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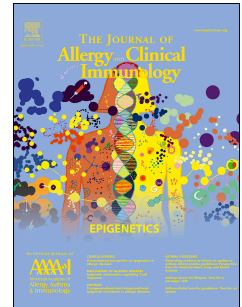
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Exposure to breast-milk triclosan and parabens and eczema phenotypes at 12-months: a nested case-control study

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of Nestlé Australia. The University of Melbourne provided funds through an internal grant scheme to allow the assessment of breast-milk parabens and triclosan for this manuscript. All bodies that have funded aspects of the MACS have had no role in interpretation and publication of study findings.

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Capsule summary

Levels of butyl-paraben in breastmilk at three months were associated with increased risk of atopic-eczema at 12 months of age, potentially due to altered gut microbiome.

Key words

Atopic-eczema

Breastmilk

Parabens

Triclosan

Conflict of interest statement

Michael Abramson holds investigator-initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has undertaken an unrelated consultancy for Sanofi. All other authors declare they have no conflicts of interest regarding this manuscript.

To the editor,

While breastfeeding has a range of health benefits, the association between breastfeeding and eczema reported between studies has been inconsistent¹. The variability in associations between breastfeeding and eczema may be due to breast-milk containing a wide range, and variable levels, of immunologically active substances, and potentially transmitting toxic substances from mother to infant, such as the antimicrobial agents triclosan and parabens². These agents are used in a range of foods and medications, but the primary source of exposure is through personal care products³. Due to ongoing consumer concerns with their safety, there are growing number of “paraben free” products becoming available. Exposure to these substances through breastmilk may inhibit the establishment of a healthy gut microbiome and impact on the child’s risk of developing allergic diseases. Alternatively, these compounds may promote proinflammatory responses, including production of thymic stromal lymphopoietin (TSLP), or induce epigenetic changes, such as altered methylation profiles⁴.

To assess if levels of breastmilk triclosan and parabens were associations with eczema and sensitisation outcomes, we conducted a nested case-control study within the Melbourne Atopy Cohort study, all of whom have a family history of asthma or allergic disease. We selected 60 children with a 3-month breastmilk sample (see Table S1 for demographic details), grouped based on their sensitisation and eczema status at 12 months. We selected 15 each of participants with: a) neither, b) atopy only (no eczema), c) non-atopic-eczema, or d) atopic-eczema (both eczema and sensitisation). Sensitisation was defined as a positive skin prick test (2mm or greater wheal) to one or more of six allergens. Eczema was defined by either a parent report of a doctor diagnosis of “eczema” or use of topical steroids for a rash excluding the nappy area. A gas chromatograph coupled with a tandem mass spectrometer was used to measure triclosan and

six parabens (See Table S2 and online supplement for further details). Due to small counts, exact logistic regression models were used, with “no atopy and no eczema” as the comparison group for all analyses. Associations were adjusted for the following factors if they had some association ($P < 0.1$) with the specific contaminant: parental age, history of smoking or eczema, maternal tertiary degree, socio-economic status based on father’s occupation, presence of older sibs and sex.

As the concentrations were above the limit of detection in 5% or less of samples, isobutyl-, isopropyl- and ethyl-paraben were excluded from the analyses (Table S3). Triclosan, methyl-, propyl- and butyl-paraben, were included, and concentrations of methyl- and propyl-paraben in breastmilk were weakly correlated (Table S4).

In the unadjusted analyses, detectable levels of methyl- and butyl-paraben in breast-milk were associated with increased risk of atopic eczema (Table 1). Adjustment for baseline factors reduced the strength of these associations, and only detectable levels of butyl-paraben were associated with increased risk of atopic eczema in the adjusted model (Table 2). Adjustment for maternal or paternal asthma or allergic rhinitis, or maternal atopy (defined as any positive [≥ 3 mm wheal] to 6 allergens tested on SPT during the first two years of child’s life), did not materially alter the association between of butyl-paraben and increased risk of atopic eczema. Similar associations were seen if atopic and non-atopic eczema groups were combined and compared to the non-eczema groups (Table S5).

Few studies have previously examined the associations between parabens or triclosan exposure and allergic disease outcomes. The results from the current study are difficult to compare directly to previous findings, as previous studies have used maternal urine levels, rather than breastmilk. While there are correlations between breastmilk, urine and even amniotic fluid levels

of parabens, these associations are not strong. Due to the short half-life (hours) of these compounds, levels will vary dramatically over time. Cross-sectional analyses in older children have supported associations between levels of triclosan, methyl-, propyl- and butyl-paraben in biological samples with increased risk of aero-allergen sensitization and asthma/wheeze^{5,6}, and increased risk of emergency department presentations for asthma (in boys)⁷.

Only two recently published longitudinal studies have been reported. Lee-Sarwar et al.⁸ found no overall associations between triclosan levels in maternal plasma during pregnancy and sensitisation or wheeze in children up to 3 years of age. However, boys whose mothers had higher paraben exposures possibly had increased risk of sensitization, a result not seen in girls. No eczema outcomes were examined in this study. Berger et al.⁹ observed that increasing levels of propyl-paraben in maternal urine collected during pregnancy were weakly associated with a reduction in probable asthma at age 7 years, while no associations were seen with aero-allergen sensitization, eczema or lung function outcomes. There was some evidence that higher levels of methyl-paraben were associated with reduction in both Th1 and Th2 cells (estimated using total CD4+ cells as the denominator) in whole blood⁹. Unfortunately, neither study reported associations with butyl-paraben due to 20-30% of samples being below the limit of detection^{8,9}. In fact, while other studies have measured levels of butyl-paraben, no previous study has reported on associations between butyl-paraben and with allergic disease outcomes, due to some participants having levels below the limit of detection^{8,9}.

Presence of parabens in breastmilk may be acting as a marker of another underlying cause of eczema in the child. Parabens are used as antimicrobial agents in some topical creams, including topical steroids used to treat eczema, and they are also used in medication suspensions (paracetamol and some antibiotics). Having a mother with active eczema or infection during

lactation may influence the risk of both having detectable levels of parabens in breastmilk and that the infant will develop eczema. While we adjusted for maternal history of ever having eczema, and this did not materially alter the association (addition or removal of maternal eczema shifted the OR from 10.6 to 12.5 with wide confidence intervals), we did not have data on active maternal eczema or infection during lactation. The concentrations of parabens and triclosan observed in breast-milk in this study were very low, and may not have been sufficient to induce alterations in the infant gut microbiome. It remains possible that their presence in breast-milk is a marker of other exposure routes (direct exposure of the infant to the products inducing the levels identified in the mothers breastmilk), or the effects seen here could be due to impacts on the immune response potentially via epigenetic changes⁴. We were also unable to assess associations with specific IgE or severity of eczema, as these data were not collected. In conclusion, while the observed association between presence of butyl-paraben in breastmilk and increased risk of atopic-eczema is plausible, these findings are preliminary due to the limited sample size and should be replicated in future studies.

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Statement of contribution

CJL and AJL conceived the study, and AJL obtained funding and wrote the first draft. XW and RYY undertook the laboratory assessments under the supervision of JFM. All authors revised the manuscript and approved the final version.

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References

1. Lodge CJ, Tan DJ, Lau MX, Dai X, Tham R, Lowe AJ, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatr* 2015; 104:38-53.
2. Hines EP, Mendola P, von Ehrenstein OS, Ye X, Calafat AM, Fenton SE. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. *Reprod Toxicol* 2015; 54:120-8.
3. Guo Y, Kannan K. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. *Environ Sci Technol* 2013; 47:14442-9.
4. Xin F, Susiarjo M, Bartolomei MS. Multigenerational and transgenerational effects of endocrine disrupting chemicals: A role for altered epigenetic regulation? *Semin Cell Dev Biol* 2015; 43:66-75.
5. Savage JH, Matsui EC, Wood RA, Keet CA. Urinary levels of triclosan and parabens are associated with aeroallergen and food sensitization. *J Allergy Clin Immunol* 2012; 130:453-60 e7.
6. Spanier AJ, Fausnight T, Camacho TF, Braun JM. The associations of triclosan and paraben exposure with allergen sensitization and wheeze in children. *Allergy Asthma Proc* 2014; 35:475-81.
7. Quiros-Alcala L, Hansel NN, McCormack MC, Matsui EC. Paraben exposures and asthma-related outcomes among children from the US general population. *J Allergy Clin Immunol* 2019; 143:948-56 e4.
8. Lee-Sarwar K, Hauser R, Calafat AM, Ye X, O'Connor GT, Sandel M, et al. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. *J Allergy Clin Immunol* 2018; 142:269-78 e15.
9. Berger K, Eskenazi B, Balmes J, Holland N, Calafat AM, Harley KG. Associations between prenatal maternal urinary concentrations of personal care product chemical biomarkers and childhood respiratory and allergic outcomes in the CHAMACOS study. *Environ Int* 2018; 121:538-49.

Table 1. Unadjusted associations* between triclosan or parabens and eczema and atopy outcomes at 1 year of age.

Molecule	N	No atopy & no eczema	Atopy (no eczema)		Non-atopic eczema		Atopic eczema	
		Proportion exposed	Proportion exposed	OR (95%CI)	Proportion exposed	OR (95%CI)	Prevalence	OR (95%CI)
Triclosan	60	46.7%	40.0%	0.77 (0.14-4.05)	40.0%	0.77 (0.14-4.05)	33.3%	0.58 (0.10-3.13)
Methyl-paraben	60	66.7%	46.7%	0.45 (0.08-2.38)	73.3%	1.36 (0.22-9.01)	100.0%	8.83 (1.07-∞)
Propyl-paraben	60	26.7%	33.3%	1.36 (0.22-9.00)	53.3%	3.02 (0.55-19.54)	46.7%	2.34 (0.42-15.02)
Butyl-paraben	60	0.0%	20.0%	4.32 (0.43-∞)	20.0%	4.32 (0.43-∞)	53.3%	19.73 (2.58-∞)

Estimates that are **bolded** have p<0.05

*Associations estimated using exact logistic regression. The no atopy or eczema group was used as the comparison group for all analyses.

Table 2. Adjusted associations* between triclosan or parabens and eczema and atopy outcomes at 1 year of age.

Molecule	N	No atopy & no eczema	Atopy (no eczema)	Non-atopic eczema	Atopic eczema
			aOR (95%CI)	aOR (95%CI)	aOR (95%CI)
Triclosan†	60	1	0.92 (0.14-5.94)	0.66 (0.09-4.07)	0.72 (0.13-3.98)
Methyl-paraben‡	60	1	0.55 (0.09-3.20)	1.02 (0.14-7.08)	6.07 (0.70-∞)
Propyl-paraben**	60	1	1.41 (0.22-9.59)	2.99 (0.53-20.31)	2.25 (0.40-14.23)
Butyl-paraben***	60	1	2.83 (0.26-∞)	3.72 (0.37-∞)	10.6 (1.36-∞)

Estimates that are **bolded** have p<0.05

*Associations estimated using exact logistic regression. The no atopy or eczema group was used as the comparison group for all analyses.

† Adjusted for maternal smoking, age and education level

‡ Adjusted for maternal and paternal eczema.

** Adjusted for maternal age

*** Adjusted for maternal and paternal smoking, older sibling(s) and maternal eczema

Online Repository material for:**Exposure to breast-milk triclosan and parabens and eczema phenotypes at 12-months: a nested case-control study**

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Participant recruitment and ethical approval

Expectant mothers were approached during antenatal care visits at the former Mercy Hospital for Women (East Melbourne, Australia) and other private consulting suites^{S1}. Children with a parent or older sibling with a history of allergic disease (asthma, eczema, allergic rhinitis or severe food allergy) were eligible for enrolment. Infants were born between 1990 and 1994. Parents provided informed consent, and the Mercy Hospital Human Ethics Committee granted ethical approval.

Outcome Definitions

Sensitisation was defined as a positive skin prick test (2mm or greater wheal) to one or more of six allergens^{S1}. A 2mm wheal was used, as in infants, the reactions on SPT are generally smaller. Eczema was defined by either a parent report of a doctor diagnosis of “eczema” or use of topical steroids for a rash excluding the nappy area^{S1}.

Analytical method:

Triclosan and parabens. One mL of breast milk samples was thawed at room temperature and spiked with 50 µL of internal standard for quantification purposes. The samples were subsequently extracted by 2 mL of acetonitrile (ACN) and centrifuged at 4000 rpm at 4°C for 10 minutes. The supernatants were then transferred and filtered using a 0.2 µm PTFE filter. The filtrates were subsequently evaporated to near dryness and reconstituted into 10mL of MilliQ water at pH=4. Subsequently samples were going through solid phase extraction (SPE) using HLB cartridges (3 cc), which was preconditioned with 1 mL of ACN and 1 mL of MilliQ water at pH=4. Samples were then loaded on the cartridges followed by washing with 1 mL of 5% ACN in MilliQ water at pH=4 solution. Cartridges were then dried for 3 min under vacuum before eluted with 2 mL of ACN. The extracts were subsequently evaporated to ~25 µL and derivatised using 70 µL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMS). The samples were then evaporated under a gentle nitrogen stream to near dryness before reconstituted into 50 µL of iso-octane prior to instrument analysis. Samples were analyzed using a TRACE GC Ultra coupled to a TSQ Quantum XLS triple quadrupole mass spectrometer equipped with a TriPlus Autosampler.

Statistical methods and measurement of possible confounding factors

A number of factors were considered as potential confounding factors in the statistical models.

Socio-economic status, based on father's occupation, was coded using the ANU-3^{S2}.

Correlations between specific breastmilk contaminants were estimated using Spearman correlation coefficients to account for samples with non-detectable levels. Correlations with $p < 0.05$ were considered significant.

Supplementary references

- S1. Lowe AJ, Lodge CJ, Allen KJ, Abramson MJ, Matheson MC, Thomas PS, et al. Cohort Profile: Melbourne Atopy Cohort study (MACS). *Int J Epidemiol* 2017; 46:25-6.
- S2. McMillan J, Jones FL. The ANU3_2 scale: a revised occupational status scale for Australia. *Journal of Sociology* 2000; 36:64-80.

Table S1. Baseline characteristics of sample.

	Proportion (n/N)	Mean (sd) or median (IQR)	Range
Female sex	50.0% (30/60)		
First born child	50.0% (30/60)		
Maternal eczema ever	45.0% (27/60)		
Paternal eczema ever	28.3% (17/60)		
Paternal smoking (ever)	43.3% (26/60)		
Maternal smoking (ever)	35.0% (21/60)		
Maternal completion of tertiary education	71.7% (43/60)		
Father's occupation status (ANU-3 scale (S1))*		44.8 (21.1)	0-91.9
Maternal age in years*		31.2 (4.6)	23-40
Paternal age in years*		32.9 (5.3)	24-48
Weeks of breastfeeding**		46 (31-64)	10-104
Weeks of exclusive breastfeeding**		18 (11-22)	1-29

Abbreviations: SD = standard deviation, IQR = interquartile range

*mean (SD)

**median (IQR)

Table S2. List of target compounds and corresponding internal standards

Target compounds	Internal standard
Triclosan	$^{13}\text{C}_{12}$ -triclosan
Methyl-paraben	$^{13}\text{C}_6$ -methyl paraben
Ethyl-paraben	$^{13}\text{C}_6$ -ethyl paraben
Isopropyl-paraben	$^{13}\text{C}_6$ -propyl paraben
Propyl-paraben	$^{13}\text{C}_6$ -propyl paraben
Butyl-paraben	$^{13}\text{C}_6$ -propyl paraben
Isobutyl-paraben	$^{13}\text{C}_6$ -propyl paraben

Table S3. Distributions of molecules assessed in breastmilk and parameterisation of exposure.

	Tested	Limit of detection (ng/mL)	% detectable	Min-max
Triclosan	60	0.072	40.0%*	0.08-23.1
Parabens				
Methyl-paraben	60	0.030	71.7%*	0.06-17.8
Propyl-paraben	60	0.042	40.0%*	0.044-.47
Butyl-paraben	60	0.022	23.3%*	0.023-0.57
Isopropyl-paraben	60	0.49	1.7%†	4.80
Ethyl-paraben	60	1.6	1.7%†	1.96
Isobutyl-paraben	60	1.4	0%†	-

† Excluded from statistical analyses

*Analyzed as detected versus not detected

Table S4. Spearman correlation** matrix between concentrations of triclosan and assessed parabens†

	Triclosan	Methyl paraben	Propyl paraben	Butyl paraben
Triclosan	1			
Methyl-paraben	0.09 (-0.18, 0.36)	1		
Propyl-paraben	-0.06 (-0.26,0.19)	0.34 (0.09,0.58)	1	
Butyl-paraben	0.09 (-0.15, 0.34)	0.19 (-0.06,0.43)	0.05 (-0.19,0.31)	1

† Samples below the LOD were assigned the value of half of the LOD

*Correlations with $p < 0.05$ are bolded.

** 95% CIs estimated using bootstrap techniques.

Table S5. Unadjusted and adjusted between triclosan or parabens and eczema at 1 year of age (regardless of sensitisation).

	N	Proportion exposed		Crude	Adjusted
		No eczema (n=30)	Eczema (n=30)	OR (95%CI)	OR (95%CI)
Triclosan†	60	43.3%	36.7%	0.76 (0.27-2.13)	0.83 (0.27-2.55)
Methyl-paraben‡	60	56.7%	86.7%	4.97 (1.39-17.8)	3.69 (0.97-14.0)
Propyl-paraben**	60	33.3%	50.0%	2.00 (0.70-5.68)	1.94 (0.67-5.65)
Butyl-paraben***	60	10.0%	36.7%	5.21 (1.28-21.2)	11.76 (1.85-74.9)

Estimates that are **bolded** have $p < 0.05$

† Adjusted for maternal smoking, age and education level

‡ Adjusted for maternal and paternal eczema.

** Adjusted for maternal age

*** Adjusted for maternal and paternal smoking, older sibling(s) and maternal eczema