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Early life exposure to phthalates and the development of childhood asthma among Canadian children

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Abstract (word count = 349)

BACKGROUND: Studies have demonstrated an association between phthalate exposure and childhood asthma, although results have been inconsistent. No epidemiological studies have examined exposure during the first year of life.

OBJECTIVE: To investigate the association between phthalate exposures in the home environment during the first year of life, and subsequent development of childhood asthma and related symptoms.

METHODS: This study used a case-cohort design including 436 randomly selected children and all additional cases of asthma at 5 years ($n_{\text{total}}=129$) and recurrent wheeze between 2 to 5 years ($n_{\text{total}}=332$) within the CHILD Cohort Study, a general population Canadian birth cohort of 3455 children. Phthalate exposure was assessed using house dust samples collected during a standardized home visit when children were 3 months of age. All children were assessed by specialist clinicians for asthma and allergy at 1, 3 and 5 years. Logistic regression was used to assess the association between exposure to five phthalates and asthma diagnosis at 5 years, and recurrent wheeze between 2 to 5 years, with further stratification by wheeze subtypes (late onset, persistent, transient) based on the timing of onset and persistence of wheeze symptoms.

RESULTS: Di(2-ethyl-hexyl) phthalate (DEHP) had the highest concentration in dust (median_{subcohort}=217 $\mu\text{g/g}$), followed by benzyl butyl phthalate (BzBP) (20 $\mu\text{g/g}$). A nearly four-fold increase in risk of developing asthma was associated with the highest concentration quartile of DEHP (OR=3.92, 95% CI: 1.87-8.24) including a positive dose-response relationship. A two-fold increase in risk of recurrent wheeze was observed across all quartiles compared to the lowest quartile of DEHP concentrations. Compared to other wheeze subtypes, stronger associations for DEHP were observed with the late onset wheezing subtype, while stronger associations for di-iso-butyl phthalate (DiBP) and BzBP were observed with the transient subtype.

DISCUSSION: DEHP exposure at 3 months, at concentrations lower than other studies that reported an association, were associated with increased risks of asthma and recurrent wheeze among children at 5 years. These findings suggest the need to assess whether more stringent regulations are required to protect children's health, which can be informed by future work exploring the main sources of DEHP exposure.

Keywords: phthalates, asthma, wheeze, children

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Introduction

Asthma is the most common chronic disease among children (Asher and Pearce 2014), with an estimated 339 million people living with asthma worldwide (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators 2017). The rapid increase in prevalence observed between the mid 1950's and the early part of this century cannot be explained by genetics alone. This has led to an increased focus on environmental factors (Asher et al. 2012), including exposure to chemicals from products and materials indoors (Parks et al. 2020), due to the increased use of synthetic materials and products in recent decades (US EPA 2017). Among those chemicals examined are phthalates, which are a group of chemicals found in a wide variety of consumer products including vinyl flooring, plastic toys, food packaging, and personal care products (Godwin 2017; Schettler 2006).

High molecular weight phthalates such as di-2-ethylhexyl phthalate (DEHP), are used primarily as plasticizers in polyvinyl chloride (Braun et al. 2013; Godwin 2017). Low molecular weight phthalates, such as diethyl phthalate (DEP), dimethyl phthalate (DMP) and dibutyl phthalate (DBP), are used as solvents and lubricants in products (Schettler 2006). Exposure can occur through all routes, with dietary ingestion being important for high molecular weight phthalates (Koch et al. 2013). Infants are also exposed by ingesting house dust due to their frequent hand to mouth activity and crawling behaviour (Bekö et al. 2013). Dermal exposure is also an important pathway for low molecular weight phthalates through the use and application of personal care products and cosmetics (Koniecki et al. 2011; Takaro et al. 2010) as well as transdermal uptake directly from air (Weschler et al. 2015). Inhalation of phthalates released from products and materials is another route of exposure (Bekö et al. 2013; Takaro et al. 2010; Yang et al. 2020).

Evidence of widespread phthalate exposure (Garí et al. 2019; Hammel et al. 2019; Navaranjan et al. 2020) and known endocrine-disrupting properties, including anti-androgenic effects of several

phthalates such as DEHP and DBP (e.g., Radke et al. 2018 *inter alia*), has led to ongoing concern about the associated chronic health effects. This concern is supported by epidemiological evidence showing an association between phthalate exposure and reproductive outcomes, neurologic and behavioral problems among children, and childhood allergy and asthma (Bølling et al. 2020; Polanska et al. 2014; Radke et al. 2019).

In recent reviews and meta-analysis, Li et al. and Bølling et al. provided evidence for an increased risk of childhood asthma associated with phthalate exposure (Bølling et al. 2020; Li et al. 2017). In particular, the strongest association was observed for postnatal exposure to DEHP, followed by BzBP (Bølling et al. 2020; Li et al. 2017). While this adds to growing concern about DEHP exposure, which has been the most commonly used phthalate, Bølling et al. concluded that further investigation was required for each phthalate since findings from epidemiological studies have been inconsistent (Bølling et al. 2020). Inconsistent findings of phthalate exposure and asthma have also been observed by sex, which is relevant given the endocrine-disrupting properties of phthalates and sex-specific mechanisms for asthma development potentially related to sex hormones (Bølling et al. 2020; Fuseini and Newcomb 2017).

Many studies used urine as their primary method for assessing exposure to phthalates. However, due to the short half-life of phthalates in the body of 5 to 48 h, urine is typically reflective of recent phthalate exposure (Frederiksen et al. 2013; Wittassek and Angerer 2008), and not necessarily long-term exposure required for careful examination of chronic health effects. House dust, which allows for the accumulation of chemicals over time, has been shown to be a representative indicator of integrated long-term exposure to semi-volatile organic (SVOCs) and non-volatile chemicals from indoor sources (Bennett et al. 2015; Whitehead et al. 2012). Four epidemiological studies have used house dust to assess exposure to phthalates and the risk of childhood asthma with the findings being

inconsistent (Ait Bamai et al. 2014; Bornehag et al. 2004; Callesen et al. 2014; Kolarik et al. 2008).

Sample sizes were also limited ($n < 400$), which reduced the certainty of risk estimates.

Epidemiological studies to date have used a cross-sectional or case-control study design, which assessed exposure to phthalates at the same time as assessment for asthma or wheeze, which was later in childhood. However, early life and particularly the first year of life presents a critical period during which there is substantial development of the respiratory and immune system (Dezateux and Stocks 1997; Stocks 1995) and environmental exposures such as mold, outdoor air pollution, antibiotic use during this period have been associated with later adverse respiratory and immune effects among children (Clark et al. 2010; Marra et al. 2006; Reponen et al. 2011; Salam et al. 2004). Thus, longitudinal studies are needed to understand the subsequent health effects later in childhood from early life exposure to phthalates. Epidemiological studies to date have also defined asthma as a single outcome, although it is well-known that asthma is a complex and heterogeneous disease (Asher et al. 2012; Chung 2011; Martinez et al. 1995; Stein et al. 1997). Using recurrent wheeze, a common symptom for asthma, one approach for examining the complexity of asthma is to examine the risk associated with wheezing subtypes characterized by the timing of onset and persistence of wheeze such as early onset, late onset or persistent wheeze (Chung 2011; Martinez et al. 1995; Stein et al. 1997), which may have different etiologies and risk factors (Granell et al. 2012).

The CHILD Cohort Study is a large Canadian population-based birth cohort designed to examine the interplay between genetics and environment in the development of allergy and asthma using extensive data collected during early life. We designed a case-cohort study nested within the CHILD Study to address several of the limitations in previous epidemiological studies, to examine phthalate exposure during infancy and childhood asthma at 5 years. Using house dust samples collected at 3-4 months from the child's home environment, as a measure of chronic exposure during early life, we tested the following hypotheses: first, exposure to phthalates at 3 months is associated with an

increased risk of asthma at 5 years and recurrent wheeze between 2 to 5 years, and secondly that the risk differs according to wheezing subtype. Due to the need to further examine sex-specific differences, we also examined the hypothesis that risk could differ according to sex.

Methods

Study population and design

This study is based on participants in the CHILD Cohort Study, a longitudinal study that followed 3455 pregnant mothers recruited from four regional health centers across Canada: Edmonton, Toronto, Manitoba (Winnipeg, Morden and Winkler), and Vancouver from 2008 to 2012. Mothers meeting the following criteria were enrolled: >18 years (>19 years in Vancouver), living within 50 km of a participating delivery hospital, and planned to deliver at a recruitment center hospital, ability to communicate in English, willing to donate cord blood, and infant was born at ≥ 35 weeks. Exclusion criteria included children born with respiratory distress syndrome or major congenital abnormalities, children of multiple births or resulting from *in vitro* fertilization, families expecting to move away from the recruitment centre within 1 year of recruitment, and children spending less than 80% of nights in their primary home.

To examine the association between phthalate exposure and asthma and related outcomes, we designed a case-cohort study nested within CHILD to have sufficient power to examine multiple health outcomes using a subset of children, while allowing for the efficient analysis of a subgroup of house dust samples to assess phthalate exposure. Figure S1 describes the study design including the inclusion and exclusion criteria and the number of children meeting these criteria. Briefly, children who had sufficient house dust collected (typically ≥ 12.5 mg of dust in each room (described below) and more than one aliquot of dust available for testing), who were genotyped and who had attended the 5-year clinical assessment were eligible for the present study (Figure S1). Genotyping was included in our eligibility

criteria to allow us to use the same population for another study examining gene-phthalate interactions and asthma risk. From this eligible population of 2,319 children, a random subcohort of 436 children was selected (Figure S1). The sample size of the subcohort was determined based on the number of asthma and recurrent wheeze cases observed, having sufficient power for comparisons between children with and without asthma or wheeze, and funding available to analyze dust samples. There were 30 children with physician diagnosed definite asthma and 61 children with recurrent wheeze between 2 to 5 years within this subcohort. All other children who were diagnosed with asthma at 5 years (n=99) and recurrent wheeze between 2 to 5 years (n=271) in the eligible cohort were added to our study population (Figure S1). This led to a total of 535 children in our analysis examining the risk of asthma at 5 years and 707 children in our analysis examining the risk of recurrent wheeze between 2 to 5 years, as demonstrated in Figure S1. Key characteristics, such as study site, sex, parental history of asthma, of those children excluded from the eligible cohort for this study were examined and found to be similar to the random subcohort. Ethics approvals for study protocols were obtained from research ethics boards in each centre and approval for these analyses was obtained from the University of Toronto Health Sciences Research Ethics Board (No. 36169). Informed consent was obtained from parents.

Data Collection

Questionnaires were administered to families at multiple time points starting from pregnancy through to age five regarding factors that could influence child's health including the home environment, socioeconomic status (SES), parental medical history and occupation, and child's nutrition. A major time point for environmental data collection was at a median of 3.4 months of age (interquartile range (IQR): 3.0-4.2), referred to as 3-4 months of age (i.e., representing the first year of life), when detailed home environment questionnaires were administered to capture information on housing characteristics such as product use, type of dwelling, age of the home, flooring type, cleaning habits, and multiple other factors discussed in detail elsewhere (Takaro et al. 2015). In addition, trained

research assistants (RAs) visited all homes at 3-4 months, mostly between 2010 to 2012, and completed detailed home environment assessments, and collected biological samples such as urine and stool from children and house dust samples.

Exposure assessment for phthalates

RAs collected dust samples from the child's sleeping area and most used living area using a standardized consumer model vacuum cleaner fitted with a depyrogenated aluminum nozzle containing two nylon mesh thimbles (DustStream, Indoor Biotechnologies) (Takaro et al. 2015). In the child's sleeping area, a combined sample was collected from the mattress sheet or cover that the child slept on and an adjacent 2m² area of carpeted flooring. In the most used room, a 2m² area of carpeted flooring was vacuumed using two new thimbles. If there was little or no carpet in the room (hardwood or laminate), the entire floor was vacuumed until sufficient dust was collected, starting with any carpet or rug in the room. The area vacuumed was measured and recorded. For every 20th home sampled, control dust samples were collected to ensure quality assurance and quality control (QA/QC) during sample collection and analysis by placing 100 mg of NIST standard reference material (SRM 2585) vacuum dust into a thimble, which was transported to homes in a closed bottle. Since there are no certified values of phthalates in NIST reference dust, concentrations measured in NIST were compared to several studies that also collected and measured phthalates in NIST 2585. The "NIST control" was obtained by opening one NIST bottle in the home while preparing vacuum heads and placing the thimbles for dust sample collection in the vacuum head. Following dust sample collection, all thimbles, including any NIST control, were transported in sterile glass bottles and stored at room temperature for no more than two weeks before being shipped to a processing lab. At the lab, large particles (food crumbs, stones) were manually removed, samples were weighed and sieved to < 150 µm, separately for samples from the bedroom and most used room. This fine dust fraction was weighed, divided into multiple aliquots and stored at -80°C until accessed for analysis.

Dust samples and NIST controls were analyzed for five common phthalates: DEP, diisobutyl phthalate (DiBP), di-*n*-butyl phthalate (DnBP), benzyl butyl phthalate (BzBP), and DEHP (see Table S1 for Chemical Abstracts Service (CAS) numbers and molecular weight). We combined equal amounts of fine dust of the previously frozen aliquots from the child's sleeping area and most used living area. Full details of the analytical procedure are provided in Supplemental Information (S1). Briefly, a subsample of 20-25 mg of the combined and homogenized dust was extracted by sonication using dichloromethane and then analyzed by gas-chromatography mass spectrometry (Agilent GC-MSD, Agilent Technologies Inc., Palo Alto, CA, USA). NIST control dust (SRM 2585) transported to homes was also analyzed 1 in every 25 house dust samples and laboratory blanks containing precleaned sodium sulfate (baked at 400°C prior to use, Baker) were analyzed 1 in every 10 house dust samples, using the same procedure as the samples (Table S2). SRM 2585 values were within the range of average values reported by other studies (Table S3), even after up to 9 years of storage suggesting minimal degradation of the house dust samples. Concentrations of phthalates measured in dust were blank corrected using the following approach: if the blank value was <5% of the measured value, no correction was needed; if the blank value was 5 - 35% of the measured level, the blank value was subtracted from the measured value; if the blank value was >35% of the measured value, the sample value was removed (Saini et al. 2015). The percentage of detected samples requiring blank correction (ranging from 0 to 59%) or removal (ranging from 0 to 2%) are reported in Table S2. Final phthalate concentrations for DEP, DnBP and DEHP were recovery corrected using the recovery of the corresponding isotopically labelled phthalate (Table S2). BzBP and DiBP concentrations were recovery corrected using an average of the recovery from the three isotopically labelled phthalates. Phthalate exposure was expressed as mass concentrations measured in dust ($\mu\text{g/g}$), to allow for comparison with other epidemiological studies which used a similar approach.

Outcome ascertainment

Children were assessed at 1, 3 and 5 years for atopic dermatitis, rhinitis, wheeze and asthma by a pediatrician with expertise in asthma and allergy (Subbarao et al. 2015). Allergic sensitization was also assessed at this time using skin prick tests (Subbarao et al. 2015). The primary outcome of interest for the current study was physician diagnosed definite asthma at 5 years that was determined consistently across all study sites by conducting a standardized interview with parents about symptoms and medication use (Dharma et al. 2018). Children were diagnosed with definite asthma if they used a daily controller medication or bronchodilator prescribed by a physician for wheeze or cough, or experienced 3 or more episodes of wheeze in the past 12 months or they had previously been diagnosed with asthma by a physician. Standardized health questionnaires were also sent to parents at several time points to capture information on child's health, including wheezing, defined as a whistling sound in the chest. Using both the health questionnaires administered at 2.5, 3, 4 and 5 years and physician assessments at 3 and 5 years, children were also classified as having recurrent wheeze (two or more episodes of wheeze) between 2 to 5 years, which was our secondary outcome of interest. Due to the large number of children diagnosed with wheeze at 1 year, which mostly resolve, and funding availability to analyze house dust samples, we examined recurrent wheeze between 2 to 5 years.

To define wheezing subtypes, children with any recurrent wheeze between 2 to 5 years were further classified separately at 3 and 5 years as having recurrent wheeze (two or more episodes of wheeze in the past 12 months) using data reported in the health questionnaires and physician assessments. Three wheezing subtypes were defined as: persistent wheezers (children with recurrent wheeze at 3 and 5 years), transient wheezers (recurrent wheeze at 3 years, but not at 5 years), and late onset (recurrent wheeze at 5 years, but not at 3 years).

Statistical Analyses

Concentrations of phthalates were right-skewed, therefore geometric means (GM) and standard deviations were calculated as part of the descriptive statistics. Only a few samples (<2% for each phthalate) were not reported due to uninterpretable chromatograms, making imputation of samples below detection unnecessary. The Spearman correlation coefficient was calculated to assess the relationship between phthalates.

To assess the association between phthalate exposure and health effects, odds ratios (OR) with 95% confidence intervals (CI) were calculated for asthma and recurrent wheeze, categorized as binary outcomes, using logistic regression. Multinomial logistic regression was used for each wheeze subtype compared to children with no wheeze. Phthalate concentrations were categorized into quartiles, defined based on the distribution observed in the random subcohort, to examine dose-response relationships which were assessed using the Cochrane-Armitage exact trend test. All models were adjusted for study site due to differences in risk factors for asthma by city observed in the CHILd cohort (Miliku et al. 2019; Parks et al. 2020), and by income because of differences in phthalate exposure previously reported in the CHILd cohort (Navaranjan et al. 2020). Several other potential confounders were assessed including sex, parental history of asthma reported at recruitment, smoking inside the home at 3 months, housing characteristics including flooring type and period the home was built. Confounders that changed the odds ratios by $\geq 10\%$ were included in the final multivariate model. To investigate differences in the risk of asthma related to phthalate exposure by sex, an interaction term between phthalate exposure and sex was included in the multivariate analysis and logistic regression analyses were further stratified by sex. All analyses were conducted using SAS version 9.4 (SAS Institute Inc.).

Results

Table 1 shows the characteristics of the case-cohort sample separately for the random subcohort, consisting largely of non-cases, the asthma cases and recurrent wheeze cases, representing our analytic subcohorts (cases vs non-cases). Children from all study sites were represented in the sample, with a slightly higher percentage of children from Manitoba (31%) in the random subcohort due to greater recruitment at that site. An equal percentage of boys and girls were present in the random subcohort. Among asthma and recurrent wheeze cases, a higher percentage (>60%) were boys, consistent with the gender prevalence of asthma during childhood. The prevalence of indoor smoking ($\leq 3\%$), as well as any reported smoking (results not shown) were low in this population. Approximately 35% of the subcohort had a parental history of asthma, while 50% or more of asthma and recurrent wheeze cases had a parental history of asthma. Based on families who provided information, approximately half reported an annual household income greater than \$100,000 (reported primarily between 2009 to 2012). A small percentage (< 3%) of families reported having vinyl flooring in their homes and families across all groups (random subcohort, and case groups) reported cleaning their homes a median of 4 times per month. Most families (> 50%) reported living in homes built prior to 1990.

Phthalate concentrations in dust

Detection frequencies of phthalates were nearly 100% of all samples analyzed (Table S4). DEHP had the highest median concentration in dust (217 $\mu\text{g/g}$ for the random subcohort), followed by BzBP (20 $\mu\text{g/g}$ for the random subcohort), while DEP had the lowest concentration (1.3 $\mu\text{g/g}$ for the random subcohort) (Table S4). Concentrations of all phthalates spanned orders of magnitude (DEP: 0.28-64 $\mu\text{g/g}$; DiBP: 0.53-661 $\mu\text{g/g}$; DnBP: 0.81-999 $\mu\text{g/g}$; BzBP: 1.22-4055; DEHP: 9.62-2675 $\mu\text{g/g}$) (Figure 1), indicating large variability of exposure via dust among children. Using Spearman correlation, significant but low ($\rho \leq 0.46$, $p < 0.05$) positive correlations were generally observed between all phthalates (Table S5).

Phthalate exposure and risk of asthma and recurrent wheeze

Figure 2 presents results for the association between phthalate exposure and risk of asthma at 5 years and any recurrent wheeze between 2 to 5 years. DEHP concentrations in the highest quartile were associated with a nearly four-fold increased risk of being diagnosed with asthma (OR=3.92, 95% CI: 1.87, 8.24). Based on the trend test, a positive dose-response relationship was observed between concentrations of DEHP and physician diagnosed asthma ($p_{\text{trend}} = <.0001$). For DEP, a significant inverse relationship was observed with asthma risk ($p_{\text{trend}} = 0.009$), whereby children with higher DEP concentrations had a reduced risk of asthma (OR_{highest quartile} = 0.48, 95% CI: 0.26, 0.88). To understand whether differences in personal care and household product (cleaning products, polishes, air freshener) use, may explain these inverse relationships, we examined the reported frequency of each of these products between children with asthma and without asthma and found no significant difference for any of the products (data not shown). Further, we conducted stratified analyses by parental history of asthma, to examine whether other changes in behavior associated with parental history affected DEP exposure and found no difference in the odds ratio for childhood asthma associated with DEP exposure by parental history. Similarly, for BzBP a reduced risk of asthma was observed in the second and third quartile of exposure (OR_{second quartile}=0.23, 95% CI: 0.11, 0.48; OR_{third quartile}=0.42, 95% CI: 0.22, 0.81). No significant association was observed between DiBP and DnBP exposure and asthma risk. Results from models additionally adjusting for all phthalates (Table S7) demonstrated similar dose-response relationships and for some phthalates, such as DEHP, with slightly higher odds ratios.

DEHP concentrations were associated with a two to three-fold increase in the risk of recurrent wheeze across all quartiles of exposure compared to the lowest quartile with a positive dose-response relationship ($p_{\text{trend}} = <.0001$). A reduced risk of recurrent wheeze was observed with DEP concentrations in the second (OR=0.52, 95% CI: 0.33, 0.84) and fourth (OR=0.59, 95% CI: 0.37, 0.93) quartiles and with

BzBP concentrations in the second quartile (OR=0.38, 95% CI: 0.23, 0.63). No associations were observed between concentrations of DnBP and DiBP and recurrent wheeze.

Differences in risk by sex

Figure 2 presents the significance of interaction terms between each phthalate and sex. Differences by sex were not statistically significant for any of the phthalates ($p_{\text{interaction}} > 0.05$), however for all phthalates in most quartiles, we observed higher odds ratios of asthma among boys compared to girls (Table S8). For instance, the odds ratio for asthma for boys in the highest quartile was 4.21 (95% CI: 1.56, 11.4) and 2.14 (95% CI: 1.00, 4.56) for DEHP and DnBP, respectively. For girls, the odds ratios were 3.75 (95%CI: 1.18, 12.0) and 0.84 (95% CI: 0.31, 2.24), respectively. For recurrent wheeze, odds ratios were generally higher among girls compared to boys for DEHP, DnBP and DiBP, and for boys compared to girls for DEP and BzBP (Table S8), although these differences between sex were also not statistically significant ($p_{\text{interaction}} > 0.05$).

Phthalate exposure and recurrent wheeze subtypes

For DiBP and BzBP a higher risk was observed with the transient wheeze subtype compared with the late onset and persistent subtypes (Table 2). For DEHP, a higher odds ratio was observed with the late onset wheeze subtype compared to the other subtypes, including over a five-fold increased risk for each quartile of DEHP exposure. No consistent, significant differences were observed between DnBP and DEP and any of the wheezing subtypes, except for reduced risks of both persistent and late onset wheeze for the highest DEP exposure quartile (Table 2).

Discussion

This study aimed to strengthen the evidence for the role of phthalate exposure in children's development of asthma by examining exposure during a critical period of development, the first 3 months of life, not considered in other epidemiological analyses, using house dust as a chronic measure

of exposure. We also explored the relationship according to wheeze subtypes to account for the heterogeneity of asthma that may be due to different etiologies and risk factors based on the onset and persistence of symptoms throughout early childhood.

We observed the highest concentration for DEHP in dust with 10-100-fold higher levels than the other phthalates measured. We observed a nearly four-fold increase in the risk of asthma associated with a median DEHP concentration of 217 $\mu\text{g/g}$, a positive association between DEHP exposure and recurrent wheeze between 2 to 5 years, both following significant positive dose-response relationships. Similar to our findings with DEHP and asthma risk, a case-control study of Swedish children, with a median DEHP concentration of 770 $\mu\text{g/g}$ in house dust, observed a three-fold increase in the risk of asthma in the highest quartile of DEHP concentrations (Bornehag et al. 2004). A case-control study in Bulgaria also observed a similar association between concentration of DEHP in dust in the highest quartile and wheezing in the past 12 months among children 2 to 7 years old, with a median concentration of 990 $\mu\text{g/g}$ (Kolarik et al. 2008). In a systematic review and meta-analysis, Li et al. reported that the strongest association between phthalate exposure and asthma risk was observed for DEHP (pooled OR= 2.71, 95% CI: 1.39-5.28) followed by BzBP (pooled OR= 2.08, 95% CI: 1.10-3.92) measured in dust collected postnatally (Li et al. 2017). We did not find an increased risk of asthma or wheeze associated with BzBP exposure at concentrations (median 20 $\mu\text{g/g}$) within the range of concentrations observed in previous studies (median: 1 to 135 $\mu\text{g/g}$).

It is noteworthy that we observed a nearly four-fold increase in the risk of asthma associated with DEHP concentrations in the highest quartile and two to three-fold increases in risk for recurrent wheeze at concentrations (median 217 $\mu\text{g/g}$) three to four-fold lower than previous studies, conducted during the early 2000's, that observed an association. In 2010, Canada enacted regulations that limited the amount of vinyl in children's toys or child-care articles to contain no more than 1000 mg/kg of DEHP, DBP or BzBP. Similarly, children's toys or child-care articles that could be placed in the mouth of a child

under four years old were limited to less than 1000 mg/kg of DNOP, diisononyl phthalate (DINP), or diisodecyl phthalate (DIDP) (Canadian Consumer Product Safety Act 2010). We did not test any products used by the families in the cohort but the phthalate concentrations presented here were collected from the most used room and bedroom, including child's mattress covers, which are known to contain plasticizers such as DEHP (Boor et al. 2017 *inter alia*).

Our study provides epidemiological evidence that is consistent with several mechanistic studies that have found an association between DEHP and asthma, by demonstrating adjuvant effects of DEHP exposure. Guo et al. demonstrated that higher concentrations of DEHP (3,000 µg/kg/day administered orally) caused increased airway hyperresponsiveness in mice sensitized with ovalbumin (OVA) (Guo et al. 2012), including changes of the lung such as airway obstruction and airway mucosa thickening at low (30 µg/kg/day) and high DEHP concentrations (3,000 µg/kg/day). Similar adjuvant effects and airway structural changes, were observed at higher concentrations of DEHP (70 mg/kg/day and 30 mg/kg/day) by Yang et al. (Yang et al. 2008) and You et al. (You et al. 2014), respectively. In contrast to studies that have observed adjuvant effects, Larsen et al. examined airway exposure to only DEHP in mice and found that extremely high concentrations (300 mg/m³) of DEHP exposure alone were associated with airway irritation, including reduced tidal volume (Larsen et al. 2007). The higher concentrations of DEHP measured in dust in our study compared to other phthalates (up to 100 times greater than some phthalates), which is also the most commonly used phthalate, as well as the biological plausibility demonstrated in several mechanistic studies, unlike other phthalates, may explain the increased risks of asthma and recurrent wheeze demonstrated with DEHP and not other phthalates in our study.

We observed an apparent lower risk of developing asthma or recurrent wheeze associated with BzBP and DEP exposure. Concentrations of these phthalates, as well as DiBP and DnBP for which we did not observe an association, were one to two orders of magnitude lower (medians: 1.3-20 µg/g for the random subcohort) than concentrations of DEHP (median 217 µg/g for the random subcohort). Other

studies have generally not observed a relationship with these phthalates or an increased risk in the case of BzBP exposure (Li et al. 2017). It is unclear why risks were reduced with BzBP exposure in our study. BzBP has been used in vinyl flooring however only a small percentage (<3%) of CHILD homes had vinyl flooring and there was no difference in the percentage of homes with vinyl flooring between children with asthma/recurrent wheeze compared to children without. DEP is commonly used in fragrance products. We hypothesized that parents with a history of asthma or allergy, a key risk factor for childhood asthma, may have limited the use of fragrance products among themselves and their infants leading to lower DEP concentrations in dust observed among children who later develop asthma. Using the questionnaire data administered in the CHILD Cohort, which asked extensively about personal care and household product (cleaning products, polishes, air freshener) use, we tested this hypothesis by examining the reported frequency of each of these products between children with asthma and without asthma and found no significant difference for any of the products (data not shown). Further, we conducted stratified analyses by parental history of asthma, to examine whether other changes in behavior associated with parental history affected DEP exposure and found no difference in the odds ratio for childhood asthma or recurrent wheeze associated with DEP exposure by parental history (data not shown). This suggests that several factors, including the greater volatility of DEP and hence generally lower concentration in dust, may have contributed to this observed inverse dose-response relationship.

We did not find any significant differences in the risk of asthma with phthalate exposure by sex, although odds ratios for almost all phthalates tended to be higher among boys compared to girls. Only a few other studies have examined differences in risk by sex with inconsistent findings. A Spanish birth cohort observed an increased risk of wheeze associated with prenatal urinary concentrations of MBzP among girls but not boys (Gascon et al. 2015), while Ku et al. (2015) observed an increased risk of wheeze associated with prenatal urinary MBzP concentrations among boys but not girls (Ku et al. 2015). The same study also observed an increased risk of childhood asthma associated with postnatal urinary

concentrations of MEHP, and the sum of DEHP metabolites (Ku et al. 2015), while a study in Norway found an increased risk of asthma associated with total DEHP urinary metabolite exposure among girls, but not boys (Bertelsen et al. 2013). These studies did not provide an explanation for their findings with sex but note that small sample sizes after stratifying may have led to imprecise estimates.

We observed stronger associations between DEHP and the late-onset wheeze subtype, and between DiBP and BzBP exposure and the transient wheeze subtypes compared to other subtypes. These findings, in comparison to our examination of asthma at 5 years as a single outcome, may indicate different mechanisms of action for different phthalates in the development of asthma, which is a heterogeneous disease. Similarly, our findings suggest that early life exposure to DEHP increases susceptibility to later wheeze development among children presenting no symptoms during early life, highlighting the importance of early life exposure and further characterization of outcomes in the examination of wheeze and asthma risk.

To our knowledge, no studies to date have examined the association specifically between phthalate exposure and the risk of different wheeze subtypes or considered plausible mechanisms leading to differences in risk by subtype. Mikeš et al. broadly examined the association between early life exposure to household chemicals using a composite score and different wheezing phenotypes using data from 3400 children in the European Longitudinal Study of Pregnancy and Childhood (ELSPAC) cohort (Mikeš et al. 2019). They found a significant association between increasing household chemical usage and persistent wheeze phenotypes (intermediate onset persistent, early onset persistent and intermediate onset transient), but not transient wheeze phenotypes (early onset transient and late onset) compared to children with no wheezing. Thus, results from Mikeš et al. as well as our study highlight the value of examining subtypes of wheeze which may be accounting for some of the heterogeneity in asthma and its association with environmental factors, including phthalates, that is not considered when asthma or wheeze diagnosis is dichotomized as 'yes or no'. More experimental studies

are also needed to better understand whether differences may be related to timing of exposures, route of exposure or different biological pathways through which different phthalates lead to respiratory effects.

To examine the generalizability of our findings to other populations, Table S9 presents a comparison of phthalate concentrations measured here, where dust samples were collected from 2010-2012, to studies published since 2010 using similar methods to collect dust. Concentrations of phthalates reported here are comparable to those of other studies from Canada and other countries. The only exception was a study by Tran et al. (2016) from Vietnam in which dust was collected in 2014; they reported concentrations an order of magnitude lower than all studies presented. The similarities in concentrations across region and over time, along with our evidence of increased risk, suggest that phthalates continue to play a potentially preventable role in the development of asthma. In particular, numerous studies have found greater phthalate exposure among populations of lower socioeconomic status (SES) (Wan et al. 2020; Wenzel et al. 2018; Ye et al. 2008). For example, Wan et al. (2020) reported 2-18 times higher air concentrations of all phthalates tested, including those examined in this study, in low SES social housing units compared to higher SES homes in Canada. This suggests that the risks of asthma associated with phthalate exposure may be higher among lower SES populations. Our study did not find a significant difference in risk of asthma when stratified by household income ($p_{\text{interaction}} > 0.05$, data not shown), possibly due to the overall higher SES represented in the CHILD cohort. However, previous work in this cohort using urine samples collected from ~1500 infants found an increase in exposure to phthalates at lower household incomes (Navaranjan et al. 2020).

One limitation of our study was that the wheeze subtypes were defined based on symptoms between 2 to 5 years instead of from birth to 5 years, based on funding available to analyze dust samples. However, since a high proportion of infants experience wheeze during early life, particularly the first year of life (Mallol et al. 2010), which resolves in later childhood (Martinez et al. 1995), an

examination of wheeze from 2 to 5 years better captures differences in symptom progression and therefore provides a good understanding of risk differences by subtypes. Recurrent wheezing was defined based on both parental report as well as physician assessments. Reliance on parental report may have resulted in misclassification of recurrent wheeze, depending on accurate recall about the past 12 months and the parent's ability to correctly identify wheeze. However, the effect of any misclassification would be minimized due to the use of multiple clinical assessments. A minor limitation is that dust samples were collected when the children were 3-4 months of age (median age of 3.4 months, IQR=3.0-4.2), which based on previous work can be representative of exposure up to about a year (Bennett et al. 2015; Whitehead et al. 2012). Thus, while our data on phthalate levels were used to represent exposure during early life (i.e., the first year of life), the data may also have captured pre-natal exposure given the likely similarity between the dust in the home during that period and at 3-4 months of age. Another limitation is that the CHILD cohort is representative of a higher SES population compared to the general population and thus would not capture risks among lower SES populations, which we speculate to be greater.

In addition to being one of the largest studies to examine the association between phthalate exposure and asthma or wheeze risk, this study had several other strengths including the use of data from a large multi-city birth cohort that captured changes in health status, particularly wheezing, over time, collected data on multiple aspects of the child's environment which were assessed and adjusted for, as appropriate, and offered increased power to investigate differences by sex. Furthermore, the use of dust samples as an indicator of longer-term exposure, and examination of exposures during infancy, which has not been examined in other epidemiological studies, were key strengths of this study. Health outcomes considered in this study were also based on clinical assessments by pediatric specialists at each study site with expertise in asthma and allergy who frequently consulted with each other to ensure consistency in the diagnoses and in all aspects of the study.

Conclusions

As the first Canadian study to investigate the relationship between exposure to phthalates, particularly during the first year of life, and the development of childhood asthma and wheeze, our results indicate a significantly increased risk of asthma at 5 years and wheeze between 2 to 5 years associated with DEHP exposure estimated during infancy from children's immediate home environment, including their sleeping area. This relationship was observed at concentrations lower than in previous studies that found an association with childhood asthma. Given the magnitude of the nearly four-fold odds ratio for DEHP and childhood asthma, the clear dose-response pattern we observed, and consistency of these findings with other epidemiological studies and mechanistic evidence, there is a need to assess whether more stringent regulations are required to protect children's health.

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Tables

Table 1 Characteristics of the case-cohort population from the CHILD Cohort Study assessed for phthalate exposure using house dust samples.^a

Characteristic	Subcohort (n=436)	Asthma cases (n=129)	Recurrent wheeze (n=332)
Study Site			
Edmonton	83 (19%)	25 (19%)	68 (20%)
Toronto	110 (25%)	36 (28%)	99 (30%)
Vancouver	108 (25%)	36 (28%)	72 (22%)
Manitoba	135 (31%)	32 (25%)	93 (28%)
Sex			
Girls	210 (48%)	45 (35%)	132 (40%)
Boys	226 (52%)	84 (65%)	200 (60%)
Any parental history of asthma (self-reported)			
Yes	158 (36%)	76 (59%)	163 (49%)
No	274 (63%)	53 (41%)	167 (50%)
Missing	4 (1%)		2 (1%)
Smoking inside home (3 months)			
Yes	12 (2.7%)	2 (1.6%)	8 (2%)
No	422 (97%)	124 (96.1%)	315 (95%)
Missing	2 (0.3%)	3 (2.3%)	9 (3%)
Annual household income (18 weeks prenatal) (\$)			
<60,000	70 (16%)	20 (15%)	55 (17%)
60000-100000	104 (24%)	39 (30%)	92 (28%)
100000-150000	123 (28%)	32 (25%)	75 (22%)
>150000	88 (20%)	28 (22%)	73 (22%)
Missing	51 (12%)	10 (8%)	37 (11%)
Any vinyl flooring in most used living area			
Yes	12 (2.5%)	4 (3%)	8 (2%)
No	423 (97%)	124 (96%)	320 (97%)
Missing	1 (0.5%)	1 (1%)	4 (1%)

Any vinyl flooring in child's sleeping area			
Yes	10 (2.5%)	3 (2%)	7 (2%)
No	425 (97%)	125 (97%)	321 (97%)
Missing	1 (0.5%)	1 (1%)	4 (1%)
Period home built			
1939 or earlier	70 (16%)	22 (17%)	48 (14%)
1940-1969	86 (20%)	26 (20%)	68 (20%)
1970-1989	94 (22%)	20 (15%)	55 (17%)
1990 or later	133 (30%)	39 (30%)	103 (31%)
Missing	53 (12%)	22 (17%)	58 (18%)
Median fine dust collected per area vacuumed (g/m ²)	0.19	0.12	0.19
Cleaning frequency (median number of times per month clean home)	4	4	4

^aThe subcohort includes 30 children with physician diagnosed definite asthma at 5 years and 61 children with recurrent wheeze between 2 to 5 years. Thus, a total of 726 unique children were included in our case-cohort study, divided into two analytic groups: 1. N= 535 children in our analysis examining the risk of asthma at 5 years (n=129 asthma cases and n=406 non asthma cases) and 2. N=707 children in our analysis examining the risk of recurrent wheeze between 2 to 5 years (n=332 recurrent wheeze cases and 375 non recurrent wheeze cases)

Table 2 Association between phthalate mass concentration in dust and wheezing subtypes (based on multinomial logistic regression comparing children of each wheeze subtype to children with no wheeze)

Phthalate concentration quartile	Late Onset (N=89) OR ^a (95% CI)	Persistent (N=72) OR ^a (95% CI)	Transient (N=118) OR ^a (95% CI)
DEP			
Q1	1.0	1.0	1.0
Q2	0.63 (0.31, 1.26)	0.44 (0.19, 1.00)	0.89 (0.47, 1.68)
Q3	0.76 (0.39, 1.47)	0.85 (0.43, 1.70)	0.79 (0.41, 1.51)
Q4	0.42 (0.20, 0.87)	0.43 (0.20, 0.94)	0.79 (0.42, 1.49)
DIBP			
Q1	1.0	1.0	1.0
Q2	0.89 (0.42, 1.90)	0.96 (0.42, 2.20)	1.20 (0.59, 2.43)
Q3	1.54 (0.77, 3.06)	1.61 (0.75, 3.45)	1.80 (0.92, 3.51)
Q4	1.30 (0.65, 2.62)	1.33 (0.61, 2.87)	1.62 (0.83, 3.16)
DNBP			
Q1	1.0	1.0	1.0
Q2	0.71 (0.34, 1.47)	1.44 (0.67, 3.11)	1.08 (0.57, 2.07)
Q3	0.94 (0.47, 1.88)	0.96 (0.42, 2.18)	1.13 (0.59, 2.14)
Q4	1.19 (0.61, 2.33)	1.35 (0.61, 2.99)	0.92 (0.47, 1.81)
BZBP			
Q1	1.0	1.0	1.0
Q2	0.49 (0.24, 1.04)	0.20 (0.07, 0.57)	0.51 (0.25, 1.03)
Q3	0.78 (0.38, 1.59)	0.70 (0.32, 1.55)	0.95 (0.48, 1.87)
Q4	0.41 (0.18, 0.94)	0.87 (0.37, 2.01)	1.10 (0.54, 2.25)
DEHP			
Q1	1.0	1.0	1.0
Q2	6.48 (2.14, 19.5)	1.59 (0.63, 4.05)	1.63 (0.77, 3.46)
Q3	5.85 (1.93, 17.8)	1.95 (0.78, 4.91)	2.26 (1.09, 4.68)
Q4	6.01 (1.99, 18.0)	2.87 (1.19, 6.93)	1.72 (0.82, 3.58)

^a Odds ratios adjusted for study site, sex, parental history of asthma and household income

Figures

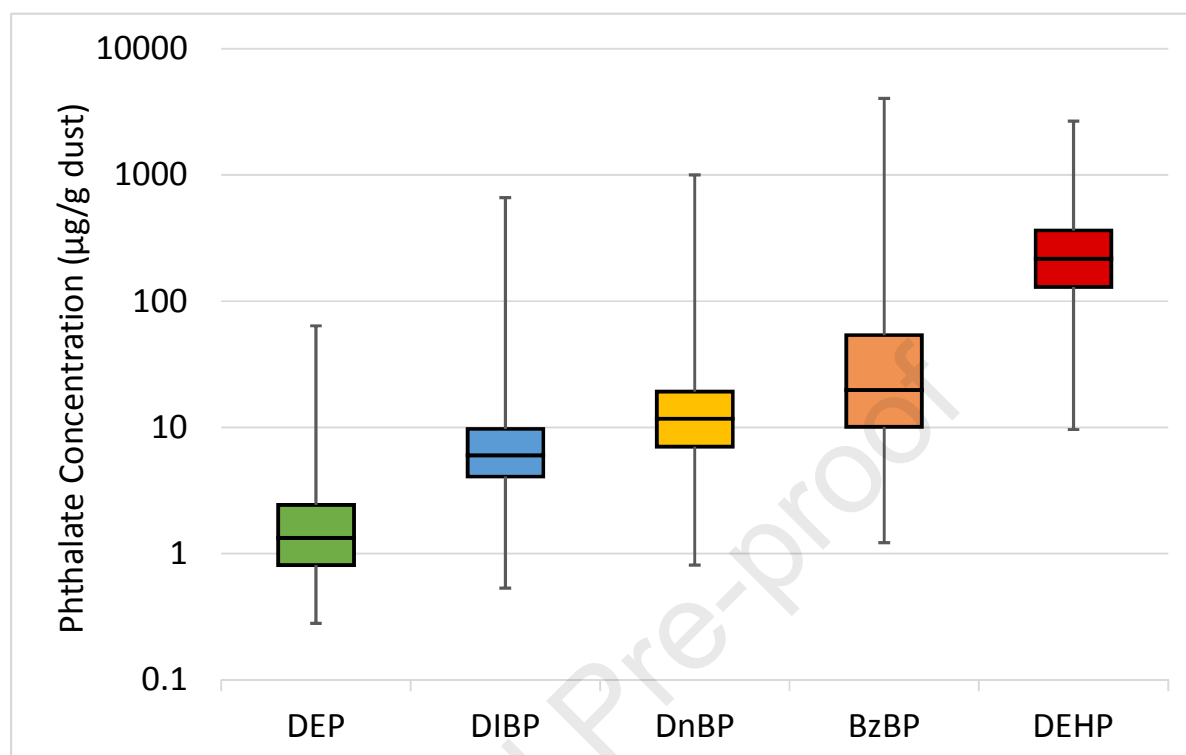


Figure 1 Boxplots showing the median (horizontal line in box), interquartile range (shaded box), and minimum and maximum (ends of vertical lines) concentrations of phthalates measured in house dust ($\mu\text{g/g}$) from 436 randomly selected children from the CHILD cohort (random subcohort). See Table S4 for corresponding numeric data.

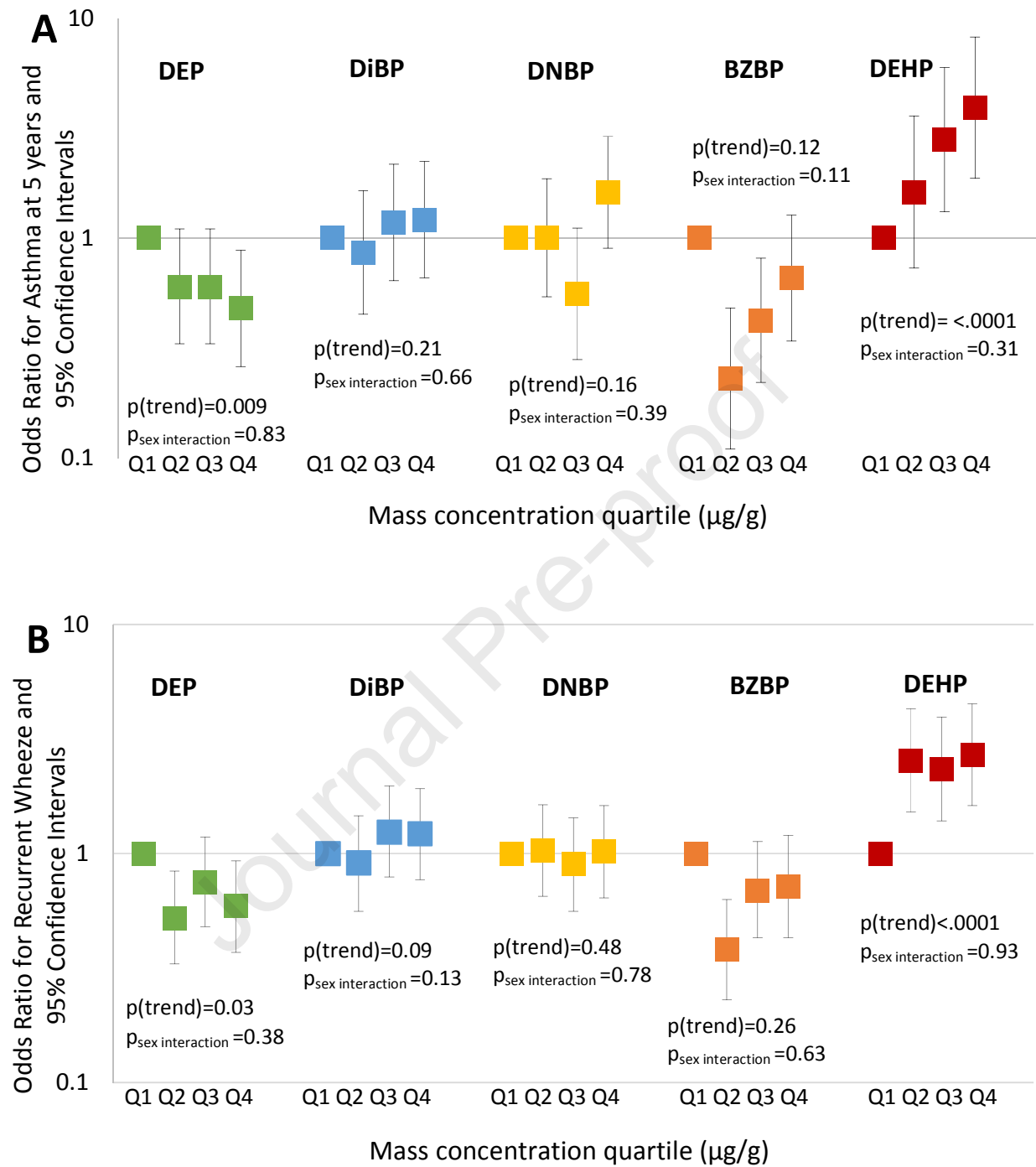


Figure 2 Association between phthalate concentration in dust and (A) asthma at 5 years and (B) recurrent wheeze between 2 to 5 years. Adjusted for study site, sex, parental history of asthma and household income. See Table S6 for corresponding numeric data. $p_{\text{sex interaction}}$ indicates significance of interaction term between each phthalate and sex. See Table S8 for logistic regression results stratified by sex.

Highlights

- DEHP, BzBP, DnBP, DiBP and DEP were detected in nearly all house dust samples
- Early life DEHP exposure was associated with a large increased risk of asthma at 5 years
- Early life DEHP exposure associated with increased risk of recurrent wheeze between 2 to 5 years
- Increased risk with DEHP observed at concentrations lower than other studies

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: