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Phthalate exposure through different pathways and allergic sensitization in preschool children with asthma, allergic rhinoconjunctivitis and atopic dermatitis

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ABSTRACT

Studies in rodents indicate that phthalates can function as adjuvants, increasing the potency of allergens. Meanwhile, epidemiological studies have produced inconsistent findings regarding relationships between phthalate exposures and allergic disease in humans. The present study examined phthalate exposure and allergic sensitization in a large group of 3–5 year old children: 300 random controls and 200 cases with asthma, rhinoconjunctivitis or atopic dermatitis as reported in questionnaires. The children were clinically examined to confirm their health status. Blood samples were analyzed for IgE sensitization to 20 allergens. Adjusted logistic regressions were used to look for associations between phthalate exposure indicators (mass fractions in dust from children's homes and daycares, metabolites in urine, and estimated daily indoor intakes from dust ingestion, inhalation and dermal absorption) and sensitization and allergic disease. No direct associations were found between phthalate exposures and asthma, rhinoconjunctivitis or atopic dermatitis. However, among children with these diseases, there were significant associations between non-dietary exposures to DnBP, BBzP and DEHP in the indoor environment (mass fractions in dust or daily indoor intakes from dust ingestion, inhalation and dermal absorption) and allergic sensitization. Some exposure pathways were more strongly associated with sensitization than others, although the results are not conclusive and require confirmation. A number of the associations depended on accounting for a child's exposure in more than one environment (i.e., daycare facility as well as home). Significant associations were not observed between phthalate metabolites in urine, which reflected exposure from diet as well as indoor pathways, and allergic sensitization.

1. Introduction

Phthalate esters are commonly used as plasticizers and are among the most frequently encountered indoor pollutants. There has been growing evidence that certain phthalate esters can function as endocrine disruptors (Braun et al., 2013; Jurewicz and Hanke, 2011; Meeker et al., 2009; Swan, 2008). The evidence for links between phthalate exposure and allergic diseases in children is inconsistent across earlier studies, which used various methodological approaches (e.g. questionnaires vs. doctor-diagnoses; phthalate dust mass fractions vs. urinary phthalate metabolites; prenatal exposure vs. early childhood exposure). Several studies

Abbreviations: ; DEP, diethyl phthalate; DiBP, di(isobutyl) phthalate; DnBP, di(n-butyl) phthalate; BBzP, benzylbutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; MEP, mono-ethyl phthalate; MnBP, mono-n-butyl phthalate; MiBP, mono-isobutyl phthalate; MBzP, mono-benzyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxylpentyl) phthalate; BHR, bronchial hyper-responsiveness; CEI, clinical examination with structured interview; DI, daily intake; DS, disease-specific; LOD, limit of detection; WQ, written questionnaire

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have reported associations between certain phthalate esters in dust or PVC materials in homes and allergic diseases in children (Bornehag et al., 2004a; Hsu et al., 2012; Jaakkola and Knight, 2008; Kolarik et al., 2008; Larsson et al., 2010). Prenatal exposure to BBzP, as measured by levels of MBzP in spot urine samples during the third trimester of pregnancy, has been associated with the child's risk of developing eczema in early childhood (Just et al., 2012a). A companion paper from the same cohort reported an association between prenatal exposure to BBzP and DnBP and the risk of asthma (Whyatt et al., 2014). A potential relationship between prenatal exposure to DEHP and BBzP and the risk of wheeze and respiratory tract infections was recently presented by Gascon et al. (in press). Hsu et al. (2012) found higher MBzP levels in asthmatic children and an increased risk of diagnosed asthma with higher quartiles, while levels of MEHP, a metabolite of DEHP, were associated with the severity of allergic rhinitis. However, in a previous study of Danish children (Callesen et al., 2014a), which used an approach similar to several of the above mentioned studies (Bornehag et al., 2004a; Hsu et al., 2012; Kolarik et al., 2008), we did not find a significant association between phthalate esters in the dust and asthma, rhinoconjunctivitis or atopic dermatitis determined by a clinical examination of the children. The only significant association was between DEHP and parent-reported current wheeze. Additionally, we found no *positive* associations between urinary concentrations of any phthalate metabolite and either asthma or rhinoconjunctivitis (Callesen et al., 2014b); the only positive association was between a metabolite of DEP and atopic dermatitis, and this was weak.

Kimber and Dearman (2010) have questioned whether phthalates themselves are the causative agents. It has been suggested that some phthalates may act as adjuvants for the agents actually responsible for allergic sensitization. Experimental studies on animals and in vitro analyses provide some evidence for the ability of phthalates to impact immune and allergic responses (Bornehag and Nanberg, 2010 and references therein; Ferguson et al., 2011; Guo et al., 2012; Lee et al., 2011; Nishioka et al., 2012) or to enhance mast cell degranulation and eosinophilic infiltration, which are important parts in the early inflammation process (Takano et al., 2006; Yanagisawa et al., 2008). In a mouse model, Simonsson et al. (2012) have shown that the skin sensitization potency of isothiocyanates increases when applied in combination with di(n-butyl) phthalate (DnBP). However, there are only a limited number of population-based studies investigating the potential impact of phthalates on immune and allergic responses. Gascon et al. (in press) did not find an association between prenatal phthalate exposure and a positive IgE response to three common allergens at age four. In recent work by Hoppin et al. (2013), the sum of DEHP metabolites was associated with allergic sensitization in adults. Furthermore, subjects with both symptoms and allergic sensitization had higher odds of BBzP exposure, as measured by urinary MBzP levels, than subjects without sensitization or with symptoms alone.

As we have previously argued (Callesen et al., 2014b), if a given pathway overwhelmingly dominates total exposure, then urinary metabolite concentrations, which represent the total intake of a phthalate ester, could fail to reveal an association between a different exposure pathway and the development/exacerbation of an allergic disease. The objective of the present paper is to assess potential associations between phthalate exposure (represented by mass fractions in dust and metabolite concentrations in urine) and allergic sensitization among children aged 3–5 years with asthma, allergic rhino-conjunctivitis and atopic dermatitis, and the role exposure pathways may play in such association.

2. Material and methods

2.1. Subjects and clinical examination

The first phase of the study included a written questionnaire (WQ) with 116 questions regarding characteristics of the building, indoor environment, family habits and occupant health with a focus on allergic diseases (asthma, rhinoconjunctivitis and eczema). The questionnaire resembled the one that was used in the original DBH study in Sweden (Bornehag et al., 2004b). The questions related to the child's health were similar to those used in the ISAAC study (Asher et al., 1995). The questionnaire was mailed in February 2008 to 17,486 families with a child aged 1–5 years living on the island of Funen, Denmark. The response rate was 63% corresponding to 11,084 families. Based on power calculations, a primary case-control group of 500 subjects was established among questionnaire responders aged 3–5 years from the municipality of Odense (main city on Funen; population 166,000) or one of its suburbs or rural neighborhoods ($n=2835$). The control group was a sample of 300 randomly selected children (random controls). The selection criteria for the 200 children in the primary case group was at least two parentally reported disease/symptoms regarding asthma, allergic rhinoconjunctivitis or eczema/atopic dermatitis on the WQ. Further details on the study population, their dwellings and prevalence of the three diseases have been described in Clausen et al. (2012).

All children underwent a clinical examination coupled with a structured interview (CEI) by the same experienced medical doctor. Weight and height were recorded and an extensive questionnaire-based interview, including medical history, time and onset of symptoms, trigger factors, family history of allergies and exposure to pets and tobacco smoke was conducted. The medical history was supplemented with the use of a video exemplifying asthma in 3–5 year olds and a demonstration of sounds indicative of asthma. The diagnostic criteria that had to be met for a child to be labeled with one of the allergic diseases during the clinical examination (asthma, rhinoconjunctivitis and atopic dermatitis) are described in detail in Callesen et al. (2014a). Children with clinically confirmed asthma, allergic rhinoconjunctivitis or atopic dermatitis were subsequently excluded from the *random control group* (and admitted in the disease-specific case groups, referred to as DS cases in what follows), leaving 243 children in the control group. Additionally, doctor-diagnosed allergic diseases, as reported by the parents using the written questionnaires, were used to obtain three new DS case groups. Children in these DS case groups based on the WQ and all children who reported in the questionnaire having "current wheeze" or "wheeze ever" were further excluded from the control group, leaving 166 children with no disease or symptoms in the *healthy control group.*

During the clinical examination a blood sample was taken and specific IgE was measured by ImmunoCap (Phadia, Allerød, Denmark) for a total of 20 allergens: i) indoor allergens (Dermatophagoides pteronyssinus, Dermatophagoides farinae, cat, horse and dog), ii) outdoor allergens (grass, common silver birch, mugwort, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata* and *Setomelanomma rostrata*) and iii) food allergens (egg white, milk, fish, wheat, peanut and soybean). Specific IgE was classified as positive when higher than 0.35 kU of antibody per liter (kU/L) (Bousquet et al., 2008; Eigenmann et al., 2013). 317 children provided a blood sample. Individuals who tested positive to at least one allergen were considered IgE positive ($n=91$; 28.7%).

2.2. Collection and analyses of dust and urine

Dust samples were vacuumed from non-floor surfaces onto a