Combined exposure to phthalate esters and phosphate flame retardants and plasticizers and their associations with wheeze and allergy symptoms among school children

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Abstract:

BACKGROUND: Phthalate esters and phosphate flame retardants and plasticizers (PFRs) are both used as plasticizers and are commonly detected in indoor environments. Although both phthalates and PFRs are known to be associated with children's wheeze and allergic symptoms, there have been no previous studies examining the effects of mixtures of these exposures.

OBJECTIVES: To investigate the association between exposure to mixtures of phthalate esters and PFRs, and wheeze and allergic symptoms among school-aged children.

METHODS: A total of 128 elementary school-aged children were enrolled. Metabolites of 3 phthalate esters and 7 PFRs were measured in urine samples. Parent-reported symptoms of wheeze, rhinoconjunctivitis, and eczema were evaluated using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. In the primary model, we created a phthalate ester and PFR mixture exposure index, and estimated odds ratios (ORs) using weighted quantile sum (WQS) regression and quantile g (qg)-computation. The two highest chemicals according to qg-computation weight %s were combined to create a combination high × high exposure estimate, with ORs calculated using the "low × low" exposure group as the reference category. Concentrations of each metabolite were corrected by multiplying this value by the sex- and body size-Standardised creatinine concentration and dividing by the observed creatinine value. All models were adjusted for sex, grade, dampness index and annual house income.

RESULTS: The odds ratio of rhinoconjunctivitis for the association between exposure to chemical mixtures according to the WQS index positive models was; OR = 2.60 (95% confidence interval [CI]: 1.38-5.14). However, wheeze and eczema of the WQS index positive model, none of the WQS index negative models or qg-computation result yielded statistically significant results. Combined exposure to the two highest WQS weight %s of “high-high” ΣTCIPP and ΣTPHP was associated with an increased prevalence of rhino-conjunctivitis, OR = 5.78 (1.81 – 18.43) to the “low × low” group.

CONCLUSIONS: Significant associations of mixed exposures to phthalates and PFRs and increased prevalence of rhinoconjunctivitis was found among elementary school-aged children in the WQS positive model. Mixed exposures were not associated with any of allergic symptoms in the WQS negative model or qg-computation approach. However, the combined effects of exposure to two PFRs suggested an additive and/or multiplicative interaction, potentially increasing the prevalence of rhinoconjunctivitis. A further study with a larger sample size is needed to confirm these results.

Keywords

Phosphate flame retardant and plasticizers, Phthalate ester, Allergy, Exposure to mixtures, Combined exposure, Children

1. Introduction[1]

Phthalate esters are a class of chemicals predominantly used as plasticizers. Di(2-ethylhexyl) phthalate (DEHP) and butyl benzyl phthalate (BBzP) are used in polychlorinated chemicals, which are found in various plastic products, toys, food containers, and housing materials, whereas di-n-butyl phthalate (DnBP) and di-i-butyl phthalate (DiBP) are used in personal care products and fragrances (Ait Bamai et al. 2014). Organophosphate triesters, also referred to as phosphorus flame retardants and plasticizers (PFRs), are a class of chemicals predominantly used as additives in flame retardants and plasticizers. 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(1-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

[1] **Abbreviations:** 3-HO-TBOEP: bis(2-butoxyethyl) 3-hydroxy-2-butoxyethyl phosphate, 3-HO-TPHP: 3-hydroxyphenyl diphenyl phosphate, 4-HO-DPHP: 4-hydroxyphenyl diphenyl phosphate, 4-HO-TPHP: 4-hydroxyphenyl diphenyl phosphate, 5-HO-EHDPHP: 5-hydroxy-2-ethylhexyl diphenyl phosphate, BBOEHEP: bis(2-butoxyethyl) 2-hydroxyethyl phosphate, BBOEP: bis(2-butoxyethyl) phosphate, BBzP: butyl benzyl phthalate, BCIPHIPP: bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate, BCIPP: bis(1-chloro-2-propyl) phosphate, BDCIPP: bis(1,3-dichloro-2-propyl) phosphate, DEHP: Di(2-ethylhexyl) phthalate, DiBP: di-i-butyl phthalate, DnBP: di-n-butyl phthalate, DNBP: dibutyl phosphate, DPHP: diphenyl phosphate, EHPHP: 2-ethylhexyl phenyl phosphate, MBzP: mono benzyl phthalate, MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate, MEHP: mono(2-ethylhexyl) phthalate, MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate, MiBP: mono-i-butyl phthalate, MnBP: mono-n-butyl phthalate, PFR: phosphate flame retardant, TBOEP: tris (2-butoxyethyl) phosphate, TCEP: tris (2-chloroethyl) phosphate, TCIPP: tris(2-chloro-*iso*-propyl) phosphate, TDCIPP: tris (1,3-dichloro-2-propyl) phosphate, TMPP: tricresyl phosphate, TNBP: tri-*n*-butyl phosphate, TPHP triphenyl phosphate, WQS: weighted quantile sum

78 phosphate (TMPP) are also used as lubricants, and tris (2-butoxyethyl) phosphate (TBOEP) is
79 often used in floor coverings, and as a plasticizer in floor finishing products and floor polish
80 (Kajiwara et al. 2011). 2-Ethylhexyl diphenyl phosphate (EHDPHP) is also used as a flame
81 retardant and plasticizer in PVC materials (Ballesteros-Gómez et al. 2015). Both phthalates and
82 PFRs are used as additives in flame retardants, plasticizers, and a variety of consumer products,
83 such as building materials, floor and wall materials, textiles, furniture, electronic equipment,
84 rubber products, textile coatings, polyurethane foam plastics, children's toys, and food
85 packaging materials (Ait Bamai et al. 2014; Ballesteros-Gómez et al. 2015; Kajiwara et al. 2011).
86 Phthalates and PFRs are not chemically bonded to the products they are used in, and can
87 migrate, leach, and evaporate into the environment (Luongo and Östman 2016). Previous
88 studies involving simultaneous measurements of phthalates and PFRs in house dust suggest
89 that they can both be commonly found in indoor environments (Kanazawa et al. 2010; Kishi et
90 al. 2017; Luongo and Östman 2016), with inhabitants potentially exposed through dust
91 ingestion, airborne particle and gas inhalation, and dermal contact. In previous studies, higher
92 concentrations of DEHP, BBzP, and DiBP were observed with the use of PVC materials, and
93 DEHP and TDCIPP were detected in carpet materials (Ait Bamai et al. 2014; Bi et al. 2018).
94 These results suggest that phthalates and PFRs share the same exposure source, and therefore
95 humans are exposed to both chemicals at the same time.
96 Recently, several studies have reported on potential health concerns related to

exposure to phthalates and PFRs, including asthma and allergies in cross-sectional studies (Ait Bamai et al. 2016; Ait Bamai et al. 2014; Bekö et al. 2015; Bi et al. 2018; Bornehag et al. 2004; Callesen et al. 2014), as well as prospective birth cohorts (Just et al. 2012; Ku et al. 2015; Whyatt et al. 2014). The results from these studies were generally consistent in their reported associations for phthalate levels in house dust or heating, ventilation, and air conditioning filter dust (Ait Bamai et al. 2016; Ait Bamai et al. 2014; Bekö et al. 2015; Bornehag et al. 2004), but reported inconsistent associations between urinary phthalate metabolite levels and asthma and allergies (Callesen et al. 2014; Just et al. 2012; Ku et al. 2015; Whyatt et al. 2014). Compared to phthalates, there have been fewer studies examining the association between PFRs and asthma and allergies. We have conducted two cross-sectional studies, and reported that increasing levels of TCIPP, TDCIPP, and TNBP in house dust, as well as their urinary metabolites, were associated with increased risk of eczema and rhinoconjunctivitis (Araki et al. 2018; Araki et al. 2014). Another study is nested case-control study, which found no difference of PFRs levels in dust collected from asthma case and control children's mattress (Canbaz et al. 2016). Several different mechanisms may underline these findings, although these are not well understood. Previous experimental studies suggested that these chemicals act as ligands of receptors involved in allergenic pathology, as adjuvants that contribute to allergies, or have immunotoxic properties that affect dendritic cells (Canbaz et al. 2017; Nishioka et al. 2012; Tanaka et al. 2013). Experimental studies of these chemicals have also

116 yielded results suggesting altered immune responses (Canbaz et al. 2017; Killilea et al. 2017;
117 Krivoshiev et al. 2018b). In addition, cross-sectional studies suggested that phthalates and
118 PFRs increase oxidative stress (Ait Bamai et al. 2019; Lee et al. 2019; Rocha et al. 2017), which
119 would lead to inflammation (Benjamin et al. 2017; Ito et al. 2007)

120 Humans are exposed to many chemicals at the same time, and health risk assessment for
121 combined exposure to multiple chemicals is a current topic in the World Health Organisation's
122 International Programme on Chemical Safety (Meek et al. 2011). However, all the
123 aforementioned previous studies examined associations between allergies and single chemical
124 exposure, with each agent analysed separately. Multivariate adjustment, achieved by including
125 several chemicals in the same model, would hypothetically provide independent associations
126 between each exposure and the relevant outcome. However, if the levels of phthalates and
127 PFRs are correlated with each other, it is not ideal to include these chemicals in the same
128 logistic regression model, as multicollinearity tends to inflate the standard errors of the
129 estimated regression coefficients. Moreover, the combined exposure effect cannot be
130 examined by the multivariable adjustment model. To date, there have been no published
131 studies reporting on mixtures or combinations of phthalates and PFRs, and their association
132 with asthma and allergies among children.

133 The major aim of this study is to find the association between mixtures of phthalate esters
134 and PFRs levels and the prevalence of allergies in children. We hypothesised that mixtures of

chemicals would increase the prevalence of wheeze, rhinoconjunctivitis, and eczema.

2. Methods

2.1 Study participants

This study was conducted among elementary school children in Sapporo, Japan, with data collected as previously described (Ait Bamai et al. 2016; Araki et al. 2018). Briefly, an initial cross-sectional study was conducted in Sapporo city in 2008. A questionnaire was distributed to 6,393 school children from 12 public elementary schools. Of the 4,408 students who responded to the questionnaire, 951 (from 832 families) were interested to participate in a home survey that environmental measurements in the following calendar year. 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. Children who transferred to different schools, including junior high schools, were excluded. Through this selection procedure, we successfully visited a total of 128 homes. The other families were unwilling to participate, did not respond, or were unable to arrange their schedules for a home visit. Overall participation proportion in this study to primary questionnaire survey was 2.9%.

2.2 Questionnaire

Information on the exposure assessment, including the collection of children's urine, was also

154 previously reported (Ait Bamai et al. 2016; Araki et al. 2018). Briefly, parents filled out the
155 International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Beasley 1998).
156 The study investigators then defined participants for wheeze, allergic rhinoconjunctivitis, and
157 eczema based on “yes” responses to descriptions of each symptom by ISAAC (Beasley 1998).
158 We classified participants as having wheeze if their parents answered ‘Yes’ to the following
159 question: ‘Has your child had wheezing or whistling in the chest in the last 12 months?’ Allergic
160 rhinoconjunctivitis was defined by the ‘Yes’ to both of following questions: (a) ‘Has your child
161 had a problem with sneezing, or a runny/blocked nose in the absence of a cold or flu in the
162 last 12 months?’ and (b) ‘Has this nose problem been accompanied by itchy, watery eyes?’
163 Eczema was defined by the ‘Yes’ to all of following questions: (a) ‘Has your child had an itchy
164 rash that has appeared and disappeared for at least 6 months?’; (b) ‘Have the aforementioned
165 itchy rashes appeared at any time during the last 12 months?’; and (c) ‘Have the
166 aforementioned itchy rashes affected one or several of the following areas: the folds of the
167 elbows, the back of knees, the front of the ankles, the underside of the buttocks, or the areas
168 around the neck, ears, or eyes?’

169 The questionnaire also included information on potential confounding factors such as
170 the child’s sex, school grade, and household income. The dampness index was generated by
171 summing the number of the signs of dampness-related problems observed in each dwellings,
172 including condensation (yes/no), mouldy odour (yes/no), visible mould (yes/no), high humidity

in the bathroom (yes/no), and problems with water leakage within the past 5 years (yes/no), for a possible score range of 0–5 (Kishi et al. 2009; Saijo et al. 2004).

2.3 Urinary measurement of phthalate esters and PFR metabolites

Details on the collection of urine samples have been reported elsewhere (Ait Bamai et al. 2015). Briefly, on the day of the 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 on the day of the urine collection and stored at −20°C until the day of analysis.

Details of the analytical procedures for the urinary phthalate esters and PFR metabolites have also been described elsewhere (Ait Bamai et al. 2015; Araki et al. 2018; Bastiaensen et al. 2019). Six urinary phthalate metabolites were measured by GC-MS/MS: mono-n-butyl phthalate (MnBP), mono-i-butyl phthalate (MiBP), mono benzyl phthalate (MBzP), mono(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). 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

192 diphenyl phosphate (4-HO-DPHP), bis(2-butoxyethyl) 3-hydroxy-2-butoxyethyl phosphate (3-
193 HO-TBOEP), 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP), 3-hydroxyphenyl diphenyl
194 phosphate (3-HO-TPHP), 5-hydroxy-2-ethylhexyl diphenyl phosphate (5-HO-EHDPHP), 2-
195 ethylhexyl phenyl phosphate (EHPHP) and TCEP. For concentrations lower than the limit of
196 quantification (LOQ), a detection frequency times LOQ value was assigned (James et al. 2002).
197 Creatinine levels in urine were determined using an enzyme-linked immunosorbent assay at
198 SRL, Inc. (Tokyo, Japan).
199 The standardised creatinine-corrected concentrations were calculated as
200 recommended in a previous report with modification (O'Brien et al. 2016). First, we calculated
201 the reference creatinine concentration using the individual creatinine clearance and fitted the
202 standardised creatinine corrected concentration according to the following equations.
203 Male (height: 90–168 cm): CE (mg/day) = Ht (6.265+0.0564 (Ht-168) Eq.(1)
204 Female (height: 90–172 cm): CE (mg/day) = 2.045 exp[0.01552(Ht-90)] Eq.(2)
205 Where CE is the creatinine clearance rate estimated as described previously (Mage et al. 2008).
206 Ht is the height of each child. For a child, the average urine volume is 1 mL/kg/h.
207 The reference creatinine concentration was calculated according to the following
208 equation:
209 Reference Creatinine (mg/L) = CE (mg/day) x 1000 (mL) / (1 mL x Wt (kg) x 24 (h))
210 Eq.(3)

211 Where Wt is the weight of an individual child.

212 Finally, the standardised creatinine-corrected concentration of each metabolite was calculated
213 by fitting to Eq.(4).

214 Standardised creatinine corrected concentration = Reference Creatinine / Observed
215 Creatinine Eq.(4)

216

217 *2.4 Data analysis*

218 Urinary phthalate and PFR metabolite levels were converted to molar concentrations (nM).
219 The metabolites of DBP, DEHP, TBOEP, TCIPP and TPHP were combined into the sum of their
220 individual concentrations, as ΣDBP (MnBP and MiBP), ΣDEHP (MEHP, MEOHP, and MECPP),
221 ΣTBOEP (BBOEP, 3-HO-TBOEP, and BBOEHEP), ΣTCIPP (BCIPP and BCIPHIPP), and ΣTPHP (DPHP
222 and 4-HO-DPHP). Correlations between each compound were analyzed by Spearman's rho.

223 For the primary analysis, we used weighted quantile sum (WQS) regression models to
224 examine the association between mixtures of chemicals and asthma and allergies. WQS is a
225 method for combining highly correlated exposures into one index, to estimate the association
226 between a chemical mixture and an outcome of interest (Carrico et al. 2015; Gennings et al.
227 2010; Romano et al. 2018). The WQS estimates the effect of the mixture as a whole and
228 calculates the impact of a single-quantile increase. This model is advantageous because it is
229 simple to implement (Keli et al., 2019). The WQS regression model was used to calculate a

weighted linear index by grouping different chemicals into ordinal quantile variables that represented the associations of a mixture of all chemicals with single health outcomes. The WQS index was created with quartiles of chemical levels, and estimated empirical weights for each chemical were included in the index. WQS assumes inference in a single direction or 'directional homogeneity' assumption. Both positive and negative associations were examined for WQS. Assuming directional homogeneity, the model would be equivalent to a generalised linear model. However, chemicals with opposite associations cannot be combined into a single exposure index (Carrico et al. 2015; Romano et al. 2018). Thus, a quantile-based g-computation (qg-computation) approach (Keil et al. 2019) was used additionally to estimate simultaneously the effects of a single-quantile exposure in both directions. The qg-computation was implemented based on a generalisation of the WQS regression, which estimates the expected ability of a change in one outcome to increase all exposures in the mixture by one quantile (Keil et al. 2019). The qg-computation also allows a valid inference regarding the contribution of an individual component to the mixture, even in the absence of directional homogeneity. The Standardised creatinine-corrected concentrations of 3 phthalates and 6 PFRs were introduced into the WQS and qg-computation models, and each model was calculated using chemical level quartiles and 200 bootstrap runs.

Individual chemical models were also examined, and ORs (95% CI) were obtained using the 1st tertile as the reference category. If the WQS regression model was statistically

249 significant, then combinations of two chemicals were modelled to examine the interactions as
250 secondary analyses. We selected the two chemicals with the two highest individual weights in
251 the qg-computation to examine combinations of two chemicals. Each chemical was divided
252 into tertiles: 1st and 2nd tertile to low and 3rd tertile to high concentrations, and chemical
253 pairs were combined into “low × low”, “low × high”, “high × low”, and “high × high” categories.
254 Logistic regression models were then used to obtain odds ratios (ORs) and 95% confidence
255 intervals (CIs) for each combination, with “low × low” as the reference category.

256 Each model was adjusted for sex, grade, annual household income included taxes
257 (coded as 1: <3 million Japanese yen, 2: 3-5 million Japanese yen, 3: 5-8 million Japanese yen,
258 4: >8 million Japanese yen; modelled as an ordinal variable), and dampness index (0-5, ordinal
259 variable) based on *a priori* evidence determined from previous studies (Ait Bamai et al. 2016;
260 Araki et al. 2018). The mean value of annual household income (2.92 million yen) was assigned
261 to missing values (14.8%).

262 Statistical analyses were performed in SPSS (Windows version 26.0J). The WQS model
263 was performed using the gWQS package (version 2.0.0) (Carrico et al. 2015), and qg-
264 computation was performed using the qgcomp package (version 1.3.0) (Keil et al. 2019), with
265 R studio (R version 3.6.1). A two-sided p-value <0.05 was considered statistically significant.

266

267 *2.5 Ethics*

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

3. Results

Participant characteristics are shown in Table 1. Of the 128 participants, 53.1% were boys, and all children were in grades 2 to 6 (age range: 7–12 years). The numbers of children with wheeze, rhinoconjunctivitis, and eczema were 29 (22.7%), 47 (36.7%), and 36 (28.1%), respectively. Seventy-two children (56.3%) had at least one of the above mentioned symptoms (one or more symptoms, hereafter).

The distributions of urinary phthalate and PFR concentrations in nM and nmol/g standardised creatinine are shown in Table 2 and ng/mL in Supplemental table S2. Among all chemicals, the level of ΣDEHP was the highest (median value of 441 nM), followed by ΣDBP (236 nM). The levels of PFRs were approximately 100x lower than phthalates, with the highest concentration observed for ΣTPHP (2.13 nM) followed by ΣTBOEP (1.88 nM). DNBP was only detected in 8.3% of samples, and was thus excluded from further analysis. The correlations between chemicals ranged from Spearman's ρ of 0.01 to 0.624, as shown in Supplemental Table S1.

The results of mixed chemical models, analysed by WQS and qg-computation, are

shown in Table 3. For one quartile change in the WQS index increased the ORs (95% CIs) for wheeze: 1.52 (0.67 – 3.50), rhinoconjunctivitis: 2.60 (1.38 – 5.14), and eczema: 1.91 (0.99 – 3.85) in the positive model. None of the negative WQS and qg-computation models were statistically significant. The individual weights for each chemical mixture component, as determined by the qg-computation approach, are shown in Figure 1. For rhinoconjunctivitis, the chemicals that showed the highest weight (%) in the positive direction were ΣTCIPP (35.0%) and ΣTPHP (24.9%). For eczema, the highest weights (%) were observed for 5-HO-EHDPHP (45.4%) and BDCIPP (42.7%).

The OR and 95% CI of single chemicals before combining 2nd and 3rd categories compared to the lowest category of phthalates and PFR were calculated, as shown in Table 4. For rhinoconjunctivitis, the 2nd and 3rd tertile of BDCIPP were associated with a significantly higher ORs of 2.95 (1.04-8.37) and 2.93 (1.04-8.28), respectively, relative to the 1st tertile, with a significant p-for trend of 0.045. The 3rd tertile of ΣTCIPP was associated with a significantly higher OR of 4.13 (1.59 – 12.90) relative to the 1st tertile, with a significant p-for trend of 0.004. The results of the combined chemical analysis are shown in Figure 2 and Supplemental Table S2. Rhinoconjunctivitis was significantly associated with the combination of ΣTCIPP and ΣTPHP, with a “high × high” group OR of 7.14 (95% CI: 2.11 – 24.15, p=0.002).

4. Discussion

306 In this study, we examined the association between asthma and allergies, and exposure to
307 mixtures of phthalates and PFRs among school-age children. Statistically significant
308 associations were observed between increased WQS index and an increased prevalence of
309 rhinoconjunctivitis and eczema. However, in the qg-computation, no significant associations
310 were observed between the mixture of all chemicals and any of the allergic symptoms. As for
311 the secondary models with combinations of highly weighted chemicals, according to the qg-
312 computation approach, the combination of “high × high” levels was yielded a significantly
313 increased OR for rhinoconjunctivitis. To our knowledge, this is the first study to examine the
314 associations between mixtures and combinations of phthalates and PFRs and prevalence of
315 allergic symptoms.

316 We have measured phthalates and PFRs in house dust and their metabolites in urine,
317 and found significant correlations between chemicals in house dust and their metabolites in
318 urine in the same cross-sectional study (Ait Bamai et al. 2016; Bastiaensen et al. 2019). These
319 findings suggest that house dust is a potential source of exposure to phthalates and PFRs,
320 leading to co-exposure to both classes of chemical. In our previous study using a single
321 chemical model, we reported that higher levels of ΣTCIPP were associated with increased ORs
322 for rhinoconjunctivitis; 3-HO-TBOEP, a metabolite of TBOEP was associated with eczema (Araki
323 et al. 2018). The findings of this study are partly in line with the previous study and provide
324 additional insight into the combined effects of these chemicals. The two chemicals with the

highest weights according to the qg-computation approach may have a potential for interaction effects. For participants with rhinoconjunctivitis in this study, the positive model of WQS index, and high levels of exposure to the combination of ΣTCIPP and ΣTPHP were associated with an elevated OR.

In combined exposure scenarios, if the effect of one exposure is added to the effect of the second exposure, the model is additive, whereas when the effect of the second exposure is multipied by the first exposure the model is multiplicative or synergism (Gordis 1996). In this study, in comparison to the "low × high" group and the "high × low" group, the ORs associated with the "high × high" group suggest the presence of additive, or effect modification in another words. When considering the ORs of ("low × high" + "high × low") as a purely additive effect, and ("low × high" * "high × low") as a purely multiplicative effect. If the mode of action of phthalates and PFRs to allergic symptoms is the same, "high × high" ORs may become similar to estimated additive effect, whereas if they have different modes of action in relation to allergic symptoms, "high × high" ORs may become like estimated multiplicatives effect. The ORs obtained using the combination of two chemicals "high × high" (rhinoconjunctivitis, OR = 7.14) were larger than the ORs calculated for the 3rd tertiles of single-chemical models (for rhinoconjunctivitis: ΣTCIPP, OR = 4.53; ΣTPHP, OR = 2.67, respectively, Table 3), and estimated additive (OR = 3.29) and multifplicable (OR = 2.25) effects, respectively (Supplemental Table S2). However, it remains unclear whether the effects are additive or

multiplicative, as the estimated additives, multiplicatives, and “high × high” ORs were similar (Supplemental Table S2). It is also possible that several different above-mentioned properties act in parallel.

The evaluation of associations between mixtures or combinations of chemicals and health outcomes is a realistic approach, as phthalates and PFRs are often detected in the same samples and concentrations of each chemicals are correlated. In this study, the associations based on the qg-computation model between the mixture of all chemicals with rhinoconjunctivitis was not significant. As shown in Figure 2, some chemicals had positive weight contributions, while others had negative contributions. These positive and negative estimates cancelled each other out in the statistical models, and consequently the associations with the overall chemical mixture became not significant. However, the WQS positive model suggested an increased prevalence of rhinoconjunctivitis, as well as associations of these symptoms with the combination of the two highest chemicals. These results should not be ignored, and continuous chemical risk assessments are needed.

Modes of action of the observed associations of mixtures of examined chemicals with allergies remain unclear. Both phthalates and PFRs may have several different modes of action such as direct pharmacological effect on receptors involved in allergenic pathology or indirect effect as adjuvants for different causative agents to influence immune and inflammatory symptoms (Bi et al. 2018). Phthalates are suggested to have adjuvant effects on allergies, as *in*

363 *vitro* and animal studies have indicated that DEHP enhances the production of allergy-related
364 molecules, and inflammatory cytokines such as IL-5, IL-6, and TNF-α (Nishioka et al. 2012;
365 Tanaka et al. 2013). DEHP and BBzP may also increase allergy by suppressing TNF-α and IFN-β
366 expression and modulate the T-cell stimulations and responses (Kuo et al. 2013). However,
367 there are few studies on the effects of PFRs. *In vitro* studies have found that TDCIPP, TPHP
368 and/or TBOEP have an immunocytotoxic effect, or may alter the immune response and induce
369 oxidative stress (Canbaz et al. 2017; Killilea et al. 2017; Krivoshiev et al. 2018b). TCIPP and TCEP
370 were found to be involved in the complement cascade along with other potent inflammatory
371 regulators (Krivoshiev et al. 2018a). One *in vivo* study of zebrafish found that TDCIPP induced
372 dose-response up-regulation of mRNA expression related to the receptor-centered gene
373 networks, such as peroxisome proliferator-activated receptor alpha (PPAR-α), estrogen
374 receptors, and glucocorticoid receptors (Liu et al. 2013). Phthalate is also known to activate
375 PPAR-α pathway and 8-OHdG levels (Ito et al. 2007), so that phthalate and PFRs share similar
376 action. Recently, we found that mixtures of PFRs were positively associated with oxidative
377 stress markers of hexanoyl-lysine, and 4-hydroxynonenal in children participating in a cross-
378 sectional study (Ait Bamai et al. 2019). Similarly, regarding phthalates, a cross-sectional study
379 of children revealed positive associations of phthalates with 8-hydroxy-2'-deoxyguanosine and
380 malondialdehyde (Lee et al, 2019;Rocha et al., 2017), suggesting that phthalates and PFRs
381 share similar properties. However, we cannot speculate on the nature of these mechanisms,

382 and even less is known for mixture effects. Another study determined that metabolites of
383 TPHP exhibited potent oestrogen receptor (ER) α and ERβ agonistic activity (Kojima et al. 2016),
384 and that the potency of the agonistic (or antagonistic) activity differed among parent
385 compounds and metabolites (i.e., TPHP and OH-DPHP) (Kojima et al. 2016). Both phthalates
386 and PFRs are readily metabolised in the human body (Greaves et al. 2016; Völkel et al. 2017).
387 But still, half-lives of chlorinated PFRs have been shown to be longer than those of, aryl- and
388 alkyl-PFRs (Wang et al., 2020). In addition, each chemical has different renal and hepatic
389 clearance, binding abilities with plasma proteins (Wang et al., 2020), which may result different
390 bioactivity. Consequently, the mode of action of each individual metabolite in the human body
391 is very complex.

392 In this study, we used standardised creatinine-corrected concentrations. These values
393 were calculated using creatinine concentrations standardised according to sex and body size.
394 Although many studies have used creatinine-corrected urinary metabolite levels as
395 independent variables or the creatinine concentration as a covariate, the methods of
396 accounting for urine dilution are still controversial. Although children comprised the target
397 population for this study, their ages ranged from 7 to 12 years. Notably, the creatinine
398 concentrations varied significantly among different age groups (Kruskal-Wallis test, $p = 0.025$)
399 and exhibited a weak, but significant positive correlation with age (Spearman's rho = 0.242, p
400 = 0.006). According to Barr et al. (2005), the age group is a significant predictor of the urinary

creatinine concentration, and thus the creatinine-corrected concentration of an analyte should be compared with a 'reference' range derived from subjects in a similar demographic group (Barr et al. 2005). Accordingly, the individual creatinine clearance is used as the reference value when fitting the standardised creatinine corrected concentration. This enabled us to control the covariate-independent, short-term multiplicative effect of hydration on urinary dilution (O'Brien et al. 2016). At the same time, it should be noted that standardized creatinine corrected concentrations were assigned to the participants with <LOQ as well. This may modify the rank of quartiles, especially for infrequently detected metabolites such as BDCIPP, and has potential for misclassification.

There are some limitations to this study. First, this is a cross-sectional study and cannot be used to infer causality or the risk of developing an allergy. Moreover, the prevalence of symptoms was 2.4 times higher among the children included in this study than in the children and dwelling characteristics observed in our initial contact in 2008 (n = 4408; Table 1). Because urine samples were collected 1 or 2 years after the initial contact, children in the 1st grade were not included in this study, and low participation frequency of 2nd grade. Moreover, the dwellings were newer buildings (Ait Bamai et al. 2014), with a higher prevalence of signs of dampness (Table 1). Therefore, we must consider the potential for selection bias as calculated prevalence ratio and participation rate given by Nohr and Liew (2018) shown in Table 1, that children with allergies and a greater interest in the home

environment may have been more likely to choose to participate in the present study. In addition, participants with allergy symptoms may change their behaviors such as more frequent vacuuming and cleaning of their house, which may result in having lower exposures through dust to chemicals that accumulate in dust. It is notable for potential selection bias, however, it is difficult to know whether this resulted in over- or under-estimation of associations or if the direction of bias would be the same for all exposures. Second, urinary metabolites were measured only once. Both phthalates and PFRs are readily metabolised and eliminated from the body within several hours to days (Greaves et al. 2016; Völkel et al. 2017). Therefore, temporal urinary metabolite concentrations may not reflect long term exposures, such as weeks or months. On the other hand, concentrations of MBzP, ΣTBOEP and ΣTCIPP were correlated with their parent compound concentrations in the house dust suggest that the potential exposure source of these compounds are housing materials (Ait Bamai et al. 2015; Bastiaensen et al. 2019). In such a case, exposure levels of BBzP and these PFRs would not vary unless the children moved or renovated their home. In addition, there may be other unmeasured exposures such as pollens and fungi, which lead to potential residual confounding. Furthermore, we observed wide confidence intervals, and may not have had enough statistical power to find significant associations, due to the relatively limited sample size; especially, the combination model that included the category of 'high x high' involved a limited number of samples. Even smaller size of each category is not enough

to make final conclusions of the mixture effect. Finally, the difference in the concentration levels of each chemical, nor its toxicological mechanism were considered in the statistical models. The concentrations of PFRs were lower than phthalates, whereas stronger associations with wheeze and allergic symptoms were found between PFRs than phthalates. Ginsberg and Belleggia estimated a hazard quotient for 7 chemicals in house dust using Monte Carlo methods, and found that DEHP and TDCIPP were among the 3 chemicals that stood out, showing elevated hazards (Ginsberg and Belleggia 2017). In this study, metabolites of TDCIPP, but not ΣDEHP, showed a high qg-computation weight. The partial consistency of this study with Ginsberg and Bellegiia's hazard quotes could be due to their measured outcomes of cancer and non-cancer. Thus, the toxic constituents influencing asthma and allergy could differ. More experimental studies of the combined effect of phthalates and PFRs on allergies are warranted.

5. Conclusions

In this study, we examined mixtures and combined exposures to phthalates and PFRs and their association with wheeze, rhinoconjunctivitis, and eczema among elementary school-aged children. WQS analysis suggests a significant positive association of mixed exposure to phthalates and PFRs with rhinoconjunctivitis; however, these associations were not significant when using the qg-computation approach. Still, the chemicals that contributed most heavily

458 to the associations with allergies and might have exerted combined effects according to the
459 qg-computation weights (%) included combined exposures to ΣTCIPP and ΣTPHP which were
460 associated with increased prevalence of rhinoconjunctivitis, respectively. These preliminary
461 findings for the associations between allergic symptoms and exposure to mixtures and
462 combined chemicals require further studies to confirm our results.
463
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641

Table 1. Characteristics of the participants.

		This study (n=128)		Primary questionnaire survey (n=4408)		prevalence ratio[a)]	participation rate[b)] (%)
		n	%		%		2.9
Sex	boys	68	53.1	1.1	48.1	1.1	3.2
	girls	60	46.9	0.9	49.6	0.9	2.7
Grade	2	14	10.9	0.0	16.4	0.0	0.0
	3	35	27.3	0.7	16.7	0.7	1.9
	4	27	21.1	1.6	17.0	1.6	4.7
	5	24	18.8	1.2	17.4	1.2	3.5
	6	28	21.9	1.2	15.8	1.2	3.4
Height (cm) (mean ± standard deviation)		137.3 ± 8.82		No information			
Weight (kg) (mean ± standard deviation)		32.1 ± 6.44		No information			
Annual household income	< 3 million	6	4.7				
(Japanese yen/year)	3-5 million	25	19.5				
	5-8 million	50	39.1				
	>8 million	28	21.9				
	missing	19	14.8				
Dampness index at home	(mean ± SD)	2.10 ± 1.25		1.04 ± 1.03			
	≤1	39	30.5	0.5	65.9	0.5	1.3
	≥2	89	69.5	2.0	34.0	2.0	5.9
Wheeze	yes	29	22.7	2.4	9.3	2.4	7.1
Rhinoconjunctivitis	yes	47	36.7	2.4	15.4	2.4	6.9
Eczema	yes	36	28.1	2.4	11.7	2.4	7.0

642 [a)], Prevalence rate = prevalence in this study (%) / prevalence in primary questionnaire survey (%)

643 [b)], Participation rate (%) = 2.9 * prevalence rate

Table 2. Distribution of phthalates and PFR metabolites in urine.

nM	n	>DL (%)	min	25%tile	50% tile	75% tile	max
ΣDBP	128	96.7	<LOQ	98.4	236	759	31500
MBzP	128	78.1	<LOQ	27.6	62.9	124	6310
ΣDEHP	128	100.0	82.3	265	442	631	29100
5-HO-EHDPHP	113	80.0%	<LOQ	0.05	0.11	0.24	3.05
BDCIPP	128	55.3%	<LOQ	<LOQ	0.20	0.29	10.2
DNBP	113	8.3%	<LOQ	<LOQ	<LOQ	<LOQ	3.69
TCEP	113	85.2%	<LOQ	0.10	0.19	0.36	1.13
ΣTBOEP	128	99.2%	<LOQ	1.08	1.88	4.29	15.1
ΣTCIPP	128	95.3%	<LOQ	0.62	0.95	2.29	42.0
ΣTPHP	128	83.6%	<LOQ	1.41	2.13	3.65	23.4
creatinine (µg/mL)	128	100%	69.9	802	1010	1430	2750
standardised creatinine corrected concentrations (nmol/g Standardised Cr.)							
ΣDBP			11.0	78.0	184	537	34700
MBzP			3.68	23.5	52.8	106	2620
ΣDEHP			58.5	179	330	512	20300
5-HO-EHDPHP			0.01	0.05	0.08	0.16	4.72
BDCIPP			0.03	0.08	0.13	0.26	6.83
TCEP			0.02	0.08	0.15	0.27	2.39
ΣTBOEP			0.14	0.77	1.63	3.40	15.2
ΣTCIPP			0.10	0.48	0.77	1.82	37.9
ΣTPHP			0.59	1.28	1.69	2.78	27.4

BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; ΣDBP, dibutyl phthalate; ΣDEHP, di(2-ethylhexyl) phthalate; DL, detection limit; DNBP dimethyl phosphate,5-HO-EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; MBzP, monobenzyl phthalate; ΣTBOEP, Σ metabolites of tris(2-butoxyethyl) phosphate; ΣTCIPP, Σ metabolites of tris(1-chloro-iso-propyl) phosphate; TCEP, tris(2-chloroethy) phosphate; ΣTPHP, Σ metabolites of triphenyl phosphate

ΣDBP (MnBP and MiBP), ΣDEHP (MEHP, MEOHP, and MECPP), ΣTBOEP (BBOEP, 3-HO-TBOEP, and BBOEHEP), ΣTCIPP (BCIPP and BCIPHIPP), ΣTPHP (DPHP and 4-HO-DPHP)

651

Table 3. Associations between allergic symptoms and chemical mixture.

	OR	95%CI		p-value		
Weighted quantile sum regression model						
Positive models						
Wheeze	1.52	0.67	3.50	0.315		
Rhinoconjunctivitis	2.60	1.38	5.14	0.004		
Eczema	1.91	0.99	3.85	0.060		
Inverse models						
Wheeze	0.96	0.83	1.79	0.109		
Rhinoconjunctivitis	0.58	0.29	1.12	0.110		
Eczema	0.54	0.24	1.18	0.131		
Quantile g-computation model	OR	95%CI		p-value	Sum of positive coefficient	Sum of negative coefficient
Wheeze	0.95	0.33	2.70	0.918	1.23	-1.28
Rhinoconjunctivitis	1.29	0.49	3.40	0.612	1.76	-1.51
Eczema	1.00	0.37	2.72	0.996	0.77	-0.78

652 Adjusted for sex, grade, annual household income and dampness index
653 OR, odds ratio; CI, confidence interval

Table 4. Single chemicals categorised into tertiles, and associations with allergic symptoms.

Allergic symptom	metabolites	1st		2nd					3rd					trend
		n		n	OR	95%CI		P-value	n	OR	95%CI		P-value	P-value
Wheeze														
	ΣDBP	43	ref	43	0.83	0.29	2.37	0.724	42	0.48	0.15	1.54	0.217	0.223
	MBzP	43	ref	43	0.29	0.08	1.10	0.069	42	1.20	0.41	3.48	0.737	0.703
	ΣDEHP	43	ref	43	0.83	0.26	2.59	0.745	42	0.75	0.23	2.37	0.620	0.623
	5-HO-EHDPHP	38	ref	38	2.43	0.70	8.43	0.162	37	3.21	0.86	11.89	0.082	0.082
	BDCIPP	45	ref	48	1.21	0.40	3.69	0.731	35	1.25	0.39	4.03	0.712	0.706
	uTCEP	38	ref	38	1.95	0.59	6.40	0.273	37	1.61	0.46	5.61	0.457	0.434
	ΣTBOEP	43	ref	43	0.89	0.28	2.81	0.846	42	1.33	0.43	4.13	0.627	0.630
	ΣTCIPP	43	ref	43	0.88	0.30	2.58	0.816	42	0.56	0.18	1.78	0.327	0.332
	ΣTPHP	43	ref	43	1.02	0.33	3.20	0.972	42	1.64	0.53	5.05	0.392	0.395
Rhinoconjunctivitis														
	ΣDBP	43	ref	43	1.39	0.53	3.61	0.502	42	0.84	0.32	2.24	0.731	0.733
	MBzP	43	ref	43	1.18	0.45	3.12	0.736	42	0.88	0.33	2.36	0.799	0.787
	ΣDEHP	43	ref	43	1.64	0.61	4.39	0.328	42	1.09	0.40	2.99	0.864	0.870
	5-HO-EHDPHP	38	ref	38	0.59	0.21	1.64	0.310	37	1.06	0.39	2.91	0.910	0.951
	BDCIPP	45	ref	48	**2.95**	**1.04**	**8.37**	**0.042**	35	**2.93**	**1.04**	**8.28**	**0.043**	**0.045**
	uTCEP	38	ref	38	0.50	0.18	1.41	0.189	37	1.37	0.48	3.88	0.558	0.642
	ΣTBOEP	43	ref	43	1.69	0.63	4.58	0.299	42	2.11	0.75	5.91	0.157	0.160
	ΣTCIPP	43	ref	43	1.31	0.46	3.73	0.614	42	**4.53**	**1.59**	**12.90**	**0.005**	**0.004**
	ΣTPHP	43	ref	43	1.67	0.60	4.63	0.322	42	2.67	0.97	7.36	0.057	0.056
Eczema														
	ΣDBP	43	ref	43	1.09	0.39	3.06	0.875	42	0.69	0.24	2.02	0.501	0.506
	MBzP	43	ref	43	0.38	0.12	1.16	0.088	42	0.83	0.29	2.36	0.726	0.741
	ΣDEHP	43	ref	43	4.91	1.47	16.48	0.010	42	2.92	0.82	10.37	0.098	0.131
	5-HO-EHDPHP	38	ref	38	1.58	0.49	5.08	0.446	37	2.59	0.79	8.46	0.116	0.115
	BDCIPP	45	ref	48	1.29	0.43	3.82	0.652	35	1.96	0.66	5.84	0.225	0.223
	uTCEP	38	ref	38	0.63	0.21	1.92	0.417	37	0.79	0.25	2.47	0.681	0.642
	ΣTBOEP	43	ref	43	0.70	0.22	2.19	0.538	42	1.85	0.63	5.42	0.260	0.220
	ΣTCIPP	43	ref	43	0.90	0.30	2.66	0.847	42	1.03	0.36	2.96	0.954	0.942
	ΣTPHP	43	ref	43	1.02	0.35	2.92	0.973	42	1.11	0.38	3.25	0.846	0.845

Odds ratio (95% CI) is calculated by logistic regression.
Adjusted for sex, grade, annual income and dampness index
Statistically significant (p<0.05) is shown in bold.
BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; ΣDBP, dibutyl phthalate; ΣDEHP, di(2-ethylhexyl) phthalate; DL, detection limit; DNBP dimethyl phosphate,5-HO-EHDPHP, 5-hydroxyethylhexyldiphenyl phosphate; MBzP, monobenzyl phthalate; ΣTBOEP, Σ metabolites of tris(2-butoxyethyl) phosphate; ΣTCIPP, Σ metabolites of tris(1-chloro-iso-propyl) phosphate; TCEP, tris(2-chloroethy) phosphate; ΣTPHP, Σ metabolites of triphenyl phosphate

Figure legend

Figure 1. Weight of each chemical according to quantile g-computation regression**.**

(A) Wheeze, (B) Rhinoconjunctivitis, (C) Eczema.

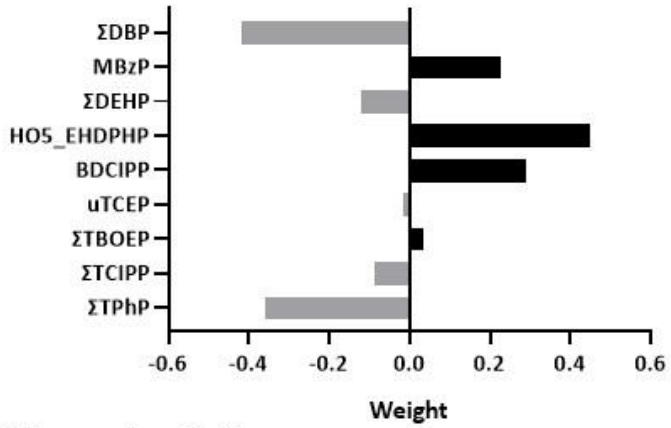
BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; ΣDEHP, di(2-ethylhexyl) phthalate; 5-HO-EHDPHP, 5-hydroxy-2-ethylhexyl diphenyl phosphate; MBzP, monobenzyl phthalate; ΣTBOEP, tris(2-butoxyethyl) phosphate; ΣTCIPP, tris(1-chloro-iso-propyl) phosphate; TCEP, tris(2-chloroethy) phosphate; ΣTPHP, triphenyl phosphate. ΣDBP (MnBP and MiBP), ΣDEHP (MEHP, MEOHP, and MECPP), ΣTBOEP (BBOEP, 3-HO-TBOEP, and BBOEHEP), ΣTCIPP (BCIPP and BCIPHIPP), and ΣTPHP (DPHP and 4-HO-DPHP).

Figure 2 Associations between combinations of ΣTCIPP and ΣTPHP with rhinoconjunctivitis

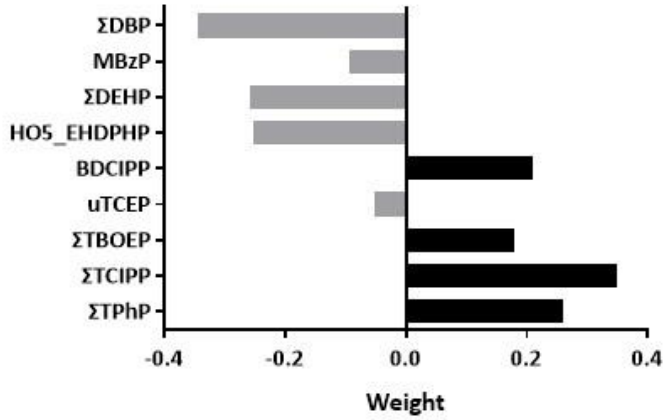
The odds ratios (ORs) and 95% confidence intervals (95% CI) of the rhinoconjunctivitis are shown as black squares and whiskers, respectively for TCIPP and ΣTPHP. The levels of each chemical were categorised as "high" or "low", with the "high" cutoff as follows: ΣTCIPP, ≥1.30 nM/g Standardised-Cr.; ΣTPHP, ≥2.29 nM/g Standardised-Cr. The ORs were calculated with the "low X low" group as the reference category, and adjusted for sex, grade, annual household income, dampness index. The X-axis indicates, from left: L × L, low × low; L × H, Low-High; H × L, high × low; and H × H, high × high. The Y-axis indicates OR (95% CI). **P <0.01

CI, confidence interval; Cr., creatinine; OR, odds ratio; ΣTCIPP, Σ metabolites of tris(1-chloro-iso-propyl) phosphate in urine; ΣTPHP, Σ metabolites of triphenyl phosphate in urine.

(A) Wheeze



(B) Rhino-conjunctivitis



(C) Eczema

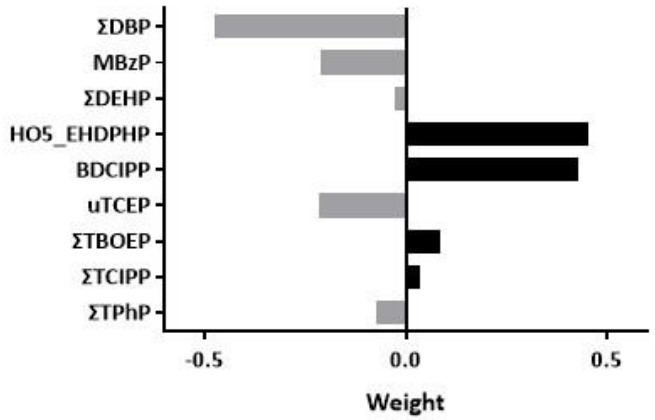


Figure 1 Weight of each chemical according to quantile g-computation regression.

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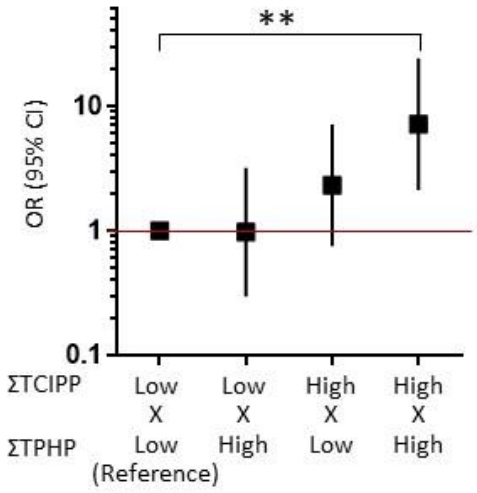

Figure 2 Associations between combinations of ΣTCIPP and ΣTPHP with rhinoconjunctivitis

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683 **Supplemental Material**

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685 **Combined exposures to phthalate esters and phosphate flame retardants and plasticizers and**
686 **their association with wheeze and allergy symptoms among school children**

687

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692

693 Table of contents

694

695 **Supplemental Table S1** Correlations between chemicals measured in urine

696

697 **Supplemental Table S2** Distribution of phthalates and PFR metabolites in urine in ng/mL

698

699 **Supplemental Table S3** Combined chemicals and their associations with allergic symptoms

Supplemental Table S1. Correlations between chemicals measured in urine.

	ΣDBP	MBzP	Σ3DEHP	5-HO-EHDPHP	BDCIPP	TCEP	ΣTBOEP	ΣTCIPP	ΣTPHP
ΣDBP	1.000	0.148	0.013	0.054	0.139	0.066	0.010	0.084	0.134
MBzP		1.000	0.138	0.209*	0.047	0.068	0.128	0.148	0.100
Σ3DEHP			1.000	0.300*	0.216*	-0.122	0.087	0.200*	0.340**
5-HO-EHDPHP				1.000	0.271**	-0.018	0.325**	0.455**	0.624**
BDCIPP					1.000	0.094	-0.013	0.081	0.340**
TCEP						1.000	-0.018	0.080	0.052
ΣTBOEP							1.000	0.074	0.251**
ΣTCIPP								1.000	0.334**
ΣTPHP									1.000

Spearman's rho, *P <0.05, **P <0.01

Supplemental Table 2. Distribution of phthalates and PFR metabolites in urine in ng/mL.

ng/mL	n	>DL (%)	min	25%tile	50% tile	75% tile	max
MBzP	128	78.1	<LOQ	7.2	16.1	31.7	1620
5-HO-EHDPHP	113	80.0%	<LOQ	0.02	0.04	0.09	1.2
BDCIPP	128	55.3%	<LOQ	<LOQ	0.07	0.09	3.3
DNBP	113	8.3%	<LOQ	<LOQ	<LOQ	<LOQ	0.78
TCEP	113	85.2%	<LOQ	0.03	0.05	0.10	0.32

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Supplemental Table S2. Combined chemicals and their associations with allergic symptoms.

	Ref (Low×Low)	Low × High				High × Low				High × High				Additive OR (L × H) + (H × L)	Multiplicative OR (L × H) * (H × L)
	n	n	OR	95%CI	P-value	n	OR	95%CI	P-value	n	OR	95%CI	P-value		
Rhino-conjunctivitis															
ΣTCIPP and ΣTPHP	63	23	0.97	0.29 3.19	0.959	23	2.32	0.75 7.13	0.142	19	**7.14**	**2.11 24.15**	**0.002**	3.29	2.25

OR (95%CI) were calculated by logistic regression model adjusted for sex, grade, annual income and dampness index

Statistically significant (p<0.05) shown in bold.

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