, few of the individual odds ratios had 95% confidence intervals that excluded the

Table 2
Distribution of urinary phthalate metabolites (ng/mL) among adults (≥18 years old) with endotoxin data, NHANES 2005–2006.

	LOD	> LOD, n (%)	GM (GSE)[a]	5th	25th	50th	75th	95th
Low Molecular-weight (LMW)								
Mono-isobutyl phthalate (MiBP)	0.3	1062 (97.3)	5.36 (0.20)	0.50	2.60	6.10	11.90	33.10
Mono-*n*-butyl phthalate (MnBP)	0.6	1089 (99.8)	20.68 (0.67)	3.20	11.10	22.50	41.2	112.7
Mono-ethyl phthalate (MEP)	0.5	1086 (99.5)	142.41 (6.58)	14.06	50.69	135.89	387.62	1911.2
Mono-*n*-methyl phthalate (MMP)	1.1	418 (38.3)	1.52 (0.05)	< LOD	< LOD	< LOD	2.70	12.50
High Molecular-weight (HMW)								
Monobenzyl phthalate (MBzP)	0.2	1077 (98.7)	8.53 (0.34)	0.79	4.10	9.50	19.80	67.61
Mono(carboxyoctyl) phthalate (MCOP)	0.7	1043 (95.6)	5.03 (0.19)	0.70	2.30	4.60	9.90	44.80
Mono(carboxynonyl) phthalate (MCNP)	0.6	981 (89.9)	2.60 (0.08)	< LOD	1.40	2.60	4.70	14.60
Mono-(3-carboxypropyl) phthalate (MCPP)	0.2	1052 (96.4)	1.89 (0.06)	0.30	1.00	2.00	3.90	11.60
Mono-cyclohexyl phthalate (MCHP)	0.6	22 (2.0)	0.44 (0.00)	< LOD	< LOD	< LOD	< LOD	< LOD
Mono-isononyl phthalate (MINP)	1.2	145 (13.3)	1.06 (0.02)	< LOD	< LOD	< LOD	< LOD	4.00
Mono-*n*-octyl phthalate (MOP)	1.8	10 (0.9)	1.32 (0.00)	< LOD	< LOD	< LOD	< LOD	< LOD
Diethylhexyl phthalate (DEHP)			83.72 (3.34)	11.85	34.30	74.95	177.10	907.00
Mono-(2-ethyl)-hexyl phthalate	1.2	739 (67.7)	3.20 (0.13)	< LOD	< LOD	2.70	7.00	41.50
Mono-2-ethyl-5-carboxypentyl phthalate	0.6	1090 (99.9)	37.15 (1.47)	5.20	15.90	32.70	76.80	385.60
Mono-(2-ethyl-5-hydroxyhexyl) phthalate	0.7	1088 (99.7)	24.77 (1.05)	3.00	9.80	22.40	54.30	302.40
Mono-(2-ethyl-5-oxohexyl) phthalate	0.7	1081 (99.1)	15.86 (0.66)	1.90	6.50	14.40	36.30	181.70

LOD: limit of detection; GM: geometric mean; GSE: geometric standard error.
[a] Phthalate levels below the LOD were assigned values of LOD/√2; these imputed values were used in the calculation of GM (GSE).

null value. Both MBzP (Odds Ratio (OR) =1.79, 95% Confidence Interval (CI)= 1.13, 2.81) and DEHP (OR=1.47, 95%CI=1.01, 2.15) had significant odds ratios for a one unit change in phthalate concentration for wheeze in the highest category of endotoxin. For asthma (Table 4), MCOP and mono-(carboxylnonyl) phthalate (MCNP) had significant interactions with endotoxin and MiBP, MnBP, MEP, and DEHP had borderline interactions with endotoxin, but none of these showed the largest odds ratio in the highest category of endotoxin. For asthma, the largest ORs were associated with the medium endotoxin level. We saw no interaction between endotoxin level and any phthalates with rhinitis or hay fever (Supplemental Tables 1 and 2). Additionally, there was no evidence of an interaction of phthalates with total dust concentration or sieved dust concentration (data not shown).

4. Discussion

Overall, home endotoxin level modified the association between specific urinary phthalates and respiratory symptoms wheeze and asthma, but not for current rhinitis or hay fever. For wheeze, the phthalates, MnBP, MCOP and DEHP had significant interactions with endotoxin; while the 95% CI for MBzP and DEHP excluded the null value in the highest category for endotoxin. For current asthma, the phthalates MCOP and MCNP had significant interactions with endotoxin while only mono-(3-carboxypropyl) phthalate had a 95% CI that excluded the null value at the highest level of endotoxin. There was no association between wheezing and increasing phthalate concentrations when endotoxin exposure was low, suggesting that phthalates may not increase the prevalence of wheeze or asthma on their own. The interaction between phthalates and endotoxin was limited to respiratory symptoms. Although rhinitis was the most prevalent symptom in our study (31%) and was well powered to see associations, we did not observe any interactions with this outcome.

Endotoxin is a prototypical inflammatory agent and is associated with the development of a variety of inflammatory diseases (Radon, 2006). In an earlier analysis from the NHANES 2005-6 data, endotoxin was associated with wheeze among all 6963 NHANES participants and this association persisted when controlling for confounders and was unrelated to allergic sensitization status (Thorne et al., 2015). However, current asthma was not associated with endotoxin, except among non-allergen sensitized individuals (Thorne et al., 2015). In our study, current asthma was related to phthalate exposure only in homes with medium or high endotoxin present; this may suggest that endotoxin alone is not associated with current asthma, but as part of an interaction between other environmental exposures including phthalates. We did not see an association with endotoxin alone in our reduced dataset.

Phthalates as a class are believed to modulate immune responses in vitro and in vivo, but the mechanisms are not well understood (Kimber and Dearman, 2010; Robinson and Miller, 2015). Additionally, few experimental studies have assessed all the phthalates evaluated here or in other human studies. While no whole animal studies have evaluated the interaction between phthalates and endotoxin, some in vitro studies of lipopolysaccharide-stimulated macrophages showed inhibition of TNF-alpha responses with DBP exposure (Hansen et al., 2015; Kim et al., 2015) and increases in IL-10 for diethyl phthalate (DEP) and dibutyl phthalate (DBP) (Hansen et al., 2015). Other studies have evaluated the association of specific phthalates on allergic mechanisms. DINP has been shown to suppress Th1 cell formation and enhance Th2 cell formation in vitro and to induce allergic asthma in mice as a result of IL-4, IL-5, and IgE production (Hwang et al., 2017). DBP *h*as been used as a sensitizing agent in studies of Th2 contact hypersensitivity responses (Larson et al., 2010). In weanling mice exposed to DEHP, DEHP acted as an adjuvant to enhance IgE and IgG1 production (Han et al., 2014). DEHP also was shown to enhance ovalbumin response as measured by IL-3 in the nasal cavity of mice (He et al., 2013). Larsen and colleagues demonstrated that longer chain phthalates (e.g., di-*n*-octyl phthalate) were more likely to have adjuvant effects than shorter chain (e.g., DBP) (Larsen et al., 2002). DEHP can influence the human nasal immune response in allergen-sensitized individuals. In human volunteers (16 with allergy and 16 without), nasal exposure to house dust and DEHP did not increase allergic symptom scores, but did influence protein expression in the nasal mucosa of allergen-sensitized individuals (Deutschle et al., 2008). In allergen-sensitized individuals, low DEHP levels in house dust were associated with increased granulocyte colony stimulating factor (G-CSF), interleukin-5 (IL-5) and IL-6; while high levels of DEHP in dust were associated with lower G-CSF and IL-6 (Deutschle et al., 2008).

The association of phthalates and allergy has been studied previously in both adults and children (Jaakkola and Knight, 2008; North et al., 2014; Robinson and Miller, 2015), though none of these studies have considered the interaction with other environmental exposures which might influence the impact of phthalates. In Taiwan, researchers evaluated environmental exposures including phthalates, endotoxin, and glucans and respiratory and allergic outcomes in 101 children aged 3–9 years (Hsu et al., 2012). In this study, case status was associated both with butyl benzyl phthalate (BBzP) and fungal glucan, though glucan was non-significant when included together in the model with

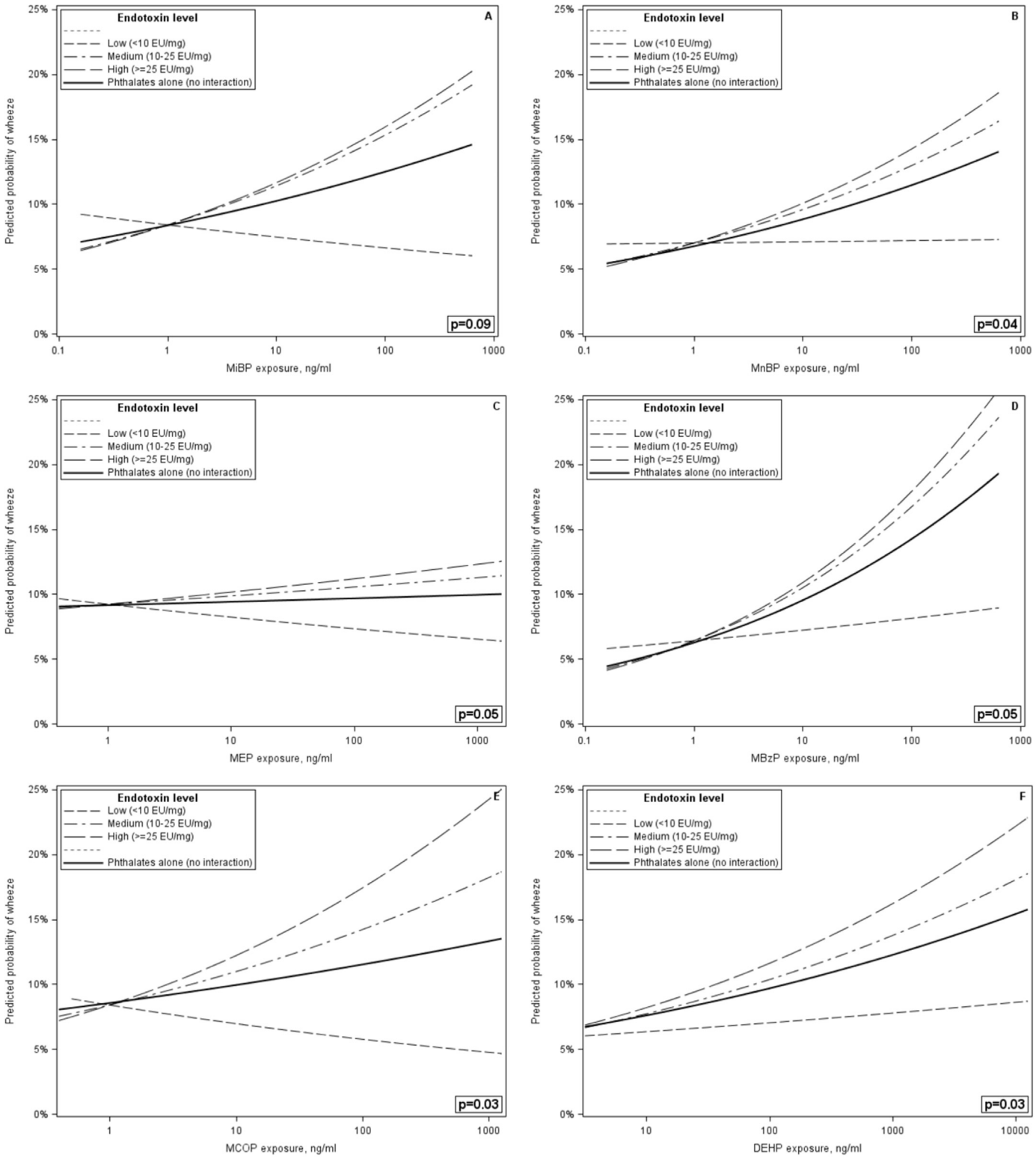

Fig. 1. Predicted probability of wheezing among adults across $\log_{10}$-transformed mono-isobutyl (A), mono-*n*-butyl (B), mono-ethyl (C), monobenzyl (D), mono(carboxyoctyl) (E), and DEHP (F) phthalate concentrations, stratified by endotoxin exposure, and controlling for age, gender, race/ethnicity, BMI, creatinine, and cotinine levels.

BBzP; the authors did not evaluate interactions.

This cross-sectional study benefitted from a large sample with data on biological levels of phthalates, house dust measures of endotoxin, as well as detailed and consistent medical information. Our study assumes a constant exposure for phthalate and endotoxin exposure across the past 12 months (the time period in which symptoms were measured), which may not accurately reflect the level of exposure during this time. Urinary phthalate concentrations are good markers of recent exposure and integrate over all sources of exposure, however, because exposures can vary day-to-day and phthalate metabolites have half-lives less than one day, biomarker levels are not consistent over time (Hauser et al., 2004; Hoppin et al., 2002; Teitelbaum et al., 2008). Endotoxin was measured in house dust, and while it is believed to be the most important source of endotoxin exposure for most individuals (Thorne

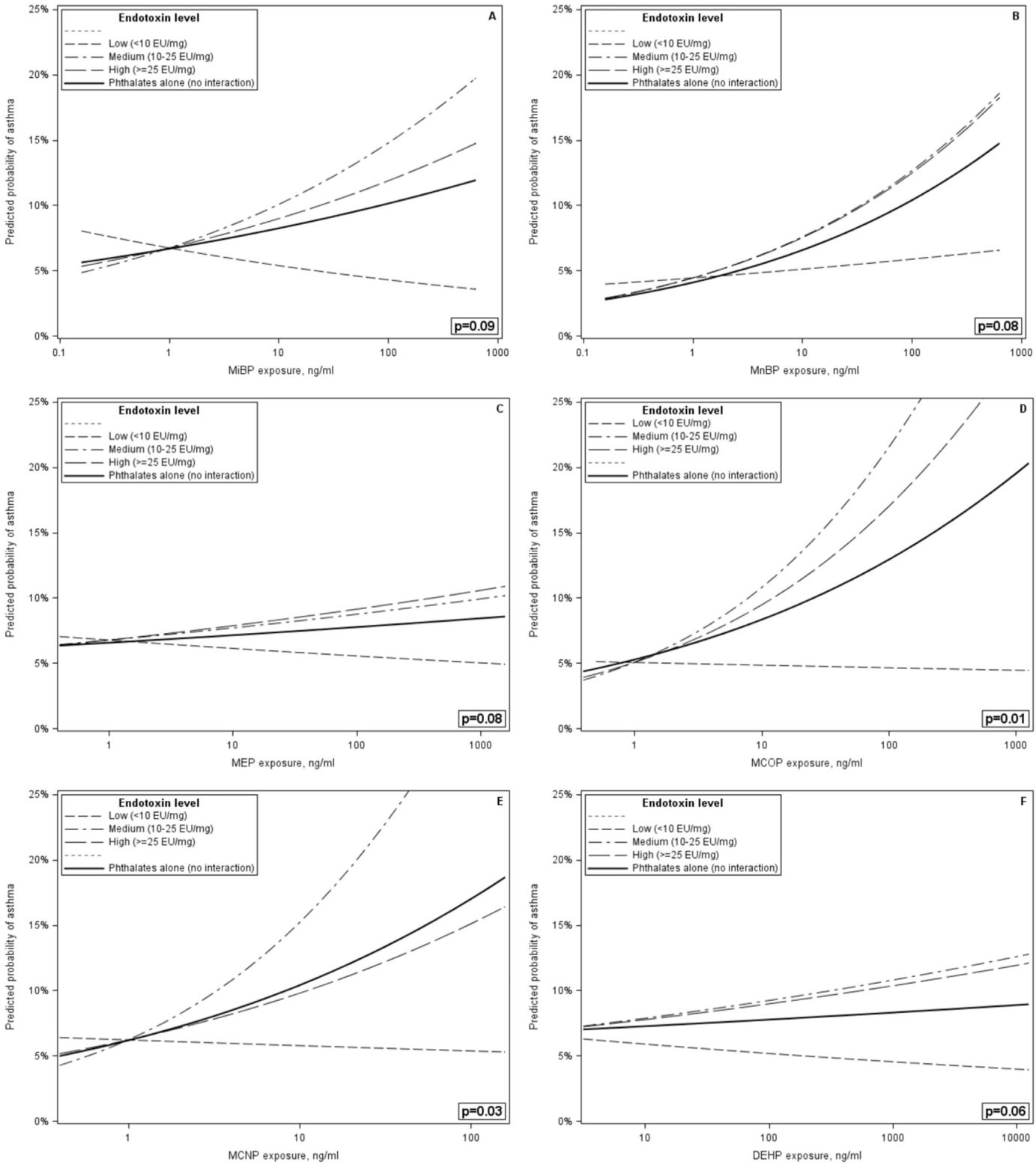

Fig. 2. Predicted probability of asthma among adults across $\log_{10}$-transformed mono-isobutyl (A), mono-*n*-butyl (B), mono-ethyl (C), mono(carboxyoctyl) (D), mono(carboxynonyl) (E), and DEHP (F) phthalate concentrations, stratified by endotoxin exposure, and controlling for age, gender, race/ethnicity, BMI, creatinine, and cotinine levels.

et al., 2005, 2015, 2009; Waser et al., 2004), home endotoxin levels may not convey a complete picture of endotoxin exposure for each individual and thus we may have some exposure misclassification for individuals who have endotoxin exposures in other settings. Moreover, endotoxin levels in dust can be affected by multiple household factors, including animals in the home, which can change over time (Chen et al., 2012; Sordillo et al., 2011). However, Abraham and colleagues have shown that within home variation of endotoxin is much less than between home variation, suggesting that use of a single measurement is appropriate (Abraham et al., 2005).

We relied on self-reported symptom information which may not be an accurate reflection of an individual's actual symptoms; however, the

Table 3
Adjusted odds ratios (ORs [95% Confidence Interval])[a] for wheeze associated with a 1-unit increase in the $\log_{10}$-transformed phthalate concentrations, both unadjusted for endotoxin and with an interaction term with endotoxin level, 1091 adults (≥18 years old), NHANES 2005–2006.

	Phthalates Alone	Low Endotoxin	Medium Endotoxin	High Endotoxin	Interaction p-value[b]
Low Molecular-weight (LMW)					
Mono-isobutyl phthalate	1.25 (0.79, 1.98)	0.88 (0.50, 1.54)	1.41 (0.83, 2.37)	1.44 (0.87, 2.39)	*0.09*
Mono-*n*-butyl phthalate	1.34 (0.80, 2.23)	1.01 (0.57, 1.78)	1.41 (0.83, 2.40)	1.49 (0.87, 2.54)	**0.04**
Mono-ethyl phthalate	1.03 (0.77, 1.38)	0.88 (0.64, 1.22)	1.08 (0.79, 1.46)	1.11 (0.81, 1.53)	*0.05*
High Molecular-weight (HMW)					
Monobenzyl phthalate	1.58 (1.04, 2.39)	1.14 (0.69, 1.88)	1.71 (1.09, 2.70)	1.79 (1.13, 2.81)	*0.05*
Mono(carboxyoctyl) phthalate	1.18 (0.82, 1.71)	0.82 (0.50, 1.33)	1.35 (0.85, 2.14)	1.52 (0.98, 2.36)	**0.03**
Mono(carboxynonyl) phthalate	1.17 (0.75, 1.82)	0.79 (0.42, 1.48)	1.34 (0.76, 2.37)	1.42 (0.81, 2.51)	0.20
Mono-(3-carboxypropyl) phthalate	1.87 (1.18, 2.96)	1.46 (0.76, 2.82)	2.35 (1.31, 4.24)	1.80 (0.97, 3.35)	0.42
Diethylhexyl phthalate[c]	1.30 (0.91, 1.86)	1.12 (0.76, 1.64)	1.38 (0.95, 2.01)	1.47 (1.01, 2.15)	**0.03**

Significant p-values ($p < 0.05$) are denoted in **bold**, borderline significant p-values ($p < 0.1$) are denoted in *italics*.
[a] Adjusted for age, gender, race/ethnicity, BMI, creatinine, and cotinine.
[b] Tests of equivalent linear slopes were conducted for each phthalate while controlling for age, gender, race/ethnicity, BMI, creatinine, and cotinine, df = 2.
[c] Sum of all DEHP metabolites.

Table 4
Adjusted odds ratios (ORs [95% CI])[a] for current asthma associated with a 1-unit increase in the $\log_{10}$-transformed phthalate concentrations both unadjusted for endotoxin and with an interaction term with endotoxin level, 1091 adults (≥18 years old), NHANES 2005–2006.

	Phthalates Alone	Low Endotoxin	Medium Endotoxin	High Endotoxin	Interacation p-value[b]
Low Molecular-weight (LMW)					
Mono-isobutyl phthalate	1.26 (0.70, 2.24)	0.79 (0.38, 1.65)	1.55 (0.82, 2.94)	1.37 (0.72, 2.59)	*0.09*
Mono-*n*-butyl phthalate	1.65 (0.87, 3.12)	1.16 (0.57, 2.37)	1.77 (0.92, 3.39)	1.75 (0.90, 3.42)	*0.08*
Mono-ethyl phthalate	1.09 (0.76, 1.58)	0.90 (0.59, 1.36)	1.15 (0.79, 1.68)	1.18 (0.79, 1.74)	*0.08*
High Molecular-weight (HMW)					
Monobenzyl phthalate	1.18 (0.70, 1.98)	0.80 (0.42, 1.53)	1.37 (0.78, 2.40)	1.27 (0.71, 2.26)	0.10
Mono(carboxyoctyl) phthalate	1.64 (1.05, 2.54)	0.96 (0.51, 1.79)	2.27 (1.34, 3.86)	1.96 (1.16, 3.32)	**0.01**
Mono(carboxynonyl) phthalate	1.77 (1.03, 3.01)	0.93 (0.41, 2.10)	2.71 (1.46, 5.03)	1.64 (0.80, 3.38)	**0.03**
Mono-(3-carboxypropyl) phthalate	2.36 (1.36, 4.10)	1.58 (0.69, 3.61)	3.21 (1.64, 6.31)	2.21 (1.05, 4.68)	0.26
Diethylhexyl phthalate[c]	1.08 (0.68, 1.69)	0.87 (0.53, 1.43)	1.19 (0.74, 1.90)	1.17 (0.73, 1.89)	*0.06*

Significant p-values ($p < 0.05$) are denoted in **bold**, borderline significant p-values ($p < 0.1$) are denoted in *italics*.
[a] Adjusted for age, gender, race/ethnicity, BMI, creatinine, and cotinine.
[b] Tests of equivalent linear slopes were conducted for each phthalate while controlling for age, gender, race/ethnicity, BMI, creatinine, and cotinine, df = 2.
[c] Sum of all DEHP metabolites.

NHANES survey uses standard questions that have been used in many studies. Because the study is cross-sectional we cannot assess whether the outcome is a result of the exposure, or if people with respiratory symptoms are more likely to be exposed to phthalates when living in homes with higher levels of endotoxin. Finally, our sample size was limited, not only to the third of NHANES participants with urinary phthalate measures, but also to the subset of those individuals who also had household dust samples. Thus our sample of 1546 adults that we analyzed previously (Hoppin et al., 2013) was reduced to 1091 individuals. The decrease in sample size between our previous and current work are due to excluding individuals missing a household dust endotoxin measurement or who had moved between the original questionnaire and dust sample collection. The sample analyzed here was slightly more likely to be non-Hispanic white (49% here vs. 46% earlier), more likely to be male (50% here vs. 49% earlier), and, probably most importantly, contained more individuals with cigarette smoke exposure as assessed by cotinine (28% with > 10 ng/mL cotinine here vs. 27% earlier). While studies have shown that smoking in the home is associated with higher levels of endotoxin (Thorne et al., 2009), it is unlikely that this small difference in the prevalence of smokers explains the observed differences because we controlled for this in our analyses. When we repeated the initial analysis (i.e. without interaction with endotoxin) on this new sample, we showed approximately the same results (Tables 3, 4) for wheeze and asthma, but with additional significant findings for current asthma with exposure to specific phthalates MCOP, MCNP, and mono-(3-carboxypropyl) phthalate (MCPP) that we did not observe earlier. For the most part, this current sample reflected the previous sample, but there were a few slight changes in the demographic characteristics that may also have influenced the differences between our results here and those reported earlier.

This is the first study to evaluate the potential interaction between endotoxin exposure in the home and phthalate metabolites and as it applies to asthma, hay fever, rhinitis, and wheeze. We utilized a large, nationally representative study with standardized measurements and controlled for multiple potential confounders. Future studies would benefit from longitudinal studies with larger sample sizes, including infants and school-aged children, better information on confounders and potentially incorporate multiple measurements of both phthalates and endotoxin levels.

Funding

This research was supported by Dr. Hoppin's start up funds at NC State University.

Human subjects

This analysis used the publicly available NHANES data https://wwwn.cdc.gov/nchs/nhanes/Default.aspx. These data have been de-identified and are publicly available. Use of these data does not constitute human subjects research.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the

online version at http://dx.doi.org/10.1016/j.envres.2018.01.017.

References

Abraham, J.H., Gold, D.R., Dockery, D.W., Ryan, L., Park, J.H., Milton, D.K., 2005. Within-home versus between-home variability of house dust endotoxin in a birth cohort. Environ. Health Perspect. 113 (11), 1516–1521.

CDC, 2015a. National Health and Nutrition Examination Survey: NHANES 2005–2006. 2012 [cited 26 May]; Available from: 〈http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm〉.

CDC, 2015b. Laboratory Procedure Manual: Phthalate Metabolites, Urine, HPLC/ESI-MS/MS. 2009 [cited 1 July]; Available from: 〈http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/PHTHTE_D.htm〉.

CDC, 2015c. Allergen Dust Collection Procedures Manual. 2006 [cited 1 July]; Available from: 〈http://www.cdc.gov/nchs/data/nhanes/nhanes_05_06/allergen_manual_06.pdf〉.

Chen, C.M., Thiering, E., Doekes, G., Zock, J.P., Bakolis, I., Norback, D., Sunyer, J., Villani, S., Verlato, G., Taubel, M., Jarvis, D., Heinrich, J., 2012. Geographical variation and the determinants of domestic endotoxin levels in mattress dust in Europe. Indoor Air 22 (1), 24–32.

Deutschle, T., Reiter, R., Butte, W., Heinzow, B., Keck, T., Riechelmann, H., 2008. A controlled challenge study on di(2-ethylhexyl) phthalate (DEHP) in house dust and the immune response in human nasal mucosa of allergic subjects. Environ. Health Perspect. 116 (11), 1487–1493.

Guo, J., Han, B., Qin, L., Li, B., You, H., Yang, J., Liu, D., Wei, C., Nanberg, E., Bornehag, C.G., Yang, X., 2012. Pulmonary toxicity and adjuvant effect of Di-(2-exylhexyl) phthalate in ovalbumin-immunized BALB/c mice. PLoS One 7 (6), e39008.

Han, Y., Wang, X., Chen, G., Xu, G., Liu, X., Zhu, W., Hu, P., Zhang, Y., Zhu, C., Miao, J., 2014. Di-(2-ethylhexyl) phthalate adjuvantly induces imbalanced humoral immunity in ovalbumin-sensitized BALB/c mice ascribing to T follicular helper cells hyperfunction. Toxicology 324, 88–97.

Hansen, J.F., Nielsen, C.H., Brorson, M.M., Frederiksen, H., Hartoft-Nielsen, M.L., Rasmussen, A.K., Bendtzen, K., Feldt-Rasmussen, U., 2015. Influence of phthalates on in vitro innate and adaptive immune responses. PLoS One 10 (6), e0131168.

Hauser, R., Calafat, A.M., 2005. Phthalates and human health. Occup. Environ. Med. 62 (11), 806–818.

Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ. Health Perspect. 112 (17), 1734–1740.

He, M., Inoue, K., Yoshida, S., Tanaka, M., Takano, H., Sun, G., Ichinose, T., 2013. Effects of airway exposure to di-(2-ethylhexyl) phthalate on allergic rhinitis. Immunopharmacol. Immunotoxicol. 35 (3), 390–395.

Hoppin, J.A., Brock, J.W., Davis, B.J., Baird, D.D., 2002. Reproducibility of urinary phthalate metabolites in first morning urine samples. Environ. Health Perspect. 110 (5), 515–518.

Hoppin, J.A., Jaramillo, R., London, S.J., Bertelsen, R.J., Salo, P.M., Sandler, D.P., Zeldin, D.C., 2013. Phthalate exposure and allergy in the U.S. population: results from NHANES 2005–2006. Environ. Health Perspect. 121 (10), 1129–1134.

Hsu, N.Y., Lee, C.C., Wang, J.Y., Li, Y.C., Chang, H.W., Chen, C.Y., Bornehag, C.G., Wu, P.C., Sundell, J., Su, H.J., 2012. Predicted risk of childhood allergy, asthma, and reported symptoms using measured phthalate exposure in dust and urine. Indoor Air 22 (3), 186–199.

Hwang, Y.H., Paik, M.J., Yee, S.T., 2017. Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium. Toxicol. Lett. 272, 49–59.

Jaakkola, J.J., Knight, T.L., 2008. The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. Environ. Health Perspect. 116 (7), 845–853.

Jaakkola, J.J., Ieromnimon, A., Jaakkola, M.S., 2006. Interior surface materials and asthma in adults: a population-based incident case-control study. Am. J. Epidemiol. 164 (8), 742–749.

Kim, H.G., Yeon, S.M., Kim, K.H., Kim, H., Park, J.I., Kang, H.J., Cha, E.J., Park, H.D., Kang, H.J., Park, T.W., Jeon, Y.H., Park, Y.I., Chang, K.T., Jung, Y.W., 2015. Estrogenic endocrine-disrupting chemicals modulate the production of inflammatory mediators and cell viability of lipopolysaccharide-stimulated macrophages. Inflammation 38 (2), 595–605.

Kimber, I., Dearman, R.J., 2010. An assessment of the ability of phthalates to influence immune and allergic responses. Toxicology 271 (3), 73–82.

Larsen, S.T., Lund, R.M., Nielsen, G.D., Thygesen, P., Poulsen, O.M., 2002. Adjuvant effect of di-n-butyl-, di-n-octyl-, di-isononyl- and di-isodecyl phthalate in a subcutaneous injection model using BALB/c mice. Pharmacol. Toxicol. 91 (5), 264–272.

Larson, R.P., Zimmerli, S.C., Comeau, M.R., Itano, A., Omori, M., Iseki, M., Hauser, C., Ziegler, S.F., 2010. Dibutyl phthalate-induced thymic stromal lymphopoietin is required for Th2 contact hypersensitivity responses. J. Immunol. (Baltim. Md: 1950) 184 (6), 2974–2984.

Mendell, M.J., Mirer, A.G., Cheung, K., Tong, M., Douwes, J., 2011. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. Environ. Health Perspect. 119 (6), 748–756.

North, M.L., Takaro, T.K., Diamond, M.L., Ellis, A.K., 2014. Effects of phthalates on the development and expression of allergic disease and asthma. Ann. Allergy Asthma Immunol.: Off. Publ. Am. Coll. Allergy Asthma Immunol. 112 (6), 496–502.

Pirkle, J.L., Flegal, K.M., Bernert, J.T., Brody, D.J., Etzel, R.A., Maurer, K.R., 1996. Exposure of the US population to environmental tobacco smoke: the Third National Health and Nutrition Examination Survey, 1988 to 1991. JAMA 275 (16), 1233–1240.

Radon, K., 2006. The two sides of the "endotoxin coin". Occup. Environ. Med. 63 (1), 73–78 (10).

Robinson, L., Miller, R., 2015. The impact of bisphenol A and phthalates on allergy, asthma, and immune function: a review of latest findings. Curr. Environ. Health Rep. 2 (4), 379–387.

Smit, L.A., Heederik, D., Doekes, G., Blom, C., van Zweden, I., Wouters, I.M., 2008. Exposure-response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. Eur. Respir. J. 31 (6), 1241–1248.

Sordillo, J.E., Alwis, U.K., Hoffman, E., Gold, D.R., Milton, D.K., 2011. Home characteristics as predictors of bacterial and fungal microbial biomarkers in house dust. Environ. Health Perspect. 119 (2), 189–195.

Teitelbaum, S.L., Britton, J.A., Calafat, A.M., Ye, X., Silva, M.J., Reidy, J.A., Galvez, M.P., Brenner, B.L., Wolff, M.S., 2008. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. Environ. Res. 106 (2), 257–269.

Thorne, P.S., Kulhankova, K., Yin, M., Cohn, R., Arbes Jr., S.J., Zeldin, D.C., 2005. Endotoxin exposure is a risk factor for asthma: the national survey of endotoxin in United States housing. Am. J. Respir. Crit. Care Med. 172 (11), 1371–1377.

Thorne, P.S., Cohn, R.D., Mav, D., Arbes, S.J., Zeldin, D.C., 2009. Predictors of endotoxin levels in U.S. housing. Environ. Health Perspect. 117 (5), 763–771.

Thorne, P.S., Mendy, A., Metwali, N., Salo, P., Co, C., Jaramillo, R., Rose, K.M., Zeldin, D.C., 2015. Endotoxin exposure: predictors and prevalence of associated asthma outcomes in the United States. Am. J. Respir. Crit. Care Med. 192 (11), 1287–1297.

Waser, M., Schierl, R., von Mutius, E., Maisch, S., Carr, D., Riedler, J., Eder, W., Schreuer, M., Nowak, D., Braun-Fahrlander, C., 2004. Determinants of endotoxin levels in living environments of farmers' children and their peers from rural areas. Clin. Exp. Allergy: J. Br. Soc. Allergy Clin. Immunol. 34 (3), 389–397.