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Exposure to organophosphate flame retardants and plasticizers is positively associated with wheeze and FeNO and eosinophil levels among school-aged children: The Hokkaido study

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ABSTRACT

Exposure to organophosphate flame retardants and plasticizers (PFRs) increases the risk of asthma and allergies. However, little is known about its association with type 2 inflammation (T2) biomarkers used in the management of allergies. The study investigated associations among urinary PFR metabolite concentrations, allergic symptoms, and T2 biomarkers. The data and samples were collected between 2017 and 2020, including school children (n = 427) aged 9–12 years living in Sapporo City, Japan, among the participants of "The Hokkaido Study on Environment and Children's Health." Thirteen urinary PFR metabolites were measured by LC-MS/MS. Allergic symptoms were assessed using the International Study of Asthma and Allergies in Childhood questionnaire. For T2 biomarkers, the peripheral blood eosinophil counts, fraction of exhaled nitric oxide level (FeNO), and serum total immunoglobulin E level were measured. Multiple logistic regression analysis, quantile-based g-computation (qg-computation), and Bayesian kernel machine regression (BKMR) were used to examine the associations between the health outcomes of the individual PFRs and the PFR mixtures. The highest concentration of PFR was Σtris(1-chloro-isopropyl) phosphates (ΣTCIPP) (Median:1.20 nmol/L). Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) was significantly associated with a high odds ratio (OR, 95%CI:1.36, 1.07–1.72) for wheeze. TDCIPP (OR, 95%CI:1.19, 1.02–1.38), Σtriphenyl phosphate (ΣTPHP) (OR, 95%CI:1.81, 1.40–2.37), and Σtris(2-butoxyethyl) phosphate (ΣTBOEP) (OR, 95%:1.40, 1.13–1.74) were significantly associated with increased odds of FeNO (≥35 ppb). ΣTPHP (OR, 95%CI:1.44, 1.15–1.83) was significantly associated with high eosinophil counts (≥300/μL). For the PFR mixtures, a one-quartile increase in all PFRs (OR, 95%CI:1.48, 1.18–1.86) was significantly associated with high FeNO (≥35 ppb) in the qg-computation model. The PFR mixture was positively associated with high FeNO (≥35 ppb) and eosinophil counts (≥300/μL) in the BKMR

Abbreviations: AOR, adjusted odds ratio; BKMR, bayesian kernel machine regression; BDCIPP, bis(1,3-dichloro-2-propyl) phosphate; BCIPP, bis(1-chloro-2-propyl) phosphate; 3-HO-TBOEP, bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate; BBOEP, bis(2-butoxyethyl) phosphate; BMI, body mass index; BAL, bronchoalveolar lavage fluid; CI, confidence interval; DNBP, di-n-butyl phosphate; DPHP, diphenyl phosphate; ELISA, enzyme-linked immunosorbent assay; ETS, environmental tobacco smoke; FeNO, fractional of exhaled nitric oxide; HO-TPHP, hydroxyphenyl diphenyl phosphate; IgE, immunoglobulin E; ISAAC, International Study of Asthma and Allergies in Childhood; IU, international unit; LOQ, limits of quantification; PFRs, organophosphate flame retardants and plasticizers; Ppb, parts per billion; QC, quality control; qg-computation, quantile-based g-computation; SG, specific gravity; TNBP, tri-n-butyl phosphate; TDCIPP, tris(1,3-dichloro-2-propyl) phosphate; TCEP, tris(chloroethyl) phosphate; Th2 cells, type 2 helper T; T2 biomarkers, type 2 inflammation; ILC2 cells, type 2 innate lymphoid; EHPHP and 5-HO-EHDPHP, Σ 2-Ethylhexyldiphenyl phosphate (ΣEHDPHP) metabolites; BCIPP and BCIPHIPP, Σ tris(1-chloro-isopropyl) phosphate (ΣTCIPP) metabolites; DPHP HO-TPHP and 4HO-DPHP, Σ triphenyl phosphate (ΣTPHP) metabolites; BBOEP BBOEHEP and 3-HO-TBOEP, Σ tris(2-butoxyethyl) phosphate (ΣTBOEP) metabolites; BCIPHIPP, 1-hydroxy-2-propyl bis(1chloro-2-propyl) phosphate; 5-HO-EHDPHP, 2-ethyl-5-hydroxyhexyl diphenyl phosphate; EHPHP, 2-ethylhexyl phenyl phosphate; BBOEHEP, 2-hydroxyethyl bis (2-butoxyethyl) phosphate; 4-HO-DPHP, 4-hydroxyphenyl phenyl phosphate.

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models. These results may suggest that exposure to PFRs increases the probability of asthma, allergies, and T2 inflammation.

1. Introduction

Organophosphate flame retardants and plasticizers (PFRs) have been widely used as flame retardants and plasticizers in a variety of products such as polyurethane foam, paints, lubricants, floor waxes, electronic and electrical products, textiles, plastics, and food packaging (Stapleton et al., 2009, Kajiwara et al., 2011, Van der Veen and De Boer 2012, Xu et al., 2016). PFRs are semi-volatile organic compounds, and as additives, they are not chemically bound to materials used in consumer products, resulting in a slow release into the ambient environment via abrasion, leaching, and volatilization (Wei et al., 2015). The presence of PFRs has been widely demonstrated in environmental matrices, including air, water, soil, and indoor dust (Araki et al., 2014, Tajima et al., 2014, He et al., 2019, Zhang et al., 2022). Using the products containing PFRs and inhaling/ingesting PFR-contaminated environmental matrices could ultimately result in human exposure. PFRs have a short biological half-life ranging from several hours to days and are rapidly metabolized and eliminated from the body (Greaves et al., 2016, Völkel et al., 2018). The "pseudo-persistent" pollutants refer to compounds that are continuously introduced into the environment, with new molecules constantly replacing those being eliminated (Daughton 2003). Owing to their widespread distribution and continuous exposure, PFRs can be considered pseudo-persistent.

Health concerns about exposure to PFRs are increasing. Exposure to PFR potentially impacts child neurodevelopment through decreased intelligence quotient and working memory scores (Castorina et al., 2017). It is also associated with inflammatory responses through high levels of oxidative stress among children (Ait Bamai et al., 2019). Our previous studies on external exposure measured the PFRs in house dust from floor or multi-surfaces. We found that tri-n-butyl phosphate (TNBP), tris(1-chloro-isopropyl) phosphate (TCIPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) significantly increased the odds of wheeze, eczema, and allergic rhinoconjunctivitis (Araki et al., 2014, Ait Bamai et al., 2018, Araki et al., 2018). However, different results have been reported in studies conducted in the United States and Sweden. No positive associations were observed between childhood asthma and the PFRs in dust from household filter systems (Bi et al., 2018) and dust from mothers' mattresses (Canbaz et al., 2016). This inconsistency may be due to the different sources of the dust samples.

The levels of urinary metabolites can reflect internal exposure not only from dust, but also from other exposure sources, including diet (Araki et al., 2018, Doherty et al., 2019). To measure internal exposure, biomonitoring of metabolites of PFRs in urine, has been recently established (Van Den Eede et al., 2013, Van Den Eede et al., 2015, Bastiaensen et al., 2018). Our previous study on the measurement of PFR metabolites in urine reported a significant increase in the children's allergic symptoms with higher concentrations of metabolites from TDCIPP, TCIPP, and tris(2-butoxyethyl) phosphate (TBOEP) (Araki et al., 2018). However, the association between urinary PFR metabolite concentration and allergic disease remains unclear.

The pathophysiology of allergic diseases includes an overreaction of type 2 immune responses driven by type 2 helper T (Th2) cells and type 2 innate lymphoid (ILC2) cells (Lloyd and Snelgrove 2018). The measurement of T2 biomarkers, such as fractional exhaled nitric oxide (FeNO) levels, blood eosinophils, and total immunoglobulin E (IgE), is recommended for the diagnosis and management of allergies (Pavord et al., 2018, Reddel et al., 2022, Goudarzi et al., 2023). An experimental study reported that eosinophil counts in mouse bronchoalveolar lavage fluid increased dramatically after inhaling TNBP (Meng et al., 2022). However, this experimental evidence was based on high doses of a single PFR exposure, whereas human exposure involves multiple PFRs at much lower levels. Whether these experimental findings can be validated in humans remains to be studied. To our knowledge, there is no epidemiological evidence of an association between exposure to PFRs and T2 biomarkers, which could better clarify the relationships among PFRs, asthma, and allergies.

The evaluation of the health effects of combined exposure to multiple chemicals has been highlighted, as humans are simultaneously exposed to different chemicals (Meek et al., 2011). Certain challenges must be addressed when considering the overall health effects of multiple chemicals. Traditional regression analysis models may be biased due to collinearity and interactions among different chemicals. Quantile-based g-computation (qg-computation) and Bayesian kernel machine regression (BKMR) analyses can identify linear and nonlinear effects, respectively, and assess the health effects of multiple exposures that were multicollinear or highly correlated (Bobb et al., 2015, Bobb et al., 2018, Keil et al., 2020). Using the abovementioned analyses, this study investigated the association among urinary individual PFR metabolites, PFR mixtures, allergic symptoms, and T2 biomarkers in 9–12 years old children. We hypothesized that increased internal exposure to PFRs would be associated with a high probability of allergic symptoms and high levels of T2 biomarkers.

2. Materials and methods

2.1. Study population

This cross-sectional study was part of the "Hokkaido Study on Environment and Children's Health", Hokkaido cohort (Kishi et al., 2011, Kishi et al., 2013, Kishi et al., 2017, Kishi et al., 2021). A flowchart of the participant selection process is presented in Supplementary Fig. S1. This ongoing birth cohort study included 20,926 pregnant women between 2003 and 2012 in Hokkaido prefecture, Japan. The face-to-face survey was conducted from September 2017 to March 2020 to collect data and samples. We contacted a total number of 1881 children born between April 2006 to January 2010, who were 9–12 years old and residing in Sapporo City and its surrounding areas at the time points of the survey. 428 of them agreed to participate in a face-to-face survey and visited selected pediatric clinics with their parents (Goudarzi et al., 2023). A questionnaire survey, urine sampling, blood sampling, FeNO measurement, and anthropometric measurements were done on the same day. In total, 427 children, available with information from the questionnaire and spot urine samples, were included in the final analysis. The questionnaire was answered by the child's parents. Of those we approached, the participation percentage was 22.8%. The selection bias may exist as the included participants were the individuals who responded to the tracking of the Hokkaido Study until the age of 9–12 and who were more convenient to visit the selected clinics. To mitigate the potential for selection bias, we approached all children aged 9–12 years, and we deliberately avoided specifying allergic diseases as study outcomes in the invitation letters. Moreover, our prior research has demonstrated that the participants in this study exhibit similar characteristics (child sex, birth weight) and allergic disease prevalence as those in the original cohort (Goudarzi et al., 2023).

2.2. Questionnaire

Parents answered a questionnaire to collect information on their children's demographics and allergic symptoms. Demographic characteristics included sex, age, physical exercise frequency, environmental tobacco smoke (ETS) status, annual household income, and maternal educational level. ETS (yes/no) is defined as a child's current exposure

to passive smoke from a mother, father, or other household members who smoke tobacco in places where the child lives. Allergic symptoms of the children, including wheeze, eczema, and allergic rhinoconjunctivitis, were defined using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Asher et al., 1995, Asher et al., 2006). Wheeze was a positive "yes" answer to the question "Has your child wheezed or whistled in the chest in the last 12 months?," and eczema symptoms were defined based on a "yes" answer to all the following three assertions: (a) "Presence of an itchy rash that comes and goes for at least 6 months" and (b) "Presence of an itchy rash on below-mentioned areas in the last 12 months" and (c) "Presence of an itchy rash on one or several of the following areas: around the neck, ears, and eyes, the folds of inside the elbows, on the back of knees, in front of the ankles, or under the buttocks." Allergic rhinoconjunctivitis symptoms were defined by positive responses to both of the following questions: (a) "Has your child experienced sneezing or a runny/stuffy nose in the absence of a cold or flu in the last 12 months?" and (b) "Was this problem accompanied by itchy or watery eyes?".

2.3. Measurement of PFR metabolites in urine

Spot urine samples of children were collected using a polypropylene cup by the research staff at pediatric clinics on the same day as the survey, and samples were transported to Hokkaido University Center for Environmental and Health Sciences in a cool box. Upon arrival, the samples were immediately dispensed into a glass tube that was cleaned with acetone, sealed with fluoric tape, wrapped in aluminum foil, and stored at − 30 °C until the day of analysis. The specific gravity (SG) of urine samples was determined at room temperature using a handheld refractometer (ATAGO T3-SE, ATAGO Co., Ltd. Japan).

Urinary metabolites of PFRs were measured at the Toxicological Center of the University of Antwerp, Belgium. The details of the PFR analysis are explained in our previous report (Bastiaensen et al., 2020). Thirteen PFR metabolites (Supplementary Table S1), di-n-butyl phosphate [DNBP], bis(1,3-dichloro-2-propyl) phosphate [BDCIPP], tris (chloroethyl) phosphate [TCEP], bis(1-chloro-2-propyl) phosphate [BCIPP], 1-hydroxy-2-propyl bis(1chloro-2-propyl) phosphate [BCIPHIPP], diphenyl phosphate [DPHP], hydroxyphenyl diphenyl phosphate [HO-TPHP], 4-hydroxyphenyl phenyl phosphate [4-HO-DPHP], bis(2-butoxyethyl) phosphate [BBOEP], 2-hydroxyethyl bis (2-butoxyethyl) phosphate [BBOEHEP], bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate [3-HO-TBOEP], 2-ethylhexyl phenyl phosphate [EHPHP], and 2-ethyl-5-hydroxyhexyl diphenyl phosphate [5-HO-EHDPHP]), were measured in urine. Urine was analyzed using an Agilent 1290 Infinity II UPLC system coupled to a 6495C triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The following quality control (QC) measures were used during the sample preparation and analytical analysis to ensure the accuracy of the results. Procedural blanks consisting of 1 mL Milli-Q water were made before sample preparation and analyzed in parallel with urine and QC samples. For every 20 urine samples, two procedural blanks, one QC-low, and one QC-medium were added. The values of the analytes measured in the procedural blanks were subtracted from those measured in the samples. Field blanks were not used, as it was not feasible to create representable field blanks during the face-to-face survey. However, external contamination of PFR metabolites in the urine samples from the environment and during sampling is negligible (Bastiaensen et al., 2021b). External QC was ensured by successful participation in inter-laboratory comparison exercises, such as the Human Biomonitoring for Europe External Quality Assurance Scheme (HBM4EU ICI/EQUAS, 2018–2021) and the External Quality Assessment Scheme for Organic Substances in Urine (OSEQAS, 2018–2023). The performance of QC measures for the PFR metabolites during the analysis of the sample baches is summarized in Supplementary Table S2.

2.4. Measurement of T2 markers

The analytical methods for T2 biomarkers have been described in our previous study (Goudarzi et al., 2023). Peripheral blood samples were collected from 417 children, and FeNO levels were measured from 422 children in pediatric clinics. Peripheral blood was then sent for analysis by a cool delivery service. Peripheral blood eosinophil counts (/μL) was measured at Daiichi Kishimoto Clinical Laboratories, Inc. (Sapporo, Japan). Serum total IgE levels (international unit, IU/mL) were measured using enzyme-linked immunosorbent assay (ELISA) at SRL, Inc. (Tokyo, Japan). FeNO levels were measured with an electrochemical sensor NIOX VERO® (Aerocrine, Stockholm, Sweden). A single-breath online method measured gas-phase FeNO (parts per billion, ppb) (American Thoracic Society, European Respiratory Society, 2005; Dweik et al., 2011). In this study, we used cut-off values to define high levels of T2 biomarkers. FeNO $\geq$ 35 ppb in children younger than 12 years of age was described to indicate eosinophilic inflammation and to support measuring airway inflammations, including asthma (Dweik et al., 2011). There is no gold standard for children's cut-off values of total IgE level and peripheral blood eosinophil counts yet. Shimazu (1994) reported that the total IgE level for healthy children (7 years and older) is less than 170 IU/ml, which has been used as a cut-off value in a national cohort study in Japan recently (Shimazu 1994, Saito-Abe et al., 2021). Nakamura (2020) reported that the eosinophil counts higher than 300/μL is related to an increased risk of asthma (Nakamura et al., 2020).

2.5. Statistical analysis

For the concentrations below the limits of quantification (LOQ), the value was imputed by the LOQ × detection frequency (James et al., 2002). DNBP and TCEP were excluded from further analysis because of the low detection frequencies (<10%). The Σ metabolite concentrations were estimated using the molar concentrations of ΣTCIPP metabolites (BCIPP and BCIPHIPP), Σ triphenyl phosphate (ΣTPHP) metabolites (DPHP, HO-TPHP, and 4HO-DPHP), ΣTBOEP metabolites (BBOEP, BBOEHEP, and 3-HO-TBOEP), Σ 2-Ethylhexyldiphenyl phosphate (ΣEHDPHP) metabolites (EHPHP and 5-HO-EHDPHP). The concentrations of Σ metabolites were used as exposure variables for assessing exposure to parent PFRs. The concentrations were corrected for individual SG values using the following formula:

$$SG\ \ corrected\ \ concentration = uncorrected\ \ concentration \times [(SG_m - 1)/(SG_i - 1)]$$

where the SG_m is the median SG of this study (1.025), and SG_i is the individual SG (Pearson et al., 2009, Meeker et al., 2012). SG reflects the ratio between the density of the urine sample and pure water. SG correction positively reduces the variability of urinary concentrations of non-persistent organic chemicals (Roggeman et al., 2022). Urinary creatinine is a chemical by-product generated from muscle metabolism, which may introduce bias to the data analysis among children owing to the developmental growth of muscle mass (Pearson et al., 2009). Conversely, SG is less likely to be influenced by individual factors compared to creatinine (Bastiaensen et al., 2021b).

The SG corrected PFR concentrations were converted to natural log scales for the downstream analyses to obtain better normality. The random forest method was used to fill in the missing values of the annual household income (missing rate: 5.6%) (Breiman 2001). Differences and correlations between demographic characteristics, PFR concentrations, and health outcomes were analyzed using the Mann–Whitney *U*, chi-square, and Spearman's rank correlation tests. Covariates retained in the adjusted models included sex, age, annual household income, body mass index (BMI), and ETS, which were selected based on our previous study (Goudarzi et al., 2023). These covariates were associated with PFR exposure, allergic symptoms, and T2 biomarkers in the bivariate tests (p

< 0.1).

Multiple logistic regression models were used to assess the relationships between individual PFRs, wheeze, eczema, allergic rhinoconjunctivitis, and T2 biomarkers. Qg-computation and BKMR examine the partial and joint effects of the PFR mixture. Qg-computation adapts the weighted quantile regression approach and enhances the causal inferential aspects using g-computation (Keil et al., 2020). Qg-computation utilizes the following equation (Keil et al., 2020):

$$Y_i = \beta_0 + \sum_{j=1}^{d} \beta_j X_{ji}^{q} + \epsilon_i$$

where Y_i is the health outcome for individual i (i = 1, 2, 3…n), β_0 denotes the model intercept, X_{ji}^{q} is a quartile version of j^{th} chemical exposure, $\sum_{j=1}^{d}\beta_j$ is the weighted quantile sum, which estimates the combined effect of increasing every exposure in the PFR mixture by one quantile simultaneously, and ϵ_i is the error term. The qg-computation also enables the evaluation of the individual contributions of the mixture and simultaneously estimates the positive or negative weight. The sum of positive and negative weights is defined to be 1.0. Each model was run for 500 iterations using bootstrapping.

Compared with qg-computation, BKMR is a non-parametric method for estimating the overall mixture effect and individual chemical impact on health outcomes. The BKMR model was executed using a kernel machine regression equation (Bobb et al., 2015, Bobb et al., 2018):

$$Y_i = h(z_{il}, \cdots, z_{im}) + x_i\beta + \epsilon_i$$

where Y_i is the health outcome for individual i (i = 1, 2, 3…n), z_{im} denotes the m^{th} chemical exposure, h is the function that fits the exposure and the outcome considering nonlinear interactions between the exposures, x_i represents the potential confounder, β is the effect of the covariates, and ϵ_i is the residual. The cumulative effect of the PFR mixture on allergic symptoms and T2 biomarkers was assessed by comparing the values when all chemical components were at their 50th percentile with values when all chemical components were at a particular percentile between the 25th and 75th percentile. The individual chemical impacts were evaluated while keeping all the remaining exposures in the mixture fixed at their median concentration. The interaction effects among PFRs on health outcomes were quantified by comparing a single PFR health risk when all other exposures were fixed at their 25th percentile to when all PFRs were fixed at their 75th percentile (Bobb et al., 2015, Bobb et al., 2018). Models were run for 10,000 iterations using a Markov-chain Monte Carlo sampler. Two-tailed tests were used for all statistical analyses, and a p-value of<0.05 was considered statistically significant. We consider a BKMR analysis statistically significant when its 95% credible interval does not overlap with zero. All statistical analyses were performed using R (Version 4.2.3) with the packages "randomForest" (version 4.7–1.1), "glm2" (version 1.2.1), "qgcomp" (Version 2.10.1), "bkmr" (Version 0.2.2) for missing value imputations, logistic regression analysis, qg-computation, and BKMR, respectively.

2.6. Ethics

After explaining the research objectives and methods to the participants, written consent was obtained from all parents, and permission was obtained from all children. The research review boards of Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environmental and Health Sciences approved this study (21–136).

3. Results

The demographic characteristics and health outcomes of the study participants are shown in Table 1. A total of 427 children aged 9–12 years were included in the study. The number of children with allergic symptoms of wheeze, eczema, and allergic rhinoconjunctivitis was 32 (7.5%), 96 (23%), and 92 (22%), respectively. For the T2 biomarker, the median (interquartile range) for FeNO, total IgE, and eosinophil counts were 17.5 (9–37) ppb, 129 (37–394) IU/mL, and 234 (130–422) counts/μL, respectively. The number of children showing FeNO ≥ 35 ppb, total IgE ≥ 170 IU/mL, and eosinophil counts ≥ 300/μL was 116 (27%), 183 (44%), and 162 (39%), respectively.

Table 1
Characteristics of study participants (n = 427).

Variable		n	%
Sex	Boys	230	53.9
	Girls	197	46.1
Age	9	140	32.8
	10	171	40.0
	11	45	10.5
	12	71	16.6
BMI (kg/m^2) (mean ± SD)		17.8 ± 2.97	
Physical exercise	yes	314	73.5
Environmental tobacco smoke	yes	167	39.1
Annual household income	< 5 million	126	29.5
(Japanese Yen/year)	5–8 million	180	42.2
	> 8 million	97	22.7
	missing	24	5.6
Wheeze	yes	32	7.5
Eczema	yes	96	22.5
Allergic rhinoconjunctivitis	yes	92	21.6
FeNO (high) (n = 422)	≥35 ppb	116	27.5
Serum total IgE (high) (n = 417)	≥170 IU/mL	183	43.9
Peripheral blood eosinophils (high) (n = 417)	≥300 counts/μL	162	38.8

The distributions of the urinary concentrations of the PFR metabolites by raw weight (before SG correction, ng/mL) and Σ weight (nmol/L) are shown in Table 2. BDCIPP, BCIPHIPP, and DPHP were detected in > 60 % of urine samples. The level of ΣTCIPP was the highest (median value of 1.20 nmol/L), followed by ΣTPHP (median value of 1.10 nmol/L). Significant correlations ($p < 0.05$) were found among all PFR metabolite groups, with Spearman's ρ ranging from 0.10 to 0.35, as shown in Supplementary Fig. S2.

In multiple logistic regression models (Fig. 1A, and Supplementary Table S3), for wheeze, a natural log unit increase in TDCIPP was significantly associated with a high adjusted odds ratio (AOR) (95% CI) of 1.36 (1.07–1.72). For FeNO ≥ 35 ppb, TDCIPP, ΣTPHP, and ΣTBOEP were significantly associated with high AOR (95% CI) of 1.19 (1.02–1.38), 1.81 (1.40–2.37) and 1.40 (1.13–1.74), respectively. For eosinophil count ≥ 300 /μL, ΣTPHP was significantly associated with a high AOR (95% CI) of 1.44 (1.15–1.83). No significant associations were observed between the concentrations of PFR metabolites and eczema, allergic rhino-conjunctivitis, or total IgE ≥ 170 IU/mL. Although the results were not statistically significant, marginal associations were observed among allergic rhino-conjunctivitis, TPHP, and TBOEP with high AOR (95% CI) of 1.25 (0.97, 1.60) and 1.22 (0.97, 1.54), respectively. FeNO ≥ 35 ppb and TCIPP, total IgE ≥ 170 IU/mL and TPHP, eosinophil count ≥ 300 /μL and TCIPP were found to have high AOR (95% CI) of 1.19 (0.98, 1.44), 1.21 (0.97, 1.52), and 1.17 (0.98, 1.39) respectively.

Qg-computation was used to analyze the mixture effect of the PFRs (Table 3 and Fig. 1 B). One-quartile increase in PFRs index was associated with significantly high odds of exceeding the cut-off level for FeNO, with values ≥ 35 ppb (AOR = 1.48, 95% CI = 1.18–1.86). No significant association was observed between the PFR mixture, wheeze, eczema, allergic rhinoconjunctivitis, total IgE, or eosinophil counts. The individual contributions of the mixtures to each chemical are shown in Fig. 1B and Supplementary Table S4. For FeNO, the chemicals with the highest weight (%) in the positive direction were ΣTPHP (47.9%), ΣTBOEP (21.0%), and TDCIPP (20.1%), while ΣEHDPHP was identified as the only one with negative weight.

The results of another mixed chemical model were analyzed using BKMR. The PFR mixture was positively associated with FeNO levels and

Table 2
Distribution of organophosphate flame retardants and plasticizers (PFRs) metabolites in urine (n = 427).

Metabolites	LOQ	>LOQ (%)	min	25th	50th	75th	95th	max
Raw concentration (Before specific gravity correction, ng/mL)								
DNBP	0.20	6.8				<LOQ	0.25	1.01
BDCIPP	0.05	63.9		<LOQ	0.11	0.29	2.61	42.9
TCEP	0.10	0.5					<LOQ	0.31
BCIPP	0.40	22.5				<LOQ	1.96	39.9
BCIPHIPP	0.05	78.0	<LOQ	0.06	0.20	0.64	2.87	83.5
DPHP	0.10	84.5	<LOQ	0.14	0.27	0.54	1.73	40.7
HO-TPHP	0.05	0.9					<LOQ	0.20
4HO-DPHP	0.20	0.0						<LOQ
BBOEP	0.05	58.1		<LOQ	0.07	0.19	0.70	4.19
BBOEHEP	0.05	53.6		<LOQ	0.06	0.23	1.31	8.32
3-HO-TBOEP	0.10	20.6				<LOQ	0.19	1.75
EHPHP	0.20	59.7		<LOQ	0.24	0.48	1.12	6.01
5-HO-EHDPHP	0.05	17.8				<LOQ	0.13	0.45
ΣMetabolites concentration (nmol/L)								
TDCIPP (based on BDCIPP)			0.10	0.10	0.34	0.91	8.17	134.0
ΣTCIPP (Σ of BCIPP, BCIPHIPP)			0.48	0.57	1.20	3.49	15.47	429.2
ΣTPHP (Σ of DPHP, HO-TPHP, 4HO-DPHP)			0.34	0.58	1.10	2.17	6.92	162.8
ΣTBOEP (Σ of BBOEP, BBOEHEP, 3-HO-TBOEP)			0.23	0.23	0.63	1.61	5.85	30.3
ΣEHDPHP (Σ of EHPHP, 5-HO-EHDPHP)			0.34	0.44	0.89	1.78	4.33	22.05
Specific gravity		100	1.003	1.018	1.025	1.029	1.034	1.044

Abbreviations: limits of quantification (LOQ).

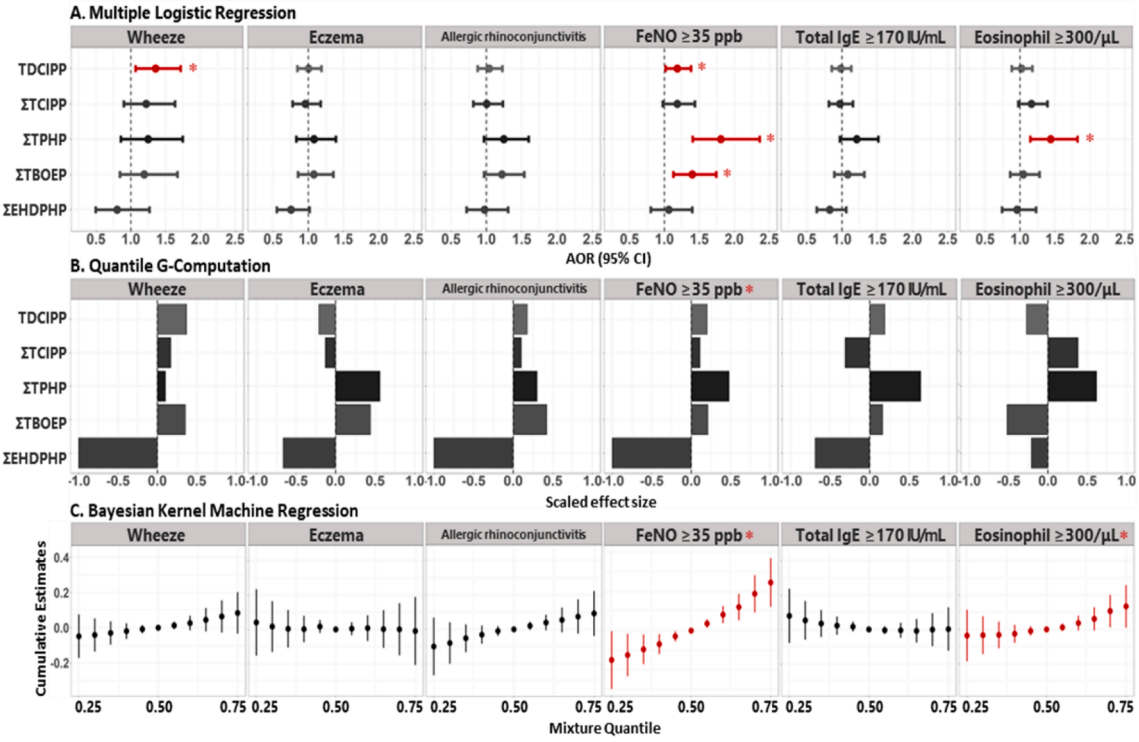

Fig. 1. Associations between allergic symptoms and type 2 inflammation (T2) biomarkers and urinary organophosphate flame retardants and plasticizers (PFRs) among school children in Hokkaido study, 2017–2020. (A)shows the results of multiple logistic regression models. Adjusted odds ratio and 95% confidence interval (CI) indicates changes in allergic symptoms and T2 biomarkers in association with a natural-log unit increase in individual PFRs; (B)shows the results of quantile g-computation. Positive and negative weights represent the partial contribution of PFRs in the mixture on health outcomes; (C)shows the results of Bayesian kernel machine regression (BKMR). The cumulative effect of PFR mixture on health outcomes was estimated when all chemical components were at their 50th percentile. All models were adjusted for sex, age, annual household income, BMI, and environmental tobacco smoke. The concentrations of urinary PFR metabolites were specific gravity adjusted and natural log transformed. An asterisk is marked next to the significant result.

eosinophil counts (Fig. 1C). The univariate estimation of the exposure–response functions on the relationship between PFRs and health outcomes are shown in Fig. 2. When all other metabolites were at their median levels, ΣTPHP was positively associated with the possibilities of FeNO ≥ 35 ppb and eosinophil counts ≥ 300/μL. Supplementary Fig. S3 shows no interaction between the PFRs, allergic symptoms, and T2 biomarkers. A sensitivity analysis was conducted to test the robustness of the main findings. The random forest method was used to fill in all missing annual household income values. We assessed the extent to which filled data could affect the findings by removing all data containing missing values and found that the results remained consistent with the original findings (data not shown).

Table 3
Associations between allergic symptoms, T2 biomarkers and organophosphate flame retardant and plasticizer (PFRs) mixture in quantile g-computation.

Quantile g-computation	Adjusted OR (95% CI)	p-value	Sum of positive coefficient	Sum of negative coefficient
Wheeze	1.59 (0.91 , 2.80)	0.105	0.77	−0.26
Eczema	1.02 (0.77 , 1.34)	0.901	0.34	−0.31
Allergic rhinoconjunctivitis	1.27 (0.94 , 1.71)	0.121	0.50	−0.18
FeNO	**1.48 (1.18 , 1.86)**	**0.001**	**0.69**	**−0.09**
Total IgE	0.96 (0.81 , 1.14)	0.664	0.13	−0.19
Eosinophil	1.17 (0.96 , 1.43)	0.124	0.52	−0.25

The adjusted odds ratio are interpreted as the effect on allergic symptoms and type 2 inflammation biomarkers (T2) of increasing every one quantile unit of exposure of PFR mixture. The models were adjusted for sex, age, annual household income, BMI, and environmental tobacco smoke.
Abbreviations: odds ratio (OR), confidence interval (CI). Bold letters indicate statistical significance ($p < 0.05$).

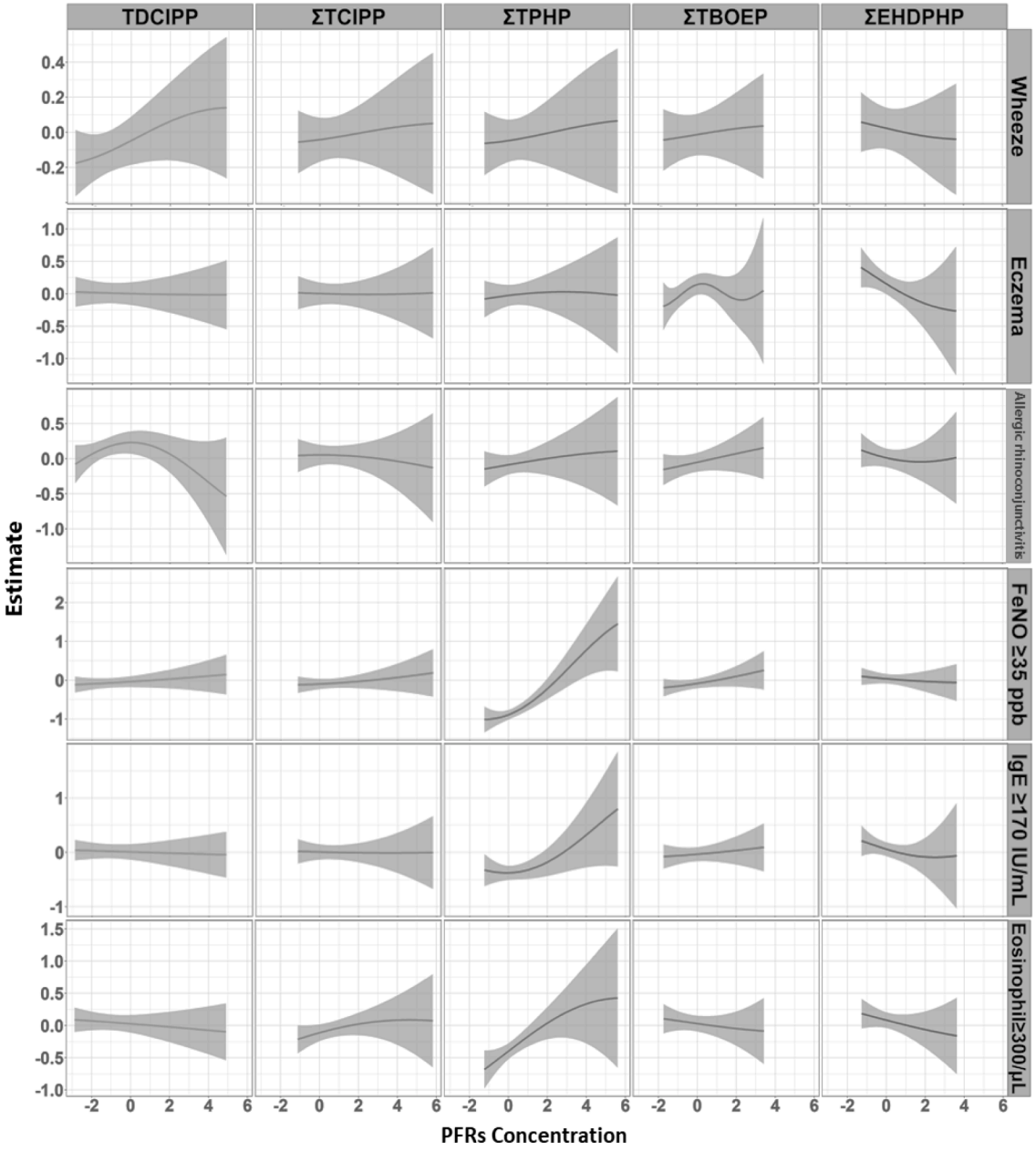

Fig. 2. Univariate exposure–response functions and 95 % credible intervals for the change in allergic symptoms, type 2 Inflammation (T2) biomarkers and urinary organophosphate flame retardants and plasticizers (PFRs) while holding all the remaining exposures in the mixture fixed at their median concentration, estimated using Bayesian kernel machine regression (BKMR). The models were adjusted for sex, age, annual household income, BMI, and environmental tobacco smoke.

4. Discussion

This study explored the associations of five individual PFRs and their mixture with allergic symptoms and T2 biomarkers using three statistical analytical methods. For individual PFRs, positive associations were observed between TDCIPP and wheeze; TDCIPP, ΣTPHP, ΣTBOEP, and FeNO $\geq$ 35 ppb; ΣTPHP and peripheral blood eosinophil counts $\geq$ 300 /μL. The exposure to a higher concentration of PFR mixture was positively associated with higher odds of having FeNO and the eosinophil counts exceeding the cut-off level, and ΣTPHP exhibited the highest contribution to the increase in T2 biomarkers. Marginal positive associations were observed with PFR levels and the occurrence of allergic rhinoconjunctivitis, and a serum total IgE $\geq$ 170 IU/mL in the individual analyses, although the results were not statistically significant. Using mixture analyses, which consider multiple exposures that exhibit multicollinearity or high correlation, can potentially reduce bias compared to single exposure analyses in determining health effects. Despite the different statistical approaches to mixtures, our findings from the qg-computation and BKMR models were generally in line with the multiple regression analysis results, verifying the robustness of our findings.

The exposure levels of PFRs in the current data are found to be comparable to those reported in our previous studies among 7-year-old and school-aged Japanese children (Araki et al., 2018, Ait Bamai et al., 2019) and in other countries, such as China, Belgium, Germany (Chen et al., 2018, Bastiaensen et al., 2021a, Van der Schyff et al., 2023). Positive associations were observed between TDCIPP and wheeze in school children aged 9–12. This result aligns with that of previous studies examining the association between PFRs and allergic diseases. Our previous research within the Hokkaido Study reported that TDCIPP in house dust is associated with increased odds of wheeze among 7-year-old children (Ait Bamai et al., 2018). Another one of our studies on a different population of Sapporo City focused on house dust and urinary metabolites of elementary school children. Here, we found that higher levels of TDCIPP in house dust increased the odds of eczema. For urinary results, TCIPP was positively associated with allergic rhinoconjunctivitis and at least one symptom of allergy; TBOEP increased the odds of eczema; and BDCIPP increased the odds of at least one symptom of allergy (Araki et al., 2018). A recent study that examined 9 PFR metabolites of school children with asthma in the United States, found that higher levels of urinary DPHP were positively associated with at least one of the daytime symptoms of asthma (Louis et al., 2023). These results indicate that PFR exposure is positively associated with allergic symptoms among children in both the general and asthmatic population.

This study observed a significant positive association between the mixture of urinary PFRs and FeNO $\geq$ 35 ppb, while the partial effects were the highest for ΣTPHP. Many studies have found that exposure to phthalates, bisphenols, hazardous metals, and parabens can increase FeNO levels in the exhaled breath (Kim et al., 2018, Mamuya et al., 2018, Junge et al., 2022, Sung et al., 2022, Wu et al., 2022). Besides the abovementioned environmental contaminants, our results provide epidemiological evidence that PFRs are associated with higher FeNO levels.

Our study found a significant positive association between the ΣTPHP and high eosinophil counts, which aligns with experimental evidence. An *in vivo* study reported that the number of eosinophils, macrophages, and neutrophils in the bronchoalveolar lavage fluid (BAL) increased drastically in asthmatic mice after TNBP exposure (Meng et al., 2022). Another experimental study also reported increased eosinophils and lymphocytes in BAL of asthmatic mice after TBOEP exposure (Win-Shwe et al., 2022). These findings indicate that PFRs may cause airway inflammation and hyperresponsiveness by increasing the eosinophil counts. They may also have a potential exacerbating effect on existing airway inflammatory diseases, particularly in individuals with asthma. Future studies in asthmatic patients are needed to validate this hypothesis.

Humans are simultaneously exposed to multiple chemicals. Both phthalates and PFRs are used as additives in consumer products and are found in house dust (Kanazawa et al., 2010, Ait Bamai et al., 2014, Araki et al., 2014, Ballesteros-Gómez et al., 2015). An experimental study reported that mice that received ovalbumin with mono-n-butyl phthalate had higher eosinophil cell counts than those that received only ovalbumin (Quoc et al., 2022). We recently examined the combined exposure to PFRs and phthalates and their associations with allergic symptoms, in which PFRs contributed more to increasing wheeze and allergic symptoms than phthalates, even though the concentration of PFRs is much lower than that of phthalates (Araki et al., 2020). This evidence indicates the need to measure the combined exposure including other environmental chemicals, and its association with eosinophils, as well as other T2 biomarkers in future research.

4.1. Strengths and limitations

There are several strengths in our research. This is the first study investigating the association between urinary PFR metabolite mixtures and T2 biomarker levels in children. This research is part of the Hokkaido Study, a well-established birth cohort collecting detailed information on mother–child pairs, enabling continuous exposure assessment in future studies. The exposure assessment included 13 PFR metabolites using a validated biomonitoring method. We conducted qg-computation and BKMR analyses to explore the associations between the mixture of PFR exposure levels, three allergic symptoms, and three T2 biomarkers. In addition to the questionnaire susceptible to recall bias, we obtained the FeNO levels, peripheral blood eosinophil counts, and serum total immunoglobulin E level for T2 biomarkers, which are objective values used in managing allergies.

This study had some limitations. Firstly, it is important to note that, due to the cross-sectional design of this study, causal relationships between exposure and outcomes could not be demonstrated. Parents of children with allergic diseases often take extra precautions to reduce exposure to environmental contaminants, minimizing the likelihood of reverse causality where allergic symptoms lead to higher exposure to PFRs. Secondly, PFR metabolites were measured from a single spot urine sample, which may not accurately reflect exposure because of the short biological half-lives and frequent exposure. Nonetheless, the short-term reproducibility of PFR metabolite concentrations improved significantly when the values were corrected for SG (Bastiaensen et al., 2021b; Roggeman et al., 2022). Thirdly, mixture models alone do not provide information on toxicological measures such as toxicity thresholds or dose–response relationships of individual PFR. Fourthly, DPHP can be a metabolite of both TPHP and EHDPHP (Van den Eede et al., 2016), which raises the possibility of overestimating TPHP and underestimating EHDPHP. Fifthly, anti-inflammatory asthma medication histories were not included because very few children taking Inhaled Corticosteroid (n = 2) and other drugs (n = 2), possibly affecting Th2 biomarkers. Sixthly, the number of children with allergic symptoms was small in this study and may not have had enough statistical power to find significant associations. Future studies with larger sample size are needed to validate our findings. Lastly, we could not incorporate potential confounders, including household dampness and mold in this study.

5. Conclusions

Our study revealed positive associations between TDCIPP and wheeze, TDCIPP, ΣTPHP, ΣTBOEP and FeNO, ΣTPHP and peripheral eosinophil counts from individual analyses. Although not statistically significant, marginal positive associations were observed between PFR levels and allergic rhinoconjunctivitis, and total IgE. The PFR mixture was positively associated with FeNO and eosinophil counts above the cut-off levels. These findings suggest that exposure to PFRs increases the possibility of asthma and allergies by enhancing the expression of T2 biomarkers. Regulating PFR-containing products, particularly in

populations with higher exposure levels, may reduce the prevalence of asthma and allergies. Further investigation is needed to assess the combined exposure effects, including other environmental chemicals that have similar effects on allergic diseases, such as phthalates and bisphenols.

CRediT authorship contribution statement

Yi Zeng: Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Validation. **Houman Goudarzi:** Methodology, Investigation, Writing – review & editing. **Yu Ait Bamai:** Methodology, Investigation, Data curation, Writing – review & editing. **Rahel Mesfin Ketema:** Investigation, Writing – review & editing. **Maarten Roggeman:** Investigation, Writing – review & editing. **Fatima den Ouden:** Investigation, Writing – review & editing. **Celine Gys:** Investigation, Writing – review & editing. **Chihiro Miyashita:** Methodology, Investigation, Writing – review & editing. **Sachiko Ito:** Methodology, Investigation, Writing – review & editing. **Satoshi Konno:** Supervision, Methodology, Writing – review & editing. **Adrian Covaci:** Funding acquisition, Methodology, Investigation, Resources, Project administration, Supervision, Writing – review & editing. **Reiko Kishi:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing. **Atsuko Ikeda-Araki:** Conceptualization, Funding acquisition, Methodology, Investigation, Resources, Project administration, Supervision, Writing – review & editing, Validation.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108278.

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