



Effects of exposure to single and multiple parabens on asthma development in an experimental mouse model and a prospective cohort study



Kristin M. Junge^{a,k,1}, Lisa Buchenauer^{a,b,1}, Sandra Strunz^{a,b,1}, Bettina Seiwert^{c,1}, Loreen Thürmann^d, Ulrike E. Rolle-Kampczyk^e, Stefan Röder^a, Michael Borte^f, Wieland Kiess^{g,h}, Martin von Bergen^{e,i}, Jan C. Simon^b, Ana C. Zenclussen^a, Torsten Schöneberg^j, Gabriele I. Stangl^k, Gunda Herberth^a, Irina Lehmann^{d,1}, Thorsten Reemtsma^{c,1}, Tobias Polte^{a,b,*,1}

^a Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Immunology, Leipzig, Germany

^b Department of Dermatology, Venerology and Allergology, Leipzig University Medical Center, University of Leipzig, Leipzig, Germany

^c Helmholtz Centre for Environmental Research - UFZ, Department for Analytical Chemistry, Leipzig, Germany

^d Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Molecular Epidemiology, Berlin, Germany

^e Helmholtz Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany

^f Children's Hospital, Municipal Hospital "St. Georg", Leipzig, Germany

^g University of Leipzig, Hospital for Children and Adolescents - Centre for Pediatric Research, Leipzig, Germany

^h University of Leipzig, LIFE - Leipzig Research Centre for Civilization Diseases, Leipzig, Germany

ⁱ University of Leipzig, Faculty of Life Sciences, Institute of Biochemistry, Leipzig, Germany

^j University of Leipzig, Medical Faculty, Rudolf Schönheimer Institute of Biochemistry, Leipzig, Germany

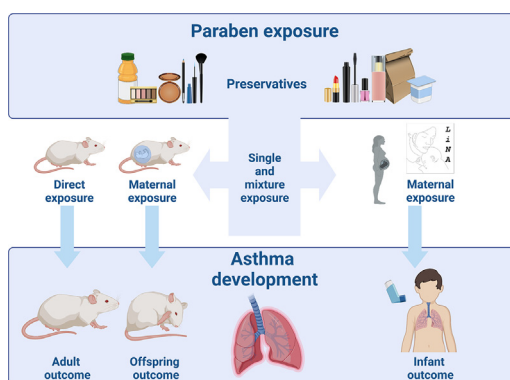
^k Institute of Agriculture and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

¹ German Center for Lung Research (DZL), associated partner, Berlin, Germany

HIGHLIGHTS

- Effects of single and multiple parabens on asthma were assessed in mice and humans.
- Adverse and preventive effects of single parabens on asthma were found in mice.
- Paraben mixtures revealed no effects in mouse or in a mother-child cohort.
- Mixtures should be considered in evaluation of individual specific disease risks.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 12 November 2021

Received in revised form 15 December 2021

Accepted 21 December 2021

Available online 30 December 2021

ABSTRACT

Parabens are widely used preservatives present in consumer products like cosmetics and food. Although several epidemiological studies suggest that early-life exposure to parabens might alter the immune response and allergy risk in childhood, the evidence with respect to asthma is not clear. Therefore, we investigated the effect of paraben exposure on asthma development in mice and humans. Using a murine asthma model the experimental data show both, an

Abbreviations: AHR, airway hyperreactivity; BAL, bronchoalveolar lavage; BKMR, Bayesian kernel machine regression; CI, confidence interval; CON, control; EtP, ethyl paraben; FHA, family history of atopy; HDM, house dust mite; H&E, haematoxylin and eosin staining; iBuP, i-butyl paraben; IFN, Interferon; IgE, immunoglobulin E; IL, interleukin; MeP, methyl paraben; nBuP, n-butyl paraben; OR, odds ratio; PrP, propyl paraben; Th1/Th2, T helper cells type 1/2.

* Corresponding author at: UFZ – Helmholtz Centre for Environmental Research Leipzig-Halle, Germany.

E-mail address: tobias.polte@ufz.de (T. Polte).

¹ These authors contributed equally to this work.

Editor: Adrian Covaci

Keywords:

Paraben exposure
Asthma
Murine model
LINA cohort
Single and mixture effect
BKMR

asthma-reducing effect after direct exposure of adult mice to n-butyl paraben (nBuP) as well as an asthma-promoting effect after maternal exposure to ethyl paraben (EtP) in the female offspring. Interestingly, exposure of mice to a mixture of EtP and nBuP starting prenatally until the end of asthma induction in the adult offspring was without effect on allergic airway inflammation. In addition, parabens were determined within the German prospective mother-child cohort LINA and their single and mixture effect on asthma development in children within the first 10 years of life was estimated by logistic and Bayesian kernel machine regression (BKMR). Both approaches revealed no adverse effects of parabens on children's asthma development, neither when stratified for being at risk due to a positive family history of atopy nor when analysed separately for sex specificity. Therefore, we conclude that although single parabens might differentially impact asthma development, an adverse effect could not be seen in a multiple paraben exposure setting. Consequently, not only the time point of exposure but also multiple exposure scenarios to parabens should be considered in the evaluation of individuals' specific disease risk.

1. Introduction

Parabens are alkyl esters with antimicrobial activity that are extensively used as preservatives in consumer product like cosmetics and food (Halla et al., 2018) and can also be found in the human environment (Wei et al., 2021). A ubiquitous exposure to single parabens was described in several studies, however it seems to be even more realistic that individuals are facing a multiple exposure scenario (Berger et al., 2020).

Parabens are considered to have endocrine disrupting activity and are therefore indicated as chemicals of emerging concern that exert adverse human health effects. Exposure to these chemicals has been linked to breast cancer (Darbre et al., 2004), reproductive malfunctions (Nishihama et al., 2016), and childhood overweight development (Leppert et al., 2020). Furthermore, several studies associated the use of paraben-containing products with allergic immunoreaction, causing contact dermatitis (Yim et al., 2014) and an increasing risk for atopic eczema (Lowe et al., 2019; Mitsui-Iwama et al., 2019; Thurmman et al., 2021).

Interestingly, with regard to allergic asthma most of the epidemiological studies did not find a correlation between exposure to parabens and the prevalence for asthma (Lee-Sarwar et al., 2018; Spanier et al., 2014; Vindenes et al., 2021). One study showed a positive association of children's urinary paraben concentrations and their asthma-related emergency department visits between 6 and 19 years (Quiros-Alcala et al., 2019), while, in the same survey, no association was found between their paraben concentrations and asthma prevalence until the age of 18 years (Spanier et al., 2014). So far, there is only one study indicating a correlation between in-utero exposure to ethyl paraben (EtP) and an increased asthma risk in children, while other parabens or their sum were without effect (Vernet et al., 2017). Another study showed even decreased odds of probable asthma in association with propyl paraben (PrP) levels (Berger et al., 2018). Thus, although several epidemiological studies suggest that exposure to parabens has the potential to alter immune functions and the risk of allergic diseases in childhood, the association to asthma is not well studied and inconsistent.

In this context, data from animal experiments could provide additional information to the sparse epidemiological data but, so far, there are no experimental in vivo studies investigating the effect of paraben exposure on asthma to clarify the inhomogeneous picture. Therefore, the current study aims to characterize the impact of paraben exposure on asthma using an experimental mouse model and epidemiological analyses from a well-characterized mother-child cohort by outlining different exposure time points and exposure scenarios.

2. Material and methods

2.1. Animals

2.1.1. Mice

Female BALB/cByJ mice (6–8 weeks of age) were obtained from the Elevage Janvier Laboratory (Le Genest St Isle, France). Mice were bred and maintained in the animal facility at the University of Leipzig (Germany) under conventional conditions with 23 °C room temperature, 60% humidity, and 12 h day/night rhythm. All mice received phytoestrogen-free diet

(C1000 from Altromin, Lage, Germany) and water ad libitum. Experiments involving induction of allergic airway inflammation included groups of 4–6 mice/cage and were performed at least two times according to institutional and state guidelines. Animal protocols used in this study were approved by the Committee on Animal Welfare of Saxony/Leipzig (Permit Number: TVV01/15, 14/18).

2.1.2. Exposure protocols to parabens

To evaluate the effect of parabens on asthma development, we exposed Balb/c mice to a representative of a short-chain (ethyl paraben, EtP) as well as a long-chain (n-butyl paraben, nBuP) paraben using different exposure protocols in an established mouse asthma model (Jahreis et al., 2018; Petzold et al., 2014; Polte et al., 2015). Both parabens used were already shown to have the most disease-affecting impact compared to other parabens of a comparable chain length (Leppert et al., 2020; Thurmman et al., 2021; Vernet et al., 2017). Doses used in the mouse model were estimated from an acceptable daily intake in humans based on No Observed Adverse Effect Levels of 1000 mg kg⁻¹ bodyweight (EtP) and 2 mg kg⁻¹ bodyweight (nBuP), respectively (EFSA, 2004; Fisher et al., 1999).

First, 6 to 8-week-old female mice were directly exposed to EtP (10 mg kg⁻¹ bodyweight/day) and nBuP (20 µg kg⁻¹ bodyweight/day) starting one week prior house dust mite (HDM) sensitization until the end of the asthma protocol (Supplementary Fig. E1A). Application was performed by subcutaneous (s.c.) injection of 0.875 mg EtP or 1.75 µg nBuP (Sigma-Aldrich Chemie GmbH, Munich, Germany) in 100 µl corn oil twice per week. Control dams received only the vehicle. In a second protocol and to study the effects of maternal exposure on the progeny, mice were exposed to EtP or nBuP starting before mating until weaning when pups were 3 weeks old (perinatal exposure, Supplementary Fig. E1B). Third, and based on the results seen in the first and second protocol, the effect of a paraben mixture of EtP and nBuP was evaluated while mice were exposed to the parabens starting one week before mating until the end of the asthma protocol induced in the offspring at 6–8 weeks (Supplementary Fig. E1C). Each exposure protocol was performed at least two times with at least 3 dams per group/condition resulting in ≥ 7 pups per group/condition (each female or male, with a maximum of 3 pups per sex per dam).

2.1.3. Asthma induction

To induce an asthma-like phenotype with airway hyperresponsiveness, eosinophilic inflammation, elevated allergen-specific IgE levels and an increased production of the T helper 2 (Th2) cytokines IL-4, IL-5 and IL-13 Balb/c mice were sensitized via the airways with 25 µg house dust mite extract (HDM, endotoxin: 1.273 EU/ml, Greer Laboratories, USA) in 40 µl saline on day 1. This was followed by 5 µg HDM given intranasally (i.n.) on days 7 to 11 and 14 to 16. Control mice received normal saline i.n. Airway hyperreactivity (AHR) was measured on day 17 and mice were sacrificed on day 18 (Supplementary Fig. 1).

2.1.4. Measurement of airway responsiveness

To measure lung resistance (R_L) mice were anesthetized (100 mg kg⁻¹ ketamine and 10 mg kg⁻¹ xylazine, Bayer, Leverkusen, Germany), intubated, and mechanically ventilated at a tidal volume of 0.2 ml and a frequency of 150 breath/min. Baseline R_L and responses to aerosolized saline

(0.9% NaCl) were measured first, followed by responses to increasing doses (2.5 to 40 mg/ml) of aerosolized methacholine.

2.1.5. Collection of bronchoalveolar lavage (BAL) fluid

To assess eosinophilic inflammation in the BAL all cells within the lavage fluid were counted using a hemocytometer. Diffquick® (Medion Diagnostics AG, Dürdingen, CH) stained cytopins were differentiated into eosinophils, macrophages, lymphocytes and neutrophils according to morphological criteria as described previously (Bickert et al., 2009; Polte et al., 2008).

2.1.6. Lung histology

Left lung was fixed in 10% formalin and stained with Haematoxylin & Eosin (H&E, MERCK, Darmstadt, Germany) to analyze inflammatory infiltrates in the airways (Petzold et al., 2014; Polte et al., 2015).

2.1.7. HDM-specific IgE assay

HDM-specific IgE serum levels were measured by ELISA according to a standard protocol. Briefly, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated overnight with 25 µg/ml HDM. After washing and blocking plates, serum was added and incubated at 4 °C overnight. Subsequently, 96-well plates were washed and 100 µl of biotin-anti-mouse IgE (BioLegend, CA, USA) was added and incubated for 1 h. The wells were washed with PBS followed incubation with avidin-horse radish peroxidase (Biolegend) for 30 min. TMB substrate solution (100 µl) was added and incubated in the dark for 30 min. The OD was determined at 450 nm using a BioTek microplate reader (BioTek Instruments, Bad Friedrichshall, Germany).

2.1.8. Cytokine production

One day after airway function test splenocytes or mediastinal lymph node cells (1×10^7 cells/ml per well) were isolated and re-stimulated in vitro with 200 µg/ml OVA in culture medium (RPMI medium supplemented with 10% FBS, 100 U/ml Penicillin, 100 µg/ml Streptomycin). After three days of culture cytokines were measured in supernatant using DuoSet® ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.2. Human cohort

2.2.1. LINA study design and sample collection

To further address the paraben-asthma relationship with epidemiological data we used the German prospective mother-child cohort LINA (Lifestyle and environmental factors and their Influence on Newborns Allergy risk). LINA recruited 622 mothers (629 children) at 34 weeks of gestation between May 2006 and December 2008 in Leipzig, Germany (Hinz et al., 2010; Weisse et al., 2013). Mothers with severe immune or infectious diseases during pregnancy were excluded from the study. Standardised self-administered questionnaires were collected annually starting in pregnancy, assessing general information, personal lifestyle, housing and environmental conditions as well as family history of atopy (FHA) and disease state (see Supplementary Table E1).

Study participation was voluntary and written informed consent was obtained by all participants. The study was approved by the Ethics Committee of the University of Leipzig (file ref # 046-2006, 160-2008, 160b/2008, 144-10-31052010, 113-11-18042011, 206-12-02072012, #169/13-ff, #150/14-ff, 008/17-ek).

2.2.2. Analysis of urinary paraben concentrations in human samples

Urinary concentrations of eight paraben species were determined in 504 maternal urine samples of the 34th week of gestation. The samples were prepared and analysed as described and validated by Schlittenbauer et al. (2016). In brief, urine samples, quality controls, and solvent blanks were thawed at room temperature, vortexed, and centrifuged. Aliquots were spiked with internal standard solution and a deconjugation standard. Hydrolysis was achieved by adding enzyme buffer solution and incubation

in an ultrasonic bath. The enzyme was removed by centrifugation/filtration using Amicon® Ultra-0.5 filters. LC-MS analysis was performed on a UPLC™ system (ACQUITY I-Class, Waters Cooperation Milford, MA, USA) coupled to a triple quadrupole mass spectrometer (Xevo TQ-S, Waters Cooperation, Manchester, UK) equipped with an electrospray ionization (ESI) source. For each analyte (MeP, EtP, nPrP, iBuP, nBuP), a quantification (Q) and a confirmation (q) MRM transition was selected. Quality criteria for positive confirmation of peaks were (i) presence of both MRM transitions, (ii) a retention time within 0.03 min, and (iii) a relative ion ratio within 50 to 150% compared to a standard. The limits of quantification (LOQ) were 0.5 µg/l for MeP and 0.1 µg/l for the others parabens (EtP, nPrP, iBuP, nBuP). Levels below the LOQ were included in the model analyses as half of the detection limit. For 498 participants valid paraben concentrations above the detection limit were obtained, for 274 of them also information about asthma prevalence until the age of 10 years as well as confounding factors were available (cases and paraben concentrations used within asthma analyses are shown in Supplementary Table E2). For iPrP, sBuP and BzP more than 70% of samples had concentrations below the LOQ and, therefore, were excluded from further analysis. For paraben analysis of mouse samples 20 µl urine and 180 µl milliQ water was mixed and the same protocol as described above was applied.

2.2.3. Asthma prevalence of children

Respiratory health of children was assessed by questionnaires asking parents annually for physician diagnosed respiratory diseases like asthma, obstructive bronchitis, wheezing etc. From that information, the prevalence for the 10-year lifetime prevalence of asthma was used for the present regression and BKMR analyses. As controls, only children who had never experienced any respiratory diseases like asthma, obstructive bronchitis or wheezing within the first 10 years were included. All model analyses with respect to paraben exposure and asthma risk were performed in all children of the sub-cohort, sex-specific as well as in children at risk for atopic diseases due to a positive family history of atopy (at least one parent positive). Analyses could not be performed separately in children without a family history of atopy (FHA) due to low case numbers of asthma positive children in that group ($n = 4$).

2.3. Statistical analysis

Experimental data sets from in vivo mouse studies were processed and analysed in GraphPad PRISM 7.02 for windows (GraphPad Software, Inc.). Data were expressed as mean $\pm$ SEM and P values of less than 0.05 were considered significant by t -test and Wilcoxon-Mann-Whitney test. With respect to the epidemiological data, only cases with complete data on prenatal urine paraben concentration, asthma prevalence within the first 10 years of life and relevant confounding factors were included in the statistical analyses ($n = 274$). The Chi2-test (categorical parameters) or Mann-Whitney U test (continuous parameters) was used to compare the analysed sub-cohort ($n = 274$) until the age of 10 years with the total cohort ($n = 629$) according general study characteristics/confounders. Logistic regression and Bayesian kernel machine regression (BKMR) models were performed with STATISTICA for Windows, Version 13 (Statsoft Inc.) and R (version 3.6.1; R development Core Team) for the bkmr package (Bobb et al., 2018), each considering the following confounders: smoking during pregnancy (as maternal log urine cotinine levels), maternal age at delivery, sex of the child (except for the analyses stratified for sex), family history of atopy (except for the analyses stratified for FHA), number of siblings, parental school education (low/medium/high) and urine creatinine levels. BKMR was performed with 50,000 iterations according to Bobb et al. (2018) and Preston et al. (2020) while paraben concentrations were ln-transformed and z-scored. BKMR analyses with respect to the asthma risk within the first 10 years of life revealed the following aspects: (A) overall/cumulative effect (pictures the comparison when all of the mixture components/all parabens were fixed at their median value, with when all of the mixture components/all parabens are at a particular (same) percentile), (B) the univariate exposure-response function (which pictures the full

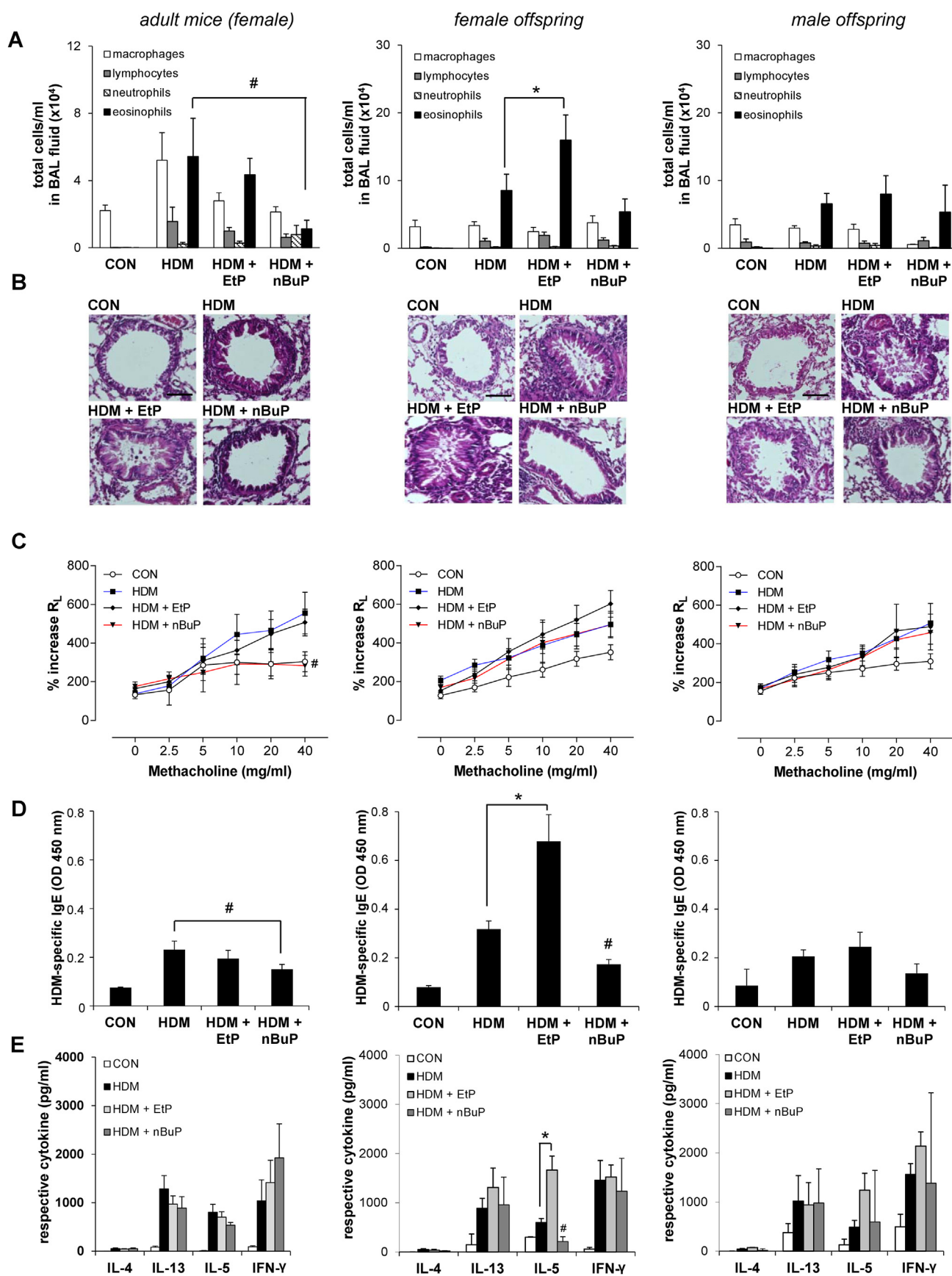


Fig. 1. Exposure to EtP or nBuP has different effects on allergic airway inflammation in a mouse asthma model. Cell number in BAL fluid (A), airway inflammation (B, H&E, $\times 100$), lung resistance (C), HDM-specific IgE (D), and cytokine (E) levels were examined in offspring from parabens-exposed dams or directly exposed adult mice. Mean $\pm$ SEM, $n \geq 7$ mice/group, $P < 0.05$, *HDM vs. EtP, #HDM vs. nBuP; BAL – bronchoalveolar lavage, HDM – house dust mite, CON – control, scale bar – 100 μ m.

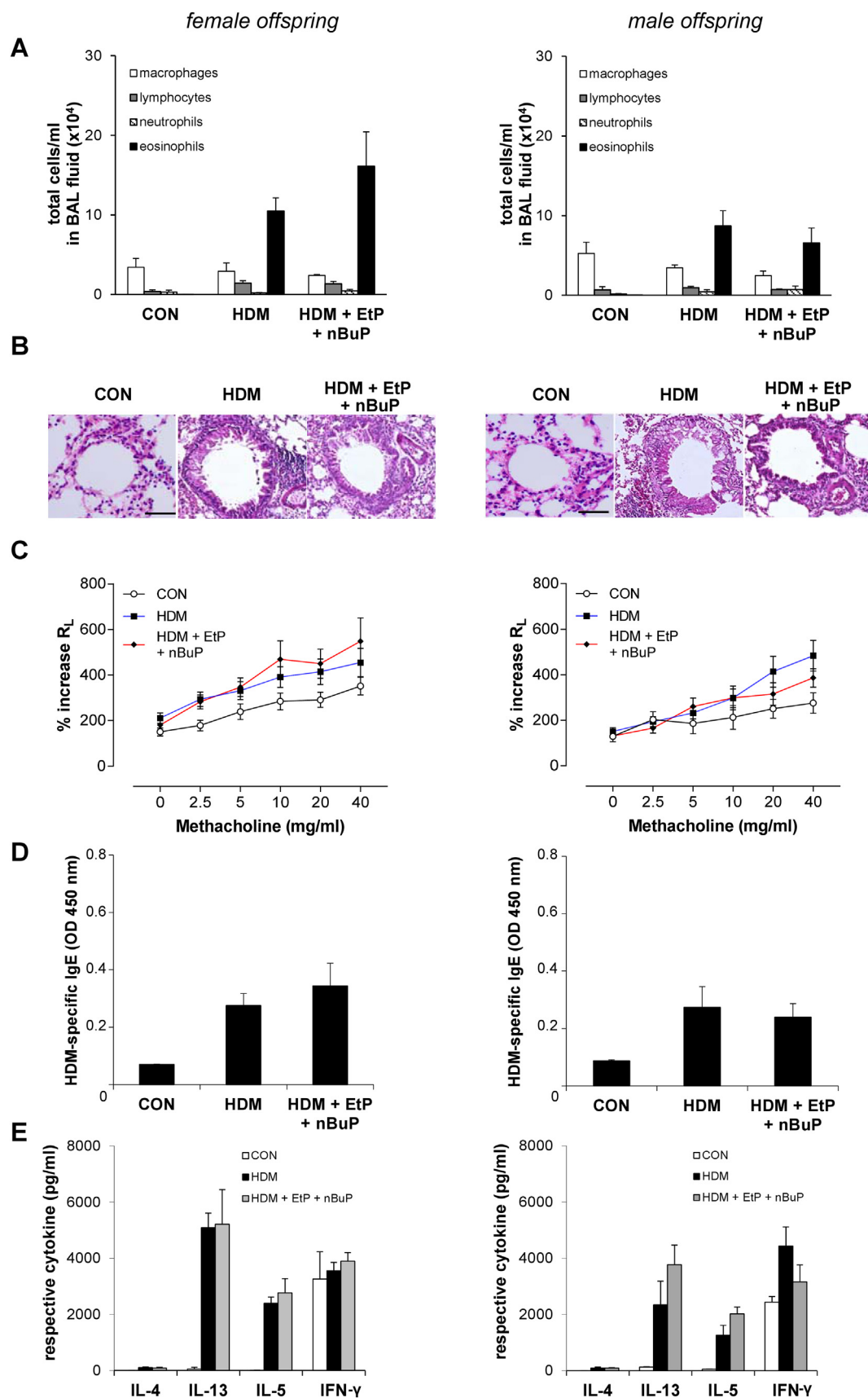


Fig. 2. Exposure to a mixture of EtP and nBuP has no effects on allergic airway inflammation in a mouse asthma model. Total cell number in BAL fluid (A), airway inflammation (B, H&E, $\times 100$), lung resistance (C), HDM-specific IgE (D), and cytokine (E) levels were examined in offspring from parabens-exposed dams. Mean $\pm$ SEM, $n \geq 7$ mice/group, scale bar - 100 μ m.

range of one mixture component/one paraben when simultaneously all other mixture components/all other parabens are held at their median concentrations) and (C) the interaction analyses, that pictures the difference in asthma risk when one mixture component/one paraben changes from its 25th to 75th percentile while all other mixture components/all other parabens are fixed at their 25th percentile, as compared to when all other mixture components/parabens are fixed at their 75th percentile.

3. Results

3.1. Effect of single exposure to EtP or nBuP on allergic asthma in a HDM-induced murine asthma model

First, adult exposure to nBuP diminished eosinophilic inflammation in the bronchoalveolar lavage (BAL) fluid (Fig. 1A) and airway inflammation in the lung as demonstrated by H&E-stained lung sections (Fig. 1B) and verified by an objective, investigator-independent computer analysis (Supplementary Fig. E2). Furthermore, nBuP exposure significantly decreased airway hyperreactivity (AHR, Fig. 1C) and HDM-specific IgE levels (Fig. 1D) and slightly reduced the production of the Th2 cytokines IL-13 and IL-5 (Fig. 1E), while exposure to EtP was without significant effect on the asthma phenotype (Fig. 1A–E).

Second, maternal paraben exposure on allergic airway inflammation in the progeny was assessed (Supplementary Fig. E1B). Thereby, exposed dams showed urine paraben levels in a comparable range to those detected in highly exposed mothers of our cohort (highest quintile urine concentration: EtP 18.1 µg/l, nBuP 5.9 µg/l; mean urine concentration in exposed mice: EtP 25.9 µg/l, nBuP 19.6 µg/l). Maternal exposure to EtP significantly increased the number of eosinophils within the BAL fluid in female offspring (Fig. 1A) and led to substantially more inflammatory infiltrates in the airways compared to female offspring from non-exposed dams (Fig. 1B). Furthermore, HDM-specific IgE levels were elevated in maternally exposed female offspring compared to control (Fig. 1D), while no significant effect on AHR was observed (Fig. 1C). Moreover, HDM-restimulated splenocytes from female offspring of EtP-exposed dams produced significantly more of the Th2 cytokine IL-5 and in trend of IL-13, while there was no significant effect on the Th1 cytokine IFN-γ (Fig. 1E). Interestingly, maternal exposure to nBuP had no significant effect on the asthma-like phenotype in female offspring (Fig. 1A–E). In addition, male offspring were not affected by any paraben (Fig. 1A–E). Taken together, within our mouse studies we observed varying effects of a single paraben exposure on asthma development depending on the applied paraben, sex and the time point of exposure.

3.2. Effect of exposure to a mixture of EtP and nBuP on allergic asthma in a murine asthma model

To further explore these contradictory effects of single EtP or nBuP at different time points, we exposed mice to a mixture of both parabens by also combining the exposure time points (Supplementary Fig. E1C). Interestingly, under these conditions we did not observe any effect of the paraben mixture on eosinophilic inflammation, AHR, antigen-specific IgE or Th2 cytokine levels in HDM-sensitized mice independent of the sex (Fig. 2A–E, Supplementary Fig. E3).

3.3. Association between prenatal exposure to single parabens and asthma development in children within the first 10 years

The epidemiological analysis was done in a subgroup of 274 LINA children with available maternal exposure data and disease outcome information until the age of 10 years. There were no differences in general characteristics comparing this sub-cohort compared to the total LINA cohort (Supplementary Table E1). Within the analysed sub-cohort, 26 children (9.5%) developed asthma within the first 10 years of life. Our logistic regression models - mimicking exposure to a single paraben - revealed neither effects in the analysed total sub-cohort nor when stratified

Table 1

Association of prenatal exposure to different parabens [µg/l] and asthma development within the first 10 years of life within the LINA cohort. Displayed are the effects for all children (A) and for those with a positive family history of atopy (FHA +; B), as well as separately for boys (C) and girls (D).

(A)								
All (n = 274)								
	Crude				Adjusted ^a			
	OR	95% CI		p-Value	OR	95% CI		p-Value
MeP	1.00	0.999	1.002	0.477	1.00	0.999	1.001	0.936
EtP	1.01	0.996	1.016	0.264	1.00	0.993	1.015	0.474
nPrP	1.00	0.999	1.005	0.289	1.00	0.997	1.004	0.649
iBuP	1.03	0.964	1.096	0.403	1.02	0.955	1.096	0.513
nBuP	1.00	0.970	1.023	0.768	0.99	0.970	1.020	0.679
(B)								
FHA + (n = 191)								
	Crude				Adjusted ^a			
	OR	95% CI		p-Value	OR	95% CI		p-Value
MeP	1.00	0.999	1.002	0.741	1.00	0.999	1.001	0.988
EtP	1.01	0.995	1.016	0.272	1.01	0.994	1.018	0.317
nPrP	1.00	0.997	1.004	0.615	1.00	0.997	1.004	0.772
iBuP	1.03	0.970	1.103	0.300	1.03	0.959	1.101	0.432
nBuP	1.00	0.974	1.021	0.809	1.00	0.974	1.020	0.784
(C)								
Boys (n = 134)								
	Crude				Adjusted ^a			
	OR	95% CI		p-Value	OR	95% CI		p-Value
MeP	1.00	0.999	1.002	0.335	1.00	0.999	1.002	0.693
EtP	1.01	0.996	1.018	0.219	1.01	0.995	1.019	0.281
nPrP	1.00	0.998	1.005	0.312	1.00	0.997	1.004	0.646
iBuP	1.04	0.974	1.109	0.242	1.05	0.975	1.134	0.191
nBuP	1.00	0.975	1.026	0.996	1.00	0.978	1.019	0.864
(D)								
Girls (n = 140)								
	Crude				Adjusted ^a			
	OR	95% CI		p-Value	OR	95% CI		p-Value
MeP	1.00	0.990	1.004	0.406	1.00	0.989	1.003	0.287
EtP	1.00	0.973	1.025	0.916	0.99	0.954	1.019	0.399
nPrP	1.00	0.990	1.010	0.978	0.99	0.983	1.007	0.387
iBuP	0.27	0.031	2.396	0.237	0.33	0.038	2.803	0.302
nBuP	0.96	0.844	1.081	0.468	0.95	0.843	1.077	0.435

CI – confidence interval, OR – odds ratio.

^a Logistic regression model adjusted for smoking during pregnancy (as urine cotinine levels), urine creatinine levels during pregnancy, number of siblings, maternal age at delivery, parental school education, family history of atopy (except B) and sex (except C and D). For specific case numbers and paraben concentrations see Supplementary Table E2.

for children being at risk due to a positive family history of atopy or when analysed separately for boys and girls (Table 1A–D). Corresponding paraben concentrations within these analysed groups are outlined in Supplementary Table E2.

3.4. Effects of multiple paraben exposure on asthma development in children within the first 10 years

Coherent with the murine experiments, we also considered a multiple exposure situation within the epidemiological data via Bayesian kernel machine regression (BKMR; (Bobb et al., 2018; Junge et al., 2021)). Interestingly, also the BKMR model did not outline an adverse effect of exposure to multiple parabens and the asthma risk in children (shown for the total sub-cohort/in children with FHA +, Fig. 3), and separately for both sexes

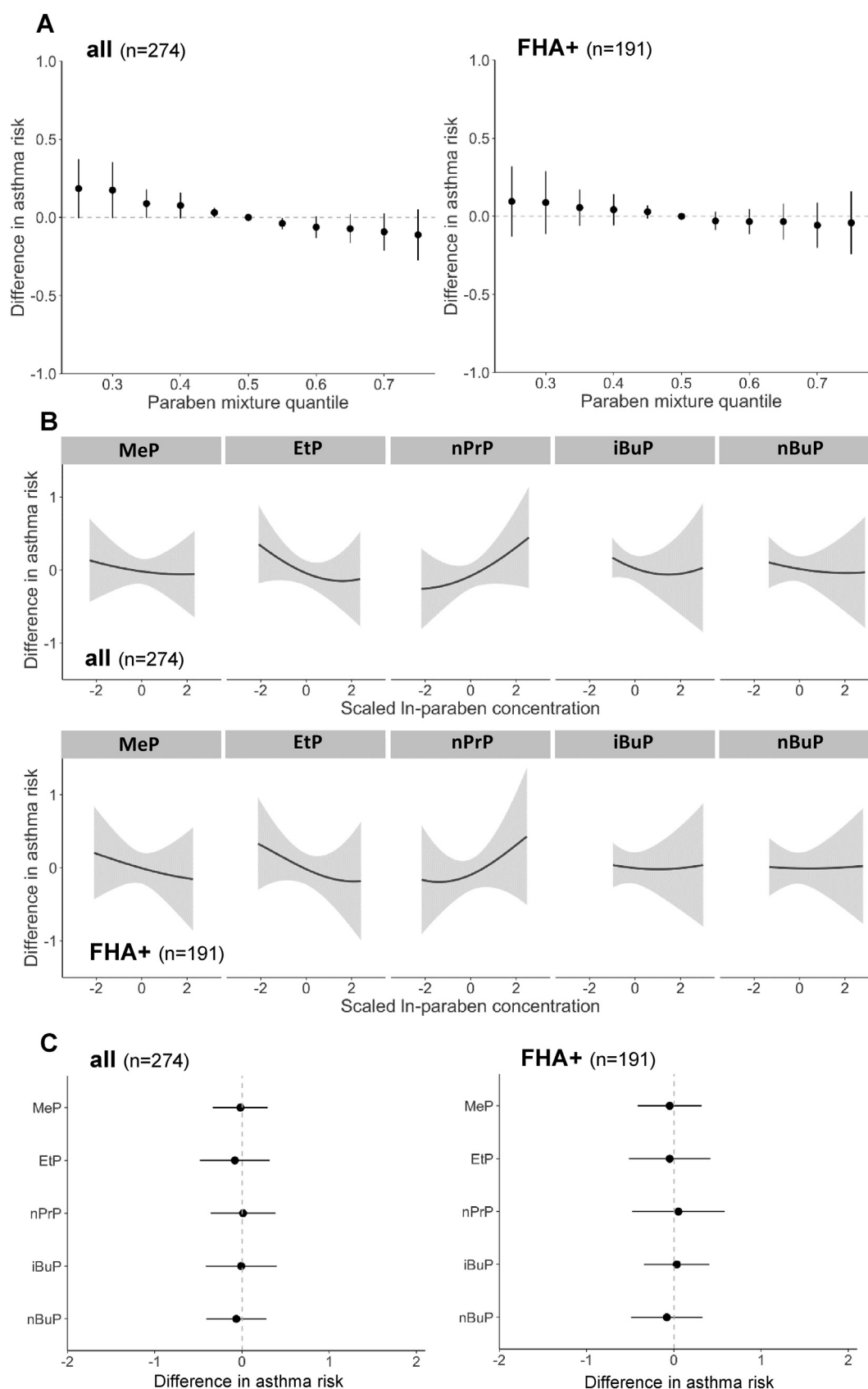


Fig. 3. Mixture analyses of prenatal paraben exposure and children's asthma risk within the first 10 years of life. Displayed are the effects for all children and for those with positive family history of atopy (FHA+) analysed by BKMR and adjusted for sex, urine cotinine during pregnancy, number of siblings, parental school education, maternal age at delivery, urine creatinine during pregnancy and FHA (except for the FHA+ sub-cohort). Shown are estimated differences in asthma risk and 95% credible intervals via (A) overall/cumulative analyses, (B) univariate exposure-response function, (C) interaction analyses.

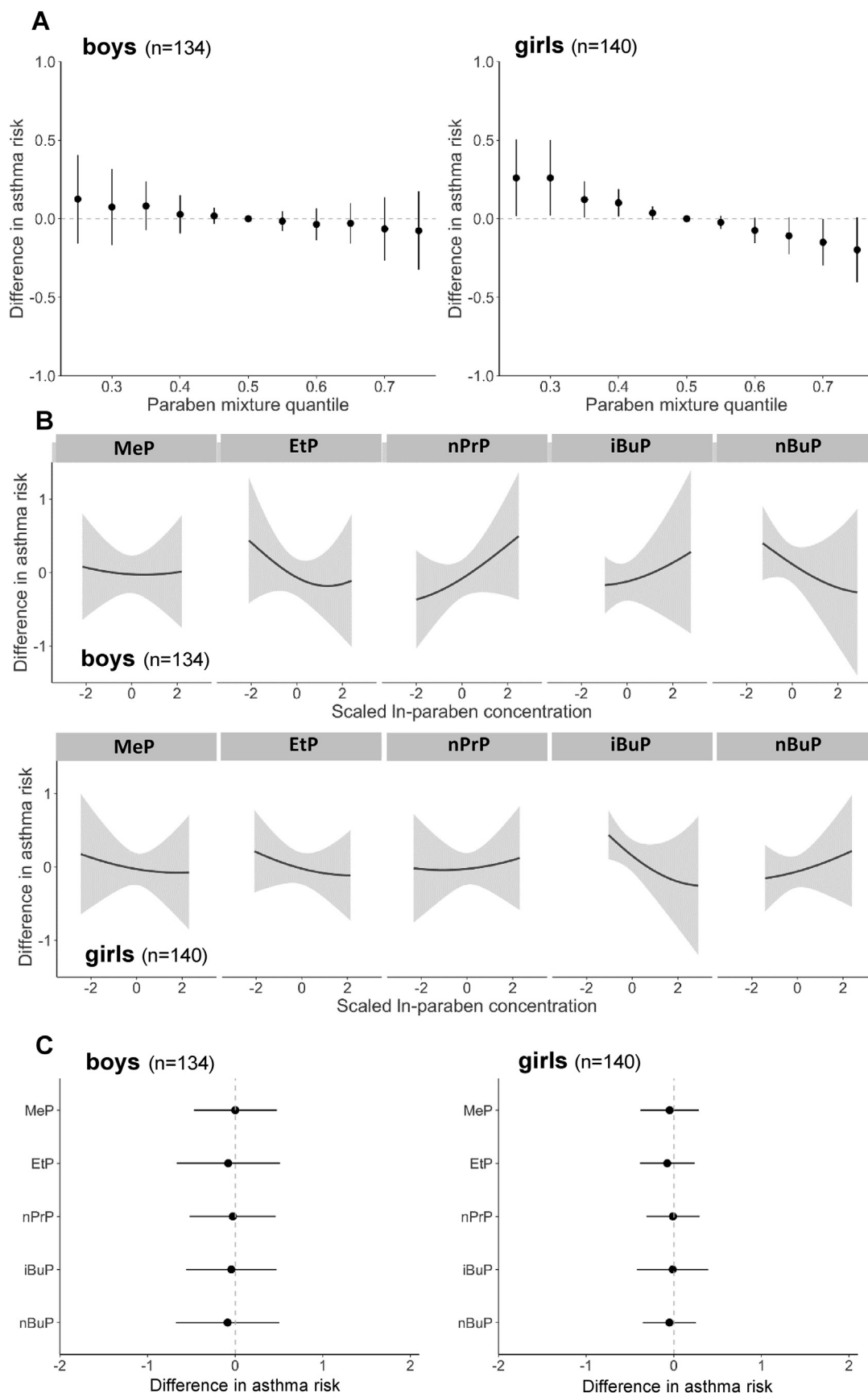


Fig. 4. Sex-specific analyses of prenatal paraben exposure and children's asthma risk within the first 10 years of life. Displayed are effects for boys/girls analysed by BKMR adjusted for urine cotinine during pregnancy, number of siblings, parental school education, and maternal age at delivery, urine creatinine during pregnancy, and FHA. Shown are estimated differences in asthma risk and 95% credible intervals via (A) overall/cumulative analyses, (B) univariate exposure-response function, (C) interaction analyses.

(Fig. 4). Furthermore, in girls this association was rather inverse when interpreting the cumulative simulation (Fig. 4A).

4. Discussion

Within this current study, experimental mouse and cohort data were assessed regarding single and multiple paraben exposure and asthma development which has not been performed earlier neither in a mouse nor in a translational setting. First, we investigated the impact of parabens in a mouse asthma model using different exposure protocols. Exposure of mice to EtP and nBuP that was estimated from an acceptable daily intake in humans based on NOAEL led to urine paraben levels in a comparable range to those detected in highly exposed mothers of our cohort. Still, it needs to be considered that humans are facing additional exposure routes (e.g. via ingestion or the lung) compared to the solely exposure via skin in our mouse model that could lead to differences in toxicokinetics (Prah et al., 2004).

Overall, our murine experimental data for single paraben exposure show both, an asthma-reducing effect after direct exposure of adult mice to nBuP as well as an asthma-promoting effect after maternal exposure to EtP in the female offspring. This effect was not observed in the male offspring demonstrating sex-specific health effects of parabens that we (Leppert et al., 2020) and others have shown previously for chemicals with endocrine disrupting activity (Chang et al., 2022; Palanza et al., 2021). The asthma-promoting effect in the offspring after maternal exposure to EtP was in contrast to most of the recent epidemiological studies that demonstrated no considerable correlation between any single paraben exposure and asthma risk (Lee-Sarwar et al., 2018; Spanier et al., 2014; Vindenes et al., 2021). However, one study showed a specific association between maternal single EtP levels and an increased asthma rate in 5-year-old children (Vernet et al., 2017) while no effects were seen for other parabens. Another study demonstrated a decreased asthma risk in association with PrP levels (Berger et al., 2018), similar to the result we observed for nBuP in our mouse asthma model. These contradictory findings of parabens might be explained by their chemical structure and different chain length. In this context, it was shown before that PrP and nBuP have a higher estrogenic activity in comparison to EtP (Routledge et al., 1998). However, the underlying mechanisms why parabens can induce different health effects have to be further explored.

Based on our observations we hypothesized that the diverse effects of EtP and nBuP might be different in a setting more comparable to the real life situation with multiple and long lasting or chronic exposure scenarios. It was outlined recently that it is of high importance also to consider a multiple exposure situation, since individuals are certainly exposed to a mixture of parabens in daily life (Huhn et al., 2021). In our experimental study exposing mice over a long period (dams and their progeny into adulthood) as well as to a mixture of both parabens showed no effect on the adult offspring asthma phenotype. This finding might suggest that in the mixed application EtP and nBuP “neutralize” each other at the biological effect level, in particular when exposure is long lasting.

With respect to our epidemiological data our findings were comparable to other cohort studies (Lee-Sarwar et al., 2018; Spanier et al., 2014; Vindenes et al., 2021): our logistic regression models – mimicking prenatal exposure to a single paraben - revealed neither effects in the analysed total sub-cohort nor when stratified for children being at risk due to a positive family history of atopy or when analysed separately for boys and girls. Furthermore, the associations in Leppert et al. (Leppert et al., 2020) showed that pregnant mothers indeed are facing a multiple paraben exposure. To address this multiple exposure scenario also in the current context, we have used a mixture approach within our epidemiological data. Bayesian kernel machine regression (BKMR) was applied for the LINA data as described earlier (Bobb et al., 2018; Junge et al., 2021) because classical regression models do not consider potential dynamic and kinetic interactions between several parabens (Bobb et al., 2018). This statistic modelling approach adds important information on just considering raw sum associations via logistic regression models (Vernet et al., 2017) by mimicking

different interacting mixture scenarios. Herein, paraben combinations did not indicate an adverse effect of prenatal paraben exposure on children's asthma risk. Moreover, in girls this association was rather inverse when interpreting the cumulative simulation. Still, this was based only on 10 female asthma cases and, therefore, should be deeper addressed in further studies. This potential mixture effect could explain why there is only little evidence from existing human epidemiological studies linking single paraben exposure and asthma risk. However, the exposure situation in cohorts can vary widely depending on the individual lifestyle but also on population- or country-specific habits (WHO, 2009) making it difficult to transfer our findings to different human environmental conditions. In addition, the recently shown adverse effect of paraben exposure on eczema risk (Thürmann et al., 2021) needs to be mentioned. Nevertheless, eczema occurs earlier in life than asthma with a potentially stronger impact of prenatal exposure. For asthma, the period after birth may play a more important role. A different or longer usage of paraben-containing products in older children may have caused different disease effects depending on the parabens exposed.

A general limitation of the LINA study is the potential bias by high rates of participating atopic parents who may have had special interest in the topic of the study. We have considered this point by always including the parental history of atopy as a confounding variable in the overall regression and BKMR models. In addition, the outcome diagnosis (asthma, as well as obstructive bronchitis or wheezing in controls) is a parental reported doctor's diagnosis that may carry some minor inaccuracies. With respect to the paraben analyses, it needs to be outlined that there are only one-time spot urine samples available in LINA. However, it was shown earlier within this cohort, that high maternal one-time urine concentrations of the analysed parabens were related to mothers usage of leave-on cosmetic products during the entire pregnancy (Leppert et al., 2020). This shows on one hand a potential source of the preservatives, and on the other hand that the measure of parabens at only one single time point could be a valid surrogate for an overall paraben exposure during pregnancy.

5. Conclusion

We investigated the effect of parabens on allergic airway inflammation after exposure at different time points using a mouse asthma model. We found an asthma-reducing effect after direct exposure of mice to nBuP and an asthma-promoting effect in the female offspring after maternal exposure to EtP. In our mother-child cohort LINA, we further examined single and mixture effects on asthma development in children within the first 10 years of life by logistic and Bayesian kernel machine regression. Both approaches revealed no adverse effects of parabens on children's asthma development. Interestingly, exposure of mice to a mixture of EtP and nBuP was without effect on allergic airway inflammation. To our best knowledge, this is the first study assessing the effects of paraben exposure using an experimental asthma model and in addition, combining this with BKMR to analyze joint effects of multiple parabens on children's asthma risk in a mother-child cohort. Our results clearly highlight the importance of the time point of exposure but also the role of mixtures that may not only implicate additive but also opposed effects, which should be considered in the evaluation of individuals' specific disease risk.

CRedit authorship contribution statement

Kristin Junge: Conceptualization, Methodology, Analysis, Writing - Original draft preparation, Visualization.

Lisa Buchenauer: Methodology, Analysis, Investigation, Visualization, Writing - Reviewing and Editing, Funding acquisition.

Sandra Strunz: Analysis, Investigation, Visualization, Writing-Reviewing and Editing.

Bettina Seiwert: Methodology, Analysis, Writing- Reviewing and Editing.

Loreen Thürmann: Analysis, Writing- Reviewing and Editing.

Ulrike Rolle-Kampczyk: Methodology, Writing- Reviewing and Editing.

Stefan Röder: Data curation, Writing- Reviewing and Editing.

Michale Borte: Resources, Writing- Reviewing and Editing.

Wieland Kiess: Resources, Writing- Reviewing and Editing.

Martin von Bergen: Methodology, Writing- Reviewing and Editing.

Jan Simon: Resources, Writing- Reviewing and Editing.

Ana Zenclussen: Resources, Writing- Reviewing and Editing.

Torsten Schöneberg: Supervision, Writing- Reviewing and Editing.

Gabriele Stangl: Supervision, Writing- Reviewing and Editing.

Gunda Herberth: Resources, Data curation, Writing- Reviewing and Editing.

Irina Lehmann: Resources, Data curation, Analysis, Writing- Reviewing and Editing.

Thorsten Reemtsma: Methodology, Analysis, Resources, Writing- Reviewing and Editing.

Tobias Polte: Conceptualization, Methodology, Analysis, Investigation, Writing- Original draft preparation, Visualization, Supervision, Funding acquisition.

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.

Acknowledgements

We thank Marita Wagner, Elena Elter, Coretta Bauer, and Melanie Bänsch for excellent technical assistance and all participants of the LINA study for contributing to the study over all the years. This work was supported by institutional funding from the Helmholtz Centre for Environmental Research – UFZ. L.B. received a PhD scholarship from the German Federal Environmental Foundation (DBU). The graphical abstract was created with BioRender.com.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.152676>.

References

- Berger, K., Eskenazi, B., Balmes, J., Holland, N., Calafat, A.M., Harley, K.G., 2018. Associations between prenatal maternal urinary concentrations of personal care product chemical biomarkers and childhood respiratory and allergic outcomes in the CHAMACOS study. *Environ. Int.* 121, 538–549.
- Berger, K., Coker, E., Rauch, S., Eskenazi, B., Balmes, J., Kogut, K., et al., 2020. Prenatal phthalate, paraben, and phenol exposure and childhood allergic and respiratory outcomes: evaluating exposure to chemical mixtures. *Sci. Total Environ.* 725, 138418.
- Bickert, T., Trujillo-Vargas, C.M., Duechs, M., Wohlleben, G., Polte, T., Hansen, G., et al., 2009. Probiotic *Escherichia coli* nissle 1917 suppresses allergen-induced Th2 responses in the airways. *Int. Arch. Allergy Immunol.* 149, 219–230.
- Bobb, J.F., Claus Henn, B., Valeri, L., Coull, B.A., 2018. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. *Environ. Health* 17, 67.
- Chang, C.H., Tsai, Y.A., Huang, Y.F., Tsai, M.S., Hou, J.W., Lin, C.L., et al., 2022. The sex-specific association of prenatal phthalate exposure with low birth weight and small for gestational age: a nationwide survey by the Taiwan maternal and infant cohort study (TMICS). *Sci. Total Environ.* 806, 151261.
- Darbre, P.D., Aljarrah, A., Miller, W.R., Coldham, N.G., Sauer, M.J., Pope, G.S., 2004. Concentrations of parabens in human breast tumours. *J. Appl. Toxicol.* 24, 5–13.
- EFSA, 2004. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the commission related to parahydroxybenzoates. *EFSA J.* 83, 1–26.

- Fisher, J.S., Turner, K.J., Brown, D., Sharpe, R.M., 1999. Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. *Environ. Health Perspect.* 107, 397–405.
- Halla, N., Fernandes, I.P., Heleno, S.A., Costa, P., Boucherit-Otmani, Z., Boucherit, K., et al., 2018. Cosmetics preservation: a review on present strategies. *Molecules* 23.
- Hinz, D., Simon, J.C., Maier-Simon, C., Milkova, L., Roder, S., Sack, U., et al., 2010. Reduced maternal regulatory T cell numbers and increased T helper type 2 cytokine production are associated with elevated levels of immunoglobulin E in cord blood. *Clin. Exp. Allergy* 40, 419–426.
- Huhn, S., Escher, B.I., Krauss, M., Scholz, S., Hackermuller, J., Altenburger, R., 2021. Unravelling the chemical exposome in cohort studies: routes explored and steps to become comprehensive. *Environ. Sci. Eur.* 33, 17.
- Jahreis, S., Trump, S., Bauer, M., Bauer, T., Thurmann, L., Feltens, R., et al., 2018. Maternal phthalate exposure promotes allergic airway inflammation over 2 generations through epigenetic modifications. *J. Allergy Clin. Immunol.* 141, 741–753.
- Junge, K.M., Buchenauer, L., Elter, E., Butter, K., Kohajda, T., Herberth, G., et al., 2021. Wood emissions and asthma development: results from an experimental mouse model and a prospective cohort study. *Environ. Int.* 151, 106449.
- Lee-Sarwar, K., Hauser, R., Calafat, A.M., Ye, X., O'Connor, G.T., Sandel, M., et al., 2018. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. *J. Allergy Clin. Immunol.* 142 (269–278), e15.
- Leppert, B., Strunz, S., Seiwert, B., Schlittenbauer, L., Schlichting, R., Pfeiffer, C., et al., 2020. Maternal paraben exposure triggers childhood overweight development. *Nat. Commun.* 11, 561.
- Lowe, A.J., Wang, X., Mueller, J.F., Abramson, M.J., Yeh, R.Y., Erbas, B., et al., 2019. Exposure to breast milk triclosan and parabens and eczema phenotypes at 12 months: a nested case-control study. *J. Allergy Clin. Immunol.* 144 (1136–1138), e6.
- Mitsui-Iwama, M., Yamamoto-Hanada, K., Fukutomi, Y., Hirota, R., Muto, G., Nakamura, T., et al., 2019. Exposure to paraben and triclosan and allergic diseases in Tokyo: a pilot cross-sectional study. *Asia Pac. Allergy* 9, e5.
- Nishihama, Y., Yoshinaga, J., Iida, A., Konishi, S., Imai, H., Yoneyama, M., et al., 2016. Association between paraben exposure and menstrual cycle in female university students in Japan. *Reprod. Toxicol.* 63, 107–113.
- Palanza, P., Paterlini, S., Brambilla, M.M., Ramundo, G., Caviola, G., Gioiosa, L., et al., 2021. Sex-biased impact of endocrine disrupting chemicals on behavioral development and vulnerability to disease: of mice and children. *Neurosci. Biobehav. Rev.* 121, 29–46.
- Petzold, S., Averbeck, M., Simon, J.C., Lehmann, I., Polte, T., 2014. Lifetime-dependent effects of bisphenol A on asthma development in an experimental mouse model. *PLoS One* 9, e100468.
- Polte, T., Hennig, C., Hansen, G., 2008. Allergy prevention starts before conception: maternofetal transfer of tolerance protects against the development of asthma. *J. Allergy Clin. Immunol.* 122 (1022–1030), e5.
- Polte, T., Petzold, S., Bertrand, J., Schutze, N., Hinz, D., Simon, J.C., et al., 2015a. Critical role for syndecan-4 in dendritic cell migration during development of allergic airway inflammation. *Nat. Commun.* 6, 7554.
- Prah, J., Ashley, D., Blount, B., Case, M., Leavens, T., Pleil, J., et al., 2004. Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers. *Toxicol. Sci.* 77, 195–205.
- Preston, E.V., Webster, T.F., Claus Henn, B., McClean, M.D., Gennings, C., Oken, E., et al., 2020. Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the project viva cohort: a mixtures approach. *Environ. Int.* 139, 105728.
- Quiros-Alcala, L., Hansel, N.N., McCormack, M.C., Matsui, E.C., 2019. Paraben exposures and asthma-related outcomes among children from the US general population. *J. Allergy Clin. Immunol.* 143 (948–956), e4.
- Routledge, E.J., Parker, J., Odum, J., Ashby, J., Sumpter, J.P., 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol.* 153, 12–19.
- Schlittenbauer, L., Seiwert, B., Reemtsma, T., 2016. Ultrasound-assisted hydrolysis of conjugated parabens in human urine and their determination by UPLC-MS/MS and UPLC-HRMS. *Anal. Bioanal. Chem.* 408, 1573–1583.
- Spanier, A.J., Fausnight, T., Camacho, T.F., Braun, J.M., 2014. The associations of triclosan and paraben exposure with allergen sensitization and wheeze in children. *Allergy Asthma Proc.* 35, 475–481.
- Thurmann, L., Herberth, G., Seiwert, B., Schlittenbauer, L., Rolle-Kampczyk, U., Roder, S., et al., 2021. Prenatal paraben exposure and atopic dermatitis-related outcomes among children. *Allergy* 76 (10), 3122–3132.
- Vernet, C., Pin, I., Giorgis-Allemand, L., Philippat, C., Benmerad, M., Quentin, J., et al., 2017. In utero exposure to select phenols and phthalates and respiratory health in five-year-old boys: a prospective study. *Environ. Health Perspect.* 125, 097006.
- Vindenes, H.K., Svanes, C., Lygre, S.H.L., Real, F.G., Ringel-Kulka, T., Bertelsen, R.J., 2021. Exposure to environmental phenols and parabens, and relation to body mass index, eczema and respiratory outcomes in the Norwegian RHINESSA study. *Environ. Health* 20, 81.
- Wei, F., Mortimer, M., Cheng, H., Sang, N., Guo, L.H., 2021. Parabens as chemicals of emerging concern in the environment and humans: a review. *Sci. Total Environ.* 778, 146150.
- Weisse, K., Winkler, S., Hirsche, F., Herberth, G., Hinz, D., Bauer, M., et al., 2013. Maternal and newborn vitamin D status and its impact on food allergy development in the german LINA cohort study. *Allergy* 68, 220–228.
- WHO, 2009. GLOBAL HEALTH RISKS: Mortality and burden of disease attributable to selected major risks. WHO Library Cataloguing-in-Publication Data.
- Yim, E., Baquerizo Nole, K.L., Tosti, A., 2014. Contact dermatitis caused by preservatives. *Dermatitis* 25, 215–231.