Associations between allergic symptoms and phosphate flame retardants in dust and their urinary metabolites among school children

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Short running title: Phosphate flame retardants and child allergic symptoms

Acknowledgments

We thank all the study participants and their family members.

This work was supported in part by a Grant-in Aid for scientific research from the Ministry of Health Labor and Welfare, Japan (H20-Kenki Ippan-009), and by the Environment Research and Technology Development Fund (5C-1151, 5-1753) of the Ministry of the Environment, Japan, and of Environmental Restoration and Conservation Agent. Michiel Bastiaensen acknowledges the funding of a PhD fellowship from the University of Antwerp.

Competing interests: The authors declare that they have no competing financial interests.

Abstract:

BACKGROUND: Phosphate flame retardants (PFRs) are ubiquitously detected in indoor environments. Despite increasing health concerns pertaining to PFR exposure, few epidemiological studies have examined PFR exposure and its effect on children's allergies.

OBJECTIVES: To investigate the association between PFRs in house dust, their metabolites in urine, and symptoms of wheeze and allergies among school-aged children.

METHODS: A total of 128 elementary school-aged children were enrolled. House dust samples were collected from upper-surface objects. Urine samples were collected from the first morning void. Levels of 11 PFRs in dust and 14 PFR metabolites in urine were measured. Parent-reported symptoms of wheeze, rhinoconjunctivitis, and eczema were evaluated using the International Study of Asthma and Allergies in Childhood questionnaire. The odds ratios (ORs) of the Ln transformed PFR concentrations and categorical values were calculated using a logistic regression model adjusted for sex, grade, dampness index, annual house income, and creatinine level (for PFR metabolites only).

RESULTS: The prevalence rates of wheeze, rhinoconjunctivitis, and eczema were 22.7%, 36.7%, and 28.1%, respectively. A significant association between tris(1,3-dichloroisopropyl) phosphate (TDCIPP) in dust and eczema was observed: OR (95% confidence interval), 1.44 (1.13-1.82) (>limit of detection (LOD) vs <LOD). The ORs for rhinoconjunctivitis (OR=5.01 [1.53-16.5]) and for at least one symptom of allergy (OR=3.87 [1.22-12.3]) in the 4th quartile of

Σtris(2-chloro-*iso*-propyl) phosphate (TCIPP) metabolites was significantly higher than those in the 1st quartile, with significant p-values for trend (P_{trend}) (0.013 and 0.024, respectively). A high OR of 2.86 (1.04-7.85) (>LOD vs <LOD) was found for hydroxy tris(2-butoxyethyl) phosphate (TBOEP)-OH and eczema. OR of the 3rd tertile of bis (1,3-dichloro-2-propyl) phosphate (BDCIPP) was higher than the 1st tertile as a reference for at least one symptom (OR=3.91 [1.25-12.3]), with a significant P_{trend} = 0.020.

CONCLUSIONS: We found that TDCIPP in house dust, and metabolites of BDCIPP, TBOEP and TCIPP were associated with children's allergic symptoms. Despite some limitations of this study, these results indicate that children's exposure to PFR may impact their allergic symptoms.

Keywords

Phosphate Flame Retardants, Allergies, House dust, Urine, Metabolites, Children

1. Introduction

Organophosphate triesters are a class of chemicals predominantly used as additives in flame retardants and plasticizers. After the manufacturer's voluntary phase-out of the use of polybrominated diphenyl esters and polybrominated biphenyls, the use of phosphorus flame retardants (PFRs) as alternative flame retardant additives is on the rise (Kajiwara et al. 2011; Le Cann et al. 2011; Bergman et al. 2012). Polyurethane foam, thermoplastics, resins, polyvinylchloride, synthetic rubbers, and textiles are some of the major products that contain tri-*n*-butyl phosphate (TNBP), tris (2-chloroethyl) phosphate (TCEP), tris(2-chloro-*iso*-propyl) phosphate (TCIPP), tris (1,3-dichloro-2-propyl) phosphate (TDCIPP), and triphenyl phosphate (TPHP) (Stapleton et al. 2009; Van den Eede et al. 2011). TNBP, TPHP, and tricresyl phosphate (TMPP) are also used as lubricants, and tris (2-butoxyethyl) phosphate (TBOEP) is often used in floor covering and as a plasticizer in floor finish products and polish (Kajiwara et al. 2011). Since PFRs have been used in many housing materials and consumer products, their presence in indoor dust has been widely studied (Garcia et al. 2007; Stapleton et al. 2009; Bergh et al., 2011; Ali et al. 2012a; Ali et al. 2012b; Dirtu et al. 2012; Dodson et al. 2012; Araki et al. 2014; Tajima et al. 2014).

However, few reports have described the associations between PFRs exposure and human health. Three studies have indicated adverse associations of reproductive function and PFR exposure, with PFR being associated with lower levels of free thyroxine and prolactin

levels, the reduced quality of semen and embryos, and the lower success of several *in vitro* fertilization outcomes, such as successful fertilization, implantation clinical pregnancy, and live birth (Meeker and Stapleton 2010; Carignan et al. 2017; Carignan et al. 2018). One birth cohort study found that maternal PFR exposure resulted in decreased intelligence quotient scores and working memory among children (Castorina et al. 2017). One case-control study found that the occurrence and severity of papillary thyroid cancer was associated with a higher exposure to TCEP (Hoffman et al. 2017). Our previous cross-sectional study examined the associations between PFR levels in house dust and inhabitants' prevalence of sick building syndrome (Kanazawa et al. 2010), as well as asthma and allergies (Araki et al. 2014). We found that the prevalence of atopic dermatitis was higher when the concentrations of TCIPP and TDCIPP were higher, and the prevalence of asthma and allergic rhinitis was higher when the concentration of TNBP was higher. However, the concentration of PFRs in house dust does not exactly reflect individual exposure levels. Moreover, no study to date has focused on children, who are considered to be more vulnerable to the development of allergic symptoms via exposure to household dust, despite living in the same environment as adults (Ait Bamai et al. 2014b).

Recently, the biomonitoring of PFR has been established, and the metabolites of the abundant PFRs in indoor dust, such as TDCIPP, TCIPP, TCEP, TPHP, TBOEP, and TNBP, were quantified as a measure of the internal exposure of individuals (Van den Eede et al. 2013; Van

den Eede et al. 2015). This study aimed to investigate the association between the PFRs in house dust and their metabolites in urine, and the prevalence of allergies in children. We hypothesized that increasing levels of TDCIPP and TCIPP in dust and their metabolites in urine are associated with an increasing risk of developing allergic symptoms.

2. Methods

2.1 Study participants

This study was conducted with elementary school-aged children (n=128) using a questionnaire and a home visit for environmental measurements, in October and November of 2009 and 2010. Details on participant selection have been reported previously (Ukawa et al. 2013; Ait Bamai et al. 2014a; Tajima et al. 2014). Briefly, an initial cross-sectional study was conducted in Sapporo city in 2008. The questionnaire was distributed to 6,393 school children from 12 public elementary schools with an additional query of whether they were interested in participating in a home survey. Of the 4,408 students who responded to the questionnaire, 951 (from 832 families) agreed to a home visit for the conduction of environmental measurements in the following calendar years. In 2009 and 2010, 681 families with children who were still attending the same elementary school as in 2008 were contacted for a home visit. Through this selection procedure, we identified a total of 128 families (18.8%) that agreed to the home visit, and we successfully adjusted a schedule for the same.

2.2 Questionnaire

When investigators visited each house for environmental measurements in 2009 or 2010, self-administered questionnaires were distributed at the 1st visit, and investigators collected the responses 2 days later, during the 2nd visit. The questionnaire included questions on children's sex, school grade, duration spent at home, duration of sleep, and parental history of allergies. Parents also filled in data pertaining to the type of dwelling, building structure, building age, renovations, floor and wall materials used, the presence of an indoor smoker at home, the presence of pets, and dampness-related signs of mold growth, moldy odor, condensation, water leakage, and high humidity levels in the bathroom.

To define children's allergies, the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used (Beasley 1998). We classified participants as having wheeze when their parents answered "Yes" to the question: "Has your child had wheezing or whistling in the chest in the last 12 months?" Allergic rhinoconjunctivitis was defined by the following questions: (a) "Has your child had a problem with sneezing, or a runny/blocked nose in the absence of a cold or flu in the last 12 months?", and (b) "Has this nose problem been accompanied by itchy, watery eyes?". Eczema was defined by the following questions: (a) "Has your child had an itchy rash that has appeared and disappeared for at least 6 months?"; (b) "Have the aforementioned itchy rashes appeared at any time during the last 12 months?";

and (c) “Have the aforementioned itchy rashes affected one or several of the following areas: the folds of the elbows, the back of knees, the front of the ankles, the underside of the buttocks, or the areas around the neck, ears, or eyes?” When a child was defined as having wheeze, eczema and/or allergic rhinoconjunctivitis, the response was ”Yes” for at least one of the before mentioned symptoms.

2.3 PFR measurements in settled home dust

Details on dust collection and PFR analysis through gas chromatography/mass spectrometry (GC/MS), together with quality control and quality assurance, have been reported elsewhere (Ait Bamai et al. 2014a; Tajima et al. 2014). Briefly, dust samples were collected from the 128 dwellings using a hand-held vacuum cleaner (National HC-V15, Matsushita Electric Works, Ltd., Osaka, Japan) equipped with a paper dust bag (Nichinichi Pharmaceutical Co., Ltd., Mie, Japan) (Kanazawa et al. 2010; Tajima et al. 2014) from the multi-surface dust present in a main living area in which all of the inhabitants spent most of their time. Samples of upper surface dust were collected from the surfaces of objects that were placed more than 35 cm above the floor, including shelves, cupboards, moldings, frames, electronics, furniture, interior materials such as wallpaper, and the ceiling. After the removal of unwanted substances, such as hair, insects, food scraps, paper, etc., the collected dust was weighted and stored in stoppered glass test tubes, sealed with fluoric-tape, wrapped with aluminum foil, and kept at -20 ℃ until the day

of analysis.

The collected dust was subjected to ultrasonic extraction with residue analysis-grade acetone (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 15 min, and then the following 11 PFRs were analyzed by GC/MS (Agilent Technologies Inc., Palo Alto, CA, USA) in SIM-SCAN mode with a DB-17 column (30 m × 0.53 mm^2 [inner diameter] × 1 μm; J & W Scientific Inc., Folsom, CA, USA) at the Osaka Occupational Health Service Center, Japan Industrial Safety and Health Association, Osaka, Japan: trimethyl phosphate (TMP), triethyl phosphate (TEP), tripropyl phosphate (TPP), TNBP, TCIPP, TCEP, tris (2-ethylhexyl) phosphate (TEHP), TBOEP, TDCIPP, TPHP, and TMPP (Tajima et al. 2014). The limit of detection (LOD) was defined as the absolute amount of analytes that yielded a signal-to-noise ratio of three (n=6) (Tajima et al. 2014). For PFR concentrations lower than the LOD, a detection frequency × LOD value was assigned (James et al. 2002). To examine the background levels of PFRs from materials used for sampling, the ethanol-soaked cotton used to wipe the vacuum cleaners, nozzles, and dust bag were extracted with acetone and analyzed to confirm that there were no PFR peaks (Kanazawa et al. 2010; Tajima et al. 2014).

2.4 Measurement of the PFR metabolites in children's urine

Details on the collection of urine samples have been reported elsewhere (Ait Bamai et al. 2015). Briefly, on the day of the 2nd home visit, morning spot urine was collected by parents

in a polypropylene container and refrigerated until our visit. Each urine sample was dispensed into a stoppered glass test tube cleaned with acetone, and stored at −20℃ until the day of analysis.

Details of the analytical procedure for the urinary PFR metabolites (Bastiaensen et al., to be submitted), together with quality control and quality assurance are given in the Supporting Information. Fourteen urinary PFR metabolites were measured by LC-MS/MS: bis(2-butoxyethyl) phosphate (BBOEP), dibutyl phosphate (DNBP), bis(2-butoxyethyl) 2-hydroxyethyl phosphate (BBOEHEP), bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate (BCIPHIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), 4-hydroxyphenyl diphenyl phosphate (4-HO-DPHP), bis(2-butoxyethyl) 3-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP), 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP), 3-hydroxyphenyl diphenyl phosphate (3-HO-TPHP), 5-hydroxy-2-ethylhexyl diphenyl phosphate (5-HO-EHDPHP), 2-ethylhexyl phenyl phosphate (EHPHP) and tris(2-chloroethyl) phosphate (TCEP). Limit of quantification (LOQ) was calculated as three times the standard deviation of procedural blank concentrations per batch. For PFR concentrations lower than the LOQ, a detection frequency × LOQ value was assigned (James et al. 2002). The creatinine level in the urine was determined using enzyme-linked immunosorbent assay at SRL, Inc. (Tokyo, Japan).

2.5 Data analysis

The distribution of allergic symptoms in children and housing characteristics were evaluated through a chi-square test, *t*-test and Mann-Whitney U test. Concentrations of PFRs in house dust and their metabolites were compared between those with and without allergic symptoms by the Mann-Whitney U test. To determine the relationships between allergies and the concentrations of PFRs in house dust and their metabolites in urine, logistic regression was performed to obtain odds ratios (ORs) with their 95% confidence intervals (CIs). The PFR levels were categorized according to the frequency of the detection of levels above the LOD: in quartiles (>75% detected), in tertiles (50–75% detected), or detected/undetected (<50% detected). The OR and 95% CI of 2^{nd}, 3^{rd}, and/or 4^{th} categories compared to the lowest category of PFR were calculated. The linear associations based on categories of PFR are examined as P for trend (P_{trend}). Each PFR was modeled separately, and subsequently they were adjusted for previously known confounding factors such as sex (boys, girls/categorical variables, boys as the reference), grade (2-6/categorical variables, 2 as the reference), income as ordinal variables (1: <3 million Japanese yen, 2: 3-5 million Japanese yen, 3: 5-8 million Japanese yen, 4: >8 million Japanese yen), and dampness index as ordinal variables (0-5). In addition, the PFR metabolites were adjusted for urinary creatinine concentration (Barr et al. 2005). The dampness index (0-5) was calculated by summing the number of the signs of dampness observed in each dwelling, such as visible mold growth, moldy odor, condensation,

and water leakage (Saijo et al. 2004; Kishi et al. 2009). The mean value of the annual household income category—2.92—was imputed to the missing value. For the urinary metabolites, ΣuTBOEP (sum of BBOEP, 3-HO-TBOEP, and BBOEHEP in urine), ΣuTCIPP (sum of BCIPP and BCIPHIPP in urine), and ΣuTPHP (sum of DPHP and 4-HO-DPHP in urine) were also considered. To calculate sum of urinary metabolites, the sum of molar concentrations (nM) were used. SPSS for Windows version 24.0 (IBM Corp., Armonk, NY, USA) was used for the analysis with the application of a significance level of $p<0.05$. For categorical models, Bonferroni's correction was applied ($p<0.05/2 = 0.025$ for tertile model, and $p<0.05/3 = 0.017$ for quartile model).

2.6 Ethics

All parents of the study participants provided written informed consent. The study protocol was approved by the ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine, and Hokkaido University Center for Environmental and Health Sciences.

3. Results

Of the 128 participants, 53.1% were boys and all of them were in grades 2 to 6. The numbers of children with wheeze, rhinoconjunctivitis, eczema were 29 (22.7%), 47 (36.7%), and 36

(28.1%), respectively. Seventy-two children (56.3%) had at least one of the before mentioned symptoms (at least one of the symptoms, hereafter). Table 1 shows the characteristics of the participants. The distributions of allergies by personal characteristics are shown in Supplemental Table S1. A higher prevalence of asthma and allergies was observed when one or both parents reported having allergies. A higher prevalence of wheeze was observed among those from low-income families, while higher prevalence rates of rhinoconjunctivitis and eczema were observed among those from high-income families. The distributions of allergies by housing characteristic are shown in Supplemental Table S2. The prevalence of wheeze was significantly higher among those living in wooden houses than those living in houses made of ferroconcrete or other materials, in newer buildings compared to older houses, and in houses with mold growth and/or that had experienced water leakage within the preceding 5 years.

The levels of PFRs in house dust and of PFR metabolites in urine are shown in Table 2. For a subset of urine samples, 5-HO-EHDPHP, EHPHP, 3HO-TBOEP, 4HO-DPHP, 3HO-TPHP, 4-HO-TPHP, DNBP, and uTCEP were not measured for batch 1. The PFR with the highest concentration in dust was TBOEP with a median value of 26.55 μg/g and a detection frequency (>LOD) of 95.3%, followed by TPHP (3.13 μg/g, 94.4%), and TCIPP (2.23 μg/g, 78.9%). The PFR metabolites with the highest urinary concentrations were those of TBOEP [BBOEHEP (0.36 ng/mL [97.7%]), and BBOEP (0.20 ng/mL [78.0%])], as well as those of TPHP [DPHP (0.31 ng/mL [80.3%])], and TCIPP [BCIPHIPP (0.18 ng/mL [93.2%])]. The detection frequencies of TMP, TEP,

TEHP, and TMPP in dust, and BCIPP, 3-HO-TPHP, 4-HO-TPHP, and DNBP in urine, were lower than 10% and were therefore not considered for further examination.

Differences in PFR levels in house dust and their metabolites are shown in Supplemental Table S5. The geometric means of TBOEP concentration in dust were significantly lower in houses of children with wheeze than in houses of those without wheeze. No other associations were found between PFR levels in dust and allergic symptoms. The associations between categorized PFRs levels in dust and their metabolites in urine, and allergic symptoms, after adjustment for potential confounding factors, are shown in Table 3 and Table 4, respectively. For PFR in dust, the OR (95% CI) of TDCIPP levels >LOD (vs <LOD) in house dust for eczema was 3.75 (1.39-10.2) as shown in Table 3 and Figure 1. For PFR metabolites in urine, the OR for rhinoconjunctivitis of the 4th quartile of ΣuTCIPP was significantly higher than the OR of the 1st quartile (OR = 5.01 [1.53-16.5]) even after Bonferroni's adjustment (p=0.008). A high OR of 2.86 (1.04-7.85) (>LOD vs <LOD) was found for TBOEP-OH and eczema. The OR for at least one of the symptoms of the 3rd tertile of BDCIPP was significantly higher than the OR of the 1st tertile (OR = 3.91 [1.24-12.3]) after Bonferroni's adjustment (p=0.0019), with a significant dose-response association (P_{trend} = 0.020). Although the p-value was not statistically significant after Bonferroni's adjustment, ORs for at least one of the symptoms of the 4th quartile of ΣuTCIPP was higher than those of the 1st quartile (OR = 3.49 [1.22-12.3]) (p=0.022), and a P_{trend} = 0.024 was observed. Figure 2 shows the association

between the PFR metabolites in urine and allergic symptoms when P_{trend} <0.1.

4. Discussion

In this study, we examined the association between children's asthma and allergies, and PFRs in house dust and their metabolites in urine. Statistically significant associations were observed between higher levels of TDCIPP in house dust and the prevalence of eczema symptoms. The urinary metabolites of ΣuTCIPP were associated with increased ORs for rhinoconjunctivitis and at least one of the symptoms. Higher levels of BDCIPP were associated with increased OR for at least one of the symptoms. To our knowledge, this is the first study to examine the association between the levels of PFR in house dust and urine and the risk of allergic symptoms in children. One limitation of using the concentration of PFR in the house as a surrogate of exposure is that the PFR levels in house dust may not accurately reflect what children are exposed to. Meanwhile, urinary levels of PFR metabolites reflect children's accurate exposure levels not only from house dust, but also from other microenvironments and exposure sources, such as diet. PFRs are readily metabolized and eliminated from the body within several hours to days (Greaves et al. 2016; Völkel et al. 2017); therefore, urinary metabolite concentrations do not reflect longtime exposure. However, the PFR levels in house dust may reflect longer exposure periods than urine metabolites. Thus, the use of PFR levels in both house dust and their metabolites in urine has strengths and limitations.

A substantial percentage of the study population had missing income data. Thus, we conducted sensitivity analyses excluding 19 samples with unavailable income data. As the variance of ORs was within 20%, the impact of imputing data was minor. Additionally, we conducted sensitivity analyses excluding 15 samples from batch 1 due to possible batch effects. This exclusion resulted in slightly higher ORs, suggesting a possible bias towards further from the null. After both exclusions, p-values became larger, which could have been influenced by the small sample size. To avoid overestimation, the results with all data are shown in Tables 3 and 4.

Because of the low detection frequency of TDCIPP, we examined the categorical model, and levels >LOD (vs <LOD) showed an OR of 3.05. However, in our dust examination, this was the only significant association observed. Because of the multiple analyses of the associations between the PFRs in dust and symptoms, the association between TDCIPP and eczema may have been obtained by chance. Meanwhile, it should be noted that TDCIPP was significantly associated with an increasing prevalence of atopic dermatitis in our previous study of 186 single family dwellings in 6 regions of Japan (Araki et al. 2014). The results of these two different population studies are similar; therefore, these associations should not be neglected. Further investigation is warranted to identify the associations between TDCIPP and eczema.

As with TDCIPP in dust, TCIPP in dust was significantly associated with atopic dermatitis

in our previous study (Araki et al. 2014). Although we did not observe any significant associations in the case of TCIPP in dust in the present study, the ΣuTCIPP metabolites were associated with rhinoconjunctivitis. Moreover, the OR of the 4th quartile of ΣuTCIPP and 3rd tertile of BDCIPP, compared to the 1st quartile, suggest a potential threshold for such an association. Typically, children spend more than 60% of their time at home, and the participants of this study spent an average of 15.2 ± 1.5 hours a day at home. Moreover, according to one Japanese study, the TCIPP and TDCIPP levels in house dust are higher than those in elementary school dust (Mizouchi et al. 2015). The participants of this study lived in Sapporo city, and commuted to school on foot; the likelihood of commuting by car or other modes of transport was low. Thus, homes may be the dominant dust exposure sources for TCIPP and TDCIPP. Besides house dust, both TDCIPP and TCIPP have also been found in market foods in Belgium and Sweden (Poma et al. 2017; Poma et al. 2018). Although their concentrations in foods, such as cereals, pastries, fish, and fats (ng/g range) are lower than those in house dust (μg/g range), the estimation of PFR ingestion from food may be equal to or even higher than that from dust ingestion (Poma et al. 2017).

Unlike the case of TCDIPP and TCIPP, TBOEP was not detected in food in previous studies (Poma et al. 2017; Poma et al. 2018). TBOEP is used as a floor polish, and in floor finish products for compressed wooden floors (Dirtu et al. 2012). Of 128 houses in Japan, 109 had wooden floors, and the level of TBOEP in floor dust was relatively high, compared to that

observed in studies conducted in other countries (Tajima et al. 2014). BBOEP, a metabolite of TBOEP in urine, was detected in 78% of the samples in this study. (Fromme et al. 2014). The corresponding value from two pooled samples of children's urine in Australia was only 6% and 0%, respectively (Van den Eede et al. 2015). Those with allergies tend to avoid the use of wall-to-wall carpets and/or tatami (Japanese style grass mat floor) and to use (compressed) wooden floor for the prevention of mite allergens. This may result in higher levels of TBOEP and TBOEP metabolites in urine, among children with allergies. TBOEP in dust showed a rather inverse association with wheeze and at least one of the symptoms, although the association was not statistically significant. However, higher levels of the metabolites of TBOEP showed a higher OR of eczema and at least one of the symptoms, although the p-values did not reached statistical significance. This discrepancy could be attributed to the fact that the metabolites in urine provide more precise data on personal exposure. However, further studies are needed to confirm the results.

The mechanisms of PFRs in allergies are still not well-known. Traditionally, TBOEP, TCIPP and TDCIPP are classified as mild-to-moderate irritants of rabbit skin (WHO 1998; 2000), while TCIPP and TDCIPP cause irritation to the skin and eyes of rats (van der Veen and de Boer 2012). Recent *in vitro* studies found that TDCIPP and TPHP have an immunocytotoxic effect, and induce oxidative stress (Canbaz et al. 2017; Killilea et al. 2017). TCIPP and TCEP were found to affect the genes involved in immune response and steroid hormone biosynthesis, and be

involved in the complement cascade along with other potent inflammatory regulators (Krivoshiev et al. 2018a). One transcriptome study found that TBOEP has potential hepatic pro-inflammatory effects, and exposure to it may alter immune response, as well as lipid and steroid hormone metabolism (Krivoshiev et al. 2018b). TDCIPP has androgen receptor and glucocorticoid receptor antagonistic activities as well as pregnane X receptor (PXR) agonistic activity; TBOEP and TCIPP also showed PXR agonist activity, and thus the authors suggested that some PFRs may have potential endocrine disrupting effects (Kojima et al. 2013). Together with our findings and those of the aforementioned *in vitro* and *in vivo* studies, the potential effects of PFRs on allergies need to be further studied.

Limitations

There are several limitations to this study. First, data obtained from cross-sectional studies cannot be used to infer causality. In addition, reverse causality may have occurred from actions that may have been taken to reduce the exposure to allergens, especially by parents of children with allergic symptoms. Allergies usually develop in early childhood. In this study, collecting dust and measuring metabolites was conducted among school-aged children. Thus, as allergic symptoms were already present, the results cannot be used to infer causality or risk of developing an allergy. In addition, the majority of the children in this study had one or two parents with a history of allergy (99 out of 128). Thus, a heredity component might have been

stronger than the effect of exposure to PFRs. Second, the parents of our study's participants may have relatively higher interest levels in their home environments. Compared to the initial survey, the prevalence of wheeze and allergies was higher, but the number of smokers at home was low (Ait Bamai et al. 2016). Moreover, houses in this study were, on average, older than houses in our original study. Older houses showed lower levels of TBOEP than newer houses (Tajima et al. 2015). This bias may shift the results towards the null. In addition, we measured PFR from upper surface dust. PFR levels in floor dust showed significant associations with allergies in our previous study (Araki et al. 2014). Thus, we may have missed a potential association between PFR and symptoms by excluding floor dust. Third, in the house dust model, there may be many other pollutants that cause allergies. Therefore, we additionally adjusted for house dust mite allergen (Der 1) and di(2-ethylhexyl) phthalate, which are well-known allergens and chemicals related to asthma and allergies. The association between TDCIPP and eczema remained significant (data not shown), so our findings were not confounded by the presence of mite allergens or phthalates. Still, there could be other exposures which could have caused a residual confounding effect. Fourth, while we collected the dust and urine samples only once. However, our study was conducted in the months of October and November; this may eliminate seasonal variations. Fifth, the limited sample size may not have enough statistical power to find significant associations. Moreover, despite statistically significant associations, the confidence intervals were wide. In addition, many

associations have been tested, which increases the chance of finding a significant value despite a lack of a true difference. Finally, although we used the internationally-validated ISAAC questionnaire, misclassification of medical outcomes may have occurred.

5. Conclusions

In this study, as we have hypothesized, the level of TDCIPP in house dust was associated with eczema, whereas the metabolites of TCIPP and TBOEP were associated with rhinoconjunctivitis, eczema, and at least one of the symptoms among elementary school-aged children. Although the detection frequency of TDCIPP was rather low, an association between its concentration in dust and eczema was found, confirming the result of a previous study conducted in a different population. The observed associations between urinary metabolites and childhood allergic symptoms are preliminary findings, and further studies are needed to confirm these results.

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Table 1 Characteristics of the participants

		Total (n=128)	
		n	%
sex	boys	68	53.1
	girls	60	46.9
grade	2	14	10.9
	3	35	27.3
	4	27	21.1
	5	24	18.8
	6	28	21.9
parental history of allergy	no	29	22.7
	mother only	48	37.5
	father only	15	11.7
	both	36	28.1
annual household income	< 3 million	6	4.7
(Japanese yens/year)	3-5 million	25	19.5
	5-8 million	50	39.1
	>8 million	28	21.9
	missing	19	14.8
wheeze	yes	29	22.7
rhinoconjunctivitis	yes	47	36.7
eczema	yes	36	28.1
at least one of the above symptoms	yes	72	56.3
		Mean/median	SD (or IQR)
height (cm)	mean±SD	137.2	±10.8
weight (kg)	mean±SD	32.1	±8.3
body mass index (kg/m^2)	mean±SD	16.8	±2.5
Duration spend at home (hour)	median, IQR	15	(15-16)
duration of sleep (hour)	median, IQR	9.5	(9.0-9.8)
birth weight (g)	mean±SD	3098.5	±419.4
breast milk (month)	median, IQR	10	(3-13)

565 IQR, inter quartile, range; SD, standard deviation

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Table 2 Distribution of PFR in dust and their metabolites in urine

dust (µg/g dust)	n	>LOD (%)	mean	SD	min	25%	50%	75%	max
TMP	128	0.0							<LOD
TEP	128	3.1	0.1	0.1				<LOD	0.5
TPP	128	96.9	1.5	2.0	<LOD	0.5	0.7	1.7	13
TNBP	128	69.5	2.1	6.3		<LOD	0.7	1.7	61
TCIPP	128	78.9	13	59	<LOD	0.8	2.2	5.0	620
TCEP	128	77.2	4.0	11.2	<LOD	0.3	1.2	2.3	92
TEHP	128	7.8	0.6	1.2				<LOD	8.7
TBOEP	128	95.3	93	220	<LOD	12	27	81	1900
TDCIPP	128	35.9	5.1	12			<LOD	4.2	73
TPHP	128	94.4	4.8	4.8	<LOD	2.0	3.1	5.5	28
TMPP	128	0.0							<LOD
urine	n	>LOQ (%)	mean	SD	min	25%	50%	75%	max
creatinine (µg/mL)	128	100.0	1000	470	70	800	1000	1400	2800
metabolites (ng/mL)									
5-HO-EHDPHP	113	78%	0.09	0.15	<LOD	0.02	0.04	0.09	1.2
EHPHP	113	65%	n.a.	n.a.					
BBOEP	128	79%	0.34	0.37	<LOD	0.09	0.20	0.47	2.4
3-HO-TBOEP	113	48%	0.01	0.01			<LOD	0.01	0.05
BBOEHEP	128	98%	0.64	0.66	<LOD	0.19	0.36	0.95	3.9
BCIPP	128	10%	0.28	0.83				<LOD	5.9
BCIPHIPP	128	95%	0.51	1.30	<LOD	0.08	0.18	0.42	13
DPHP	128	80%	0.51	0.60	<LOD	0.17	0.32	0.64	5.7
4-HO-DPHP	113	41%	0.69	0.87	<LOD	0.18	0.22	0.92	4.3
3-HO-TPHP	113	2%	n.a.	n.a.					
4-HO-TPHP	113	3%	n.a.	n.a.					
BDCIPP	128	57%	0.12	0.32		<LOD	0.07	0.09	3.3
DNBP	113	8%	0.05	0.01				<LOD	0.78
uTCEP	113	85%	0.07	0.06	<LOD	0.03	0.05	0.10	0.32

BBOEHEP, bis(2-butoxyethyl)-(2-hydroxyethyl) phosphate; BBOEP, bis(2-butoxyethyl) phosphate; BCIPHIPP, bis(1,3-dichloro-2-propyl) phosphate; BCIPP, bis(2-chloro-iso-propyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; DNBP dimethyl phosphate, DPHP, diphenyl phosphate; LOD, limit of detection; LOQ, limit of quantification; 4-HO-DPHP, 4-hydroxydiphenyl phosphate; 5-HO-EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; 3-HO-TPHP, 3-hydroxytriphenyl phosphate; 4-HO-TPHP, 3-hydroxytriphenyl phosphate; PFR, phosphate flame retardants; TBOEP, tris(2-butoxyethyl) phosphate; 3-HO-TBOEP; tris(2-(3-hydroxy)butoxyethyl) phosphate; TCEP, tris(2-chloroethyl) phosphate; TCIPP, tris(2-chloro-iso-propyl) phosphate; TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; TEP, triethyl phosphate; TEHP tris(2-ethylhexyl) phosphate; TMP, trimethyl phosphate; TMPP, tricresyl phosphate; TNBP, tributyl phosphate; TPHP, triphenyl phosphate; TPP, tripropyl phosphate; uTCEP, tris(2-chloroethy) phosphate in urine

n.a., not applicable

Table 3 Associations between PFR in dust and allergies

	1st	2nd				3rd				4th				P for
		OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value	trend
wheeze														
Q-TPP	Ref.	0.50	0.10	2.57	0.408	3.64	0.99	13.4	0.051	0.89	0.21	3.77	0.871	0.506
T-TNBP	Ref.	0.67	0.23	1.99	0.471	0.64	0.21	1.99	0.440					0.424
Q -TCIPP	Ref.	1.04	0.28	3.91	0.955	1.26	0.33	4.83	0.734	1.52	0.42	5.53	0.521	0.493
Q -TCEP	Ref.	1.98	0.48	8.08	0.342	0.12	0.02	0.71	0.019	2.00	0.52	7.71	0.312	0.818
Q-TBOEP	Ref.	0.54	0.16	1.83	0.323	0.30	0.08	1.10	0.069	0.53	0.14	2.04	0.359	0.172
D-TDCIPP	Ref.	0.85	0.31	2.36	0.754	-				-				-
Q-TPHP	Ref.	0.51	0.14	1.93	0.325	0.67	0.19	2.40	0.540	0.86	0.26	2.84	0.809	0.874
rhino-conjunctivitis														
Q-TPP	Ref.	0.68	0.23	2.05	0.495	1.19	0.40	3.57	0.752	0.64	0.19	2.14	0.471	0.713
T-TNBP	Ref.	0.46	0.17	1.24	0.125	0.57	0.21	1.53	0.263					0.259
Q -TCIPP	Ref.	0.33	0.10	1.04	0.058	0.46	0.15	1.43	0.182	0.96	0.30	3.02	0.945	0.945
Q -TCEP	Ref.	1.70	0.50	5.83	0.398	1.95	0.67	5.66	0.220	2.28	0.69	7.49	0.175	0.150
Q-TBOEP	Ref.	0.79	0.26	2.39	0.679	0.54	0.17	1.67	0.281	0.40	0.12	1.32	0.134	0.105
D-TDCIPP	Ref.	0.81	0.34	1.90	0.621	-				-				-
Q-TPHP	Ref.	1.13	0.37	3.42	0.827	1.25	0.42	3.78	0.689	0.39	0.12	1.28	0.122	0.178
eczema														
Q-TPP	Ref.	0.93	0.27	3.14	0.901	0.92	0.26	3.30	0.900	0.64	0.17	2.44	0.512	0.530
T-TNBP	Ref.	1.65	0.57	4.73	0.355	1.00	0.34	2.98	0.997					0.997
Q -TCIPP	Ref.	1.53	0.47	5.05	0.481	0.73	0.20	2.66	0.638	0.88	0.25	3.10	0.843	0.591
Q -TCEP	Ref.	1.59	0.43	5.86	0.487	1.28	0.40	4.15	0.676	1.91	0.52	6.97	0.326	0.396
Q-TBOEP	Ref.	1.01	0.30	3.39	0.990	0.40	0.10	1.54	0.183	1.38	0.38	4.97	0.622	0.961
D-TDCIPP	**Ref.**	3.75	1.39	10.2	0.009	-				-				-
Q-TPHP	Ref.	2.82	0.81	9.74	0.102	0.70	0.20	2.50	0.582	0.69	0.19	2.46	0.563	0.228
at least one of the symptoms														
Q-TPP	Ref.	0.87	0.29	2.56	0.795	2.29	0.74	7.12	0.152	0.81	0.25	2.58	0.715	0.827
T-TNBP	Ref.	0.82	0.31	2.14	0.687	0.80	0.31	2.07	0.652					0.653
Q -TCIPP	Ref.	0.87	0.29	2.55	0.794	0.87	0.29	2.60	0.800	1.15	0.36	3.69	0.811	0.855
Q -TCEP	Ref.	4.12	1.18	14.4	0.027	1.60	0.54	4.77	0.396	2.06	0.63	6.73	0.233	0.374
Q-TBOEP	Ref.	0.99	0.32	3.02	0.985	0.43	0.14	1.33	0.143	0.57	0.17	1.85	0.347	0.166
D-TDCIPP	Ref.	0.90	0.40	2.06	0.811	-				-				-
Q-TPHP	Ref.	1.57	0.51	4.78	0.432	0.72	0.23	2.19	0.557	0.96	0.32	2.85	0.935	0.623

Adjusted for sex, grade, annual income, and dampness index

D-, dichotomized; Q-, quartile; Ref., reference; T-, tertile; TBOEP, tris(2-butoxyethyl) phosphate; TCEP, tris(2-chloroethy) phosphate; TCIPP, tris(2-chloro-iso-propyl) phosphate; TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; TNBP, tributyl phosphate; TPHP, triphenyl phosphate; TPP, tripropyl phosphate; "-", not examined

Table 4 Associations between PFR metabolites in urine and allergic symptoms

	1st	2nd				3rd				4th				p-for trend
		OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value	
wheeze														
Q-5HO-EHDPHP	Ref.	0.80	0.16	4.07	0.788	1.42	0.35	5.83	0.624	2.66	0.65	10.9	0.173	0.160
Q-BBOEP	Ref.	0.85	0.22	3.29	0.819	0.77	0.21	2.74	0.683	1.54	0.45	5.33	0.492	0.589
D-TBOEP-OH	Ref.	1.18	0.42	3.30	0.755									
Q-BBOEHEP	Ref.	0.34	0.08	1.39	0.133	0.47	0.12	1.88	0.286	1.34	0.38	4.77	0.651	0.600
Q-BCIPHIPP	Ref.	3.74	0.90	15.6	0.070	2.59	0.61	11.1	0.199	2.53	0.59	10.9	0.211	0.364
Q-DPHP	Ref.	1.38	0.39	4.92	0.620	0.95	0.23	3.98	0.943	2.00	0.45	8.84	0.362	0.479
D-4HO-DPHP	Ref.	0.43	0.15	1.22	0.111									
T-BDCIPP	Ref.	1.35	0.43	4.29	0.607	1.95	0.55	6.86	0.298					0.299
Q-uTCEP	Ref.	1.15	0.30	4.40	0.843	0.61	0.14	2.62	0.506	1.96	0.50	7.57	0.332	0.512
Q-ΣuTBOEP	Ref.	0.39	0.10	1.62	0.197	0.38	0.10	1.52	0.173	1.86	0.51	6.82	0.349	0.443
Q-ΣuTCIPP	Ref.	1.38	0.36	5.19	0.638	2.54	0.67	9.55	0.169	1.14	0.28	4.62	0.858	0.640
Q-ΣuTPhP	Ref.	1.35	0.38	4.82	0.643	1.08	0.26	4.40	0.916	1.77	0.40	7.83	0.451	0.540
rhino-conjunctivitis														
Q-5HO-EHDPHP	Ref.	1.58	0.43	5.84	0.494	0.82	0.24	2.77	0.746	1.31	0.40	4.26	0.655	0.835
Q-BBOEP	Ref.	0.39	0.12	1.27	0.117	0.41	0.13	1.27	0.121	1.42	0.48	4.20	0.524	0.619
D-TBOEP-OH	Ref.	0.85	0.35	2.09	0.729									
Q-BBOEHEP	Ref.	0.41	0.13	1.37	0.148	0.94	0.30	2.93	0.914	0.87	0.27	2.82	0.822	0.831
Q-BCIPHIPP	Ref.	0.57	0.17	1.91	0.361	0.99	0.31	3.18	0.991	1.83	0.59	5.65	0.291	0.178
Q-DPHP	Ref.	0.76	0.24	2.46	0.650	2.32	0.66	8.07	0.188	2.13	0.62	7.32	0.232	0.132
D-4HO-DPHP	Ref.	0.74	0.31	1.77	0.501									
T-BDCIPP	Ref.	0.76	0.29	2.02	0.586	2.02	0.70	5.85	0.193					0.215
Q-uTCEP	Ref.	0.49	0.14	1.68	0.257	0.60	0.18	1.95	0.394	1.59	0.48	5.27	0.450	0.457
Q-ΣuTBOEP	Ref.	0.64	0.20	2.07	0.460	0.81	0.26	2.53	0.723	1.88	0.57	6.26	0.302	0.268
Q-ΣuTCIPP	**Ref.**	1.31	0.40	4.27	0.651	1.06	0.31	3.59	0.931	5.01	1.53	16.5	0.008	**0.013**
Q-ΣuTPhP	Ref.	0.97	0.31	3.05	0.962	1.76	0.51	6.05	0.367	1.93	0.56	6.66	0.296	0.233
Eczema														
Q-5HO-EHDPHP	Ref.	0.51	0.10	2.62	0.421	1.45	0.39	5.37	0.577	2.80	0.76	10.4	0.123	0.103
Q-BBOEP	Ref.	1.34	0.38	4.71	0.645	0.56	0.15	2.14	0.399	2.17	0.68	6.94	0.190	0.377
D-TBOEP-OH	**Ref.**	**2.86**	**1.04**	**7.85**	**0.041**									
Q-BBOEHEP	Ref.	0.91	0.23	3.59	0.898	1.46	0.40	5.39	0.568	2.76	0.77	9.96	0.121	0.080
Q-BCIPHIPP	Ref.	0.82	0.21	3.29	0.784	2.85	0.79	10.3	0.111	1.73	0.47	6.39	0.411	0.163
Q-DPHP	Ref.	0.46	0.13	1.62	0.227	0.68	0.18	2.63	0.578	1.25	0.36	4.35	0.721	0.654
D-4HO-DPHP	Ref.	1.14	0.44	2.96	0.783									
T-BDCIPP	Ref.	1.59	0.55	4.56	0.392	1.92	0.57	6.45	0.291					0.283
Q-uTCEP	Ref.	0.73	0.20	2.68	0.633	0.96	0.26	3.48	0.950	1.08	0.29	4.00	0.909	0.835
Q-ΣuTBOEP	Ref.	1.24	0.34	4.56	0.745	1.06	0.29	3.96	0.926	3.48	0.94	12.9	0.062	0.082
Q-ΣuTCIPP	Ref.	2.42	0.64	9.18	0.192	3.38	0.90	12.6	0.070	1.87	0.50	6.98	0.349	0.329
Q-ΣuTPhP	Ref.	0.62	0.19	2.07	0.439	0.60	0.16	2.29	0.453	0.99	0.28	3.49	0.983	0.974
atleast one of the symptoms														
Q-5HO-EHDPHP	Ref.	1.52	0.37	6.22	0.561	1.32	0.40	4.35	0.653	3.33	0.96	11.6	0.059	0.082
Q-BBOEP	Ref.	0.60	0.19	1.88	0.378	0.51	0.17	1.51	0.223	3.82	1.07	13.6	0.039	0.121
D-TBOEP-OH	Ref.	1.40	0.56	3.49	0.465									
Q-BBOEHEP	Ref.	0.51	0.17	1.57	0.241	0.98	0.30	3.16	0.974	1.37	0.43	4.36	0.593	0.410
Q-BCIPHIPP	Ref.	0.72	0.22	2.31	0.578	1.88	0.58	6.09	0.290	1.76	0.55	5.62	0.340	0.138
Q-DPHP	Ref.	0.58	0.19	1.76	0.337	0.83	0.24	2.84	0.766	2.00	0.60	6.68	0.258	0.214
D-4HO-DPHP	Ref.	0.65	0.27	1.55	0.332									
T-BDCIPP	**Ref.**	**1.66**	**0.65**	**4.25**	**0.289**	**3.91**	**1.25**	**12.3**	**0.019**					**0.020**
Q-uTCEP	Ref.	0.69	0.21	2.19	0.523	0.89	0.27	2.97	0.850	1.95	0.54	7.07	0.309	0.299
Q-ΣuTBOEP	Ref.	0.66	0.21	2.02	0.465	0.94	0.30	2.89	0.912	3.49	0.97	12.6	0.056	0.060
Q-ΣuTCIPP	**Ref.**	2.60	0.81	8.33	0.107	3.08	0.97	9.74	0.056	3.87	1.22	12.3	0.022	**0.024**
Q-ΣuTPhP	Ref.	0.75	0.25	2.28	0.613	0.70	0.21	2.29	0.555	1.63	0.50	5.34	0.420	0.448

581 Adjusted for sex, grade, annual income, dampness index, and creatinine

582 BBOEHEP bis(2-butoxyethyl)-(2-hydroxyethyl) phosphate; BBOEP, bis(2-butoxyethyl) phosphate; BCIPHIPP, bis(1,3-
583 dichloro-2-propyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; DNBP dimethyl phosphate, D-,
584 dichotomized; DPHP, diphenyl phosphate; CI, confidence interval; 4-HO-DPhP, 4-hydroxydiphenyl phosphate; 5-HO-
585 EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; OR, odds ratio; PFR, phosphate flame retardants; Q-, quartile; Ref.,
586 reference; ΣuTBOEP, Σ metabolites of tris(2-butoxyethyl) phosphate in urine; ΣuTCIPP, Σ metabolites of tris(2-chloro-iso-
587 propyl) phosphate in urine, ΣuTPHP; Σ metabolites of triphenyl phosphate in urine; T-tertile,

588 ΣuTBOEP, ΣuTCIPP, ΣuTPHP are sum of molar concentrations (nM) "-", not examined

Figure legends

Figure 1 Association between TDCIPP in house dust and eczema.

The levels of TDCIPP in house dust were dichotomized as either >LOD (D1) or <LOD (D2), and the odds ratio (OR) and 95% confidence interval (95% CI) of the prevalence of eczema are shown as black squares and whiskers, respectively. The OR of D2 was calculated for undetected levels as a reference (D1), and adjusted for sex, grade, annual income, and dampness index.

TDCIPP, tris(1,3-dichloroisopropyl) phosphate

Figure 2 Associations between PFR metabolites in urine and allergic symptoms

The odds ratios (ORs) and 95% confidence intervals (95% CI) of the allergic symptoms are shown as black squares and whiskers, respectively for (A) ΣuTCIPP with rhinoconjunctivitis; (B) TBOEP-OH, (C) BBOEHEP, and (D) ΣuTBOEP with eczema; (E) 5HO-EHDPHP, (F) BDCIPP, (G) ΣuTBOEP and (H) ΣuTCIPP with at least one of the symptoms. The levels of ΣuTCIPP in urine were categorized in quartiles as Q1 (≤ 0.62 nM), Q2 (0.63 - 0.95 nM), Q3 (0.96 - 2.27 nM), Q4 (> 2.27 nM). The levels of TBOHP-OP were categorized as dichotomized D1 ≤ LOQ, D2 > LOQ. The levels of BBOEHEP were categorized in quartiles as Q1 (≤ 0.09 ng/mL), Q2 (0.10 - 0.20 ng/mL), Q3 (0.21 - 0.47 ng/mL), Q4 (> 0.47 ng/mL). The levels of ΣuTBOEP were categorized in quartiles as Q1 (≤ 0.99 nM), Q2 (1.0 - 1.7 nM), Q3 (1.8 - 3.8 nM), Q4 (> 3.8 nM). The levels of 5HO-EHDPHP were categorized in quartiles as Q1 (≤ 0.62 ng/mL), Q2 (0.63 - 0.95 ng/mL), Q3 (0.96 - 2.27 ng/mL), Q4 (> 2.27 ng/mL). The levels of BDCIPP were categorized in tertiles as T1 (≤ 0.03 ng/mL), T2 (0.04 - 0.09 ng/mL), T3 (> 0.09 ng/mL). The ORs were calculated for the 1^{st} quartile, 1^{st} tertile, or 1^{st} dichotomized (undetected levels)

as reference, and adjusted for sex, grade, annual income, dampness index, and creatinine level. When compared to the reference, the statistical significances of the p values were $p<0.017$ for the quartile model and $p<0.025$ for the tertile model based on Bonferroni's correction. Statistical significances are marked "*".

BBOEHEP, bis(2-butoxyethyl)-(2-hydroxyethyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; CI, confidence interval; 5-HO-EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; OR, odds ratio; ΣuTBOEP, Σ metabolites of tris(2-butoxyethyl) phosphate in urine; ΣuTCIPP, Σ metabolites of tris(2-chloro-iso-propyl) phosphate in urine

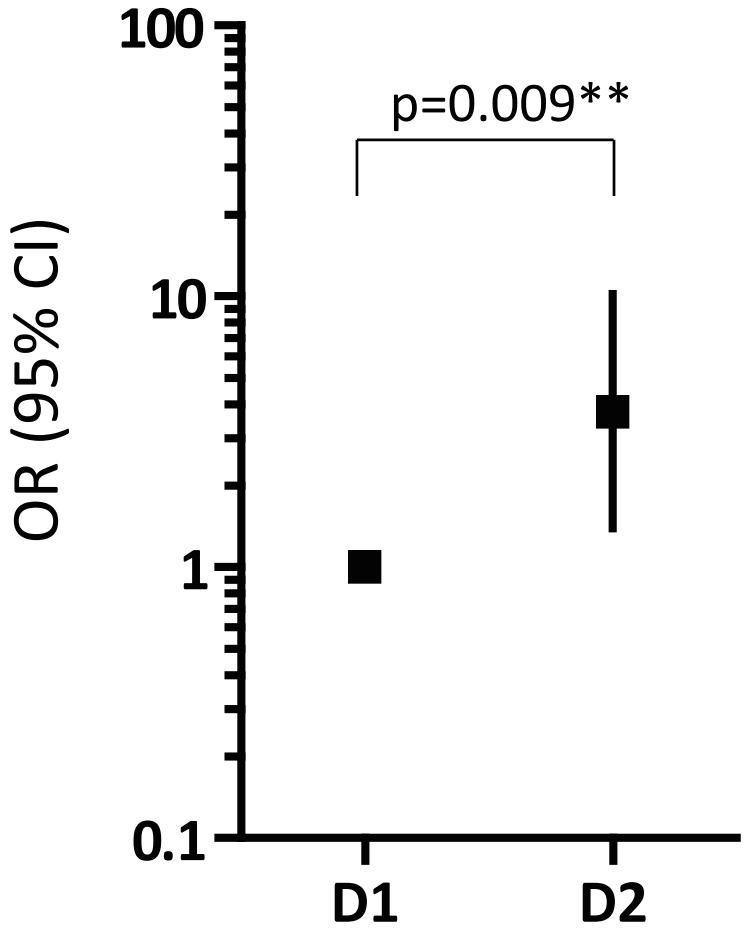

Figure 1 TDCIPP in house dust and its association with eczema

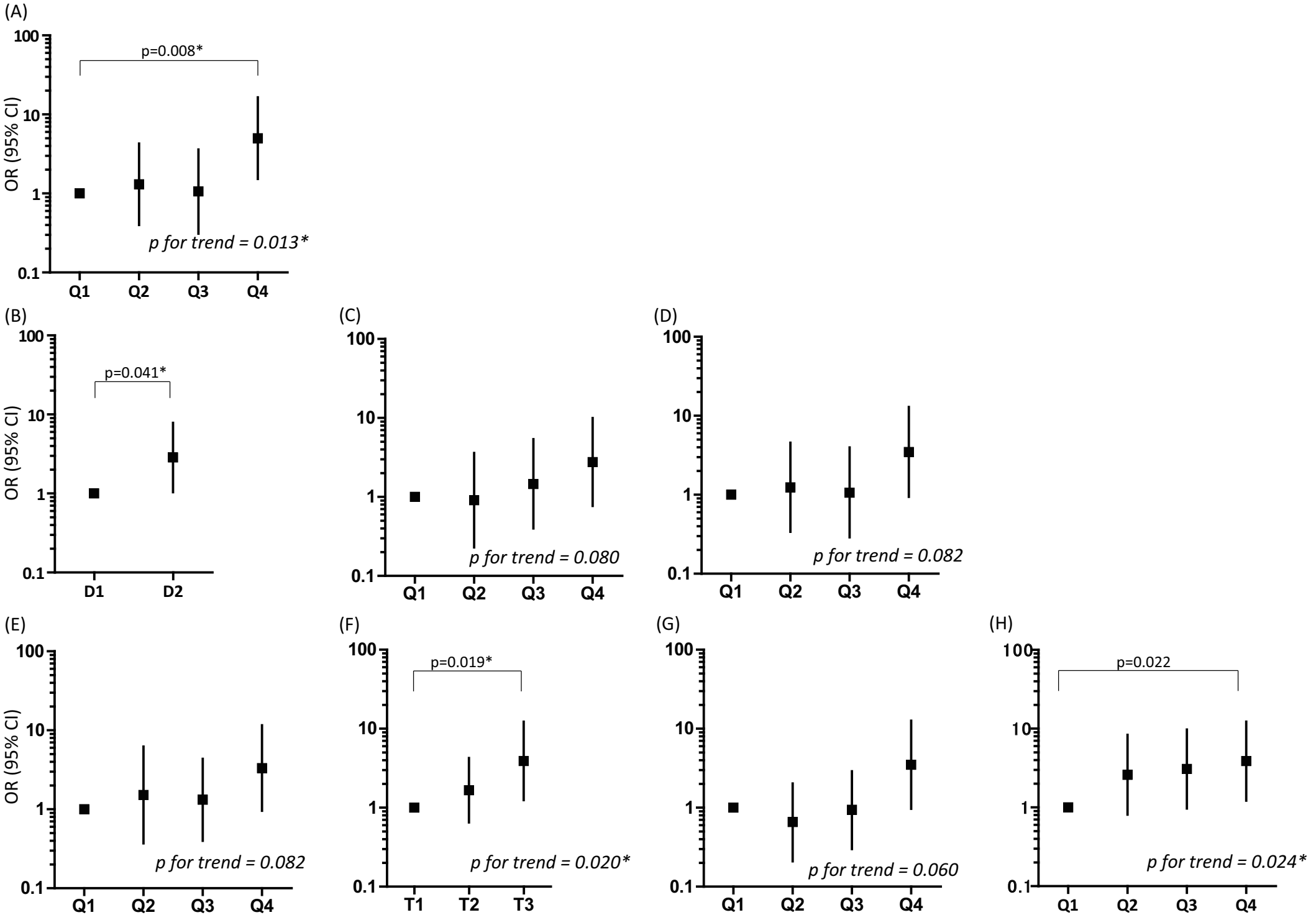

Figure 2 PFR metabolites in urine and their association with allergic symptoms

Supplemental Material

Associations between allergic symptoms and phosphate flame retardants in dust and their urinary metabolites among school children

Atsuko Araki, Michiel Bastiaensen, Yu Ait Bamai, Nele Van den Eede, Toshio Kawai, Tazuru Tsuboi, Rahel Mesfin Ketema, Adrian Covaci, Reiko Kishi

Table of contents

Measurement of metabolites of organophosphate flame retardants in urine

Fourteen urinary PFR metabolites were measured using the analytical procedure described below. The procedure was adapted for urine as matrix from a similar method for the analysis of the same compounds in wastewater (Been et al., 2017). The validated procedure has been submitted to Analytical and Bioanalytical Chemistry.

Metabolites of interest included bis(2-butoxyethyl) phosphate (BBOEP), dibutyl phosphate (DNBP), bis(2-butoxyethyl) 2-hydroxyethyl phosphate (BBOEHEP), bis(1-chloro-2-propyl) phosphate (BCIPP), bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate (BCIPHIPP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), diphenyl phosphate (DPHP), 4-hydroxyphenyl diphenyl phosphate (4-HO-DPHP), bis(2-butoxyethyl) 3-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP), 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP), 3-hydroxyphenyl diphenyl phosphate (3-HO-TPHP), 5-hydroxy-2-ethylhexyl diphenyl phosphate (5-HO-EHDPHP), 2-ethylhexyl phenyl phosphate (EHPHP) and tris(2-chloroethyl) phosphate (TCEP). All compounds were quantified using an 8-point linear calibration curve (0.08 to 20 ng in calibration vials), except BCIPP and HO-DPHP for which the calibration ranged from 0.4 to 100 ng in standard. Internal standard mixture was always spiked at 5 ng for all internal standards. EHPHP, 4-HO-TPHP and 3-HO-TPHP were only assessed on a qualitative basis, due to unsatisfactory validation results.

Chemicals

TCEP was purchased from Chiron AS (Trondheim, Norway). DNBP and DPHP were purchased from Sigma-Aldrich, Bornem, Belgium). All other individual standards were custom synthesized by Dr. Vladimir Belov (Max Planck Institute, Göttingen, Germany). Internal standard mixture included TCEP-D12, BBOEHEP-D4, BBOEP-D4, BDCIPP-D10, TBOEP-D6 (all custom synthesis), DPHP-D10 and TPHP-D15 (bought from Sigma-Aldrich). Other chemicals used in this procedure were methanol (LC-grade), potassium hydroxide and potassium phosphate (all purchased from Merck, Darmstadt, Germany); formic acid, ammonium acetate and β-glucuronidase deconjugation enzyme (lypophilized powder from *E. coli*, >10 000 000 unit/g) (from Sigma-Aldrich). Ultrapure water (UPW) was obtained from a PURELAB Flex system (ρ = 18.2 MΩ/cm, Elga Veolia, Tienen, Belgium).

Sample preparation and instrumental analysis

For sample preparation, 2 mL urine samples were spiked with mass labelled reference standards (IS mixture, 2.5 ng/mL) followed by the addition of 1.5 mL of phosphate buffer (1 M, pH 6) and 100 µl of deconjugation enzyme solution (2 mg/mL at pH 6). Samples were then incubated for 2 hours at 37°C. Subsequently, target analytes were acidified with 100 µL formic acid and extracted using solid phase extraction. Samples were loaded onto Bond-Elut C18 cartridges (3 mL, 200 mg, Agilent, Santa Clara,

USA), previously conditioned with 3 mL of methanol and 2 mL of UPW. Cartridges were washed using 1.5 mL of UPW and eluted using 3 mL of methanol. Before the eluates were evaporated to near dryness under a gentle stream of nitrogen, 50 µl of UPW was added as keeper solvent. Final extracts were then reconstituted in 100 µl of UPW:methanol (50/50, v/v) mixture, filtered with 0.2 µm centrifugal filters (nylon, VWR International, Leuven, Belgium) and transferred to injection vials with glass inserts for LC-MS/MS analysis.

Instrumental analysis was carried out on an Agilent 1290 Infinity liquid chromatography system coupled to an Agilent 6460 Triple Quadrupole mass spectrometer (LC-MS/MS, Santa Clara, CA, USA) with an electrospray ionization (ESI) source. Separation of the metabolites was performed on a Phenomenex Kinetex Biphenyl column (2.1 x 100 mm, 2.6 µm; Torrance, CA, USA), at a column temperature of 40 °C. Mobile phases consisted of UPW with 2% methanol (A) and methanol with 2% UPW (B), both with 5mM ammonium acetate. The mass spectrometer was operated in dynamic multiple reaction monitoring (dMRM) in positive to negative switching ionization mode, with time segment set at 1 minute for each compound.

Urine samples analysis

Urine samples were analysed in three batches (see Table SI-1). Batch 1 contained 15 samples that were measured in preliminary screening for BBOEP, BBOEHEP, BCIPP, BCIPHIPP, DPHP, BDCIPP and DNBP. All metabolites were measured in batch 2 (n=64) and batch 3 (n=49). Limit of quantification (LOQ) was calculated as three times the standard deviation of procedural blank concentrations per batch. PFR metabolite concentrations below LOQ were filled up with a value of LOQ * detection frequency.

Quality assurance and quality control

Various measures were taken to guarantee precise and accurate measurements. During sample preparation 1 procedural blank extraction (with UPW instead of urine matrix) was carried out per 6 urine samples. Contamination from the laboratory environment and from materials and reagents used during the analytical procedure was generally low, as reflected by LOQs calculated from PFR metabolite concentrations in procedural blanks.

Quality control samples (QC) consisted of mid (4 ng) or high (10 ng) spiked UPW extractions and were analyzed in parallel to urine samples and procedural blanks. Four QC samples were analyzed per 50 samples per level. Average QC accuracies at both levels and method recoveries for each compound were summarized in Table SI-2.

The performance of the instrumental LC-MS/MS analysis was continuously monitored through random

injection of standards (every 20 injections) and injection of solvent blanks (to assess carry-over between injections).

External quality control was assessed through participation to inter-laboratory comparison exercises such as HBM4EU ICI/EQUAS (2018) and OSEQAS (2018).

References

1. Been F, Bastiaensen M, Lai FY, van Nuijs AL, Covaci A. Liquid Chromatography–Tandem Mass Spectrometry Analysis of Biomarkers of Exposure to Phosphorus Flame Retardants in Wastewater to Monitor Community-Wide Exposure. Analytical Chemistry. 2017, 89: 10045-10053.
2. Bastiaensen M, Xu F, Been F, Van den Eede N, Covaci A. Simultaneous Determination of Fourteen Urinary Biomarkers of Exposure to Organophosphate Flame Retardants by LC-MS/MS. Analytical and Bioanalytical Chemistry

Supplemental Table S1: Overview of the analysis in batches and corresponding limits of quantification (LOQ) and detection frequencies (% >LOQ). LOQs are given in ng/mL.

		5-HO-EHDPHP	BBOEP	3-HO- TBOEP	BBOEHEP	BCIPP	BCIPHIPP	DPHP	4-HO-DPHP	BDCIPP	DNBP	TCEP
Batch 1	LOQ		0.73		0.02	0.1	0.10	0.29		0.40	0.15	
(n=15)	% >LOQ		7%		87%	13%	60%	53%		27%	20%	
Batch 2	LOQ	0.031	0.106	0.002	0.003	0.956	0.005	0.148	0.572	0.042	0.391	0.020
(n=64)	% >LOQ	64%	88%	44%	100%	11%	98%	78%	31%	55%	6%	84%
Batch 3	LOQ	0.007	0.049	0.001	0.004	0.484	0.001	0.099	0.422	0.038	0.090	0.019
(n=49)	% >LOQ	96%	90%	52%	100%	8%	100%	92%	52%	68%	6%	86%
Total	% >LOQ	78% (n=113)	79% (n=128)	48% (n=113)	98% (n=128)	10% (n=128)	95% (n=128)	80% (n=128)	41% (n=113)	57% (n=128)	8% (n=128)	85% (n=113)

Supplemental Table S2: Average performance of quality control (QC) samples and method recovery (%) per compound of interest. All compounds were spiked at 10 ng and 4 ng, except BCIPP and 4-HO-DPHP who were spiked at 50 ng and 20 ng.

	5-HO-EHDPHP	**EHPHP**	**BBOEP**	**3-HO-TBOEP**	**BBOEHEP**	**BCIPP**	**BCIPHIPP**	**DPHP**	**4-HO-DPHP**	**3-HO-TPHP**	**4-HO-TPHP**	**BDCIPP**	**DNBP**	**TCEP**
Average accuracy high spike (10 ng)	268%	n.a.	110%	134%	103%	249%	101%	101%	167%	n.a.	n.a.	104%	250%	74%
Average accuracy mid spike (4 ng)	270%	n.a.	89%	128%	100%	208%	100%	100%	125%	n.a.	n.a.	100%	123%	77%
Method recovery (4 ng)	107%	104%	109%	104%	105%	85%	101%	101%	107%	100%	93%	99%	102%	112%

Supplemental Table S3 Personal characteristics and allergic symptoms

			wheeze (n=29)			rhinoconjunctivitis (n=47)			eczema (n=36)			at least one of the symptoms (n=72)		
		n	Yes %	NO %	p-value	Yes %	NO %	p-value	Yes %	NO %	p-value	Yes %	NO %	p-value
sex	boys	68	23.5%	76.5%	0.835	35.3%	64.7%	0.854	30.9%	69.1%	0.556	55.9%	44.1%	1.000
	girls	60	21.7%	78.3%		38.3%	61.7%		25.0%	75.0%		56.7%	43.3%	
grade	2	14	21.4%	78.6%	0.492	21.4%	78.6%	0.260	28.6%	71.4%	0.177	64.3%	35.7%	0.354
	3	35	20.0%	80.0%		42.9%	57.1%		28.6%	71.4%		48.6%	51.4%	
	4	27	33.3%	66.7%		29.6%	70.4%		37.0%	63.0%		40.7%	59.3%	
	5	24	12.5%	87.5%		29.2%	70.8%		37.5%	62.5%		41.7%	58.3%	
	6	28	25.0%	75.0%		50.0%	50.0%		10.7%	89.3%		32.1%	67.9%	
height	cm	128	136.9±10.8	137.4±10.9	0.769	138.4±11.9	136.5±10.1	0.341	137.8±11.2	137.8±11.2	0.285	138.8±11.2	135.2±10.1	0.066
weight	kg	128	33.1±8.6	31.8±8.2	0.445	32.2±8.6	32.0±8.1	0.904	32.5±8.6	32.5±8.6	0.402	33.4±8.6	30.3±7.6	**0.035**
BMI	kg/m2	128	17.4±2.4	16.6±2.5	0.105	16.5±2.3	16.9±2.5	0.388	16.8±2.5	16.8±2.5	0.865	17.1±2.5	16.4±2.4	0.084
Duration spend at home (hour）		128	15.1±1.5	15.3±1.4	0.566	15.2±1.7	15.1±1.2	0.887	15.1±1.5	15.3±1.5	0.431	15.1±1.5	15.3±1.4	0.551
duration of sleep	hour	128	9.4±0.6	9.3±0.6	0.448	9.3±0.6	9.3±0.6	0.928	9.3±0.6	9.3±0.6	0.956	9.3±0.6	9.4±0.6	0.474
birth weight	g	128	3093±491	3099±399	0.946	3124±374	3082±445	0.590	3105±448	3094±410	0.894	3121±416	3067±425	0.476
breast milk	month	128	11.5±82.	9.9±8.5	0.370	6.4%	9.2%	**0.047**	8.5%	8.4%	0.571	7.9%	9.0%	0.461
parental history of allergy	no	29	0.0%	100.0%	**0.011**	10.3%	89.7%	**0.007**	10.3%	89.7%	0.098	17.2%	82.8%	**<0.001**
	mother only	48	29.2%	70.8%		41.7%	58.3%		31.3%	68.8%		64.6%	35.4%	
	father only	15	33.3%	66.7%		40.0%	60.0%		40.0%	60.0%		80.0%	20.0%	
	both	36	27.8%	72.2%		50.0%	50.0%		33.3%	66.7%		66.7%	33.3%	
annual household income	< 3 million	6	66.7%	33.3%	**0.015**	16.7%	83.3%	**0.006**	16.7%	83.3%	**0.017**	83.3%	16.7%	**0.002**
	3-5 million	25	12.0%	88.0%		20.0%	80.0%		20.0%	80.0%		40.0%	60.0%	
	5-8 million	50	16.0%	84.0%		28.0%	72.0%		24.0%	76.0%		42.0%	58.0%	
	>8 million	28	35.7%	64.3%		57.1%	42.9%		53.6%	46.4%		78.6%	21.4%	
	missing	19	21.1%	78.9%		57.9%	42.1%		15.8%	84.2%		73.7%	26.3%	

P-values are calculated by chi-square text and t-test

Supplemental Table S4 Housing characteristics and allergic symptoms

		Total		wheeze (n=29)			Rhino-conjunctivitis (n=47)			eczema (n=36)			at least one of the symptoms (n=72)		
				yes	no	p-value	yes	no	p-value	yes	no	p-value	Yes	NO	p-value
		n	%	%	%		%	%		%	%		%	%	
type of dwelling	single family	60	46.9%	25.0%	75.0%	0.398	33.3%	66.7%	0.469	25.0%	75.0%	0.556	60.0%	40.0%	0.477
	multi family	68	53.1%	30.9%	69.1%		39.7%	60.3%		30.9%	69.1%		52.9%	47.1%	
building structure	wood	59	46.1%	23.7%	76.3%	**0.020**	35.6%	64.4%	0.855	23.7%	76.3%	0.331	61.0%	39.0%	0.373
	ferroconcrete, others	69	53.9%	31.9%	68.1%		37.7%	62.3%		31.9%	68.1%		52.2%	47.8%	
occupancy	rental	31	24.2%	29.0%	71.0%	0.082	32.3%	67.7%	0.670	29.0%	71.0%	1.000	67.7%	32.3%	0.152
	owner	97	75.8%	27.8%	72.2%		38.1%	61.9%		27.8%	72.2%		52.6%	47.4%	
building age (years)	<=5	34	27%	26.5%	73.5%	**0.049**	41.2%	58.8%	0.618	26.5%	73.5%	0.864	52.9%	47.1%	0.269
	6～ 10	30	23%	23.3%	76.7%		33.3%	66.7%		23.3%	76.7%		43.3%	56.7%	
	11～ 20	34	27%	32.4%	67.6%		29.4%	70.6%		32.4%	67.6%		61.8%	38.2%	
	> 21	30	23%	30.0%	70.0%		43.3%	56.7%		30.0%	70.0%		66.7%	33.3%	
renovation within a	Yes	31	24%	16.1%	83.9%	0.629	38.7%	61.3%	0.832	16.1%	83.9%	0.110	54.8%	45.2%	1.000
year	No	97	76%	32.0%	68.0%		36.1%	63.9%		32.0%	68.0%		56.7%	43.3%	
Wall to wall carpet	Yes	11	9%	36.4%	63.6%	0.711	27.3%	72.7%	0.745	36.4%	63.6%	0.502	54.5%	45.5%	1.000
	No	117	91%	27.4%	72.6%		37.6%	62.4%		27.4%	72.6%		56.4%	42.7%	
Wooden flooring	Yes	105	82%	25.7%	74.3%	**0.013**	36.2%	63.8%	0.814	25.7%	74.3%	0.208	53.3%	46.7%	0.172
	No	23	18%	39.1%	60.9%		39.1%	60.9%		39.1%	60.9%		69.6%	30.4%	
PVC flooring	Yes	11	9%	18.2%	81.8%	0.123	45.5%	54.5%	0.531	18.2%	81.8%	0.727	72.7%	27.3%	0.346
	No	117	91%	29.1%	70.9%		35.9%	64.1%		29.1%	70.9%		54.7%	45.3%	
PVC wall paper	Yes	113	88%	29.2%	70.8%	0.328	37.2%	62.8%	1.000	29.2%	70.8%	0.555	57.5%	42.5%	0.581
	No	15	12%	20.0%	80.0%		33.3%	66.7%		20.0%	80.0%		46.7%	53.3%	
PVC ceiling	Yes	110	86%	28.2%	71.8%	0.240	37.3%	62.7%	0.799	28.2%	71.8%	1.000	57.3%	42.7%	0.614
	No	18	14%	27.8%	72.2%		33.3%	66.7%		27.8%	72.2%		50.0%	50.0%	
Condensation	Yes	92	72%	28.3%	71.7%	0.164	38.0%	62.0%	0.687	28.3%	71.7%	1.000	59.8%	40.2%	0.236
	No	36	28%	27.8%	72.2%		33.3%	66.7%		27.8%	72.2%		47.2%	52.8%	
Moldy odor	Yes	19	15%	36.8%	63.2%	0.137	36.8%	63.2%	1.000	36.8%	63.2%	0.410	78.9%	21.1%	**0.044**
	No	109	85%	26.6%	73.4%		36.7%	63.3%		26.6%	73.4%		52.3%	47.7%	
Mold growth	Yes	98	77%	28.6%	71.4%	**0.023**	37.8%	62.2%	0.829	28.6%	71.4%	1.000	61.2%	38.8%	0.058
	No	30	23%	26.7%	73.3%		33.3%	66.7%		26.7%	73.3%		40.0%	60.0%	
High humidity in	Yes	32	25%	28.1%	71.9%	0.808	34.4%	65.6%	0.834	28.1%	71.9%	1.000	62.5%	37.5%	0.538
bathroom	No	96	75%	28.1%	71.9%		37.5%	62.5%		28.1%	71.9%		54.2%	45.8%	
Water leakage	Yes	28	22%	39.3%	60.7%	0.009	35.7%	64.3%	1.000	39.3%	60.7%	0.157	78.6%	21.4%	**0.009**
	No	100	78%	25.0%	75.0%		37.0%	63.0%		25.0%	75.0%		50.0%	50.0%	
Housing pet	Yes	43	34%	27.9%	72.1%	1.000	30.2%	69.8%	0.334	27.9%	72.1%	1.000	60.5%	39.5%	0.573
	No	85	66%	28.2%	71.8%		40.0%	60.0%		28.2%	71.8%		54.1%	45.9%	
Smoker	Yes	34	27%	29.4%	70.6%	0.151	32.4%	67.6%	0.679	29.4%	70.6%	0.828	58.8%	41.2%	0.841
	No	94	73%	27.7%	72.3%		38.3%	61.7%		27.7%	72.3%		55.3%	44.7%	
Mechanical	Yes	60	47%	23.3%	76.7%	0.298	40.0%	60.0%	0.582	23.3%	76.7%	0.325	50.0%	50.0%	0.213
ventilation usage	No	68	53%	32.4%	67.6%		33.8%	66.2%		32.4%	67.6%		61.8%	38.2%	

P-values are calculated by chi-square text

Supplemental Table S5 Levels of PFR in dust and urine between with and without allergic symptoms

	wheeze (n=29)			rhinoconjunctivitis (n=47)			eczema (n=36)			at least one of the symptoms (n=72)		
	Yes GM	NO GM	p-value	Yes GM	NO GM	p-value	Yes GM	NO GM	p-value	Yes GM	NO GM	p-value
Dust (μg/g dust)												
TPP	1.13	0.89	0.137	0.83	1.01	0.355	0.86	0.97	0.867	0.94	0.93	0.646
TNBP	0.54	0.84	0.117	0.71	0.79	0.664	0.70	0.78	0.817	0.70	0.84	0.427
TCIPP	2.25	2.19	0.520	2.00	2.34	0.632	2.18	2.22	0.811	2.11	2.34	0.985
TCEP	1.26	1.22	0.908	1.25	1.21	0.730	1.29	1.20	0.708	1.25	1.19	0.627
TEHP	0.39	0.40	0.835	0.45	0.37	0.102	0.42	0.38	0.387	0.43	0.35	0.105
TBOEP	**14.4**	**34.6**	**0.025**	29.8	27.6	0.619	22.9	30.9	0.816	23.0	37.2	0.086
TDCIPP	0.69	1.04	0.249	0.82	1.03	0.381	1.39	0.82	0.239	0.92	0.98	0.593
TPHP	3.48	3.18	0.907	2.81	3.53	0.186	3.05	3.32	0.322	3.16	3.36	0.486
dust weight (mg)	230	168	0.085	169	187	0.759	225	165	0.063	196	182	0.155
Urine												
5HO-EHDPHP	0.05	0.05	0.584	0.05	0.05	0.807	0.06	0.05	0.314	0.06	0.04	0.130
BBOEP	0.24	0.21	0.537	0.21	0.24	0.585	0.26	0.20	0.178	0.24	0.19	0.230
TBOEP-OH	0.01	0.01	0.762	0.01	0.01	0.819	**0.01**	**0.01**	**0.012**	0.01	0.01	0.389
BBOEHEP	0.36	0.35	0.655	0.33	0.40	0.387	**0.48**	**0.32**	**0.024**	0.39	0.32	0.363
BCIPHIPP	0.21	0.21	0.952	0.20	0.24	0.625	0.22	0.21	0.705	0.23	0.19	0.438
DPHP	0.33	0.36	0.699	0.33	0.38	0.298	0.35	0.35	0.992	0.37	0.33	0.446
4HO-DPHP	0.49	0.62	0.175	0.58	0.61	0.745	0.62	0.58	0.995	0.62	0.55	0.552
BDCIPP	0.07	0.07	0.529	0.06	0.07	0.556	0.08	0.06	0.221	**0.07**	**0.06**	**0.045**
uTCEP	0.06	0.05	0.589	0.05	0.06	0.666	0.06	0.05	0.969	0.06	0.05	0.556

P-vaules are calculated by Mann-Whitney U test
GM, geometric mean

Supplemental Table S6 Associations between PFR and allergic symptoms (linear models)

Compounds	wheeze				rhinoconjunctivitis				eczema				at least one of the symptoms			
	OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value	OR	95% CI		P-value
dust[a]																
TPP	1.07	0.63	1.84	0.792	0.78	0.49	1.27	0.321	0.63	0.36	1.12	0.116	0.86	0.51	1.43	0.551
TNBP	0.78	0.53	1.15	0.206	0.84	0.61	1.16	0.293	0.93	0.66	1.31	0.688	0.91	0.66	1.25	0.572
TCIPP	1.01	0.74	1.38	0.944	0.94	0.72	1.23	0.676	1.01	0.77	1.34	0.919	0.96	0.74	1.26	0.795
TCEP	1.08	0.75	1.55	0.676	1.21	0.88	1.68	0.238	1.21	0.86	1.70	0.284	1.23	0.87	1.75	0.238
TBOEP	0.80	0.60	1.07	0.132	0.96	0.75	1.23	0.761	0.88	0.68	1.14	0.345	0.82	0.63	1.06	0.132
TDCIPP	0.94	0.74	1.19	0.601	0.99	0.81	1.21	0.923	**1.44**	**1.13**	**1.82**	**0.003**	1.07	0.87	1.30	0.538
TPHP	1.24	0.73	2.11	0.435	0.65	0.40	1.05	0.081	0.89	0.55	1.44	0.645	0.96	0.60	1.54	0.874
urine[b]																
5-HO-EHDPHP	1.27	0.75	2.13	0.371	1.07	0.69	1.65	0.758	1.58	0.95	2.64	0.078	1.62	0.98	2.68	0.061
BBOEP	1.22	0.74	2.02	0.430	1.13	0.73	1.74	0.588	1.27	0.79	2.02	0.322	**1.70**	**1.02**	**2.83**	**0.043**
3-HO-TBOEP	1.13	0.80	1.60	0.491	0.89	0.65	1.21	0.458	**1.54**	**1.07**	**2.20**	**0.019**	1.14	0.81	1.61	0.444
BBOEHEP	0.98	0.65	1.47	0.927	1.08	0.76	1.52	0.665	1.23	0.84	1.81	0.287	1.13	0.79	1.59	0.505
BCIPHIPP	1.07	0.72	1.59	0.740	1.29	0.92	1.83	0.143	1.18	0.82	1.71	0.374	1.35	0.93	1.98	0.117
DPHP	1.18	0.58	2.43	0.646	1.62	0.91	2.86	0.099	1.01	0.55	1.86	0.977	1.39	0.76	2.52	0.285
4-HO-DPHP	0.61	0.33	1.11	0.104	1.00	0.64	1.58	0.992	1.23	0.75	2.02	0.404	1.02	0.62	1.65	0.952
BDCIPP	1.12	0.65	1.95	0.681	1.20	0.77	1.89	0.416	1.14	0.70	1.87	0.588	1.39	0.83	2.32	0.207
uTCEP	1.35	0.71	2.54	0.358	1.21	0.69	2.14	0.501	1.03	0.55	1.91	0.935	1.36	0.72	2.56	0.348
ΣuTBOEP	1.14	0.68	1.93	0.620	1.19	0.76	1.87	0.444	1.39	0.85	2.27	0.194	1.44	0.89	2.34	0.134
ΣuTCIPP	1.02	0.64	1.63	0.921	**1.60**	**1.07**	**2.39**	**0.022**	1.09	0.73	1.63	0.670	1.46	0.96	2.21	0.074
ΣuTPHP	0.89	0.35	2.26	0.813	1.49	0.72	3.07	0.285	0.76	0.34	1.70	0.507	1.00	0.46	2.16	0.993

Each levels were loge transformed and included into the model separately

a, Adjusted for sex, grade, annual income, parental allergy, and dampness index

b, Adjusted for sex, grade, annual income, parental allergies, dampness index, and creatinine

BBOEHEP bis(2-butoxyethyl)-(2-hydroxyethyl) phosphate; BBOEP, bis(2-butoxyethyl) phosphate; BCIPHIPP, bis(1,3-dichloro-2-propyl) phosphate; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; DNBP dimethyl phosphate, DPHP, diphenyl phosphate; CI, confidence interval; 4-HO-DPhP, 4-hydroxydiphenyl phosphate; 5-HO-EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; OR, odds ratio; PFR, phosphate flame retardants; ΣuTBOEP, Σ metabolites of tris(2-butoxyethyl) phosphate in urine; ΣuTCIPP, Σ metabolites of tris(2-chloro-iso-propyl) phosphate in urine, ΣuTPHP; Σ metabolites of triphenyl phosphate in urine; TBOEP, tris(2-butoxyethyl) phosphate; 3-HO-TBOEP; tris(2-(3-hydroxy)butoxyethyl) phosphate; TCEP, tris(2-chloroethy) phosphate; TCIPP, tris(2-chloro-iso-propyl) phosphate; TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; TNBP, tributhyl phosphate; TPHP, triphenyl phosphate; TPP, tripropyl phosphate; uTCEP, tris(2-chloroethy) phosphate in urine

ΣuTBOEP, ΣuTCIPP, ΣuTPHP are sum of molar concentrations (nM)

133
134