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

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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

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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