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Respiratory health in children and indoor exposure to (1,3)-ß-D-glucan, EPS mould components, and endotoxin

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ABSTRACT

Background

For a long time, exposure to mould and dampness derived microbial components was considered as risk factor for the development of respiratory diseases and symptoms. Some recent studies suggested that early childhood exposure to mould components such as (1,3)-ß-D-glucan and Extracellular Polysaccharides (EPS) may protect children from developing allergy.

Objective

We investigated the association of the exposure to (1,3)-ß-D-glucan, EPS as well as endotoxin on asthma and allergies in 6 year old children.

Methods

This investigation is the follow-up of an originally nested case-control study among three European birth cohorts. Children from two ongoing birth cohort studies performed in Germany (n=358) and one in The Netherlands (n=338) were selected. Levels of (1,3)-ß-D-glucan, EPS and endotoxin were measured in settled house dust sampled from children's mattress and living-room floor, when the children were on average 5 years old. At the age of 6 years, health outcome information was available for 678 children.

Results

In the two German subsets, domestic EPS and endotoxin exposure from children's mattresses was significantly negatively associated with physician-diagnosed asthma (OR (95% Confidence Interval per IQR increase), **0.60(0.39-0.92)** and **0.55(0.31-0.97)**, respectively). In addition, EPS exposure was inversely related to physician-diagnosed allergic rhinitis

(**0.50(0.31-0.81)**). For the Dutch population, no associations were observed between exposure to microbial agents and respiratory health outcomes.

Conclusion

We found inverse associations between domestic exposure to EPS and endotoxin from children's mattresses and doctor-diagnosed asthma and rhinitis in German but not in Dutch school children. The reasons for the differences between countries are not clear.

INTRODUCTION

The effect of visible mould and mould components in indoor environment on asthma and allergic diseases in children has been widely discussed in the recent years. Several studies have investigated the associations but the results were not conclusive.

Some studies have shown that visible mould in homes increases the risk of physician diagnosed asthma and wheezing in children (1-6). A birth cohort study in the U.S. concluded that one year old children of asthmatic and allergic mothers who were exposed to high levels of *Penicillium*, a common species of mould, were at significantly higher risk for wheeze and persistent cough (7). Another U.S. study showed that exposure to dust born *Aspergillus, Alternaria* and *Aureobasidium* at 3 months of age was associated with the development of physician diagnosed allergic rhinitis within the first 5 years of life (8).

Few studies measured bio-components of mould, such as (1,3)-ß-D-glucan and Extracellular Polysacchardies (EPS) as surrogates for mould exposure (3, 9). (1,3)-ß-D-glucan are non-allergenic water-insoluble structural cell wall components of most fungi. The biological active poly-glucose molecule may account for up to 60% of the weight of the fungal cell wall (10). However, (1,3)-ß-D-glucan are also part of the structure of plant materials, including pollen and cellulose, as well as soil bacteria; therefore, the level of mould exposure may be overestimated by using (1,3)-ß-D-glucan as a surrogate. Fungal Extracellular Polysaccharides (EPS) are stable carbohydrates secreted or shed during fungal growth and have antigenic specificity at the genus level. In contrast to the findings on visible mould and measured specific mould species, longitudinal studies showed that exposure to (1,3)-ß-D-glucan and EPS was inversely associated with wheezing symptoms and parental reported physician diagnosed asthma in children (3, 5, 11). In addition, one case-control study reported that

elevated levels of (1,3)-ß-D-glucan and EPS exposure from mattress dust were associated with a lower prevalence of allergic sensitisation in 2-4 year-old children (9). However, the mechanism of these inverse effects is not yet understood. Different ways of assessing mould exposure could explain the conflicting results. Haas et al. reported that visible mould growth was significantly correlated with the concentration of fungal spores (12). As opposed to the latter, a U.S. cohort study did not observe a correlation between (1,3)-ß-D-glucan exposure and visible mould (3, 5).

Early exposure to mould components compared with the exposure later in life also showed different impact on allergic health outcomes (13). The immune response of newborns is dominated by Th2 cells and a shift to Th1-mediated immune response takes place during early childhood. It has been hypothesized that exposure to (1,3)-ß-D-glucan and EPS may have a similar impact on the development of immune system of infants as early endotoxin exposure (3, 14, 15). Endotoxins are cell wall components of the outer membrane of gram-negative bacteria. They are ubiquitous and can be found in normal indoor environments as constituents of house dust. Exposure to endotoxin has been suggested to have strong immune-stimulatory properties (16, 17). In support of the 'hygiene hypothesis' (18, 19), previous studies showed that there is a lower prevalence of allergic sensitisation and physician diagnosed asthma in children who were exposed to higher levels of endotoxin at home (9, 11, 20). It was hypothesized that microbial products such as endotoxin could affect the development of children's immune system early in life and play a crucial role in the development of tolerance to allergens ubiquitous in natural surroundings (21, 22).

We investigated prospectively the associations between exposure to mould components and endotoxin in settled house dust on respiratory and allergic health outcomes in 6 year old children using the data from two German birth cohorts and one Dutch birth cohort. This study

is a continuation of the work that has been done within the AirAllerg study (9, 23). Earlier AirAllerg investigations were based on health outcomes measured *before* exposure assessment. However, in the present analysis, health outcomes out of the 6 year follow-up were now available *after* exposure assessment.

MATERIALS AND METHODS

Study design and study population

Three European birth cohort studies were included in this investigation, the German LISA Life-style Related Factors on the Immune System and the Development of Allergies in Childhood), GINI (German Infant Nutritional Intervention) studies and the Dutch PIAMA (Prevention and Incidence of Asthma and Mite Allergy) study. LISA is a population based birth cohort study. A total of 3097 neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases (24). A total of 5991 mothers and their newborns were recruited into the GINI study between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated in the interventional study arm of the GINI study investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life (25). All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated in the non-interventional arm. Detailed descriptions of the LISA and GINI studies were published elsewhere ((24) and (25), respectively). For the PIAMA study, a total of 4,146 pregnant women were recruited in 1996-1997 during their second trimester of pregnancy from a series of communities in the North, West, and Centre of The Netherlands. Non-allergic pregnant women were invited to participate in a "natural history" study arm. Pregnant women identified as allergic through the screening questionnaire were primarily allocated to an intervention arm with a random subset allocated to the natural history arm. The intervention involved the use of mite-impermeable mattress and pillow covers.

The three European birth cohorts described above were part of a collaborative nested case-control study (AirAllerg) within European birth cohorts (LISA, GINI and PIAMA) using the

data on allergic sensitisation that have been collected at age 4 in The Netherlands and at age 2 and 3 in Germany (see **figure 1** and **supplementary material 1**). The target population size was approximately 180 sensitized children and 180 not sensitized children as controls in each country. The controls were not matched by any criteria. Based on serum IgE determination, cases were defined as children who were sensitized to common aeroallergens. As it turned out the number of children sensitised to aeroallergens was not reached in Germany and the Netherlands, the cases were supplemented with children sensitized to food. Allergen panels differed between the cohorts, but specific IgE to egg white, milk, house dust mites, cat, tree and grass pollens were measured in all cohorts. Families should not have moved 6 months prior to the AirAllerg house dust samplings. However, in Germany it was not possible to strictly follow this criterion; only 76% of the German participants fulfilled the criterion of not moving home. For the current investigation, 317 sensitized and 379 non-sensitized children were selected from the GINI, LISA and PIAMA birth cohort studies. At the age of 6 years, health end point data is available from 346 and 332 of the German and Dutch participants of the AirAllerg study.

Questionnaire data

In the German and Dutch population, information on respiratory and allergic disorders, as well as history of moving home, and visible mould in the child's home was collected at age 6, using self-administered questionnaires. An online supplement is provided to display the exact health outcome definitions within the 6 year follow-up of both subsets (see **supplementary material 2**). Information on parental educational level, family history of allergic diseases, smoking during pregnancy, and breast feeding were collected using self-administered questionnaires during the first year of life.

Dust collection

Between January 2002 and May 2003, trained fieldworkers collected house dust samples during home visits when the study children were on average 5 (LISA and PIAMA) and 6 (GINI) years old. A detailed description of the analysis and collection of the house dust samples was provided elsewhere (23). In brief, dust sampling was conducted using a common standard operation procedure of the AirAllerg study in the cool seasons. During the home visit, two settled house dust samples from the child's mattress and the living-room floor were collected by vacuuming. After dust sampling, the filters and the dust were stored at -20°C until extraction.

Dust extraction and analysis

Dust, including filters, was extracted sequentially as described previously (14). The first supernatant was used to measure endotoxin by a chromogenic kinetic Limulus Amoebocyte Lysate test (26). The second supernatant was used to measure EPS of *Aspergillus* and *Penicillium spp*. by a sandwich enzyme immunoassay (27). (1,3)-ß-D-glucan was measured in the third supernatant with a (1,3)-ß-D-glucan-specific inhibition enzyme immunoassay (28). The detection limits of the assay were 0.05 EU/ml, 3.3 µg/ml and 0.9 EPSU/ml for endotoxin, (1,3)-ß-D-glucan and EPS of *Aspergillus* and *Penicillium spp*., respectively. Exposures were expressed as both per g of sampled dust (concentration) and per m^2 of sampling surface area (load). Samples of (1,3)-ß-D-glucan and EPS lower than the detection limits were assigned a value of two-thirds of the respective detection limit (11).

Statistical analysis

Distributions of the biocontaminant levels in house dust samples were highly skewed and therefore described using median and quartiles. Spearman's rank correlation coefficient was used to calculate the correlations. The skewed variables were log transformed for further

analysis. Generalized additive models using local regression smoothing operation were fitted to assess the relationship of the associations between continuous indoor bio-contaminants exposure and the logit of the binary health outcomes. Since most associations were linear, all exposure variables were used as continuous variables without transformation in further analyses.

Logistic regression models were used to determine associations between microbial exposure from children's mattresses and living room floors and allergic health outcomes. The confounders we adjusted for in logistic regression models, were selected based on previous literature. For the German subset, confounders included in all models were sex, parental allergy, parental education, current pet ownership, breast feeding, case-control status in the AirAllerg study and season of dust sampling. Total amount of dust and endotoxin was additionally adjusted for current domestic exposure to environmental tobacco smoke (ETS). Visible mould exposure was adjusted for sex, parental allergy, parental education, outdoor activity in summer, breastfeeding, maternal smoking during pregnancy, study type and case or control status. Within the Dutch subset, confounders included in all models were sex, parental allergy, parental education, current domestic exposure to environmental tobacco smoke (ETS), current pet ownership, breast feeding, AirAllerg case-control status and season of dust-sampling. Since the AirAllerg study is not a population based sample and selected based on sensitisation status, we adjusted for case-control status in order to avoid bias. Being a case or a control within the study population not only affect the health outcomes in terms of allergic diseases and symptoms, but also the exposure and is therefore a confounder which we took into account for the current investigation.

The results are presented as odds ratios with 95% CIs for an interquartile range increase in microbial exposure. We focused on exposure from children's mattresses due to a considerable

amount of non-detectable values out from living-room floor dust samples. The analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Study population

A total number of 346 German and 332 Dutch children with information on domestic microbial exposure and respiratory and allergic health outcomes were included in the analysis. Baseline characteristics and health outcomes assessed at the age of 6 years are presented in **table 1**. There are some significant differences between the German and the Dutch subset. A higher percentage of the Dutch children were exposed to visible mould and reported of having a pet at home compared to the German cohort. A considerable amount of the Dutch but only a small number of the German children have visited a day care within the first year of life. The prevalence of physician-diagnosed respiratory infections in the past 12 months at the age of 6 years was five times higher among the German compared to the Dutch population. The German children reported more often physician-diagnosed allergic rhinitis and rhinoconjunctivitis whereas the Dutch children showed a higher prevalence of nocturnal dry cough. The seasons of dust sampling differed considerably between Germany and The Netherlands.

Table 1: Description of the German and Dutch AirAllerg study population at age 6

	LISA & GINI	PIAMA	p-value
Subjects n	**358**	**332**	
Cohort type			
LISA	138 (39%)		
GINI	220 (61%)		
Male sex	204/358 (57%)	186/332 (56%)	0.859
Parental allergy[1]	294/358 (82%)	260/332 (78%)	0.246
Parental education[2]			
High	198/358 (55%)	193/332 (56%)	0.502
Medium	106/358 (30%)	110/332 (33%)	0.360
Low	54/358 (15%)	29/332 (9%)	**0.014**
Visible mould in any room (6 years)	56/323 (17%)	108/329 (33%)	**< 0.001**
Dwelling considered as damp (6 years)	10/339 (3%)	Not available	Not available
Any Pets in child's home (6 years)	86/345 (25%)	133/326 (41%)	**< 0.001**
Day-care attendance			
1st year	5/318 (1%)	83/330 (25%)	**< 0.001**
2nd year	29/306 (8%)	85/323 (26%)	**< 0.001**
3rd year	68/319 (19%)	133/326 (41%)	**< 0.001**
4th year	257/324 (72%)	244/324 (75%)	0.260
Breastfeeding[3]	179/333 (54%)	201/328 (61%)	0.060
Smoking in child's home (6 years)	72/344 (21%)	89/331 (27%)	0.084
Maternal smoking during pregnancy	50/357 (14%)	45/328 (14%)	0.985
Moving home[4] (6 years)	39/346 (11%)	13/330 (4%)	**< 0.001**
Physician-diagnosed asthma[5]	17/343 (5%)	27/328 (8%)	0.119
Physician-diagnosed allergic rhinitis[5,6]	47/342 (14%)	24/327 (7%)	**0.010**
Allergic respiratory symptoms[5]			
Rhinoconjunctivitis	48/343 (14%)	28/327 (7%)	**0.036**
Wheezing	43/341 (13%)	48/331 (15%)	0.546
Dry cough (PIAMA: Nocturnal dry cough)	56/343 (16%)	80/330 (24%)	**0.014**
Physician-diagnosed infections (upper airways)[5]	275/342 (80%)	47/329 (14%)	**< 0.001**
Dust sampling season[7]			
Autumn	48/358 (13%)	101/332 (30%)	**< 0.001**
Winter	57/358 (16%)	113/332 (34%)	**< 0.001**
Spring	253/358 (71%)	118/332 (36%)	**< 0.001**

Data are presented as n (%) N

[1] GINI&LISA: defined as asthma and/or hay fever and/or eczema (at least one parent)

PIAMA: defined as asthma and/or allergy to house dust (mite) or pets, and/or hay fever in at least one parent

[2] GINI&LISA: categorised according to the German educational system as less than, equal to and more than grad 10 for low, medium and high, respectively.

PIAMA: defined as the highest attained educational level of mother and father; low: primary school, lower vocational or lower secondary education; medium: intermediate vocational education or intermediate/ higher secondary education; and high: higher vocational education and university

[3] GINI&LISA: defined as exclusive breastfeeding during the first 4 months of life

PIAMA: defined as any breastfeeding at the age of three months

[4] GINI: moving home in the last 24 months, LISA and PIAMA: moving home in the last 12 months

[5] in the past 12 months

[6] GINI&LISA: defined as hay-fever and/or allergic rhinitis (all-season), PIAMA: defined as hay fever ever

Amount of dust sampled, (1,3)-ß-D-glucan, EPS and endotoxin levels

The number of samples below the limit of detection (LOD), the median and interquartile range of total amount of dust, mould components and endotoxin measured from domestic dust samples are presented in **Table 2**. Wilcoxon-Tests showed significant differences in biocontaminant levels measured between the cohorts. Endotoxin and (1,3)-ß-D-glucan loads as well as (1,3)-ß-D-glucan concentrations from children's mattresses in Germany were significantly higher compared to the Dutch sample. There were weak correlations between the biocontaminant levels from children's mattresses and living-room floor for both, surface load and per gram of dust (GINI&LISA: < 0.25, PIAMA: < 0.13, Spearman Correlation Coefficient). The correlations between (1,3)-ß-D-glucan, EPS and endotoxin from mattress dust samples are weak when expressed as units per gram of collected dust; however, the correlations are stronger when defined as surface loads (**table 3**).

Table 2: Biocontaminant levels measured from children's mattress and living-room floor

	GINI&LISA	PIAMA	
	Median[1]		Wilcoxon test p-value
Subjects n	**358**[2] n<LOD / median (25^{th}-75^{th} percentile)	**332**[2] n<LOD / median (25^{th}-75^{th} percentile)	
Child's mattress dust per m^2 of surface			
Amount of dust mg·m^{-2}	0 / **257** (139 – 471)	0 / **247** (148 - 366)	0.322
Endotoxin EU·m^{-2}	2 / **3053** (1521 – 6015)	0 / **2356** (1461 - 4208)	**0.003**
(1,3)-ß-D-glucan µg·m^{-2}	0 / **421** (238 – 865)	0 / **380** (199 - 625)	**0.002**
EPS EPSU·m^{-2}	6 / **1008** (4458 – 25904)	5 / **8257** (3890 - 17310)	0.026
Child's mattress dust per gram of dust			
Endotoxin EU·g^{-1}	2 / **12222** (7379 – 21337)	0 / **10608** (6550 - 17366)	0.021
(1,3)-ß-D-glucan µg·g^{-1}	0 / **1859** (1277 – 2396)	0 / **1662** (1135 - 2205)	**0.002**
EPS EPSU·g^{-1}	6 / **40792** (24235 – 65371)	5 / **34696** (20364 - 58156)	0.021
Living-room floor dust per m^2 of surface			
Amount of dust mg·m^{-2}	0 / **200** (52 – 523)	22 / **104** (31 - 564)	0.040
Endotoxin EU·m^{-2}	14 / **3749** (1034 – 10212)	23 / **2299** (441 - 14224)	0.126
(1,3)-ß-D-glucan µg·m^{-2}	0 / **445** (114 – 1267)	7 / **177** (59 - 1417)	0.024
EPS EPSU·m^{-2}	28 / **8113** (1076 – 32188)	70 / **2009** (154 - 33251)	**< 0.001**
Living-room floor dust per gram of dust			
Endotoxin EU·g^{-1}	14 / **19400** (10104 – 32678)	23 / **18196** (9522 - 32106)	0.451
(1,3)-ß-D-glucan µg·g^{-1}	0 / **2229** (1703 – 3114)	7 / **2137** (1519 - 2994)	0.130
EPS EPSU·g^{-1}	28 / **39344** (18290 – 76367)	70 / **20330** (3896 - 61555)	**< 0.001**

[1] Data are presented as n<LOD / median (25^{th}-75^{th} percentile), unless otherwise stated

[2] 2 subjects more than in Gehring et al. 2007 (n=356)

Table 3: Correlation (Spearman's Rho) between the measured microbial components*

CHILDREN'S MATTRESS

GINI & LISA				
	Dust	**(1,3)-ß-D-glucan**	**EPS**	**Endotoxin**
Dust	1.00			
(1,3)-ß-D-glucan	***0.86***	1.00	**0.04**	**0.24**
EPS	***0.76***	***0.67***	1.00	**0.22**
Endotoxin	***0.63***	***0.66***	***0.60***	1.00

PIAMA				
	Dust	**(1,3)-ß-D-glucan**	**EPS**	**Endotoxin**
Dust	1.00			
(1,3)-ß-D-glucan	***0.78***	1.00	**0.13**	**0.15**
EPS	***0.70***	***0.63***	1.00	**0.07**
Endotoxin	***0.59***	***0.54***	***0.51***	1.00

LIVING-ROOM FLOOR

GINI & LISA				
	Dust	**(1,3)-ß-D-glucan**	**EPS**	**Endotoxin**
Dust	1.00			
(1,3)-ß-D-glucan	***0.94***	1.00	**0.21**	**0.26**
EPS	***0.87***	***0.89***	1.00	**0.24**
Endotoxin	***0.87***	***0.88***	***0.82***	1.00

PIAMA				
	Dust	**(1,3)-ß-D-glucan**	**EPS**	**Endotoxin**
Dust	1.00			
(1,3)-ß-D-glucan	***0.95***	1.00	**0.36**	**0.42**
EPS	***0.90***	***0.89***	1.00	**0.49**
Endotoxin	***0.93***	***0.93***	***0.87***	1.00

*Amount of dust sampled (**per m^2**), endotoxin, (1,3)-ß-D-glucan and extracellular polysaccharide (EPS) levels **per g of dust** (upper triangular matrix) and ***per m^2*** (lower triangular matrix) for **children's mattress** and **living-room floor** exposure

Associations between mould components and endotoxin and respiratory diseases and symptoms

Adjusted, logistic regression models showed non-consistent results in the German and Dutch subsets. In the German population, EPS and endotoxin exposure from children's mattresses is significantly negatively associated with physician-diagnosed asthma (OR (95% Confidence Interval per IQR), **0.60(0.39-0.92)** and **0.55(0.31-0.97)**, respectively). EPS exposure was additionally inversely related to physician-diagnosed allergic rhinitis (**0.67**(**0.49-0.92**)) (**table 4a**). Further stratification for parental allergy showed similar effects in children with allergic parents, however, the confidence intervals are wide. No effect on respiratory symptoms was observed. For the Dutch population, we could not find any effect of exposure to biocontaminants on any health outcomes assessed (**table 4b**). The associations between

exposure from living room-floor and assessed health outcomes were similar to exposure from children's mattresses, however not significant (data not shown), this may be due to a higher number of non-detectable values for living room-floor dust samples compared to mattress dust samples.

In both samples, (1,3)-ß-D-glucan, EPS, endotoxin, and total amount of dust were highly correlated. Mutual adjustment for microbial exposure did not change the observed effects.

Table 4a: Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and ln-transformed (1,3)-ß-D-glucan (µg/m^2), EPS (EPSU/m^2), endotoxin loads (EU/m^2) and total amount of mattress dust. Results are presented as odds ratios and 95% CI.

GERMAN BIRTH COHORTS

						CHILDREN WITH PARENTAL ALLERGY				
6 years	**N**	**(1,3)-ß-D-glucan*** (µg/m²)	**EPS*** (EPSU/m^2)	**Endotoxin*** (EU/m^2)	**Amount of dust*** (mg/m^2)	**n**	**(1,3)-ß-D-glucan *** (µg/m²)	**EPS*** (EPSU/m^2)	**Endotoxin*** (EU/m^2)	**Amount of dust*** (mg/m^2)
		$OR_{adj}$$^{+}$	**$OR_{adj}$$^{+}$**	**$OR_{adj.}$$^{++}$**	**$OR_{adj.}$$^{++}$**		**$OR_{adj}$$^{+}$**	**$OR_{adj}$$^{+}$**	**$OR_{adj.}$$^{++}$**	**$OR_{adj.}$$^{++}$**
Allergic diseases										
Asthma	17	0.76 (0.40 – 1.45)	**0.60 (0.39 – 0.92)**	**0.55 (0.31 – 0.97)**	0.65 (0.35 – 1.21)	15	0.59 (0.30 – 1.19)	**0.5 (0.31 – 0.81)**	**0.46 (0.25 – 0.85)**	0.54 (0.27 – 1.08)
Allergic rhinitis	47	0.69 (0.45 – 1.05)	**0.67 (0.49 – 0.92)**	0.71 (0.48 – 1.04)	0.71 (0.47 – 1.08)	42	**0.58 (0.37 – 0.91)**	**0.66 (0.47 – 0.93)**	**0.60 (0.40 – 0.92)**	**0.63 (0.40 – 0.99)**
Respiratory symptoms										
Rhino-conjunctivitis	48	0.74 (0.49 – 1.12)	0.77 (0.56 – 1.07)	0.78 (0.53 – 1.15)	0.81 (0.52 – 1.24)	45	0.71 (0.46 – 1.09)	0.81 (0.58 – 1.15)	0.70 (0.47 – 1.06)	0.83 (0.52 – 1.31)
Wheezing	43	0.78 (2.35 – 11.54)	1.02 (0.71 – 1.48)	0.82 (0.54 – 1.24)	0.81 (0.52 – 1.27	36	0.68 (0.42 – 1.09)	0.92 (0.62 – 1.38)	0.69 (0.43 – 1.1)	0.76 (0.46 – 1.25)
Dry Cough	56	0.78 (0.53 – 1.13)	0.93 (0.68 – 1.27)	0.89 (0.63 – 1.26)	0.92 (0.63 – 1.34)	45	**0.65 (0.43 – 0.98)**	0.84 (0.58 – 2.55)	0.82 (0.55 – 1.22)	0.76 (0.50 – 1.16)

* Per interquartile range increase in ln-transformed exposure

+ Adjusted for sex, (parental allergy), parental education, current pet ownership, breastfeeding, AirAllerg case-status, season of dust-sampling

++ Adjusted for sex, (parental allergy), parental education, current ETS exposure at home, current pet ownership, breastfeeding, AirAllerg case-status, season of dust-sampling

Table 4b: Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and ln-transformed mattress dust (1,3)-ß-D-glucan (µg/m²), EPS (EPSU/m^2) and endotoxin levels (EU/m^2). Results are presented as odds ratios and 95% CI.

DUTCH BIRTH COHORT

						CHILDREN WITH PARENTAL ALLERGY				
6 years	**n**	**(1,3)-ß-D-glucan*** (µg/m²)	**EPS*** (EPSU/m^2)	**Endotoxin*** (EU/m^2)	**Amount of dust*** (mg/m^2)	**n**	**(1,3)-ß-D-glucan *** (µg/m²)	**EPS*** (EPSU/m^2)	**Endotoxin*** (EU/m^2)	**Amount of dust*** (mg/m^2)
		OR_{adj}+	**OR_{adj}+**	**OR_{adj}+**	**OR_{adj}+**		**OR_{adj}+**	**OR_{adj}+**	**OR_{adj}+**	**OR_{adj}+**
Allergic diseases										
Asthma	27	1.28 (0.72-2.29)	1.24 (0.78-1.96)	1.51 (0.94-2.42)	1.28 (0.76-2.17)	22	1.28 (0.67-2.43)	1.29 (0.77-2.15)	1.49 (0.89-2.50)	1.25 (0.70-2.23)
Hay fever	23	0.83 (0.42-1.63)	1.00 (0.61-1.65)	0.61 (0.35-1.07)	0.88 (0.49-1.60)	22	0.79 (0.39-1.60)	1.00 (0.59-1.70)	0.59 (0.33-1.05)	0.86 (0.47-1.59)
Respiratory symptoms										
Rhino-conjunctivitis	28	1.11 (0.62-1.97)	1.19 (0.75-1.90)	0.96 (0.61-1.51)	1.01 (0.62-1.65)	27	1.07 (0.59-1.95)	1.17 (0.73-1.87)	0.93 (0.59-1.48)	0.99 (0.60-1.65)
Wheezing	47	0.82 (0.53-1.28)	1.02 (0.74-1.42)	1.11 (0.77-1.59)	1.00 (0.68-1.49)	40	0.92 (0.56-1.50)	1.12 (0.77-1.64)	1.21 (0.82-1.80)	1.14 (0.73-1.77)
Nocturnal dry Cough	79	0.88 (0.61-1.25)	1.03 (0.79-1.34)	1.05 (0.78-1.41)	0.94 (0.68-1.29)	69	0.93 (0.63-1.38)	1.18 (0.87-1.61)	1.10 (0.79-1.52)	0.98 (0.69-1.39)

* Per interquartile range increase in ln-transformed exposure

+ Adjusted for sex, (parental allergy), parental education, current ETS exposure at home, current pet ownership, breastfeeding, AirAllerg case-status, season of dust-sampling

Associations between visible mould exposure and endotoxin and respiratory diseases and symptoms

We further investigated the effect of visible mould exposure on allergic respiratory disorders. A total number of 56 homes (17%) in Germany and 108 homes (33%) in the Netherlands were reported of having visible mould. We could not observe any association between visible mould exposure and any health outcome assessed within the German and Dutch sample (**table 5**).

Table 5: Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and visible mould in any room at home. Results are presented as odds ratios and 95% CI.

6 years	**GERMANY Visible mould (n=56)** $OR_{adj.*}$	**THE NETHERLANDS Visible mould (n=108)** $OR_{adj.**}$
Allergic diseases		
Asthma	1.03 (0.26-4.16)	1.14 (0.48-2.70)
Allergic rhinitis[+]	1.77 (0.79-3.99)	1.60 (0.62-4.14)
Respiratory symptoms		
Rhinoconjunctivitis	1.36 (0.56-3.26)	0.58 (0.22-1.53)
Wheezing	1.29 (0.52-3.21)	1.28 (0.65-2.49)
Dry Cough	1.27 (0.59-2.76)	1.24 (0.71-2.15)

Germany

* adjusted for sex, parental allergy, parental education level, outdoor activity (hours), breastfeeding, maternal smoking during pregnancy, AirAllerg case-status

The Netherlands

** adjusted for sex, parental allergy, parental education, current ETS exposure at home, current pet ownership, breastfeeding, study arm, AirAllerg case-status

[+] Germany: physician-diagnosed allergic rhinitis, The Netherlands: hay fever

DISCUSSION

Although we investigated birth cohort studies with a longitudinal design, exposure and health outcome assessment were only measured at one time point between the age of 5 and 6. However, in contrast to earlier AirAllerg investigations, we were now able to measure health outcomes after exposure assessment. A further reason for the present study design is that before and after the age of 6 years, the German and Dutch birth cohorts have different time points of follow-ups., e.g. PIAMA was investigated every year while the intervals for the GINI and LISA were less regular. To have at least one common, comparative time point with a standardized exposure and health outcome measurement, we determined the 6 year follow-up as a common reference.

Our results showed a mixed picture of the relationship between exposure to biocontaminant levels at home and the risk of respiratory diseases and symptoms in the three birth cohorts. In the German population, exposures to total amount of dust, (1,3)-ß-D-glucan, EPS and endotoxin from children's mattresses were associated with a lower risk of respiratory diseases. In contrast, in the Dutch sample, there was no association between domestic microbial exposures and any health outcomes assessed. To our knowledge, this investigation is the first study which reports the effects of exposure to domestic mould components on allergic and respiratory health in school age children.

Within the German sample, exposure to higher levels of (1,3)-ß-D-glucan and EPS at home from children's mattresses was inversely related to the risk of respiratory diseases. It was considered that exposure to mould components, such as (1,3)-ß-D-glucan and EPS, may have immune stimulatory properties (9, 11, 14). An U.S. birth cohort study observed that exposure to high levels of (1,3)-ß-D-glucan from settled house dust in the first year of life was

associated with a persistent decreased risk for recurrent wheezing among genetically predisposed children until the age of 3 years (3, 5). Douwes et al. observed a statistically significant protective effect of EPS-*Pen/Asp.* exposure from living-room floor dust at the age of three months on persistent wheeze in the first 4 years of life in the whole Dutch PIAMA study population (11). In the previously, AirAllerg case-control investigation, higher amounts of mattress dust have been reported to decrease the risk of allergic sensitisation to inhalant allergens in 2-4 year old children (9). Compared to the German sample, we could not observe any effect of exposure to mould components on the risk of respiratory diseases and symptoms within the Dutch sample. We also investigated the exposure of visible mould at home and the risk of respiratory disorders. There are a number of studies, considering visible mould as a risk factor for respiratory diseases and symptoms among children (1, 2, 4-6). We found no association between visible mould and respiratory disorders within the German and Dutch population. However, there was no evidence that the mould components are associated with visible mould. This is in agreement with a recent cohort study in the U.S. which did not observe a correlation between (1,3)-ß-D-glucan and EPS mould components and visible mould (3, 5). Further, it is known that (1,3)-ß-D-glucan derives also from many other sources than mould, such as pollen or plants which may explain the differences. Since indoor environment consists of a variety of indoor and outdoor sources, not only the measured ones, a clear assignment to the observed health effects is difficult.

In addition, our investigation showed, that exposure to higher levels of endotoxin at home from children's mattresses was inversely related to the risk of asthma within the German population. In support of the 'hygiene hypothesis', which postulated an inverse effect of household size and siblings on the risk of hay fever (18, 19), there was a considerable number of epidemiological studies in the past, investigating the effect of living on a farm and the risk of allergic disorders (for a review, see (29)). The farm environment contains a high amount of

microbes, including endotoxin (30). Endotoxin has been suggested to have strong immune-stimulatory properties. It may therefore be capable to enhance the TH1 cells dominated immune response and suppress the TH2 cells dominated allergic response in newborns and infants (16, 17). Born and growing up on a farm was protective against the risk of developing hay fever and allergic sensitisation early in life and some recent studies suggested that these protective effects are persistent until adulthood ((29, 31)). A protective effect on respiratory and atopic disorders in children was also observed for domestic endotoxin exposure in non-farming environments. Children who were exposed to high level of endotoxin at home showed a lower prevalence of physician-diagnosed asthma and allergic sensitisation in the first years of life (9, 11, 20, 32). A recent investigation of a U.S. birth cohort study showed that exposure to gram-negative bacterial biomarker endotoxin was inversely associated with asthma and allergic sensitisation at school age (32). The inverse association of exposure to high levels of endotoxin at home and the risk of asthma could be also observed in our German sample.

The major strengths of our study are the comparison of three European birth cohort studies with a similar study design and a standardized exposure measurement from two different countries. We observed that endotoxin and (1,3)-ß-D-glucan loads as well as (1,3)-ß-D-glucan concentrations from children's mattresses in Germany were significantly higher compared to the Dutch sample. Further, the percentage of children exposed to visible mould was higher among the Dutch sample which could indicate the presence of an increased exposure to microbial components other than the measured ones. Moreover, the "population density" outside the domestic area may also have different impact on the children's exposure to microbial contaminants. In our study, the German children were all recruited from within and around Munich whereas the Dutch children were recruited from several communities all over The Netherlands. In a recent PIAMA investigation, Caudri et al. presented the number of

addresses per square kilometre, as a proxy for the degree of urbanisation ((33)). As to our study population, 87% of the Dutch children and 94 % of the German children live in an area with more than 1500 addresses in a circular buffer with a 1000 meter radius. We investigated whether the degree of population density was associated with an increase in microbial exposures. However, there was no clear association between biocontaminants, measured from children's mattresses and living-room floor exposure and the number of addresses in a circular buffer with a 1000 meter radius.

A limitation of the current study is to have only a single dust sampling over a period of 6 years. Dust samples of a single time point cannot represent the overall exposure, as the microbial components in house dust samples may change over time. A previous AirAllerg investigation showed that the within-home variance of endotoxin, (1,3)-ß-D-glucan and EPS measurements was small compared to the between-home variance (34) However, some investigations looked at variations over time and performed repeatability analyses within and between-homes. Heinrich and colleagues concluded that a single dust sampling and analysis of endotoxin is representative of the exposure to these components for at least a period up to one year (35). To take into account the importance of early life exposure to biocontaminants on the developing immune system, we restricted analysis to those children who never changed residential location since birth. We observed that although associations between exposure to microbial components and physician-diagnosed asthma as well as allergic symptoms were getting smaller within the German subset, exposure to domestic (1,3)-ß-D-glucan, EPS and total amount of dust from children's mattresses was getting more pronounced for the risk of physician-diagnosed allergic rhinitis. Within the Dutch subset, we could observe a significantly inverse effect of exposure to domestic endotoxin from children's mattresses to the risk of physician-diagnosed hay fever. The results indicate that a single biocontaminant

measurement provides a reasonable proxy of the levels that were present since early life, at least among those children who never changed residential location.

Furthermore, the prevalence of early day-care attendance as another source of exposure to microbial contaminants differs considerably between the German and the Dutch sample: 2% of the German children, but 25% of the Dutch children have visited a large scale day-care institution within the first year of life. The difference remains up to the age of 4 years. A number of studies observed a higher infection rate among children with early day care ((36), (37)), which can be confirmed for the Dutch PIAMA children in a recent investigation. Early day-care and the presence of older siblings was associated with more airway symptoms until the age of 4 years ((33)). At the age of 6 years, infection rates among the Dutch PIAMA children were considerably lower than for the German children. Therefore, the impact of indoor exposure at home at the age of 6 on the developing immune system may be attenuated within the Dutch subset due to a higher amount of multiple exposures early in life. However, when restricting analysis to those children who did not attend a large-scale day-care facility during the first year of life, we could not observe any effect on respiratory health at school age.

Based on our study design, we cannot exclude the possibility of reverse causation. A considerable amount of German and Dutch parents (82% and 78%, respectively) have allergic diseases and they may therefore more frequently remove mould or dust, especially when having children diagnosed with allergic disorders. However, there is only little literature on cleaning habits in relation to the levels of mould components or endotoxin in settled house dust and no indication of a greater variability in dust amount (38-40). In our study, levels of (1,3)-ß-D-glucan, EPS and the total amount of dust from children's mattresses were not different between allergic and non-allergic parents, except a significantly lower endotoxin

load from homes of genetically predisposed children in Germany. Further, seasonal variation as a possible factor of influence on the actual microbial exposure could also be excluded. House dust sampling was performed during the cold season (October till April) only and the differences in the endotoxin loads between the sampling months were not statistically significant for the German subset. In PIAMA, the amount of dust per square metre and the (1,3)-ß-D-glucan levels per g of dust, both for the mattress of the child, were significantly associated with the month of dust collection. However, given the large overall variability in exposure levels between the homes, the seasonal variation as a reason for the biased results can be neglected.

Considering all of the potential reasons for the non-consistent findings in the German and Dutch population discussed above, we cannot provide a sufficient explanation for the observed differences.

Conclusion

Domestic microbial exposure showed different effects on allergic disorders among the German and the Dutch sample. We found inverse associations between domestic exposure to EPS and endotoxin from children's mattresses and doctor-diagnosed asthma and rhinitis in German but not in Dutch school children. The reason for the differences between countries is not clear and requires further study.

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Figure 1

Figure 1: Study design and study population