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Domestic mould and microbial agents in relation to allergic diseases in children
- Epidemiological approach and results from epidemiological studies

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Summary

Every day we are facing a complex variety of microbial agents and components in indoor environments of whom a major part derives from fungal and bacterial origin. Exposure to residential mould has repeatedly been found to be an environmental hazard and a risk factor for human health: Living in a damp and mouldy environment was consistently associated with respiratory disorders including asthma, wheeze and allergic rhinitis. On the other hand, recent studies in children have been shown that early life exposure to increased levels of fungal and bacterial agents was inversely related to the development of allergy.

However, little is known about the causal agents provoking or arresting the development of allergic, respiratory disorders in children. To draw a causal relationship is hindered by the variability of microbial components in indoor air and a reliable and valid exposure assessment as well as analyses methods. Moreover, a considerable part of existing studies are cross-sectional and based on a single time point of health assessment only. Therefore, it is first important to specify mould exposure as detailed as possible. Secondly, prospective, population based birth cohort studies should be given more weight as they can better assign the temporal sequence of causality and ideally assess multiple follow-ups. Moreover, the collaboration of birth cohort studies with a similar design has substantial impact on the power and the relevance of the findings.

In the first publication of this thesis I performed a comprehensive, systematic review on residential mould exposure and allergic health outcomes in children, followed by a meta-analysis. I observed that exposure to visible mould at home was consistently associated with an increased risk for asthma, wheeze and allergic rhinitis. In contrast, there was a tendency of a decreased risk of allergic health outcomes in relation to elevated levels of mould derived components. However, the evidence is mainly based on cross-sectional studies. In the second publication I performed meta-analyses in European birth cohorts to investigate whether a damp and/or mouldy environment early in life is associated with the development of asthma and allergic disorders later in life. I was able to look at different time points of health outcome assessment between birth and 10 years of age in a large, prospective dataset of 8 European birth cohort studies. Our main findings indicated that early life exposure to visible mould and/or dampness significantly increased the risk of allergic rhinitis symptoms up to 10 years of age. We also found a modest and significantly increased risk of early asthma (<3 years) and a non-significantly increased risk for later asthma outcomes (6-8 and 3-10 years). No association was observed for sensitisation against aero-allergens or mould allergens at school age. In a third publication, I addressed the microbial pollution in mattress dust in school age children from three European birth cohorts in Germany and the Netherlands. I aimed to investigate whether the protective effects of microbial pollution on asthma and allergic diseases observed in studies among farm children could be also confirmed in children from urban areas. Within the German sample, exposure to higher levels of mould derived components including (1,3)-ß-D-glucan and Extracellular Polysaccharides (EPS) as well as bacterial endotoxin were inversely related to the risk of respiratory diseases, whereas there was no association among the Dutch children. It was suggested that different life-style factors such as day care attendance or other microbial sources, apart from the measured ones, might be related to the different observations in allergic health outcomes. Nevertheless, this study in children from an urban area could partly confirm the findings from the so-called ‘farm studies’, suggesting protective effects on asthma and allergy in relation to elevated microbial exposure.

In conclusion, this thesis confirms and extends the existing literature on health effects of fungal and microbial exposure at home during childhood. It is among the first to examine the exposure to residential visible mould in prospective, population-based birth cohorts. Further, I was able to confirm that exposure to elevated levels of microbial agents had also protective effects on asthma among school children from urban areas and that this effect is not restricted to farm children. However, the various environmental hazards indoor and the complex interplay between environmental and genetic factors hampers to identify a causal relationship between exposure to mould, microbial agents and allergic disorders. It is suggested recently, that the diversity of microbial pollution at home might play a more decisive role than just the quantity of microbial agents. Elaborated analyses techniques including molecular methods might help to identify at least patterns of causal agents in relation to harmful or protective health effects.

Future research should address interventions in homes of children with asthma, with pre- and post-evaluation measures of mould at genus and species levels. Politics, civil engineering and health care professionals should cooperate in a greater extent in order to ensure healthy indoor air quality and to not only get rid of visible mould but also preventing it in the first place, especially in susceptible populations. Lastly, the impact of exposure to mould and microbial agents on the development of diseases beyond respiratory health is surely an important issue of further research.

Zusammenfassung

Mikroorganismen, vor allem Schimmelpilze und Bakterien sind ein allgegenwärtiger und natürlicher Bestandteil unserer Atemluft im Innenraum. Zahlreiche epidemiologische Studien postulieren einen Zusammenhang zwischen Schimmelpilzexposition im Wohnraum und dem Auftreten diverser gesundheitlicher Beschwerden, darunter Atemwegserkrankungen wie Asthma, Asthma-Symptome (keuchende Ausatmung) oder allergische Rhinitis. Im Gegensatz dazu stehen Studien, die Hinweise darauf geben, dass eine höhere mikrobielle Belastung in der Wohnumgebung des Kindes, vor allem in den ersten Lebensjahren, vor der Entwicklung einer allergischen Erkrankung schützen kann. Jedoch sind die ursächlichen Faktoren einer schädlichen wie auch protektiven Wirkung noch unklar.

Zahlreiche Faktoren limitieren einen Kausalitätsnachweis: Zum einen repräsentieren Schimmelpilze die mikrobielle Vielfalt im Innenraum nur partiell, zum anderen haben auch die vorhandenen Methoden der Expositionsbestimmung einen limitierenden Einfluss. Darüber hinaus basieren die Ergebnisse der meisten Studien auf Querschnittsuntersuchungen und nur auf einem Zeitpunkt der Expositionserfassung, was einen Kausalitätsnachweis erschwert. Um zumindest den formalen Anforderungen einer Kausalitätsaussage zu entsprechen, sollten epidemiologische Studien den Risikofaktor „Schimmelexposition“ so spezifisch wie möglich definieren. Prospektive Geburtskohorten eignen sich besonders die Entstehung eines Asthmas oder allergischen Erkrankung zu erfassen, da im Falle mehrerer Untersuchungszeitpunkte eine zeitlich vorausgegangene Exposition klar definiert werden kann. Darüber hinaus kann eine gemeinsame Auswertung von Daten zu vergleichbaren Geburtskohorten die Aussagekraft und Relevanz der Forschungsergebnisse erhöhen.

Ziel der ersten Publikation dieser Dissertation war es, auf Basis der Ergebnisse eines systematischen Reviews und nachfolgender Meta-Analyse, die Auswirkungen einer Schimmelpilzexposition im Wohnraum auf die Atemwegsgesundheit von Kindern zu untersuchen. Ein sichtbarer Schimmelschaden war mit einem signifikant erhöhten Risiko für Asthma, Asthma-Symptome und allergische Rhinitis verbunden. Jedoch zeigte sich auch, dass Kinder mit einer höheren Belastung an Schimmelpilzbestandteilen im Hausstaub wie zum Beispiel (1,3)-*ß*-D-Glucan weniger gefährdet waren, eine allergische Erkrankung zu entwickeln. In einer zweiten Veröffentlichung konnte ich das Ergebnis des Reviews in einer Meta-Analyse von 8 europäischen Geburtskohorten bestätigen. Aufgrund des prospektiven Studiendesigns war es möglich, die Auswirkungen einer frühen Schimmelexposition im Wohnraum auf das spätere Auftreten von Asthma und allergischer Rhinitis zu verschiedenen Zeitpunkten zwischen Geburt und 10 Jahren zu untersuchen. Kinder, die in den ersten zwei Lebensjahren in einer Wohnung mit Schimmelproblemen aufwuchsen, zeigten ein signifikant erhöhtes Risiko für eine allergische Rhinitis in der späteren Kindheit zwischen 3 und 10 Jahren und auch im frühen Schulalter zwischen 6 und 8 Jahren. Des Weiteren könnten wir auch für Asthma in der Kindheit ein (nicht signifikant) erhöhtes Risiko feststellen. Eine frühe Exposition gegenüber Schimmel und/oder Feuchtigkeit im Innenraum war jedoch nicht mit einem erhöhten Risiko für eine allergische Sensibilisierung gegenüber Inhalationsallergenen im frühen Schulalter zwischen 6 und 8 Jahren assoziiert. In einer dritten Studie mit Kindern von zwei deutschen und einer niederländischen Geburtskohorte konnte ich den protektiven Effekt einer erhöhten mikrobiellen Belastung auf Asthma und allergische Rhinitis bei Kindern bestätigen. Das Besondere an diesem Studienergebnis liegt darin begründet, dass die Kinder in einer urbanen Umgebung aufgewachsen sind, denn für Kinder von Bauernhöfen

oder aus einer ländlichen Umgebung ist dieser Zusammenhang bereits gut belegt. Eine höhere Belastung an Schimmelpilzkomponenten (1,3)-ß-D-glucan, Extrazellulären Polysacchariden (EPS) sowie Endotoxin von gram-negativen Bakterien zeigte einen inversen Effekt in Bezug auf Asthma und allergische Rhinitis, allerdings nur innerhalb der deutschen Kohorten. Andere Lebenstilfaktoren wie eine erhöhte Inanspruchnahme von Kinderkrippen in den Niederlanden oder andere, nicht gemessene mikrobielle Kontaminanten im Innenraum könnten einen Einfluss auf die länderspezifischen Unterschiede haben.

Diese drei Publikationen bestätigen und bereichern bereits veröffentliche Untersuchungen zu Gesundheitswirkungen von Schimmelpilzexposition im Wohnraum bei Kindern. Darüber hinaus ist sie eine der ersten Studien, die die möglichen Gesundheitsauswirkungen einer Schimmelpilzexposition prospektiv in europäischen Geburtskohorten untersucht. Ich konnte weiterhin feststellen, dass die protektiven Effekte einer erhöhten mikrobiellen Belastung im Hausstaub auf Asthma und Allergien sich nicht nur auf Schulkinder aus dem ländlichen Raum beschränken. Die Komplexität der Bioaerosole im Innenraum sowie die Exposition gegenüber weiteren Umweltfaktoren und dem Einfluss der genetischen Disposition erschweren jedoch einen Kausalitätsnachweis im Hinblick auf das Entstehen von Asthma und allergischen Erkrankungen im Kindesalter. Neuere Untersuchungen geben Hinweise darauf, dass möglicherweise die Diversität der mikrobiellen Belastung im Innenraum eine entscheidende Rolle spielt. In dem Zusammenhang könnten Analysemethoden wie zum Beispiel die quantitative Polymerase-Kettenreaktion (qPCR) helfen, die Zusammensetzung der mikrobiellen Spezies in der Raumluft oder im Hausstaub besser zu charakterisieren.

Zukünftige Studien sollten sich auch mit den Auswirkungen von Sanierungsmaßnahmen im Wohnraum von Kindern mit einer Asthmaerkrankung beschäftigen. Vor und nach der Entfernung beziehungsweise Sanierung von Schimmel oder Feuchtigkeitsschäden sollten Konzentrationen und auch die Artenzusammensetzung von Schimmelspezies bestimmt werden, vor allem im Zusammenhang mit Gesundheitsauswirkungen. Um eine gesunde Raumluft in Wohnräumen zu garantieren, sollten Vertreter aus Politik, Gesundheitswesen sowie dem Bauingenieurswesen verstärkt zusammenarbeiten um Schimmel oder Feuchtigkeitsschäden schon im Vorfeld zu verhindern. Abschließend sollten neben Atemwegserkrankungen auch andere Krankheitsbilder bei Kindern diskutiert werden, die möglicherweise mit einer Exposition gegenüber Schimmelpilzen in Verbindung stehen können.

1. Domestic mould and microbial agents: state of the art

Asthma and allergy are still a global burden and one of most frequent chronic diseases diagnosed during childhood [1-6]. These diseases have a substantial impact on the quality of life in children and adults [7] and are also associated with considerable health care expenditures. It is suggested that heredity is linked to the development of allergic diseases, however, not exclusively but rather implicating an environmental exposure component [8].

1.1 Allergy and respiratory diseases: definitions and nomenclature

Atopy and allergic sensitisation

'Allergy is a hypersensitivity reaction initiated by immunologic mechanisms' [9], according to the nomenclature proposal established by the European Academy of Allergology and Clinical Immunology (EAACI). 'Atopic' subjects have the natural tendency to become sensitized against common environmental exposure and to respond with an enhanced Immunoglobulin E antibody production compared to their healthy peers [10, 11]. In most cases, an allergic reaction is mediated by antibodies from the Immunoglobulin E isotype (IgE) to allergens from the indoor or outdoor environment. Although not yet fully understand, there are also cases of non-IgE-mediated respiratory outcomes, imitating allergic reactions [9].

The process of sensitisation against aero-allergens including pollen, pet and mite allergens or mould proteins is a major risk factor for later developing symptoms in the mucosal membranes of the airways [9]. However, the reasons for the 'switch' towards an allergic immune response, dominated by a T-Helper cell type 2 immune response with the respective cytokine milieu is still not yet fully understand. Some studies have been shown that allergens might have the potential to injure airway epithelium in such way, that the epithelial barrier is damaged leading to allergic sensitisation. It is suggested that healthy subjects obtain a tolerance to allergic sensitisation due to increased cell regulation within the mucosal surface in the airways [12].

Asthma

Asthma is a complex, chronic disease and the WHO characterized asthma as having symptoms of recurrent attacks of breathlessness, bronchial obstruction and wheezing [9]. According to the WHO, over 235 million people worldwide are currently suffering from asthma [9]. In a recent international investigation, the mean prevalence of an asthma diagnose was reported with 10.8% for 6 to 7 year old children with similar patterns for the asthma symptom wheeze [2]. The underlying causes of asthma are still not revealed whereas genetic predispositions and exposure to environmental pollutants and irritants are suggested to play an important role [10, 11].

In about 80 percent of childhood asthma and about 40%-50% adult asthma cases, the development of asthma is mediated by IgE antibodies against aero-allergens [7], also called 'extrinsic' asthma. However, allergic sensitisation is not an essential precondition for asthma or asthma-like symptoms. It is suggested that 10%-30% asthma cases in childhood are attributed to the non-allergic, 'intrinsic' form. However, in both variants, bronchial obstruction and systemic inflammation are the predominant features [12]. In contrast to allergic asthma, the non-allergic form was observed to induce increased neutrophilic inflammation responses

[13]. The intrinsic form was found to be strongly associated with occupational exposure in animal farming environments with increased exposure to microbial agents in adults [14-16]. The aetiology of non-allergic asthma in children is not well defined, but it is suggested that viral infections might be an important trigger [6, 17, 18].

Allergic rhinitis

Allergic rhinitis or hay fever are often used interchangeably and are characterised by inflammation of the mucous membrane of nose, often in combination with the eyes and induced after exposure to allergens [19, 20]. Reported symptoms are runny, blocked or itchy nose, mostly in combination with itchy watery eyes [21]. It is one of the most common allergic disorders and the current prevalence worldwide ranged from 8.5% to 14.6% among children and adolescents as reported from the latest ISAAC survey [22]. Allergic sensitisation to aero-allergens is an important risk factor next to family history and more pronounced for allergic rhinitis than for asthma [23]. In general, allergic rhinitis symptoms can occur seasonal, induced by exposure to outdoor allergens such as pollen or perennial triggered by mostly indoor derived allergens including house dust mites, pet or mould allergens [19]. Although it is not well evaluated in children populations, there are also forms of non-allergic rhinitis which are not mediated by the IgE-isotype but mimic the clinical phenotype. It is suggested that infections, physical or chemical agents might play a role in the development of non-allergic rhinitis symptoms [19]. Moreover, there is a large body of evidence that allergic rhinitis is an important risk factor for asthma and they are often co-morbidities [20]. The connection between both should therefore be carefully considered in future studies.

1.2 Exposure to mould and microbial agents

Every day we are facing a complex variety of microbial agents in indoor environments with a major part deriving from fungal or bacterial origin. ‘The enormous diversity of the Fungal Kingdom is well recognized’ [24] and fungal species are ubiquitous in indoor and outdoor surroundings: In ‘healthy’ indoor environments, the predominant part of fungi is presented by the outdoor air genera *Cladosporium*, *Penicillium* and *Aspergillus* [25, 26]. However, once there is dampness or visible mould, the composition of the fungal profile is shifting mainly to ‘indicator mechanisms’: Species such as *Penicillium chrysogenum*, *Penicillium expansum*, *Aspergillus versicolor*, *Aspergillus penicillioides* and also *Stachybotrys chartarum* were reported to be detected typically in moisture damaged environments [27, 28].

Several studies assessed mould derived components such as (1,3)-β-D-glucan and Extracellular Polysaccharides (EPS) in house dust samples as surrogates for mould exposure [29-31]. (1,3)-β-D-glucan may account for up to 60% of the dry weight of the cell wall of fungi. However, (1,3)-β-D-glucan can be also part of the structure of plant materials, including pollen and cellulose, as well as soil bacteria [29, 32, 33] and a fungal exposure might therefore be overestimated by measurement of (1,3)-ß-D-glucan. Fungal Extracellular Polysaccharides (EPS) are heat stable and water-soluble non-branched carbohydrates. During fungal growth, EPS are secreted in the environment. These polysaccharides have antigenic specificity at the genus level [34] except for EPS from *Aspergillus* and *Penicilium spp*. which are cross reactive [35]. Compared to (1,3)-β-D-glucan there are only a few studies on exposure to extra cellular polysaccharides. As there is no evidence for EPS inducing inflammatory reactions at present [36] the exposure amount might serve as a good marker for fungal biomass contamination in indoor environment [35].

Endotoxin is often used as a surrogate for bacterial exposure in a number of studies [37-40]. Endotoxins are part of the outer membrane of gram-negative bacteria and are composed of proteins, lipids and lipopolysaccharides (LPS) [13, 40]. The term endotoxin refers to biological active LPS from cell walls or cell wall fragments [31, 41]. Endotoxin has been found to be very heat resistant and stable within the environment which emphasizes its persistent biological activity over prolonged periods [40]. Increased endotoxin levels within the dwelling have shown to be associated with the presence of moisture damage and fungal spores [42]. This applies especially to agricultural settings with animal stables which have been observed to transport the microbial components into the home environment [43]. Increased levels of endotoxin were also found in non-farming and urban settings with varying levels [31]. The presence of pets, carpets, dampness, mould or air-conditioning has a considerable influence on the endotoxin levels indoors [13, 44].

In general, total exposure to microbial agents during childhood and later on depends on the different microenvironments such as workplace, school, kindergarten and residential area. Further, personal factors such as air-conditioning, ventilation, frequency of airing, fungal exposure from outside, building materials, characteristics of the substrate and type of moisture or water damage have also a substantial impact on the individualized exposure amount [45]. Several strategies have been developed in the past in order to determine the relevant, inhaled exposure of residential mould or microbial pollution.

1.3 Exposure assessment of mould and microbial agents

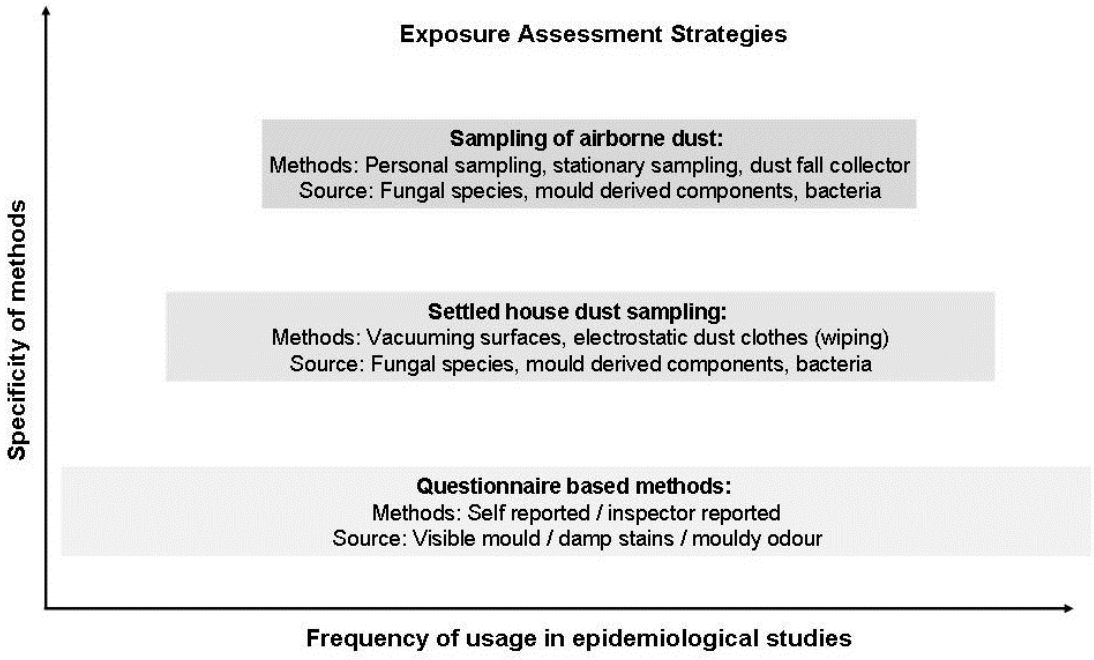

Figure 1: Different strategies for assessing mould and microbial exposure according to usage and specificity in epidemiological studies

There are several strategies for assessing visible mould and microbial exposure in indoor environments (Figure 1). The choice for a specific exposure assessment method and the analysis method can have a considerable impact on the interpretation of the findings later on. A common, simpler method attended with low costs and feasible within a large group of participants is to assess domestic visible mould or dampness by means of questionnaires. This common approach might lead to misclassification if moulds were not reported in spite of being present (e.g. hidden behind furniture) or not aerosolized and therefore not relevant for inhaled exposure. In order to reflect the potential inhaled amount, some studies measured mould exposure via settled house dust or air sampling [30, 46-48]. Settled house dust is usually assessed by vacuuming a predefined area from mattresses or the floor, following a standardized protocol. This procedure can be performed either by trained fieldworkers or by the study participants themselves [49-51]. However, a limitation of this method is that some particles in settled house dust are too heavy to become airborne and therefore might not be inhalable. As a consequence, some epidemiological studies considered air sampling methods in order to mirror the actual exposure to inhalable components [52-54]. These methods are very costly and work intensive and the concentrations of fungal and microbial agents within the air are very unstable and showed considerable variation [26]. Recently introduced assessment methods try to overcome the disadvantages from previous sampling methods. In a recent publication on determining indoor air dust concentration, passive sampler consisting of an electrostatic cloth showed good results in handling, costs and reproducibility [50]. However, the universal application on a wider range of allergens and in large epidemiological studies has to be evaluated in further studies.

The analyses methods of mould or microbial agents from settled house dust or airborne dust have limiting factors themselves (Figure 2). Cultivation methods are common but these are time consuming, expensive and the validity was critically discussed because of ‘overgrowing’ of some species and the strong seasonality effect. Further, this method applies only to living fungal and bacterial species [55-57]. Recent studies suggested that non-viable fungal fragments have also allergenic potential and are of biological relevance. The smaller fragments might deposit much deeper in the lung than fungal spores and therefore may also contribute to mould-related health disorders [24, 57-59]. Countable methods by microscope have the advantage to include also the non-viable components of microbial agents, however, could give no information at the genus level. Therefore, elaborated and cost intensive analysis techniques including assayable or molecular methods are performed in order to identify specific agents of fungal and microbial exposure at the genus level [26, 57]. These methods have also the potential to evaluate the diversity level of microbial pollution in indoor environments [39].

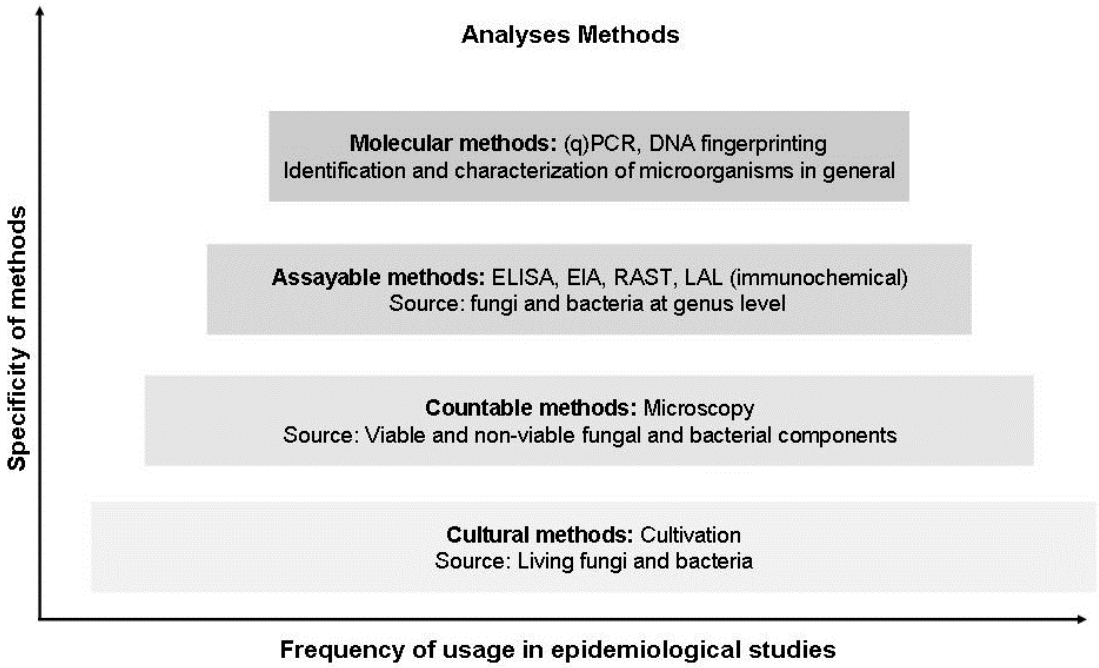

Figure 2: Different methods of analysing mould and microbial exposure according to usage and specificity

In conclusion, the assessment of settled or airborne dust is considered to be an objective method, but on the other hand costly, work intense and often not feasible in larger cohorts. Moreover, fungal species may only partly represent the true pollutant mixture in the home and to draw a causal relationship is hindered by the variability of microbial components in indoor air and the limitations of assessment strategies and analyses methods. Therefore, apart from being a subjective method and the limitations mentioned above, the questionnaire-based assessment of visible mould might still serve as a fair indicator that the indoor environment is out of balance [25].

1.4 Health effects of mould and microbial agents

Available data are indicating that at least 20% of the homes worldwide have dampness problems or visible mould [60]. There is a large body of literature which found consistent associations of living in a damp and mouldy environment in relation to adverse respiratory and allergic health disorders in children from different regions worldwide [31, 42, 60-62]. Occupational exposure to high levels of bacteria or mould derived allergens was linked to allergic diseases including asthma and allergic rhinitis among adults [63]. On the other hand, being exposed to increased levels of microbial agents early in life was observed to protect children from allergic diseases [39, 46, 47, 51, 64-66]. However the knowledge of relevant exposure or causal agents in relation to health disorders – harmful and protective – is still limited.

We know that there are several biological pathways suggested between exposure to microbial agents in relation to allergic health outcomes. Allergic respiratory diseases are

characterized by inflammatory reactions of the mucosal membrane of the upper and lower airways and eyes against environmental agents, dominated by a T-Helper cell type 2 immune response [6]. Fungal species are able to induce inflammatory reactions [42, 67, 68] and several fungi and isolated mycotoxins were linked to inflammatory responses in vitro [69-71]. Further, sensitisation to mould allergens was linked to severe asthma [72] and associations between exposure to spores of *Penicillium* indoors and an increased risk of respiratory disorders in children were described in epidemiological studies [73, 74]. Other biological active components such as (1,3)-ß-D-glucan or endotoxin were also suggested to have pro-inflammatory properties and to be associated with allergic and non-allergic respiratory symptoms [29, 30, 46, 47, 64, 75]. Further, these properties are not limited on viability and the amount of (1,3)-β-D-glucan from non-viable sources are suggested to be equally important [13, 40, 76]. By contrast, exposure to Extracellular polysaccharides (EPS) was not linked to health effects in existing literature, but is often used as indicator for fungal biomass [35, 48].

Interestingly, epidemiological studies on exposure to mould and bacterial derived components revealed conflicting results. Studies in occupational settings with high levels of exposure to endotoxin or mould allergens reported an increased risk for non-atopic asthma in adult subjects [63]. As opposed to this, some studies among farm children observed that elevated levels of endotoxin early in life protected from allergic disorders including asthma and allergic rhinitis [13, 65, 77]. Similar was found for elevated levels of mould components including (1,3)-ß-D-glucan and EPS, also among children without a farming background [39, 46, 47, 51, 64-66]. These inverse effects on allergic diseases were explained by the so-called ‘hygiene hypothesis’ [78] stating that early expo sure to a rich microbial environment including viruses may decrease the risk for allergic diseases later in life by stimulating the immune system towards an non-allergic immune response. Although a plausible explanation, this hypothesis could not be confirmed in all studies [66] and exposure to mould or bacterial derived components could not be confined to specific agents. It was recently suggested that the composition and the diversity might play a more important role than the quantity of microbial exposure levels, and assuming different health effects from different fungal or microbial profiles [39]. Therefore, further research on the specific exposure types is needed. Molecular techniques including qPCR analyses or DNA finger printing might help in order to better characterise the indoor environment and to give evidence about the diversity of the microbial pollution. Moreover, more weight should be given to longitudinal birth cohort studies because of the prospective study design and the use of evaluation and validation criteria also in epidemiological studies could emphasize, but also mitigate the study results.

2. Specific aims and results

Aims

This thesis is based on the hypothesis that exposure to mould at home is associated with allergic, respiratory disorders in children. The main objectives of this thesis are as follows:

- To review systematically the existing literature on specific types of residential mould exposure assessment in relation to allergic disorders in children, separated by epidemiological study design. In addition, meta-analyses to quantify the associations were performed.

- To study whether early exposure to visible mould and dampness at home is associated with the development of asthma and allergic rhinitis as main health outcomes in children later in life in a collaboration of 8 European birth cohorts.

- To analyse the effect of exposure to mould and bacteria derived components measured in settled house dust in relation to asthma and allergic rhinitis in school age children from three European birth cohorts.

This thesis contains three manuscripts, accepted and published within the European Respiratory Journal and the Allergy Journal. For all three publications I developed the research question, study design, performed the statistical analyses, and interpreted the results. After the preparation of the first draft, the manuscripts were sent to the co-authors and finalized based on their comments and suggestions. Once I got response from the journals with the permission to resubmit the drafts, the manuscripts were again edited based on the discussions with my supervisor as well as the reviewers' comments and resubmitted to the respective journal, together with the responses to the reviewers.

Study population and Methods

The three manuscripts are based on three different study populations. For the systematic review, the literature research in pubmed identified 1398 peer reviewed scientific publications of the past 30 years worldwide on exposure to mould and allergic, respiratory health outcomes in children. In total, 61 studies with comparable health outcome and exposure information were included. We restricted the study population to children only (between birth and 17 years) and the findings were analysed separated by epidemiological study design. The findings were additionally quantified with the help of meta-analyses. For this purpose, we focused on studies which used logistic regression models with similar adjustment for risk factors. To summarize the effect estimates among appropriate studies and to account for the heterogeneity between the different studies, random effect models were applied.

The meta-analysis on the association between early exposure to visible mould at home (birth to 2 years) in relation to the development of allergic diseases was performed in 31,742 children from 8 ongoing European birth cohorts. The birth cohorts were selected based on their comparability regarding exposure and health outcome assessment. Exposure to mould and allergic health outcomes were assessed by parental questionnaires at different time points from birth up to 10 years of age. Exposure was defined as parent-reported mould and/or dampness in any room of the home during the first two years of life. We determined seven health end points: "Early childhood asthma" (0-3 years), "school age asthma" (6-8 years), and "ever asthma" at any time between 3 and 10 years of life. School age and childhood "symptoms of allergic rhinitis" was defined as sneezing attacks, runny, blocked and

itchy nose without having a cold. Sensitisation against aero-allergens and mould allergens was available for five of the eight cohorts and defined as having specific IgE (Immunoglobulin E) of at least 0.35 kU/L to at least one of the measured aero-allergens (cat dander, dog dander, mite, mould allergens, grass or tree pollen) between 6 and 8 years. Meta-analyses with fixed and random effect models were applied to account for the heterogeneity between different cohorts. The number of the studies included in each analysis varied, based on the outcome data available for each cohort.

For the third manuscript I investigated the association of exposure to mould derived (1,3)-ß-D-glucan and EPS as well as bacterial endotoxin in relation to asthma and allergic rhinitis at early school age. This investigation was the follow-up of an originally nested case-control study among three European birth cohorts (AirAllerg). A more detailed description of the study design and the study population is provided elsewhere [51, 79]. Children from two ongoing birth cohort studies performed in Germany (GINIplus and LISAplus, n=358) and one in The Netherlands (PIAMA, 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. Information on respiratory and allergic disorders, as well as visible mould in the child's home was collected at age 6, using self-administered questionnaires. At the age of 6, health outcome information was available for 678 children. Earlier AirAllerg investigations were based on health outcomes measured before exposure assessment. However, in the present analysis, we were able to look at the health outcomes after exposure assessment.

Results

In the first publication of this thesis I performed a comprehensive, systematic review on residential mould exposure and allergic health outcomes in children. Compared to previous investigations, mould exposure was specified into visible mould, measured fungal spores and mould derived components including (1,3)-ß-D-glucan and Extracellular polysaccharides (EPS). We found that exposure to visible mould at home was consistently associated with an increased risk for asthma, wheeze and allergic rhinitis, regardless of the study design. These findings were also confirmed by the results of the meta-analyses later on. Exposure to fungal spores measured from airborne or settled house dust was found to increase the risk for asthma and wheeze in children up to one year, but there are inconclusive results at later ages. In contrast, there was a tendency of a decreased risk of allergic health outcomes in relation to elevated levels of mould derived components. We additionally evaluated the results of the systematic review according to the "Bradford Hill criteria" for assessing evidence of causation. The evaluation supports the findings of our review, especially with regard to aspects such as strength of an association, temporal relationship, biological gradient, plausibility and coherence, however, the evidence is mainly based on cross-sectional studies. Nevertheless, this study revealed that further research is needed in order to disentangle and specify the effects of mould exposure in indoor environments to a greater extent.

In the second publication I performed meta-analyses to investigate whether a damp and mouldy environment early in life is associated with the development of asthma, allergic rhinitis and sensitization to aero-allergens later in life in a large, prospective dataset of 8 European birth cohort studies. We were able to look at different time points of health outcome assessment between birth and 10 years of age. Our main findings indicated that early life exposure to visible mould and/or dampness during the first two years of life significantly increased the risk of allergic rhinitis symptoms up to 10 years of age. We also found a modest and significantly increased risk of early asthma (<3 years) and a non-

significantly increased risk for later asthma outcomes (6-8 and 3-10 years). No association was observed for sensitisation against aero-allergens or mould allergens at school age (6-8 years). However, a damp and mouldy environment might be also associated with non-allergic forms of asthma as observed among adults [14-16]. After stratifying children by their atopic status, there was a positive, however not statistically significant association for non-IgE-mediated asthma at early school age compared to the children with increased IgE levels to aero-allergens in five birth cohorts. The mechanisms of non-allergic form of asthma in children is not well understood to date, but it is suggested to result from similar inflammatory changes, mainly by the production of antibodies of the IgG, IgA, and IgM isotype after inhalation of large amounts of mould protein [7, 17]. We further observed an increased risk of visible mould exposure in relation to asthma and symptoms of allergic rhinitis in children with allergic parents, but not in children without this hereditary component. This underscores the recommendation to ensure healthy indoor air quality and to both remove visible mould or signs of moisture and actively prevent its formation in the first place, especially in susceptible populations.

In a third publication, I aimed to investigate whether the protective effects of an increased microbial pollution on allergic diseases observed in studies among farm children could be also confirmed in school children from urban areas in Germany and the Netherlands. The results revealed a mixed picture of the relationship between exposure to biocontaminant levels at home and the risk of respiratory diseases and symptoms. Within the German sample, exposure to higher levels of mould derived components including (1,3)-ß-D-glucan and Extracellular Polysaccharides (EPS) as well as bacterial endotoxin from children's mattresses was inversely related to the risk of respiratory diseases including asthma and allergic rhinitis. In contrast, there was no association observed among the Dutch children. The reasons for the differences are not quite clear and require further study, however, 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 a wider range of microbial agents. In addition, different life-style factors such as a higher day care attendance in the Netherlands might be also related to the different observations in allergic health outcomes. Furthermore, compared to the two previous manuscripts, current visible mould exposure at school age was not associated with the allergic health outcomes in children from both countries. In conclusion, these findings underscore that the true fungal and microbial pollution at home is considered to be very heterogeneous and specific. The complex interplay of different microbial sources associated with personal behaviour might end up in individual microbial profiles with specific exposure, not only at home, and health responses later on [39]. Nevertheless, this study in children from an urban area could partly confirm the findings from the so-called 'farm studies', suggesting protective effects on allergy in relation to elevated microbial exposure.

3. Conclusion and outlook

This thesis confirms and extends the existing literature on health effects of mould and microbial exposure at home during childhood. The systematic review on the literature in the past 30 years revealed that a mouldy home environment was consistently associated with allergic health outcomes including asthma, wheeze and allergic rhinitis among children and adolescents, regardless of epidemiological study design. Additionally, the results were evaluated according to the "Bradford Hill criteria which supports the findings, especially with regard to aspects such as strength of an association, temporal relationship, biological gradient, plausibility and coherence. However, the evidence is mainly based on cross-sectional studies. For the first time, I could reinforce these findings in a collaborative study among 8 European prospective birth cohorts: Early exposure to visible mould at home during the first two years of life increased the risk for symptoms of allergic rhinitis at different time points between birth and 10 years of age and was also associated with asthma prevalence during childhood. In contrast, we could also partly confirm that exposure to elevated levels of mould and bacteria derived components might protect from asthma and allergic rhinitis in childhood, not only among children with farming or rural background.

Although we could confirm harmful as well as protective effects of residential exposure to mould and mould related agents in relation to allergic disorders, there are a number of factors which have to be considered carefully. This thesis revealed that 'mould' exposure is just an umbrella term for a broader spectrum of specific exposure types, viable and non-viable, which are in turn often associated with different health outcomes. Dampness and moisture damage is not only an important precondition for mould growth, but favours the spread of microbial agents in general. The release activity of mould spores and the impact on health is further depending on personal behaviour and synergistic or repressive effects of the overall microbial pollution indoors [26, 41]. A recent cross-sectional study in farm children concluded that not single agents, but rather the diversity of microbial pollution might play a decisive role [39]. Up to now, this could not be confirmed for children living in urban or rural areas. In order to identify at least patterns of agents, evoking harmful or protective effects on the development of allergic diseases, more attention should be given to elaborated analyses techniques, including molecular methods (e.g. quantitative polymerase chain reactions (qPCR)).

Apart from the specification of exposure type, the development of allergic diseases is based on a complex interplay between environmental and genetic factors. There are a number of genes identified to be associated with the development of asthma and allergies in genome wide association studies [80, 81]. Mutations in the toll-like receptor (TLR) pathway genes, especially TLR 4 which is the major signalling receptor for Lipopolysacchardies (LPS), were observed to alter the response to environmental, microbial exposure [82]. Moreover, recent publications are suggesting that genetic variants in genes related to the cell protection controlling the inflammatory and antioxidative systems (e.g., GSTP1 gene) may modify the risk for allergic respiratory diseases including asthma and rhinitis as it was observed for ambient air pollution [83-85]. There might be also a joint effect of residential mould or microbial exposure and genetic variants in the pathogenesis of allergic diseases.

The various environmental hazards indoors and the complex interplay between environmental and genetic factors hampers to identify a causal relationship between exposure to mould, microbial agents and allergic disorders. Only few epidemiological studies have evaluated their findings according to validation criteria and one can speculate about the

proportion of studies which found no association and in turn, have never been published. However, the significance of future studies on environmental exposure in relation to allergic disorders can be strengthened by regarding factors such as strength of an association, plausibility, consistency and specificity, but should also include the genetic evidence as a new criterion. In relation to that, there is limited knowledge about interventional trials on mould remediation in relation to allergic disorders. A recent review on housing intervention concluded that there is sufficient evidence for reducing respiratory symptoms after remediation activities, but the validity of the included studies is impaired by differences in local regions [86]. Moreover, exposure to mould and microbial agents in indoor environments is not only considered to affect respiratory health. A damp and mouldy environment during childhood might be also embedded in a broader spectrum of children's health. In two recent publications from a German birth cohort (LISAplus) it could be shown that maternal smoking during pregnancy was associated with hyperactivity and inattention problems at 10 years [87] and exposure to environmental tobacco smoke was also suggested to influence insulin resistance later in childhood [88]. The impact of exposure to mould and microbial agents on the development of diseases beyond respiratory health is surely an important issue of further research.

In conclusion, this thesis confirmed that growing up in a damp and mouldy environment at home increases the risk for later asthma and allergic health disorders among children. An increased risk was especially seen for children with allergic parents. Further, the effect of mould remediation on respiratory health in children is still under-investigated. Future research should assess interventions in homes of children with asthma, with pre- and post-evaluation measures of mould at genus and species levels. Politics, civil engineering and health care professionals should cooperate in a greater extent in order to ensure healthy indoor air quality and to not only get rid of visible mould but also preventing it in the first place, especially in susceptible populations. This is highly recommended, especially against the backdrop that the impact of exposure to mould and indoor contaminants during childhood might not only affect respiratory health but also the health development in children in general and should get more attention in future research.

4. References

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5 Systematic Review on Mould and Allergy in Children

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Association between domestic mould and mould components, and asthma and allergy in children: a systematic review

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ABSTRACT: Critical reviews over the past 10 yrs have found increased respiratory and allergic health outcomes for children living in damp and mouldy environments. However, recent studies have suggested that early childhood exposure to specific mould components may actually protect children from developing allergy.

We conducted a systematic review of observational studies published in English from January 1980 to July 2010. This review was conducted according to systematic guidelines for Meta-analyses of Observational Studies in Epidemiology (MOOSE). The literature was searched using a computerised bibliographic database, PubMed. In order to increase the quality of the reviewed studies, meta-analyses of the effects of visible mould exposure on allergic health outcomes were performed and we evaluated the findings according to the Bradford Hill criteria for evidence of causation.

The literature search identified 1,398 peer-reviewed scientific publications, and 61 studies that fulfilled the inclusion criteria were included in this review. We observed increased risks of allergic respiratory health outcomes in children exposed to visible mould and mould spores. These findings were confirmed by the results of the meta-analysis and in line with the evaluation criteria according to Bradford Hill. Visible mould was positively associated with asthma (OR 1.49 (95% CI 1.28–1.72)), wheeze (OR 1.68 (95% CI 1.48–1.90)) and allergic rhinitis (OR 1.39 (95% CI 1.28–1.51)). However, there was a tendency of lower risk for allergic health outcomes in children exposed to mould-derived components such as (1,3)-β-D-glucan and extracellular polysaccharides.

These findings suggest that home environments with visible mould and mould spore exposure increase the risk of allergic respiratory health outcomes in children. However, further investigations are needed to examine the effects of exposure to mould-derived components as the current literature is inconclusive. In order to disentangle the different effects of overall microbial exposure on children's health, research should focus on specific microbial markers in the home, in combination with new assessment techniques including molecular methods.

KEYWORDS: Allergy, asthma, biomarkers, moulds, systematic review, wheeze

Numerous studies have analysed the relationship between living in a damp and mouldy environment and effects on respiratory health. Reviews conducted in the past 10 yrs have found an increased risk of respiratory and allergic health outcomes in children with a parent-reported damp and mouldy home environment. A review of the European studies (NORD DAMP) published prior to 1998 concluded that there was strong evidence for an association between dampness at home and increased risk of respiratory and allergic symptoms in children and young adults [1], which was also confirmed in a subsequent review (EUROEXPO) of studies published from 1998–2000 [2]. In 2004, the Institute of Medicine (IOM) of the US National Academy of Sciences reviewed studies published up to late 2003 and concluded that there is sufficient evidence for an association between exposure to dampness and mould and wheezing symptoms in children. Similar associations were also observed for physician-diagnosed asthma and asthma symptoms [3]. Subsequent epidemiological studies have strengthened the evidence for a positive association

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between home dampness and new-onset asthma in children aged up to 7 yrs [4]. The only meta-analysis to date [5] found a positive association between exposure to dampness or visible mould in the home and wheezing symptoms in children (OR combined estimate 1.53 (95% CI 1.39–1.68)). Recently, the World Health Organization (WHO) presented guidelines for the protection of public health from dampness- and mould-derived risks and concluded that there was sufficient epidemiological evidence that dampness and mould were associated with an increased risk of respiratory symptoms and exacerbation of asthma in children and adults [6]. However, this review was neither systematic nor were combined quantitative effect estimates given.

While the previous reviews and publications focused mainly on self- or parent-reported indoor exposure to dampness, visible mould and mould spores, there are also some recent studies which used measured mould components, such as (1,3)-β-D-glucan and extracellular polysaccharides (EPS) in house dust samples as surrogates for mould exposure [6, 7]. (1,3)-β-D-glucans are nonallergenic water-insoluble structural cell wall components of most fungi. The biological active polyglucose molecule may account for up to 60% of the weight of the fungal cell wall [7]. However, (1,3)-β-D-glucans are also part of the structure of plant materials, including pollen and cellulose, as well as of soil bacteria. Therefore, the level of mould exposure may be overestimated by using (1,3)-β-D-glucan as a surrogate. Fungal 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, longitudinal studies have shown that exposure to (1,3)-β-D-glucan and EPS was inversely associated with the development of wheezing symptoms and reported physician-diagnosed asthma in children [8–12]. In addition, one case–control study reported that elevated levels of (1,3)-β-D-glucan and EPS exposure from mattress dust were associated with a lower prevalence of allergic sensitisation in 2–4-yr-old children [13]. The mechanism of these negative associations is not yet understood. It has been hypothesised that exposure to (1,3)-β-D-glucan and EPS may have a similar impact on regulating the development of the infant immune system as does endotoxin exposure during the perinatal period.

Previous investigations (NORDDAMP, EUROEXPO, IOM and WHO) [1–3, 6] have summarised the main findings of the studies reviewed here. However, only the work of FISK *et al.* [5] also provided quantitative summaries. Furthermore, almost all of the previous reviews failed to distinguish between exposure to visible mould at home, measured mould spores and mould-derived components. Finally, several publications have been published since the inclusion deadline for the most recent meta-analysis by FISK *et al.* [5] in 2007.

There is a strong need for a comprehensive and specific review that distinguishes mould and dampness exposure into visible mould, mould spores and measured mould components. Although some investigations have also included endotoxin exposure, we concentrated on mould exposure specifically. Additionally, meta-analyses were used to quantitatively assess the exposure–response relationships. While previous reviews investigated a broad range of health outcomes in adults and children, we have restricted our analysis to children and the development of allergic diseases and symptoms. Lastly, birth cohort and cohort studies with a prospective design were given more weight than cross-sectional investigations as they can better assign the temporal sequence. To account for the different value of each epidemiological design we have presented the results according to their epidemiological study design.

METHODS

This review was conducted following the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines for meta-analyses of observational studies [14]. The literature was searched using a computerised bibliographic database, PubMed, with the free text search terms listed in table 1.

Inclusion criteria were: observational study, human study population, English, publication date between January 1, 1980 and July 1, 2010, and study population recruited from community. The review included publications that specifically assessed exposure to mould and mould-derived components for children at home. This included inspector- or subject-reported visible mould, measured airborne or dust-borne fungal genera, and measured specific biomarkers of mould species such as (1,3)-β-D-glucan and EPS within the domestic area. Exposure to dampness, and exposure to dampness or mould as well as endotoxin, were excluded from the current review to ensure a specific exposure definition. Studies that did not evaluate asthma or allergic health outcomes were also excluded. Further hand searches were conducted using citations from the previous systematic reviews [3–6] and personal files, published until July 1, 2010.

Longitudinal studies, cross-sectional studies and case–control studies were included. We restricted the health outcomes to physician-diagnosed allergic diseases including asthma, allergic rhinitis or hay fever and eczema, as well as allergic symptoms

TABLE 1 Terms used to search PubMed

1) β-glucan
2) EPS (extracellular polysaccharides)
3) *Cladosporium*
4) *Penicillium*
5) *Aspergillus*
6) *Alternaria*
7) Mould spores
8) Mould
9) Endotoxin
10) Visible mould
11) Mould components
12) Biocontaminants
13) Sensitisation
14) Allergy
15) Asthma
16) Wheezing
17) Hay fever
18) Allergic rhinitis
19) Itchy, runny, blocked nose
20) Respiratory
21) Eczema
22) Itchy skin rash
23) 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
24) 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22
25) 23 and 24

such as wheezing, itchy, blocked or running nose without having a cold, itchy skin rash and allergic sensitisation to inhalant allergens. Each relevant article underwent standardised data extraction.

Statistical analysis

In order to increase the quality of the reviewed studies, we have reported the results of a quantitative meta-analysis for the exposure–response relationships between exposure to visible mould and asthma, wheeze and allergic rhinitis. The specific risk factor and outcome definitions of each investigation included in the meta-analysis are listed in tables 2–4. To summarise the effect estimates among appropriate studies, we used random effect models to account for the heterogeneity between different studies. The results are presented as forest plots with central point estimates and confidence intervals of odds ratios, and summarise the intensity of increased risk of asthma, wheeze and allergic rhinitis with exposure to visible mould. In order to assess possible publication bias, which may lead to an overestimation of the health effects, funnel plots were performed.

Statistical analyses were performed using the statistical software R, version R 2.9.1 (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The literature search identified 1,398 peer-reviewed scientific publications, out of which 36 reported relevant exposures and health outcomes in suitable study populations (fig. 1). Hand searching of previously published reviews and personal files identified 25 additional publications. In total, 61 investigations are included in this review. The funnel plots for the quantitative assessment of the exposure–response relationship between visible mould and asthma showed a symmetric shape. However, there was a higher publication rate for studies that found positive associations between exposure to visible mould and wheeze or allergic rhinitis (see online supplement 1).

Of the 1,398 peer-reviewed scientific publications identified through PubMed, 1,366 were excluded. A large number were background papers such as comments and reviews, laboratory experimental and animal studies or genetic studies (n=727). Studies that lacked essential information about the exposure–response relationship, had objectives other than to investigate the relationship between exposure to mould and allergic health outcomes, or that examined only adult study populations were also excluded (n=622). Finally, studies that focused solely on exposure to endotoxin were not considered in this systematic review (n=17).

Results from the systematic review: birth cohort studies

The birth cohort findings are summarised in online supplement 2. Exposure to domestic visible mould increased the risk for wheezing in children. No effects were observed for allergic rhinitis and allergic sensitisation. The findings also suggested that exposure to higher levels of mould components may decrease the risk of allergic disorders.

Visible mould exposure

Wheeze

Of the nine publications evaluating the longitudinal effect of early exposure to visible mould at home and wheezing in the first 3 yrs of life, seven studies observed a significant positive association [8, 12, 36, 41, 42, 48, 49]. In one US birth cohort study (the Cincinnati Childhood Allergy and Air Pollution Study) from IOSSIFOVA *et al.* [8], the reported increase in risk was persistent from 1–3 yrs of age. BAKER *et al.* [50] observed no effect of current exposure to visible mould and wheezing at 6 months of age, and TISCHER *et al.* [30] also found no effect in children followed until 6 yrs of age from Germany and the Netherlands.

Other health outcomes

Three studies investigated the effect of exposure to visible mould on allergic rhinitis [30, 45, 46]; one study reported findings on allergic sensitisation [48] and one on physician-diagnosed asthma [30]. However, no significant associations were found.

Exposure to mould spores

Only one birth cohort study reported findings on the association between exposure to mould spores and allergic health outcomes. Exposure to certain dust-borne fungal species such as *Alternaria*, *Aspergillus*, *Aureobasidium* as well as nonsporulating genera and total dust-borne fungal mass were significantly positively associated with physician-diagnosed allergic rhinitis or hay fever at 5 yrs of age [45].

Exposure to mould components

Two European and two US birth cohort investigations studied the effect of exposure to mould components on the risk of allergic health outcomes. IOSSIFOVA *et al.* [12] reported that exposure to low levels of (1,3)-β-D-glucan from children's primary activity room was associated with a higher risk of recurrent wheeze (OR 3.04 (95% CI 1.25–7.38)) in 1-yr-old children, high levels were protective (OR 0.39 (95% CI 0.16–0.93)). However, this could not be confirmed at 3 yrs of age in the same birth cohort [8]. The two European studies did not observe an effect of exposure to (1,3)-β-D-glucan on asthma, wheezing or allergic rhinitis symptoms in school-aged children [9, 30].

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study and a follow-up of the European AirAllerg collaboration reported significant inverse effects of exposure to higher levels of EPS on asthma, wheeze and allergic rhinitis in 4–6-yr-old children. Within the PIAMA cohort, there was also an inverse effect on allergic sensitisation status [9, 30].

Results from the systematic review: cohort studies (not recruited at birth)

The cohort study findings are summarised in the online supplement 2. There was no clear exposure–response relationship between exposure to visible mould and asthma. However, the findings did suggest that domestic visible mould may have a harmful effect on wheeze. Exposure to airborne mould spores was associated with wheezing in children aged up to 1 yr.

Visible mould exposure

Asthma

Three studies investigated the relationship between exposure to visible mould and physician-diagnosed asthma, and overall results were inconclusive. Studies from the USA and Finland [21, 51] found no associations, while a second US study

TABLE 2 Risk factor and health outcome definition: visible mould and asthma

First author [ref.]	Location	Definition of exposure	Definition of outcome	Age yrs	Children n	OR central estimate (95% CI)	Type of estimate
ANTOVA [15]	Pollution and the Young Study, multiple locations	Visible mould	Asthma ever	6–12	57099 (pooled)	**1.35 (1.20–1.51)**	aOR
		Mould ever				1.36 (1.19–1.56)	aOR
		Recent mould				1.23 (1.07–1.41)	aOR
DALES [16]	Canada	No. of mould sites	DD asthma	5–8	13495		
		0 *versus* 1				1.40 (1.16–1.68)	cOR
		0 *versus* 2				**1.67 (1.27–2.19)**	cOR
DONG [17]	China	Visible mould	DD asthma (ever)	6–13	10784	1.54 (1.22–1.94)	aOR
			Current asthma			**1.69 (1.15–2.48)**	aOR
PONSONBY [18]	Tasmania, Australia	Mould in child's room i.r.	Asthma	7	6378	1.26 (0.87–1.81)	aOR
		Mould (excl. bathroom) p.r.				**1.20 (0.96–1.51)**	aOR
SPENGLER [19]	Russia	Presence of moulds	DD asthma	8–12	5951	**2.82 (1.63–4.88)**	aOR
			Asthma symptoms			1.98 (1.53–2.55)	aOR
FREEMAN [20]	USA	Any mould	DD asthma	8.1–10.9	4634	**1.54 (1.27–1.87)**	aOR
		Any mould		<6	240	**3.30 (1.57–6.97)**	aOR
BRUNEKREEF [21]	USA	Mould or mildew (age 7–11 yrs)	DD Asthma	8–12	4625	**1.27 (0.93–1.74)**	aOR
DONG [22]	China	Visible mould	DD asthma	1–6	3945	**1.56 (1.13–2.16)**	aOR
			Current asthma			1.89 (1.22–2.94)	aOR
BRUNEKREEF [23]	The Netherlands	Visible mould (1987)	Asthma	6–12	1051	**1.12 (0.39–3.38)**	aOR
		Visible mould (1989)	Asthma		3344	**1.53 (1.04–2.28)**	aOR
WARMAN [24]	USA	Visible mould on walls	DD asthma	5–11	1772	**3.26 (2.38–4.45)**	cOR
		Visible mould on walls, ceilings or windows	DD asthma			2.66 (2.04–3.48)	cOR
CHEN [25]	Taiwan	Mould patches	DD asthma	7–12	1452	**1.55 (0.78–3.09)**	aOR
			Asthma symptoms			1.56 (0.90–2.69)	aOR
LI [26]	Taiwan	Visible mould/mildew	DD asthma	8–12	1340	**1.12 (0.72–1.74)**	aOR
ZHENG [27]	China	Mould or fungi	DD Asthma	6–10	1209		
		Family ceiling				**1.8 (1.1–2.9)**	aOR
		Child's bedroom				1.8 (1.0–3.2)	aOR
MAIER [28]	USA	Visible mould	DD asthma	5–9	925	**1.3 (0.9–1.9)**	cOR
DIJKSTRA [29]	The Netherlands	Damp stains and mould	Asthma	6–12	775	**1.56 (0.50–4.87)**	cOR
TISCHER [30]	Germany and The Netherlands	Visible mould (Germany)	DD asthma	6	358	**1.03 (0.26–4.16)**	aOR
		Visible mould (The Netherlands)			332	**1.14 (0.48–2.70)**	aOR
VERHOEFF [31]	The Netherlands	Visible mould	DD Asthma ever	6–12	516		
		Living room p.r.				**2.95 (1.34–6.25)**	cOR
		Child's bedroom p.r.				1.88 (0.74–4.78)	cOR
		Living room i.r.				1.83 (0.81–4.13)	cOR
		Child's bedroom i.r.				0.99 (0.31–3.14)	cOR
DALES [32]	Canada	Ever mould/mildew	DD asthma	10	403	**0.91 (0.42–1.95)**	aOR
PEKKANEN [33]	Finland	Visible mould	DD Asthma	1–7	362	**1.24 (0.73–2.11)**	aOR
		Mould spots living room				4.01 (1.12–14.32)	aOR
		Visible mould living room				1.95 (0.69–5.47)	aOR
FAGBULE [34]	Nigeria	Mould growth	Current asthma	5.5	280	**0.48 (0.30–0.79)**	aOR
LI [35]	Taiwan	Visible mould	Asthma	7–15	46	**1.02 (0.39–2.69)**	aOR

aOR: adjusted odds ratio; DD: physician-diagnosed; cOR: crude odds ratio; p.r.: parental reported; i.r.: inspector reported. Data in bold were included within the meta-analysis.

TABLE 3 Risk factor and health outcome definition: visible mould and wheeze

First author [ref.]	Location	Definition of exposure	Definition of outcome	Age#	Children n	OR central estimate (95% CI)	Type of estimate
Antova [15]	Pollution and the Young Study, multiple locations	Visible mould	Current wheeze	6–12	57099	**1.43 (1.36–1.49)**	aOR
		Mould ever				1.44 (1.35–1.53)	
		Recent mould				1.46 (1.31–1.61)	
Dales [16]	Canada	No. of mould sites	Wheeze	5–8	13495		
		0 *versus* 1				1.42 (1.26–1.59)	cOR
		0 *versus* 2				**1.73 (1.45–2.06)**	cOR
Dong [17]	China	Visible mould	Current wheeze	6–13	10784	**1.65 (1.25–2.17)**	aOR
Spengler [19]	Russia	Presence of moulds	Wheeze	8–12	5951	**1.52 (1.19–1.94)**	aOR
Brunekreef [21]	USA	Mould/mildew (age 7–11 yrs)	Persistent wheeze (age 8–12 yrs)	8–12	4625	**1.79 (1.44–2.32)**	aOR
Emenius [36]	Sweden (BAMSE cohort)	Visible mould (age 1 yr)	Recurrent wheeze (age 2 yrs)	1–2	4089	**1.5 (1.0–2.22)**	aOR
Dong [22]	China	Visible mould	Current wheeze	1–6	3945	**2.07 (1.56–2.75)**	aOR
Brunekreef [23]	The Netherlands	Visible mould (1987)	Wheeze	6–12	1051	**1.34 (0.58–3.26)**	aOR
		Visible mould (1989)	Wheeze		3344	**1.90 (1.41–2.54)**	aOR
Li [26]	Taiwan	Dampness and mould	Wheeze	8–12	1340	**1.20 (0.73–1.99)**	aOR
Strachan [37]	UK	Mould p.r.	Wheeze	6.5–7.5	1000	**3.70 (2.22–6.15)**	cOR
		Mould i.r.			330	3.25 (1.60–6.60)	cOR
Strachan [38]	UK	Mould in bedroom	Severe wheeze	13–18	961	**1.25 (0.67–2.31)**	aOR
Maier [28]	USA	Visible mould	Wheeze	5–9	925	**1.20 (0.70–1.90)**	cPR
Alper [39]	Turkey	Dampness and mould (age 7 yrs)	Persistent wheeze (age 0–6 yrs)	0–7	858	**2.53 (1.30–4.87)**	cOR
			Early wheeze (age 0–3 yrs)			2.37 (1.52–3.69)	cOR
			Early transient wheeze			2.28 (1.34–3.87)	cOR
			Late-onset wheeze (age 3–6 yrs)			2.46 (1.29–4.66)	cOR
Dijkstra [29]	The Netherlands	Damp stains and mould	Wheeze	6–12	775	**1.54 (0.59–4.00)**	cOR
Cho [40]	USA (Cincinnati Childhood Allergy and Air Pollution Study)	Mould class 2 *versus* 0 (age 8 months)	Recurrent wheeze (age 1 yr)	8–12 months	640	**2.1 (1.2–3.6)**	aOR
Iossifova [12]	USA (Cincinnati Childhood Allergy and Air Pollution Study)	Visible mould (age 8 months)	Recurrent wheeze (age 1 yr)	8–12 months	574		
		Low *versus* none				1.18 (0.73–1.91)	aOR
		High *versus* none				4.44 (1.36–12.05)	aOR
Schroer [41]	USA (Cincinnati Childhood Allergy and Air Pollution Study)	Mould exposure (age 8 months)	Wheezing (age 1 yr)	8–24 months	570	1.22 (0.79–1.86)	aOR
			Wheezing (age 2 yrs)			2.12 (1.25–3.60)	aOR
			Persistent wheezing (age 2 yrs)			**2.47 (1.27–4.80)**	aOR
Iossifova [8]	USA (Cincinnati Childhood Allergy and Air Pollution Study)	Visible mould (age 8 months)	Wheezing with API (age 3 yrs)	8 months–3 yrs	483		
		Low *versus* none				1.68 (0.96–2.94)	aOR
		High *versus* none				**7.08 (2.22–12.60)**	aOR
Karvonen [42]	Finland (Protection Against Allergy: Study in Rural Environments)	Mould spots (age 2 months) i.r.	DD wheezing (age 1 yr)	2–12 months	396	0.99 (0.38–2.58)	aOR
			Wheezing (age 1 yr)			0.81 (0.31–2.12)	aOR
		Visible mould (age 2 months) i.r.	DD wheezing (age 1 yr)			**1.39 (0.57–3.39)**	aOR
			Wheezing (age 1 yr)			1.98 (0.90–4.35)	aOR
		Mould in kitchen (age 2 months) i.r.	DD wheezing (age 1 yr)			1.06 (0.41–2.71)	aOR
			Wheezing (age 1 yr)			1.96 (0.89–4.31)	aOR
		Mould living area (age 2 months) i.r.	DD wheezing (age 1 yr)			3.92 (1.54–10.00)	aOR
			Wheezing (age 1 yr)			1.22 (0.43–3.45)	aOR
		Mould child's room (age 2 months) i.r.	DD wheezing (age 1 yr)			5.22 (1.48–18.35)	aOR
			Wheezing (age 1 yr)			1.92 (0.48–7.60)	aOR
Rosenbaum [43]	USA (Assessment of Urban Dwellings for Indoor Toxics)	Visible mould (age 3 months) i.r.	Wheeze (age 1 yr)	3–12 months	103	**0.90 (0.35–2.29)**	cOR

aOR: adjusted odds ratio; cOR: crude odds ratio; p.r.: parental reported; i.r.: investigator reported; API: asthma predictive index; DD: physician-diagnosed. #: presented in yrs, unless otherwise stated. Data in bold were included within the meta-analysis.

TABLE 4 Risk factor and health outcome definition: visible mould and allergic rhinitis

First author [ref.]	Location	Definition of exposure	Definition of outcome	Age yrs	Children n	OR central estimate (95% CI)	Type of estimate
ANTOVA [15]	Pollution and the Young Study, multiple locations	Visible mould	Hay fever ever	6–12	57099	**1.35 (1.18–1.53)**	aOR
		Mould ever				1.48 (1.34–1.62)	aOR
		Recent mould				1.47 (1.35–1.61)	aOR
DONG [17]	China	Visible mould	DD allergic rhinitis (ever)	6–13	10784	**1.21 (0.97–1.50)**	aOR
BRUNEKREEF [21]	USA	Mould/mildew (age 7–11 yrs)	Hay fever (8–12 yrs)	7–12	4625	**1.57 (1.31–1.87)**	aOR
DONG [22]	China	Visible mould	DD allergic rhinitis	1–6	3945	**1.20 (0.72–1.99)**	aOR
IBARGOYEN-ROTETA [44]	Spain	Mould on walls (age 1 yr)	Allergic rhinoconjunctivitis (age 5–8 yrs)	1–8	3360	**1.34 (0.64–2.79)**	aOR
CHEN [25]	Taiwan	Mould patches	DD allergic rhinitis	7–12	1452	**1.48 (1.03–2.12)**	aOR
LI [26]	Taiwan	Visible mould/mildew	Allergic rhinitis symptoms	8–12	1340	**1.27 (0.96–1.68)**	aOR
BIAGINI [45]	USA (Cincinnati Childhood Allergy and Air Pollution Study)	Visible mould	Allergic rhinitis	1	495		
		Low *versus* none				1.2 (0.6–2.5)	aOR
		High *versus* none				**3.2 (0.7–14.8)**	aOR
STARK [46]	USA	Mould/mildew (age 1 yr)	DD allergic rhinitis or hay fever (age 5 yrs)	1–5	405	1.28 (0.74–2.22)	cHR
KOSKINEN [47]	Finland	Mould present	Rhinitis	⩽7	57	**8.01 (0.77–83.82)**	aOR
				7–15	147	**1.77 (0.69–4.53)**	aOR
LI [35]	Taiwan	Visible mould	Allergic rhinitis	7–15	45	**3.50 (1.00–12.34)**	aOR

aOR: adjusted odds ratio; DD: physician-diagnosed; cHR: crude hazard ratio. Data in bold were included within the meta-analysis.

FIGURE 1. Flow chart of the study selection process.

reported that current exposure to mildew was significantly inversely related to physician-diagnosed asthma at 12 yrs of age [52]. However, this was only found for children with a wheezing phenotype.

Wheeze

Three cohort studies, all from the USA, investigated the effect of visible mould exposure on wheezing in children. BRUNEKREEF *et al.* [21] reported a significantly positive association between domestic mould and persistent wheeze in 12-yr-old children. BELANGER *et al.* [53] observed an increased risk among 1-yr-old children who were genetically predisposed to allergic diseases, while a second study found no association among children in the same age range (although this may have been due to inadequate power as the study included only 103 children) [43].

Other health outcomes

Reported visible mould during pregnancy was a risk factor for physician-diagnosed atopic eczema in 2–9-month-old Japanese infants without parental allergy [54]. A US study of 12-yr-old schoolchildren reported a significant increased risk of hay fever when exposed to self-reported domestic mould [21].

Exposure to mould spores

Three US studies investigated the relationship between exposure to airborne mould spores and wheezing in 1-yr-old children. GENT *et al.* [55] and ROSENBAUM *et al.* [43] reported an increased risk of wheeze in 1-yr-old infants, when exposed to airborne *Penicillium* ($\geqslant$1,000 and 120–1270 cfu·m^{-3}, respectively). A subsequent US study on infants found a positive association between exposure to airborne total fungi sampled at 3 months and wheeze at 1 yr [53]. A German cohort study found an increased risk for sensitisation against grass (immunoglobulin E) in 3-yr-old children when exposed to airborne *Aspergillus* genera [56].

Results from the systematic review: case–control studies

There was no clear direction observed for the effect of visible mould or mould spores on measured allergic health outcomes among studies with a case–control design (online supplement 2, case–control studies). However, in studies with a larger sample size, there was a tendency for an increased risk of

asthma when exposed to domestic visible mould. In contrast, the findings suggested that mould component exposure was inversely associated with risk of allergic health outcomes.

Visible mould exposure

Asthma

Five case–control studies investigated the relationship between exposure to visible mould and asthma. One study from China with 1,209 subjects [27], and two studies from Europe [31, 33] reported an increased risk of physician-diagnosed asthma with exposure to visible mould, in children up to school age. This association could not be confirmed by LI and HSU [35] in a small population of 46 Taiwanese school children. A Nigerian case–control study of 5-yr-old schoolchildren reported protective effects on current asthma for mould growth at home [34].

Other health outcomes

Two European studies investigated the effect of visible mould exposure on wheezing [36, 38], but no association was observed. A study of 3-yr-old children from New Zealand also found no association between visible mould exposure and atopic dermatitis [57]. One small study from Taiwan reported a significant increased risk for allergic rhinitis in school-aged children [35].

Exposure to mould spores

Asthma

Four studies investigated the effect of mould spore exposure on physician-diagnosed asthma among children. One small study from Taiwan reported positive associations between exposure to airborne *Cladosporium* and asthma in school-aged children [35]. However, three publications from Europe could not find an association between higher levels of dust-borne fungal species and asthma [58–60].

Allergic rhinitis

One European study from Germany [58] with 272 subjects, reported a higher risk of allergic rhinitis symptoms with exposure to total fungi, *Cladosporium* and *Penicillium* (>200,000, >35,000 and >55,000 cfu·g^{-1}, respectively). A similar finding was observed in a Danish cohort: children sensitised to house dust mites had a significantly higher risk of allergic rhinitis when exposed to dust-borne *Cladosporium* >35 cfu·mg^{-1} [59]. In contrast, higher levels of airborne *Penicillium* and total fungi measured in summer, were found to be protective against allergic rhinitis in a small Taiwanese study of school-aged children [35].

Other health outcomes

Two studies investigated the effect of dust-borne mould spore exposure on physician-diagnosed eczema and eczema symptoms. While there was no association observed within the German population [58], there was an increased risk for eczema in Swedish children sensitised to house dust mites but not to aeroallergens [59]. Two German studies looked at the association between exposure to domestic mould spores and the risk of allergic sensitisation to inhalant allergens (immunoglobulin E). While JOVANOVIC *et al.* [61] found no association, JACOB *et al.* [58] reported a higher risk of sensitisation against inhalant allergens when exposed to *Cladosporium* and *Aspergillus* (>35,000 and 0–25,000 cfu·g^{-1} and above, respectively). No association was found between exposure to dust-borne mould genera and wheezing phenotype in a German study [58].

Mould components exposure

Three European studies investigated the effect of mould component exposure on allergic disorders. Exposure to EPS was found to significantly reduce the risk of physician-diagnosed asthma [62] and atopic wheeze [10], while exposure to (1,3)-β-D-glucan was significantly inversely related to sensitisation against inhalant allergens among 2–4-yr-old children [13].

Results from the systematic review: cross-sectional studies

A large number of cross-sectional studies reported increased risk of asthma and wheeze when exposed to domestic visible mould. However, the results for other allergic health outcomes such as allergic rhinitis, atopic eczema and atopic sensitisation were less conclusive (online supplement 2, cross-sectional studies). Only two investigations considered the effects of mould component exposure, and they suggested that higher levels of EPS might decrease the risk of allergic health outcomes in children.

Visible mould exposure

Asthma

A total of 30 cross-sectional studies were included, out of which 15 investigated the effect of exposure to visible mould on asthma in school-aged children. There were 10 studies [15–20, 22–24, 63] with sample sizes above 1,500 subjects. Out of these, nine observed a significantly increased risk of asthma. A study from Tasmania, Australia did not observe an association [18], but this may have been due to the young age of the children (7 yrs). No association was found in the remaining studies, which had smaller sample sizes (403–2,720) [25, 26, 28, 29, 32].

Wheeze

The picture with wheeze was similar to that with asthma: nine out of 15 cross-sectional studies found that exposure to mould at home was associated with a higher risk of wheeze in children. This was especially true among studies with a larger sample size [15–17, 19, 22, 23, 37, 39, 64]. However, five studies did not find any association [26, 28, 29, 65, 66]. In one Spanish study, an increased risk of wheezing was observed only in nonatopic schoolchildren [67].

Allergic rhinitis

Two out of eight studies investigated the relationship between visible mould exposure and allergic rhinitis and observed positive associations. The Pollution and the Young (PATY) study reported a significantly increased risk for hay fever in 6–12-yr-old children when exposed to visible mould at home [15]. Two Asian studies from Singapore also reported higher risks for rhinitis and rhinoconjunctivitis among 1–12-yr-old children [25, 65]. The remaining six studies did not observe any statistically significant exposure–response relationships [17, 22, 26, 44, 47].

Atopic eczema

Four studies investigated the relationship between exposure to visible mould and atopic eczema. One German study by SCHÄFER *et al.* [68] observed a significantly increased risk of atopic eczema in a sample of 6-yr-old children. However, no association was observed for the remaining three studies [25, 47, 65].

Atopic sensitisation

Two investigations examined the association between domestic visible mould exposure and atopic sensitisation in school-aged children. ANTOVA *et al.* [15] observed an increased risk of sensitisation against inhalant allergens in a pooled analysis of >58,000 children. In a smaller German study of 1,235 children, exposure to visible mould was found to increase the risk of sensitisation against mugwort, dust mites and cat (assessed by skin-prick test) among 5–7-yr-old children [68].

Exposure to mould spores

Allergic rhinitis

There were only two cross-sectional studies that investigated the effect of mould spores on the risk of allergic health outcomes in childhood. A small study from Australia reported that exposure to airborne *Penicillium* and airborne *Cladosporium* was significantly positively associated with asthma and wheeze, respectively [69]. Exposure to airborne *Penicillium* was significantly related to sensitisation (skin-prick test) to *Penicillium* mix, *Aspergillus* mix, house dust and dog dander. Higher levels of airborne *Cladosporium* were also associated with sensitisation to *Aspergillus* mix and exposure to airborne *Aspergillus* was suggested to increase the risk for sensitisation against inhalant allergens [69]. SALO *et al.* [70] could not find any association between dust-borne *Alternaria alternata* and physician-diagnosed asthma.

Exposure to mould components

There were only two investigations of one cross-sectional study in Germany, Austria, Switzerland and The Netherlands (the Prevention of Allergy–Risk Factors for Sensitization Related to Farming and Anthroposophic Lifestyle study). KARADAG *et al.* [71] and colleagues found that exposure to EPS from children's mattresses was negatively associated with physician-diagnosed eczema but not with eczema symptoms. In the second investigation by EGE *et al.* [72], EPS was found to significantly decrease the risk of asthma ever and current wheeze. However, there was no effect on atopic sensitisation against inhalant and food allergens. The associations were less conclusive for exposure to (1,3)-β-D-glucan. KARADAG *et al.* [71] found an association with decreased risk of atopic eczema symptoms.

Results of the meta-analysis for the association between visible mould exposure and asthma, wheeze and allergic rhinitis

A total of 21, 19 and 10 publications of different study designs on exposure to domestic visible mould in relation to asthma, wheeze and allergic rhinitis health outcomes, respectively, were included in the meta-analysis. The summary estimates illustrate that exposure to visible mould at home was significantly positively associated with asthma, wheeze and allergic rhinitis (OR 1.49 (95% CI 1.28–1.72), OR 1.68 (95% CI 1.48–1.90) and OR 1.39 (95% CI 1.28–1.51), respectively). Forest-plots in figure 2 illustrate the odds ratios with their 95% confidence intervals and provide a summary estimate for the association between the investigated exposure–response relationships.

Owing to the limited number of studies that investigated the relationship between exposure to mould-derived components and allergic health outcomes, it was not possible to aggregate the results to perform a meta-analysis.

DISCUSSION

This systematic review included 61 publications. The most commonly reported health outcomes were asthma, wheeze and allergic rhinitis. There was a statistically significant increased risk of asthma (OR 1.49 (95% CI 1.28–1.72)), wheeze (OR 1.68 (95% CI 1.48–1.90)) and allergic rhinitis (OR 1.39 (95% CI 1.28–1.51)) in children when exposed to visible mould. There were fewer studies on exposure to airborne, dust-borne mould spores or measured mould components. While mould spore exposure was found to increase the risk for asthma and wheeze in children at a younger age, our review suggests, however, that mould components such as (1,3)-β-D-glucans and EPS do not increase the risk for allergic health outcomes.

This systematic review on the health impact of visible mould, mould spores and mould-derived components in children provided a comprehensive overview on the literature over the past 30 yrs, which, for the first time, is combined with a quantitative assessment of the reviewed studies. The only previous meta-analysis to examine the health effects of dampness and mould exposure was by FISK *et al.* [5], which reported a significant positive association with wheezing symptoms in children and adults. These prior findings are consistent with those of the present meta-analysis. However, we aimed to go beyond the work of FISK *et al.* [5] and specifically addressed issues such as specificity of exposure, study design, study population, validation criteria and validity of exposure assessment.

Studies after the inclusion deadline for the meta-analysis of FISK et al. [5]

A number of studies were considered here that had not been published in time to be included in the previous meta-analysis of FISK *et al.* [5]. For the association between exposure to mould and wheeze, there were 19 additional publications and for asthma there were 17 additional studies. Furthermore, we identified studies published before 2006 that were not part of the meta-analysis of FISK *et al.* [5], supporting the application of a systematic approach. FISK *et al.* [5] looked at the association between dampness and mould exposure in relation to a number of different upper respiratory tract symptoms, whereas we focused on (physician-diagnosed) allergic rhinitis exclusively. Although we constrained exposure and health outcome definition and limited the analysis to children only, we did reach a higher number of publications than the meta-analysis in 2007 [5].

Specification of type of exposure

Previous investigations, including the work from FISK *et al.* [5], have often assessed dampness, water leakage, mould, mould spores, mould odour and mould-derived exposure as a common exposure type. In order to specify the type of exposure, we defined three different kinds of mould exposure to account for conflicting study results in the past: domestic visible mould, measured airborne fungal species and measured mould-derived components assessed by house dust sampling. While there is a good correlation suggested between visible mould exposure and the concentration of fungal spores [73], recent literature indicated that exposure to mould-derived

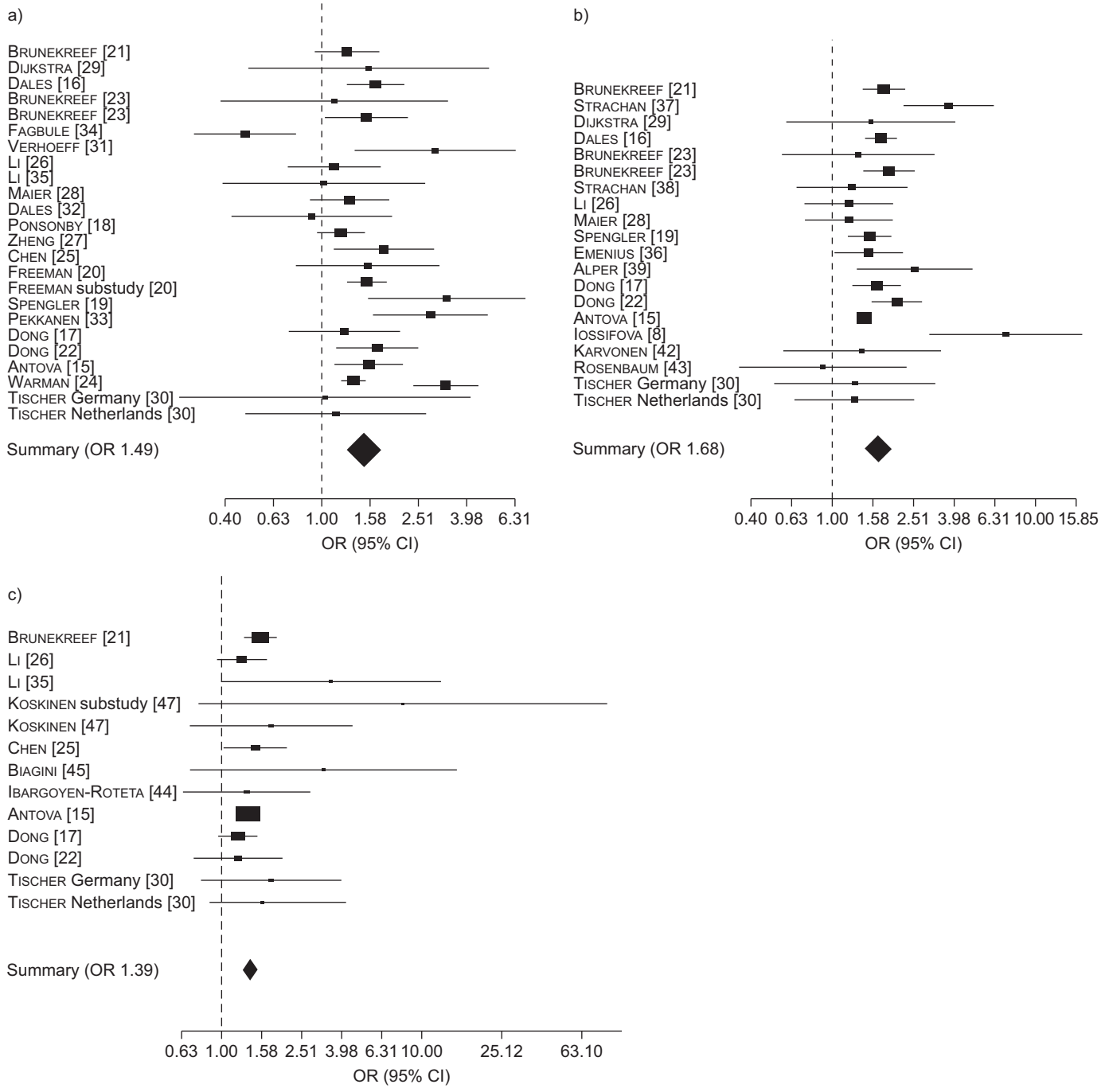

FIGURE 2. Odds ratios and 95% confidence intervals for the association between visible mould and a) asthma, b) wheeze and c) allergic rhinitis from original studies and from a meta-analysis (combined effect) performed using the random effects model. For each study, the size of the box is proportional to the precision (inverse of variance) of the study. The combined estimate from the meta-analysis is indicated by the diamond-shaped box (labelled ''Summary'') at the bottom of the figure.

components might have a different impact on children's health and may not measure a single kind of exposure. This hypothesis was supported by a US cohort study that did not find a correlation between (1,3)-β-D-glucan exposure and visible mould [8, 12]. This might be partly due to the fact that (1,3)-β-D-glucan is 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)-β-D-glucan as a surrogate [7]. EPS are stable carbohydrates secreted or shed during fungal growth and have antigenic specificity at the genus level but cannot represent exposures to all of the fungal species in an indoor environment. Furthermore, it has been suggested that mould-derived components such as (1,3)-β-D-glucan or EPS can protect children from developing allergic disorders, as shown in recent longitudinal investigations [8, 9, 12]. A protective tendency of mould-derived components on allergic diseases was also confirmed by this study. It has been proposed that

early exposure to indoor microbial elements may have strong immune-stimulatory properties, as has been suggested for endotoxin in several studies [74–76]. The present review revealed that there are still not enough data on exposure to mould-derived components to perform combined analyses, which would be required to make a more definite statement on the impact of exposure to these components.

Study design

Compared to FISK *et al.* [5], we further addressed different types of study design. Nearly half (41%) of the publications included in this review were cross-sectional studies, and a considerable proportion of these had large sample sizes. In contrast, the proportion of cohort studies and case–control studies is lower (14 and 23%, respectively) and with considerably fewer study subjects. Compared with cross-sectional studies, it was not possible to determine a clear direction of the investigated exposure–response relationships, which might be partly due to lack of power within the original studies. Nevertheless, birth cohort studies and cohort studies not recruited at birth might be given more weight as they can better assign the temporal sequence and presumably the important perinatal exposure window. However, due to the limited number of (birth) cohort studies and short follow-up time, we were not able to quantify them separately in a meta-analysis. In future, combined investigations focused on longitudinal studies exclusively may be able to assess causality over a longer time period. This is currently ongoing in the frame of the Environmental Health Risks in European Birth Cohorts initiative (www.enrieco.org).

Study population

In contrast to previous investigations, the current review focused on studies in children only, as it is suggested that the exposure–response relationship alters with ageing; and the development of allergic diseases and symptoms occurs during early childhood. Furthermore, the incidence of allergic diseases in adults may be provoked by different triggers, for example due to occupational exposure and causing nonallergic rather than allergic responses [76].

Validation criteria

The interpretation of the results from this review is based on systematic, validated criteria in terms of the search for eligible publications and also interpretation and analysis. We performed a reasonable and replicable systematic search using the electronic database PubMed in order to make the process transparent. Until now, there has been no review on the association between mould exposure and allergic health outcomes in children according to systematic search criteria. In addition to the meta-analysis on mould exposure and allergic health outcomes, we evaluated the results of the systematic review according to the Bradford Hill criteria for assessing evidence of causation [77], which are discussed in detail later on.

Validity of exposure assessment

Visible mould exposure at home was assessed mainly by questionnaire. Although this method is convenient and favourable, questionnaire-based methods are difficult to validate against microbial measurements [40, 48]. Numerous studies validated self-reported visible mould questions against inspector-reported observations [36, 31, 78–81] and did not find any evidence for over- or underreporting of dampness and mould by occupants. Further, against the backdrop of fungal diversity, it is not clear whether the obviously visible mould or unknown, invisible species contribute to the observed effects in children's health [69, 82]. The most ideal exposure assessment for exposure to mould or mould-derived components would be repeated sample collections through a mobile personal air sampler. However, individual biological measurements are costly and therefore usually not feasible, especially in larger (birth) cohort studies. Some studies collected fungal species or mould-derived components by means of settled house dust or air samples. While these methods are generally considered more standardised, in that they follow a protocol and reduce the risk of systematic biases such as reverse causation compared with questionnaire-based methods, there are some shortcomings. To begin with, sampling methods vary considerably between the studies. Dust sampling from floors or mattresses using a vacuum cleaner provides a crude mixture of different particle sizes [10, 83, 84], but some of the dust fraction may never become airborne and might not have an effect on children's health. Hence some investigations sampled specific airborne dust fraction in domestic environments [43, 46, 53, 55, 56, 61]. However, this requires considerable time and cost resources. Recently, new exposure assessment methods have been developed. Passive airborne sampling (''pizza box''), electrostatic dust-fall collector or electrostatic dust clothes [85–87] can be used for a broad range of allergen measurements. These newly developed exposure assessment methods might be a valuable substitute for existing methods in terms of cost and work.

In addition to the meta-analysis on mould exposure and allergic health outcomes, we evaluated the results of the systematic review according to the Bradford Hill criteria [77]. Epidemiological studies typically examine associations between exposure and health outcomes, while the Bradford Hill criteria are suggested to assess the causal nature of an observed association on the basis of nine categories [88, 89]. These nine criteria should not be used as a checklist, but instead highlight important aspects of an investigation. According to these criteria, the evaluation supports the findings of the meta-analysis, especially with regard to aspects such as strength of association, temporal relationship, biological gradient, plausibility and coherence. Further research is needed to examine exposure specification against the backdrop of microbial diversity in indoor environments (see online supplement 3 for an extensive description).

Limitations

The timing of health outcome assessment is crucial in epidemiological studies. Some birth cohorts included in this review were too young to classify wheezing symptoms into transient, persistent and late-onset wheezing. Five out of six studies had an age range of 6 months to 3 yrs. There might be children having transient symptoms of asthma at an early age, but who are not a risk of developing clinical symptoms later during childhood. However, the follow-up time was too short to allow monitoring of disease development over a long period. Therefore, findings from birth cohort studies at younger ages should be interpreted with caution and the results may be of a short-term rather than a long-term character.

Although we specified mould exposure in terms of three different exposure sources, namely visible mould, airborne or dust-borne measurement of mould spores, and measured mould components from settled house dust, a clear assignment to the observed health effects is difficult. While there is a good correlation suggested between visible mould exposure and the concentration of fungal spores [73], exposure to mould-derived components might have different impacts on children's health. Indoor environments consist of a variety of indoor and outdoor sources, not only the measured ones. Visible mould or measured mould spore and mould-derived component exposure might only partly represent the actual microbial pollution at home. A recent study on predictors of bacterial and fungal biomarkers in house dust concluded that home characteristics such as dampness or visible mould explain variation in microbial exposure levels only partially [90]. Moreover, a study from Finland indicated that a considerable part of the measured microbial pollution from mattresses is human-derived (up to 88%) rather than from environmental sources, and varies in addition to that from other sampling locations [91]. Therefore, to draw a causal relationship is complicated by the variability of microbial biomarkers and their suspected effects on children's health. In conclusion, further research measuring specific biomarkers in the home should be emphasised.

Conclusion

The reviewed studies on visible mould exposure indicated an increased risk for allergic respiratory symptoms in children. These findings were confirmed by the meta-analysis results; exposure to visible mould was significantly associated with a higher risk of allergic respiratory disorders including asthma, wheezing and allergic rhinitis in children. Furthermore, the results of this meta-analysis are consistent with the evaluation of causation according to the Bradford Hill criteria. In order to disentangle the different effects of overall microbial exposure in children's health, research on specific microbial markers in the home, in combination with new assessment techniques such as recently developed molecular methods, should be followed. In this context, more weight needs to be given to studies with longitudinal design as they can better assign the temporal sequence; especially studies with a long follow-up and multiple time-point measurements to account for the variation of the complex microbial milieu over time.

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STATEMENT OF INTEREST

None declared.

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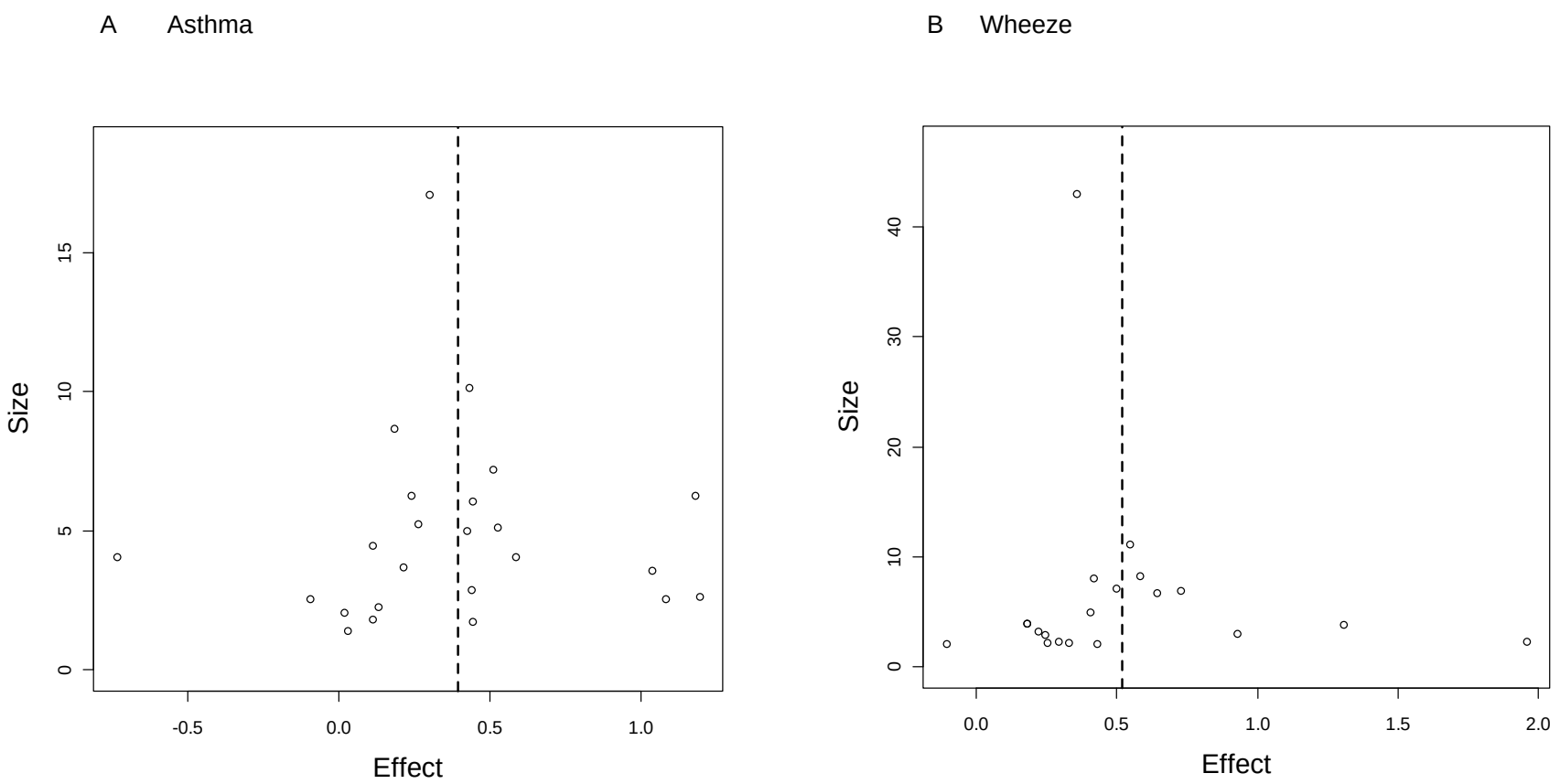
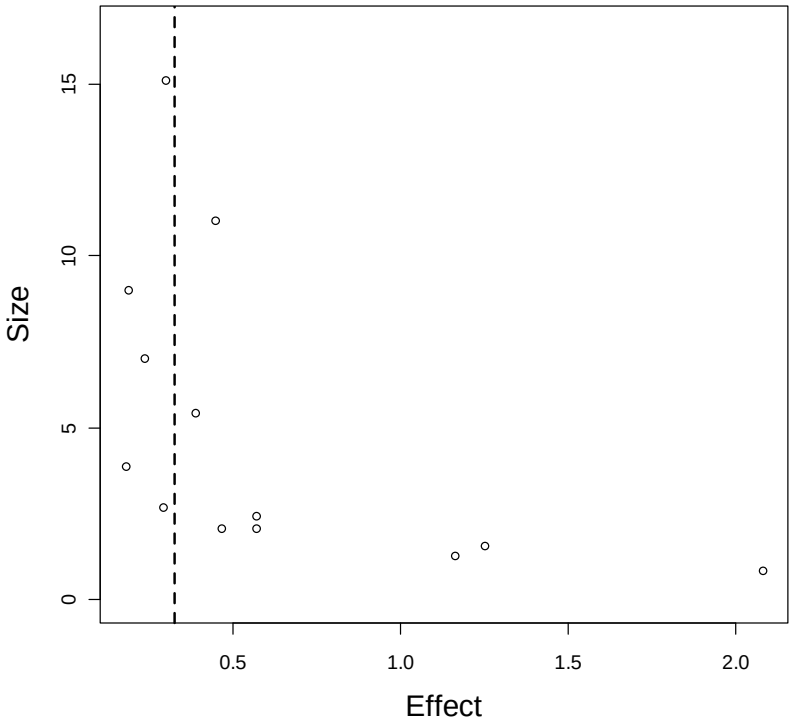
SUPPLEMENTARY MATERIAL

1) Funnel plots



Online Supplement 1: Funnel plots to check the existence of publication bias in meta-analyses. The vertical dashed line represents the mean of all study results. A symmetric shape suggests a balanced study publication which displays the study results with a natural statistical variance. An asymmetric shape indicates a publication bias which means that there are some not-published studies with no or contradictory results.

C Allergic Rhinitis



SUPPLEMENTARY MATERIAL

2) Results of the systematic review by epidemiological study design

BIRTH COHORT STUDIES

VISIBLE MOULD EXPOSURE								
Author, Year, Country (Study Acronym)	**Design**	**N**	**Exposure & time (age)**	**Outcome & time (age) (in odds ratios unless indicated otherwise)**	**Confounders adjusted/ Other factors in the multivariate model**	**Stratified analysis**	**Significance level for respiratory symptoms & diseases**	**Significance level for sensitization**
Emenius et al., 2004, Sweden (BAMSE)	Population-based	4089	Visible mould (1y) (inspector reported)	(Q) Recurrent wheezing (2y): 1.5(1.0-2.2)	Gender, parental allergy, maternal smoking, breast feeding, building age		+	
Baker and Henderson, 1999, U.K. (ALSPAC)	Population-based	1954	Visible mould (6 m) (parental-reported)	(Q) Wheeze (6m): n.s. **cOR**			n.s.	
Cho et al., 2006, U.S. (CCAAPS)	Population-based (enriched*)	640	Mould class 2 vs. 0 (8 m) (parental-reported)	(Q) Recurrent wheeze (1y): 2.1(1.2-3.6) **RR** (Q) Recurrent wheeze with SPT(+, any) (1y): 4.7(2.1-10.5) **RR** (Q) Recurrent wheeze with SPT(+, aero) (1y): 6.0(2.2-14.2) **RR** (Q) Recurrent wheeze with SPT(+, mould) (1y): 0.6(0.1-4.0) **RR** Sensitization aeroallergens (SPT) (1y): 1.6(0.9-3.0) **RR**	Mould class, house dust mite, income		+ + + n.s.	n.s.
Iossifova et al., 2007, U.S. (CCAAPS)	Population-based (enriched*)	574	Visible mould (8 m) (parental-reported) low vs. none high vs. none Visible mould (8 m) (parental-reported) low vs. none high vs. none Visible mould (8 m) (parental-reported) low vs. none high vs. none	(Q) Recurrent wheeze (1y): 1.18(0.73-1.91) 4.44(1.36-12.05) (Q) Recurrent wheeze with SPT(+, any) vs. no wheeze with SPT(-) (1y): 1.29(0.57-2.90) 9.51(2.34-38.63) (Q) Recurrent wheeze with SPT(+, any) vs. no wheeze with SPT(+) (1y): 2.64(0.89-7.86) 42.47(4.70-384.14)	Race, number of siblings, parental asthma, maternal smoking, lower respiratory tract condition, upper respiratory tract condition, visible mould, (1,3)-ß-D-glucan, endotoxin		 n.s. + n.s. + n.s. +	
Schroer et al., 2009, U.S. (CCAAPS)	Population-based (enriched*)	570	Mould exposure (8 m) (parental-reported)	(Q) Wheezing (1y): 1.22(0.79-1.86) (Q) Wheezing (2y): 2.12(1.25-3.60) (Q) Persistent wheezing (2y): 2.47(1.27-4.80)	Gender, daycare attendance, genotype, race, DEP, ETS, mould		n.s. + +	
Biagini et al.,	Population-	495	Visible mould (1y)	(Q) Allergic rhinitis (1y): 1.2(0.6-2.5)	Gender, maternal		n.s.	

2006, U.S. (CCAAPS)	based (enriched*)		(parental-reported) low vs. none	(Q) Rhinitis (1y): 1.1(0.8-1.6)	education, cat and dog ownership, daycare	n.s.
			high vs. none	(Q) Allergic rhinitis (1y): 3.2(0.7-14.8)	attendance, breast	n.s.
				(Q) Rhinitis (1y): 1.7(0.7-3.8)	feeding and number of diaries returned	n.s.
Iossifova et al., 2009, U.S. (CCAAPS)	Population-based (enriched*)	483	Visible mould (8 m) (parental-reported) low vs. none	(Q) Wheezing with SPT(+, any) (3y): 1.86(0.86-4.00)	Race, number of siblings, maternal smoking, lower respiratory tract symptoms, upper respiratory tract symptoms, visible mould, (1,3)-ß-D-glucan, endotoxin	n.s.
			high vs. none	6.16(1.38-27.44)		+
			Visible mould (8 m) (parental-reported) low vs. none	(Q) Wheezing with API=1 (Asthma Predictive Index) (3y): 1.68(0.96-2.94)		n.s.
			high vs. none	7.08(2.22-12.60)		+
Stark et al., 2005, U.S.	Population-based	405	Mould/mildew (1y) (parental-reported)	(Q) Physician-diagnosed allergic rhinitis or hay fever (at 5y): 1.28(0.74-2.22) **HR** (crude)		n.s.
Karvonen et al., 2009, Finland (PASTURE)	Population-based	396	Mould spots indoor (2m) (inspector reported)	(Q) Physician-diagnosed wheezing (1y): 0.99(0.38-2.58)	Gender, siblings, maternal education, cat and/or dog ownership, maternal smoking during pregnancy, parental allergy, study cohort, place of residence	n.s.
				(Q) Wheezing apart from cold (1y): 0.81(0.31-2.12)		n.s.
				(Q) Nocturnal cough apart from cold (1y): 0.74(0.30-1.85)		n.s.
			Visible mould indoor (2m) (inspector reported)	(Q) Physician-diagnosed wheezing (1y): 1.39(0.57-3.39)		n.s.
				(Q) Wheezing apart from cold (1y): 1.98(0.90-4.35)		n.s.
				(Q) Nocturnal cough apart from cold (1y): 1.13(0.51-2.53)		n.s.
			Mould in the kitchen (2m) (inspector reported)	(Q) Physician-diagnosed wheezing (1y): 1.06(0.41-2.71)		n.s.
				(Q) Wheezing apart from cold (1y): 1.96(0.89-4.31)		n.s.
				(Q) Nocturnal cough apart from cold (1y): 0.94(0.40-2.21)		n.s.
			Mould in the main living area (2m): (inspector reported)	(Q) Physician-diagnosed wheezing (1y): 3.92(1.54-10.00)		+
				(Q) Wheezing apart from cold (1y): 1.22(0.43-3.45)		n.s.
				(Q) Nocturnal cough apart from cold (1y): 1.73(0.69-4.30)		n.s.
				(Q) Physician-diagnosed wheezing (1y):		

				5.22(1.48-18.35)		+
			Mould in child's bedroom (2m) (inspector reported)	(Q) Wheezing apart from cold (1y): 1.92(0.48-7.60)		n.s.
				(Q) Nocturnal cough apart from cold (1y): 1.17(0.30-4.65)		n.s.
Tischer et al., 2010, Germany & the Netherlands (AirAllerg)	Population-based (enriched*)	358 D 332 N	Visible mould (6y)	(Q) Physisician-diagnosed astma: GERMANY: 1.03(0.26-4.16)	Sex, parental allergy, parental education, outdoor activity (hours), breastfeeding, maternal smoking during pregnancy, current ETS, pets at home, AirAllerg case status	n.s.
				NETHERLANDS: 1.14(0.48-2.70)		n.s.
				(Q) Wheezing (6y): GERMANY: 1.29(0.52-3.21)		n.s.
				NETHERLANDS: 1.28(0.65-2.49)		n.s.
				(Q) Physician-diagnosed allergic rhinitis (6y): GERMANY: 1.77(0.79-3.99)		n.s.
				NETHERLANDS: 1.60(0.62-4.14)		n.s.
				(Q) Rhinoconjunctivitis (6y): GERMANY: 1.36 (0.56-3.26)		n.s.
				NETHERLANDS: 0.58 (0.22-1.53)		n.s.
Jedrychowski et al., 2007, Poland	Population-based (enriched*)	275	Indoor moulds (3y) (parental-reported)	(Q) Wheezing episodes (over 6 months) (3y): 3.22(1.37-7.54) **IRR**	Sex, maternal allergy, maternal education, older siblings, ETS, HDM-levels, house dampness	+
MOULD SPORES EXPOSURE						
Stark et al., 2005, U.S.	Population-based	405	Air-borne (child's bed room) (3 m) **[cfu/m^3]**	(Q) Physician-diagnosed allergic rhinitis or hay fever (at 5y): **Survival analysis (HR)**	Water damage or mould/mildew in year 1, African-Amercian ethnicity, maternal Alternaria IgE > 0.35 U/mL, sex, birth date in fall	
			Aspergillus Cladosporium Nonsporulating	1.10(0.43.2.80)		n.s.
			Penicillium	1.25(0.43-3.64)		n.s.
			Yeasts	0.55(0.17-1.81)		n.s.
			Total airborne	0.69(0.23-2.06)		n.s.
				0.79(0.24-2.60)		n.s.
			Dust-borne (child's bed room) (3 m) **[cfu/m^3]**	0.83(0.28-2.43)		n.s.
			Alternaria Aspergillus Aureobasidium	2.34(1.12-4.91)		+
			Cladosporium	2.57(1.22-5.40)		+
			Coelomyces	3.12(1.50-6.50)		+
			Fusarium	1.88(0.81-4.35)		n.s.
			Nonsporulating	0.93(0.36-2.38)		n.s.
			Penicillium	1.81(0.76-4.34)		n.s.
			Ulocladium	2.45(1.15-5.22)		+
			Wallemia	1.51(0.63-3.64)		n.s.
			Yeasts	1.04(0.37-2.95)		n.s.
			Zygomycetes	1.73(0.80-3.75)		n.s.

			Total dust-borne	2.90(1.37-6.09)		+
				0.87(0.31-2.44)		n.s.
			Dust-borne *Alternaria*	3.13(1.51-6.47)	Water damage or	+
			Dust-borne *Aspergillus*	**Cox regression (RR)**	mould/mildew in	
			Dust-borne	1.40(0.61-3.23)	year 1, African-	n.s.
			Aureobasidium	3.27(1.50-7.14)	Amercian ethnicity,	+
			Dust-borne yeasts	3.04(1.33-6.93)	maternal Alternaria	+
				2.67(1.26-5.66)	IgE > 0.35 U/mL,	+
					sex, birth date in fall, any lower respiratory infection in year	
MOULD COMPONENTS EXPOSURE						
Douwes et al., 2006, The Netherlands (PIAMA)	Population-based (enriched*)	696	(1,3)-ß-D-glucan from living-room floor (3 m) (settled house dust) **[µg / m^2]**	(Q) Physician-diagnosed asthma (4y):	Gender, region,	
				Medium exposure: 0.63(0.27-1.48)	parental	n.s.
				High exposure: 0.70(0.30-1.60)	education, ETS,	n.s.
				(Q) Current wheeze (4y):	other children in	
				Medium exposure: 1.50(0.77-2.94)	the household	n.s.
				High exposure: 0.76(0.34-1.72)		n.s.
				(Q) Early transient wheeze (0-4y):		
				Medium exposure: 0.89(0.46-1.71)		n.s.
				High exposure: 0.57(0.28-1.16)		n.s.
				(Q) Persistent wheeze (0-4y):		
				Medium exposure: 1.16(0.52-2.62)		n.s.
				High exposure: 0.43(0.15-1.21)		n.s.
				(Q) Physician-diagnosed asthma (4y):		
				Medium exposure: 0.85(0.32-2.27)	Gender, region,	n.s.
				High exposure: 2.22(0.55-8.97)	parental	n.s.
					education, ETS, other children in the household, endotoxin, EPS, Total dust	
			EPS from living-room floor (3 m) (settled house dust) **[EPSU / m^2]**	(Q) Physician-diagnosed asthma (4y):		
				Medium exposure: 0.78(0.40-1.55)		n.s.
				High exposure: 0.42(0.18-0.99)	Gender, region,	-
				(Q) Current wheeze (4y):	parental	
				Medium exposure: 1.28(0.70-2.32)	education, ETS,	n.s.
				High exposure: 0.63(0.30-1.32)	other children in	n.s.
				(Q) Early transient wheeze (0-4y):	the household	
				Medium exposure: 0.99(0.56-1.76)		n.s.
				High exposure: 0.67(0.36-1.23)		n.s.
				(Q) Persistent wheeze (0-4y):		
				Medium exposure: 1.07(0.53-2.16)		n.s.
				High exposure: 0.37(0.15-0.96)		-
				(Q) Physician-diagnosed asthma (4y):		

				Medium exposure: 1.19(0.50-2.82) High exposure: 0.39(0.10-1.59) Sensitisation inhalant allergens (IgE): 0.40(0.18-0.91)	Gender, region, parental education, ETS, other children in the household, (1,3)-ß-D-glucan, endotoxin, Total dust	n.s. n.s. -	
Iossifova et al., 2007, U.S. (CCAAPS)	Population-based (enriched*)	574	(1,3)-ß-D-glucan from children's primary activity room (8 m) (settled house dust) **[µg/g]**	(Q) Recurrent wheeze (1y): 1st quartile (3-22 µg/g): 3.04(1.25-7.38) 2nd quartile (23-60 µg/g): 1.29(0.99-1.67) 3rd quartile (61-133 µg/g): 0.82(0.65-1.05) 4th quartile (134-900 µg/g): 0.39(0.16-0.93)	Race, number of siblings, parental asthma, maternal smoking, lower respiratory tract condition, upper respiratory tract condition, visible mould, (1,3)-ß-D-glucan, endotoxin	 + n.s. n.s. -	
			(1,3)-ß-D-glucan from children's primary activity room (8 m) **[µg/g]** (settled house dust) **[µg/g]**	(Q) Recurrent wheeze with SPT(+, any) vs. no wheeze with SPT(-) (1y): 1st quartile (3-22 µg/g): 4.89(1.02-23.57) 2nd quartile (23-60 µg/g): 1.23(0.79-1.92) 3rd quartile (61-133 µg/g): 0.59(0.38-0.92) 4th quartile (134-900 µg/g): 0.13(0.03-0.61)		 + n.s. - -	
			(1,3)-ß-D-glucan from children's primary activity room (8 m) (settled house dust) **[µg/g]**	(Q) Recurrent wheeze with SPT(+, any) vs. no wheeze with SPT(+) (1y): 1st quartile (3-22 µg/g): 160.51(4.85-5311.00) 2nd quartile (23-60 µg/g): 2.54(0.97-6.62) 3rd quartile (61-133 µg/g): 0.17(0.05-0.57) 4th quartile (134-900 µg/g): 0.00(0.00-0.07)		 + n.s. - -	
Iossifova et al., 2009, U.S. (CCAAPS)	Population-based (enriched*)	483	(1,3)-ß-D-glucan from children's primary activity room (8 m) (settled house dust) **[µg/g^e]**	(Q) Wheezing with SPT(+, any) (3y): 1st quartile (0.35-22.0 µg/g^e): 1.91(0.18-20.56) 2nd quartile (22.1-60.0 µg/g^e): 0.97(0.72-1.31) 3rd quartile (60.1-133.0 µg/g^e): 0.80(0.54-1.18) 4th quartile (133.1-960.0 µg/g^e): 0.47(0.13-1.71)	Race, number of siblings, maternal smoking, lower respiratory tract symptoms, upper respiratory tract symptoms, visible mould, (1,3)-ß-D-glucan, endotoxin	 n.s. n.s. n.s. n.s.	
			(1,3)-ß-D-glucan from children's primary activity room (8 m) (settled house dust) **[µg/g^e]**	(Q) Wheezing with API=1 (Asthma Predictive Index) (3y): 1st quartile (0.35-22.0 µg/g^e): 3.44(0.50-23.52) 2nd quartile (22.1-60.0 µg/g^e): 1.14(0.87-1.50) 3rd quartile (60.1-133.0 µg/g^e): 0.91(0.70-1.17) 4th quartile (133.1-960.0 µg/g^e): 0.61(0.24-1.59)		 n.s. n.s. n.s. n.s.	

Tischer et al., 2010, Germany & the Netherlands (AirAllerg)	Population-based (enriched*)	358 D 332 N	(1,3)-ß-D-glucan from children's mattress (5y) (settled house dust) **[mg/m^2]**	(Q) Physician-diagnosed asthma (6y): GERMANY: 0.76 (0.40-1.45) NETHERLANDS: 1.28 (0.72-2.29) (Q) Wheezing (6y): GERMANY: 0.78 (0.35-11.54) NETHERLANDS: 0.82 (0.53-1.28) (Q) Physician-diagnosed allergic rhinitis: GERMANY: 0.69 (0.45-1.05) NETHERLANDS: 0.83 (0.42-1.63) (Q) Rhinoconjunctivitis: GERMANY: 0.74 (0.49-1.12) NETHERLANDS: 1.11 (0.62-1.97))	Sex, parental allergy, parental education, outdoor activity (hours), breastfeeding, maternal smoking during pregnancy, current ETS, pets at home, AirAllerg case status	 n.s. n.s. n.s. n.s. n.s. n.s. n.s. n.s.
			EPS from children's mattress (5y) (settled house dust) **[mg/m^2]**	(Q) Physician-diagnosed asthma (6y): GERMANY: 0.60 (0.39-0.92) NETHERLANDS: 1.24 (0.78-1.96) (Q) Wheezing (6y): GERMANY: 1.02 (0.71-1.48) NETHERLANDS: 1.02 (0.74-1.42) (Q) Physician-diagnosed allergic rhinitis: GERMANY: 0.67 (0.49-0.92) NETHERLANDS: 1.00 (0.61-1.65) (Q) Rhinoconjunctivitis: GERMANY: 0.77 (0.56–1.07) NETHERLANDS: 1.19 (0.75-1.90)		 - n.s. n.s. n.s. - n.s. n.s. n.s.

*** enriched:** stands for studies which has over selected subjects who are at risk of developing allergic diseases for their study population.
RR = Relative Risks
HR = Hazard Ratio
IRR = Incident Risk Ratio
ETS = Environmental Tobacco Smoke
IgE = Immunoglobulin E, immune reaction in the serum
SPT = Skin Prick Test Reaction

COHORT STUDIES (not recruited at birth)

VISIBLE MOULD EXPOSURE								
Author, Year, Country (Study Acronym)	**Design**	**N**	**Exposure & time (age)**	**Outcome & time (age) (in odds ratios unless indicated otherwise)**	**Confounders adjusted/ Other factors in the multivariate model**	**Stratified analysis**	**Significance level for respiratory symptoms & diseases**	**Significance level for sensitization**
Brunekreef et al., 1989, U.S.	Population-based	4625	Mould or mildew (7-11 y) (parental-reported)	(Q) Persistent wheeze (8-12 y): 1.79(1.44-2.32) (Q) Hay fever (8-12 y): 1.57(1.31-1.87) (Q) Physician-diagnosed asthma (8-12 y): 1.27(0.93-1.74)	Sex, age, city of residence, parental education, maternal smoking		+ + n.s.	
McConnel et al., 2002, U.S.	Population-based	3535	Mildew (at ø 12.5 y) (parental-reported)		Ethnicity, residence, age groups, sex	(Q) Physician-diagnosed asthma at ø 16 years, stratified **by wheeze**: YES: 0.6(0.4-0.9) **RR** NO: 1.1(0.8-1.6) **RR**	- n.s.	
Jaakola et al., 2005, Finland	Population-based	1916	Visible mould (1-6 y) (parental-reported) *Poisson-Regression*	(Q) Physician-diagnosed asthma (1-6 y): 0.65(0.24-1.72) **IRR**	Sex, age, breast-feeding, parental education, single-parent or guardian, maternal smoking during pregnancy, ETS, gas cooking, furry or feathery pets, type of child care		n.s.	
Miyake et al., 2007, Japan (OMCHS)	Population-based	865 (pairs)	Visible mould in the kitchen (pregnancy) (parental-reported)	(Q) Physician-diagnosed atopic eczema (2-9 m): 1.86(1.08-3.15)	Maternal age, gestation, income, parental education, parental allergy, time of delivery, older siblings, baby's sex, baby's birth weight	(Q) Physician-diagnosed atopic eczema at 2-9 m, stratified **by parental allergy**: YES: 1.23(0.55-2.56) NO: 2.93(1.27-6.75)	+	
Belanger et al., 2003, U.S.	Population-based (enriched*)	849	Mould in the living room area (1 y) (parental-reported)		Sex, maternal education, ethnicity. ETS, mite allergen, cockroach allergen, cat allergen, dog	(Q) Wheeze at 1 y, stratified **by maternal asthma**: YES: 2.51(1.37-4.62) NO: 1.22(0.80-1.88)	+ n.s.	

					allergen, gas stove, wood stove, respiratory illness		
Rosenbaum et al., 2009, U.S. (AUDIT)	Population-based (enriched*)	103	Visible mould (3 m) (inspector reported)	(Q) Wheeze (1y): 0.90(0.35-2.29) **cOR**			n.s.
MOULD SPORES EXPOSURE							
Gent et al., 2002, U.S.	Population-based (enriched*)	880	Air-borne (main living area (4 m) **[cfu/m^3]**	(Q) Wheeze (1 y):	Sex, maternal allergy, multifamily home, heating system, ethnicity, air conditioner		
			Penicillium	Low (1-499 cfu/m^3): 1.11(0.87-1.42)			n.s.
				Medium (500-999 cfu/m^3): 1.29(0.65-1.48)			n.s.
				High (≥ 1000 cfu/m^3): 2.15(1.34-3.46)			+
			Cladosporium	Low (1-499 cfu/m^3): 0.92(0.69-1.22)			n.s.
				Medium (500-999 cfu/m^3): 0.95(0.61-1.49)			n.s.
				High (≥ 1000 cfu/m^3): 0.91(0.53-1.56)			n.s.
			”Other” mould	Low (1-499 cfu/m^3): 0.97(0.75-1.26)			n.s.
				Medium (500-999 cfu/m^3): 0.91(0.49-1.68)			n.s.
				High (≥ 1000 cfu/m^3): 1.02(0.49-2.11)			n.s.
Belanger et al., 2003, U.S.	Population-based (enriched*)	849	Air-borne (main living room) (ø 3 m) **[cfu/m^3]**		Sex, maternal education, ethnicity, ETS, mite, cockroach, cat and dog allergen, nitrogen dioxide	(Q) Wheeze at 1 y, stratified **by maternal asthma**:	
						YES: 1.23(1.01-1.49)	+
			Total fungi			NO: 1.10(0.99-1.23)	n.s.
Müller et al., 2002, Germany (LARS)	Population-based (enriched*)	475	Air-borne (children’s room) (3y) **[cfu/m^3]**	Sensitization to grass (IgE) (3y): 5.28(1.02-27.1)	ETS, parental atopy		+
			Aspergillus				
Rosenbaum et al., 2009, U.S. (AUDIT)	Population-based (enriched*)	103	Air-borne (main living room (3 m) **[cfu/m^3]**	(Q) Wheeze (1y):	Season, maternal smoking during pregnancy, ETS, day care, endotoxin levels, *insurance, mother’s education, race, carpet*		
			Total fungi	2nd quartile (269-571 cfu/m^3): 3.64(0.67-19.65)			n.s.
				3rd quartile (572-1214 cfu/m^3): 3.64(0.67-19.65)			n.s.
				4th quartile (1215-4770 cfu/m^3): 0.96(0.19-4.84)			n.s.
			Aspergillus	Low (16-64 cfu/m^3): 1.27(0.41-3.98)			n.s.
				High (65-2604 cfu/m^3): 1.58(0.43-5.79)			n.s.
			Penicillium	Low (16-119 cfu/m^3): 1.80(0.50-6.55)	*Insurance, mother’s education, sex*		n.s.
				High (120-1270 cfu/m^3): 6.18(1.34-28.46)			+

	Cladosporium	Low (16-191 cfu/m^3): 2.11(0.51-8.74)	*Insurance, sex, age at visit, mother's age*	n.s.
		High (192-1715 cfu/m^3): 2.28(0.41-12.67)		n.s.
	Acrodontium	Detected (16-478 cfu/m^3): 1.72(0.49-6.03)		n.s.
	Alternaria	Detected (16-191 cfu/m^3): 0.96(0.27-3.45)	*Mother's education, sex, age, mother's age*	n.s.
	Basidiomycetes	Low (16-63 cfu/m^3): 0.76(0.24-2.40)		n.s.
		High (64-2191 cfu/m^3): 0.77(0.24-2.49)		n.s.
	Hyaline unknown	2nd quartile (34-142 cfu/m^3): 0.44(0.11-1.68)		n.s.
		3rd quartile (143-381 cfu/m^3): 0.64(0.18-2.32)		n.s.
		4th quartile (382-2159 cfu/m^3): 0.71(0.20-2.52)		n.s.
	Yeast	Low (16-64 cfu/m^3): 0.61(0.19-1.96)		n.s.
		High (65-413 cfu/m^3): 0.76(0.23-2.57)		n.s.
	Dark unknown[§]	Low (16-79 cfu/m^3): 1.37(0.44-4.21)		n.s.
		High (80-604 cfu/m^3): 1.01(0.27-3.74)		n.s.

***enriched:** stands for studies which has over selected subjects who are at risk of developing allergic diseases for their study population
[§] Non-sporulating fungi with dark hyphae
IRR = Incident Risk Ratio
RR = Relative Risks
cOR = Crude Odds Ratios
ETS = Environmental Tobacco Smoke
IgE = Immunoglobulin E, immune reaction in the serum
SPT = Skin Prick Test Reaction

CASE-CONTROL STUDIES

VISIBLE MOULD EXPOSURE								
Author, Year, Country (Study Acronym)	**Design**	**N**	**Exposure & time (age)**	**Outcome & time (age) (in odds ratios unless indicated otherwise)**	**Confounders adjusted/ Other factors in the multivariate model**	**Stratified analysis**	**Significance level for respiratory symptoms & diseases**	**Significance level for sensitization**
Zheng et al., 2002, Republic of China	Case-control (6-10 y, case: (dd) asthma, matched by sex, age)	1209	Mould or fungi: (parental-reported) Family ceiling Child's bedroom	(Q) Physician-diagnosed asthma: 1.8(1.1-2.9) 1.8(1.0-3.2)	Sex, age, ethnicity, parental allergy, full-term pregnancy, breastfeeding		+ +	
Strachan and Carey, 1995, U.K.	Case-control (13-18 y, case: wheezing attacks, limitation of speech in the past year)	961	Mould in the bedroom (parental-reported)	(Q) Severe wheeze: 1.25(0.67-2.31)	Sex, year of birth, housing tenure, gas for cooking, maternal smoking, paternal smoking, type of pillow, type of quilt, age mattress		n.s.	
Verhoeff et al., 1995, the Netherlands	Case-control (6-12 y, case: chronic wheeze, cough, shortness of breath, asthma)	516	Visible mould (parental-reported) Living room Child's bedroom Visible mould (inspector reported) Living room Child's bedroom	(Q) Physician-diagnosed asthma (ever): 2.95(1.34-6.52) **cOR** 1.88(0.74-4.78) **cOR** 1.83(0.81-4.13) **cOR** 0.99(0.31-3.14) **cOR**		**Sensitized to mite/mould**: Visible mould (parental-reported) 1.93 (0.85-4.41) **cOR** Visible mould (inspector reported) 2.61 (1.21-5.64) **cOR**	+ n.s. n.s. n.s.	
Purvis et al., 2005, New Zealand (ABC)	Case-Control (3.5 y, case: birth weight ≤ 10th centile, matched by gestational age)	550	Mould in ceilings / walls (parental-reported)	(Q) Atopic dermatitis: 1.25(0.65-2.38) **cOR**	Parental atopy, breast feeding, history of wheeze, rash or runny nose at 1 year		n.s.	
Emenius et al., 2004, Sweden (BAMSE)	Case-control (1-2 y, case: wheezing, matched by age)	540	Mould spots on surface materials in wet areas (2 m) (inspector reported)	(Q) Recurrent wheezing (2y): 1.0(0.5-1.7)	Gender, parental allergy, maternal smoking, breast feeding, building age		n.s.	

Pekkanen et al., 2007, Finland	Case-Control (12-84 m, case: diagnosed asthma, matched by age, sex, municipality)	362	Visible mould (inspector reported) Main living area: Mould spots Visible mould (inspector reported)	(Q) Physician-diagnosed asthma: 1.24(0.73-2.11) 4.01(1.12-14.32) 1.95(0.69-5.47)	Parental asthma, paternal education, siblings, pets, day-care attendance	(Q) Physician-diagnosed asthma, stratified **by atopy** (IgE): YES: 4.74(0.94-24.01) NO: 1.08(0.32-3.64) (Q) Physician-diagnosed asthma, stratified by age: Older: 0.81(0.34-1.91) Younger: 1.96(0.87-4.38)	n.s. + n.s.
Fagbule and Ekanem, 1994, Nigeria	Case-control (ø 5.5 y, case: current asthma, matched by sex, age, SES)	280	Mould growth elsewhere (parental-reported)	(Q) Current asthma: 0.48(0.30-0.79)	Town of residence		-
Li and Hsu, 1997, Taiwan	Case-control (7-15 y, case: asthma, atopic status)	46	Visible mould (parental-reported)	(Q) Asthma: 1.02(0.39-2.69) (Q) Allergic rhinitis: 3.50(1.00-12.34)	Age, parental education, nr. of smokers, gas cooking		n.s. +
MOULD SPORES EXPOSURE							
Jovanovic et al., 2004, Germany	Case-control (9-11 y, case: allergy history)	397	Air-borne **[cfu/m^3]** AND dust-borne **[cfu/m^2]** (children's mattress & bedroom floor) Total fungi	Sensitization to mould (IgE): n.s.			n.s.
Jacob et al., 2002, Germany (INGA)	Case-control (5-14 y, case: atopic or physician-diagnosed asthma)	272	Dust-borne (living room floor) **[cfu/g]**: Total moulds **IQR** **> 90th percentile** *Cladosporium* *Penicillium* *Aspergillus* Total moulds > 200.000 (CFU/g)	Sensitization inhalant allergens (IgE): 48.750-200.000 (CFU/g): 1.56(0.85-2.86) > 200.000 (CFU/g): 1.67(0.65-4.29) 5.000-35.000 (CFU/g): 1.15(0.67-1.95) > 35.000 (CFU/g): 2.93(1.17-7.36) 5.000-55.000 (CFU/g): 1.09(0.64-1.84) > 55.000 (CFU/g): 1.38(0.54-3.51) LOD*-25.000 (CFU/g): 2.11(1.22-3.65) > 25.000 (CFU/g): 1.76(0.73-4.28) (Q) Physician-diagnosed asthma (ever): 0.47(0.06-3.90)	Age, sex, residential region, parental education, parental atopy		 n.s. n.s. n.s. + n.s. n.s. + n.s. n.s.

		(Q) Persistent wheezing:	
		0.82(0.10-7.13)	n.s.
		(Q) Physician-diagnosed hay-fever (ever):	
		2.14(0.39-11.8)	n.s.
		(Q) Red eyes/runny, congested nose:	
		11.3(1.23-103.1)	+
		(Q) Physician-diagnosed eczema (ever):	
		1.65(0.57-4.81)	n.s.
		(Q) Itchy rash:	
		1.46(0.54-3.96)	n.s.
	Cladosporium	(Q) Physician-diagnosed asthma (ever):	
	> 35.000 (CFU/g)	0.52(0.06-4.27)	n.s.
		(Q) Persistent wheezing:	
		1.18(0.47-2.94)	n.s.
		(Q) Physician-diagnosed hay-fever (ever):	
		1.89(0.35-10.3)	n.s.
		(Q) Red eyes/runny, congested nose:	
		15.5(2.08-1154.0)	+
		(Q) Physician-diagnosed eczema (ever):	
		0.54(0.12-2.44)	n.s.
		(Q) Itchy rash:	
		1.18(0.41-3.39)	n.s.
	Penicillium	(Q) Physician-diagnosed asthma (ever):	
	> 55.000 (CFU/g)	1.74(0.35-8.73)	n.s.
		(Q) Persistent wheezing:	
		2.55(0.44-14.7)	n.s.
		(Q) Physician-diagnosed hay-fever (ever):	
		2.57(0.54-12.3)	n.s.
		(Q) Red eyes/runny, congested nose:	
		17.6(1.69-183.4)	+
		(Q) Physician-diagnosed eczema (ever):	
		1.21(0.83-3.88)	n.s.
		(Q) Itchy rash:	
		0.62(0.17-2.24)	n.s.
	Aspergillus	(Q) Physician-diagnosed asthma (ever):	
	> 25.000 (CFU/g)	1.29(0.27-6.18)	n.s.
		(Q) Persistent wheezing:	
		2.15(0.41-11.4)	n.s.
		(Q) Physician-diagnosed hay-fever (ever):	
		Not estimable	
		(Q) Red eyes/runny, congested nose:	
		Not estimable	
		(Q) Physician-diagnosed eczema (ever):	
		2.16(0.80-2.52)	n.s.
		(Q) Itchy rash:	

				1.47(0.55-3.93)			n.s.
Wickman et al., 1992, Denmark	Case-control (3-17 y, case: sensitization status, SPT to mite or inhalant allergens)	175	Dust-borne (living room floor) **[cfu/mg] ≥ median**	(Q) Physician-diagnosed bronchial asthma: (Q) Physician-diagnosed atopic eczema: (Q) Physician-diagnosed allergic rhinitis: (Q) Physician-diagnosed bronchial asthma: (Q) Physician-diagnosed atopic eczema: (Q) Physician-diagnosed allergic rhinitis:		**HDM-sensitized**: **cOR** Total genera (≥ 35 cfu): 2.0(0.4-9.1) *Alternaria*: 1.5(0.3-7.4) *Cladosporium*: 0.3(0.0-1.5) Total genera (≥ 35 cfu): 0.4(0.1-1.3) *Alternaria*: 4.8(1.2-21.1) *Cladosporium*: 2.1(0.7-7.1) Total genera (≥ 35 cfu): 0.8(0.2-2.6) *Alternaria*: 0.7(0.2-2.7) *Cladosporium*: 3.5(1.0-12.2) **Aeroallergen-sensitized**: **cOR** Total genera (≥ 35 cfu): 0.9(0.3-3.0) *Alternaria*: 1.0(0.3-3.4) *Cladosporium*: 2.1(0.6-7.4) Total genera (≥ 35 cfu): 1.1(0.3-3.6) *Alternaria*: 1.5(0.4-5.3) *Cladosporium*: 0.9(0.3-3.2) Total genera (≥ 35 cfu): 0.3(0.1-1.1) *Alternaria*: 1.1(0.3-4.5) *Cladosporium*: 0.8(0.2-3.1)	n.s. n.s. n.s. n.s. + n.s. n.s. n.s. + n.s. n.s. n.s. n.s. n.s. n.s. n.s. n.s. n.s.
Hyvärinen et al., 2007, Finland	Case-control (1-7 y, case: new cases of physician-diagnosed asthma, matched by sex, age, place of residence)	72	Dust-borne **[cfu/g]** (dust bag, parents) Mesophilic actinomycetes Ergosterol Mesophilic fungi Xerophilic fungi	(Q) Physician diagnosed current asthma: 1.18(0.99-1.42) 1.12(0.97-1.30) 1.08(0.95-1.23) 1.11(0.94-1.31)	Parental asthma, paternal education, number of siblings, having livestock, moisture damage, daycare attendance		n.s. n.s. n.s. n.s.
Li and Hsu, 1997, Taiwan	Case-control (7-15 y, case: asthma, atopic status)	46	Air-borne **[cfu/g]** from living room floor and children's room *Aspergillus*	(Q) Asthma: 1.55(0.71-3.36) Summer (S) 0.69(0.28-1.73) Winter (W)	Age, parental education, nr. of smokers, gas cooking		n.s. n.s.

			Penicillium	0.61(0.21-1.81) (S) 0.56(0.17-1.84) (W)		n.s. n.s.	
			Cladosporium	1.88(1.07-3.30) (S) 4.14(1.17-14.67) (W)		+ +	
			Yeast	1.30(0.63-2.68) (S) 3.26(0.83-12.81) (W)		n.s. n.s.	
			Total fungi	0.77(0.13-4.47) (S) 4.93(0.63-38.72) (W)		n.s. n.s.	
				(Q) Allergic Rhinitis:			
			Aspergillus	1.00(0.43-2.34) (S) 2.58(0.87-7.60) (W)		n.s. n.s.	
			Penicillium	0.24(0.07-0.89) (S) 0.60(0.17-2.18) (W)		- n.s.	
			Cladosporium	1.56(0.79-3.07) (S) 1.12(0.39-3.27) (W)		n.s. n.s.	
			Yeast	0.94(0.44-1.98) (S) 0.77(0.20-2.96) (W)		n.s. n.s.	
			Total fungi	0.08(0.01-0.91) (S) 2.40(0.29-19.47) (W)		- n.s.	
MOULD COMPONENTS EXPOSURE							
Gehring et al., 2007, Germany, the Netherlands, Sweden (AIRALLERG)	Case-Control (2-4 y, matched by sensitization status)	1052	(1,3)-*ß*-D-glucan from children's mattresses (5-7 y) (settled house dust) **[µg/m^2]**	Sensitization inhalant allergens (IgE) (2-4 y): 0.81(0.71-0.93)	Sex, parental allergy, parental education, study design, endotoxin, EPS		-
			EPS from children's mattresses (5-7 y) (settled house dust) **[EPSU/m^2]**	-	Sex, parental allergy, parental education, study design, endotoxin, (1,3)-*ß*-D-glucan		n.s.
			(1,3)-*ß*-D-glucan from children's mattresses (5-7 y) (settled house dust) **[µg/g]**	n.s.			n.s.
			EPS from children's mattresses (5-7 y) (settled house dust) **[EPSU/g]**	+			n.s.
Schram-Bijkerk et al., 2005, Austria, Germany, the Netherlands,	Case-control (5-13 y, case: atopic or non-atopic wheeze)	879	(1,3)-*ß*-D-glucan from children's mattresses (settled house dust) **[µg/g]**	(Q) Atopic wheeze: 0.77(0.58-1.01)	Country, age, sex, older siblings, parental education, ETS, maternal smoking	n.s.	

Switzerland (PARSIFAL)			EPS from children's mattresses (settled house dust) **[EPSU/g]**	(Q) Atopic wheeze: 0.79(0.63-0.98)	during pregnancy	-
Douwes et al., 1999, the Netherlands	Case-control (6-12 y, case: respiratory symptoms)	60	EPS-*Asp/Pen* from living room floor (settled house dust) **[EPSU/mg]**	(Q) Physician-diagnosed asthma: 9.5(0.9-103.5)	Der p 1	n.s.
			Child's bedroom floor **[EPSU/mg]**	(Q) Physician-diagnosed asthma: 0.1(0.0-0.7)		-
			Childrens's mattress **[EPSU/mg]**	(Q) Physician-diagnosed asthma: 0.8(0.2-3.9)		n.s.

cOR = Crude Odds Ratios
ETS = Environmental Tobacco Smoke
IgE = Immunoglobulin E, immune reaction in the serum
SPT = Skin Prick Test Reaction
LOD = Limit of detection

CROSS-SECTIONAL STUDIES

VISIBLE MOULD EXPOSURE								
Author, Year, Country (Study Acronym)	**Design**	**N**	**Exposure & time (age)**	**Outcome & time (age) (in odds ratios unless indicated otherwise)**	**Confounders adjusted/ Other factors in the multivariate model**	**Stratified analysis**	**Significance level for respiratory symptoms & diseases**	**Significance level for sensitization**
Antova et al., 2008, North America, Eastern and Western Europe (PATY)	Pooled analysis of original cross-sectional based studies (6-12 y)	12 studies 57099	Visible mould (parental-reported)	**Combined ORs**: (Q) Hay fever ever: 1.35(1.18-1.53) (Q) Asthma ever: 1.35(1.20-1.51) (Q) Current wheeze: 1.43(1.36-1.49) (Q) Nocturnal dry cough: 1.30(1.22-1.39) Sensitization to inhalant allergens: 1.33(1.23-1.44)	Age, sex, parental education, nationality, household crowding, gas for cocking, unvented heater, post/prenatal ETS exposure, birth order, ever had a pet		+ + + + +	
			Mould “ever” (parental-reported)	(Q) Hay fever ever: 1.48(1.34-1.62) (Q) Asthma ever: 1.36(1.19-1.56) (Q) Current wheeze: 1.44(1.35-1.53) (Q) Nocturnal dry cough: 1.26(1.16-1.38) Sensitization to inhalant allergens: 1.38(1.26-1.52)			+ + + + +	
			“Recent” mould (parental-reported)	(Q) Hay fever ever: 1.47(1.35-1.61) (Q) Asthma ever: 1.23(1.07-1.41) (Q) Current wheeze: 1.46(1.31-1.61) (Q) Nocturnal dry cough: 1.23(1.12-1.34) Sensitization to inhalant allergens: 1.29(1.12-1.49)			+ + + + +	
Lee et al., 2003, Taiwan	Cross-sectional (6-15 y)	35036	Visible mould (parental-reported)	(Q) Physician-diagnosed asthma	Age, parental education, number of siblings, maternal smoking during pregnancy	(Q) Asthma, stratified **by gender** GIRLS: 1.20(1.01-1.41) BOYS: 1.27(1.10-1.47)		
Dales et al., 1991, Canada	Cross-sectional (5-8 y)	13495	Nr. of mould sites (parental-reported) 0 vs. 1 0 vs. 2	(Q) Wheeze: 1.42(1.26-1.59) **cOR** 1.73(1.45-2.06) **cOR**	Age, sex, race, parental education, gas cooking, nr. of smokers in home, hobbies, sex of respondent, region of residence		+ +	
			0 vs. 1 0 vs. 2	(Q) Physician diagnosed asthma: 1.40(1.16-1.68) **cOR** 1.67(1.27-2.19) **cOR**			+ +	
Dong et al., 2008, China	Cross-sectional (6-13 y)	10784	Visible mould (parental-reported)	(Q) Physician-diagnosed asthma (ever): 1.54(1.22-1.94) (Q) Current asthma (past 2 y):	Age, sex, breastfeeding, living in the city,		+	

				1.69(1.15-2.48) (Q) Current wheeze: 1.65(1.25-2.17) (Q) Physician-diagnosed allergic rhinitis (ever): 1.21(0.97-1.50)	schooling, house type, area of residence, number of rooms, distance to traffic pollution source near by house, indoor coal use, current ETS exposure, ETS exposure in the first 2 years and during pregnancy, pets at home, home decorations, parental education, parental atopy	+ + n.s.
Ponsonby et al., 2000, Tasmania	Cross-sectional (7y)	6378	Mould in child's room (inspector observed) Mould (excluding bathroom) (parental-reported)	(Q) Asthma: 1.26(0.87-1.81) (Q) Asthma: 1.20(0.96-1.51)	Sex, family asthma, breastfeeding, gas heater, ETS, active smoking in baby's room, maternal education, number of residents	n.s. n.s.
Spengler et al., 2004, Russia	Cross-sectional (8-12 y)	5951	Presence of moulds (parental-reported)	(Q) Physician-diagnosed asthma: 2.82(1.63-4.88) (Q) Wheeze: 1.52(1.19-1.94) (Q) Asthma symptoms: 1.98(1.53-2.55) (Q) Physician-diagnosed any allergy: 1.51(1.25-1.82)	Age, sex, preterm birth, parental atopy, parental education, ETS exposure	+ + + +
Tham et al., 2007, Singapore	Cross-sectional (1.5-6 y)	4759	Visible mould in child's room (parental-reported) *Cox proportional hazard regression*	(Q) Wheeze: 1.34(0.91-1.96) **PR** (Q) Rhinitis: 1.55(1.16-2.07) **PR** (Q) Rhinoconjunctivitis: 2.38(1.51-3.75) **PR** (Q) Eczema: 1.28(0.83-1.97) **PR** (Q) Flexural rash: 1.15(0.70-1.88) **PR**	Age, sex, race, SES, ETS, parental atopy, respiratory infections, food allergy	n.s. + + n.s. n.s.
Freeman et al., 2003, U.S.	Cross-sectional (8.1 – 10.9 y)	4634	Any mould (parental-reported)	(Q) Physician-diagnosed asthma: 1.54(1.27-1.87)	Multiple mould sites, damp bathroom, ETS, furry pets, roaches	+
	preschool age	240	Any mould (parental-reported)	(Q) Physician-diagnosed asthma: 3.30(1.57-6.97)		+
Dong et al., 2008, China	Cross-sectional (1-6 y)	3945	Visible mould (parental-reported)	(Q) Physician-diagnosed asthma: 1.56(1.13-2.16) (Q) Physician-diagnosed allergic rhinitis: 1.20(0.72-1.99) (Q) Current asthma: 1.89(1.22-2.94) (Q) Current wheeze: 2.07(1.56-2.75)	Age, sex, breast feeding, living in the city, school, house type, area of residence, nr. or	+ n.s. + +

					rooms, distance to traffic, pollution source, indoor coal use, ETS, ETS < 2y of age, smoking during pregnancy, pet keeping, home decoration, parental education, parental atopy	
Ibargoyen-Roteta et al., 2007, Spain	Cross-sectional (5-8 y)	3360	Mould on walls (1y) (parental-reported)	(Q) Current allergic rhinoconjunctivitis: 1.34(0.64-2.79)	Age, sex, response language	n.s.
Brunekreef, 1992, The Netherlands	Cross-sectional (6-12 y)	1051	Visible mould (parental-reported)	Study 1 (1987): (Q) Wheeze (6-12 y): 1.37(0.58-3.26) (Q) Asthma (6-12 y): 1.12(0.38-3.38)	Sex, age, height, weight, ETS, (sources of) nitrogen dioxide in the home, parental education	n.s. n.s.
		3344	Visible mould (parental-reported)	Study 2 (1989): (Q) Wheeze (6-12 y): 1.90(1.41-2.54) (Q) Asthma (6-12 y): 1.53(1.04-2.28)		+ +
Chong Neto and Rosario, 2008, Brazil	Cross-sectional (12-15 m)	3003	Mould/mildew (parental-reported)	(Q) Wheezing: 1.13 **PReg**, p=0.003	Sex, parental asthma, sibling asthma, age of day care attendance, pets at home during pregnancy, bathroom in the home, colds, atopic dermatitis, up-to-date immunization	+
Garcia-Marcia et al., 2005, Spain	Cross-sectional (9-12 y)	2720	Mould stains (parental-reported)	(Q) Atopic (SPT+) Wheezing: 1.86(0.81-4.24) (Q) Non-atopic (SPT-) Wheezing: 2.70(1.16-6.30)	Sex, parental asthma, maternal smoking 1st year of child's life	n.s. +
Warman et al., 2009, U.S.	Cross-sectional (5-11 y)	1772	Visible mould on walls (parental-reported)	(Q) Physician-diagnosed asthma: 3.26(2.38-4.45) **cOR**		+
			Visible mould on walls, ceilings or windows (parental-reported)	(Q) Physician-diagnosed asthma: 2.66(2.04-3.48) **cOR**		+
Chen et al., 2003, Taiwan	Cross-sectional (7-12 y)	1452	Mould patches (parental-reported)	(Q) Asthma symptoms: 1.56(0.90-2.69) (Q) Physician-diagnosed asthma: 1.55(0.78-3.09) (Q) Allergic rhinitis symptoms: 1.44(1.02-2.05) (Q) Physician-diagnosed allergic rhinitis:	Sex, stuffed toys, cockroaches, floor blankets, ETS, exposure to	n.s. n.s. +

				1.48(1.03-2.12) (Q) Eczema symptoms: 1.45(0.81-2.62) (Q) Physician-diagnosed eczema symptoms: 1.70(0.82-3.50)	incense	+ n.s. n.s.	
Li and Hsu, 1996, Taiwan	Cross-sectional (8-12 y)	1340	Visible mould/ mildew (parental-reported)	(Q) Physician-diagnosed asthma: 1.12(0.72-1.74) (Q) Allergic rhinitis (symptoms): 1.27(0.96-1.68) (Q) Wheeze: 1.20 (0.73-1.99)	Age, sex, parental education, ETS, use of gas stove	n.s. n.s. n.s.	
Schäfer et al., 1999, Germany	Cross-sectional (5-7 y)	1235	Dampness and mould (parental-reported)	Sensitization grass (SPT): 1.49(0.85-2.61) Sensitization birch (SPT): 0.75(0.28-2.00) Sensitization mugwort (SPT): 2.86(1.29-6.35) Sensitization alternaria (SPT): 1.65(0.69-3.93) Sensitization dust mites (SPT): 3.37(1.63-6.96) Sensitization cat (SPT): 3.19(1.11-5.74)	Sex, parental education, family size, parental atopy, smoking during pregnancy, location, observer		n.s. n.s. + n.s. + +
Strachan et al., 1990, U.K.	Cross-sectional (6.5-7.5 y)	1000	Mould (parental reported)	(Q) Wheeze: 3.70(2.22-6.15) **cOR**		+	
		330	Inspector reported (children's room)	(Q) Wheeze: 3.25(1.60-6.60) **cOR**		+	
Maier et al., 1997, U.S.	Cross-sectional (5-9 y)	925	Visible mould (parental-reported)	(Q) Physician-diagnosed asthma: 1.3(0.9-1.9) **cPR** (Q) Wheezing: 1.2(0.7-1.9) **cPR**		n.s. n.s.	
Alper et al., 2006, Turkey	Cross-sectional (7y)	858	Dampness and mould (parental-reported)	(Q) Early wheeze (0-3y): 2.37(1.52-3.69) **cOR** (Q) Early transient wheeze: 2.28(1.34-3.87) **cOR** (Q) Persistent wheeze(0-6y): 2.53(1.30-4.87) **cOR** (Q) Late-onset wheeze(3-6y): 2.46(1.29-4.66) **cOR**		+ + + +	
Dijkstra et al., 1990, The Netherlands	Cross-sectional (6-12 y)	775	Damp stains and mould (parental-reported)	(Q) Wheeze: 1.54(0.59-4.00) **cOR** (Q) Asthma: 1.56(0.50-4.87) **cOR**		n.s. n.s.	
Schäfer et al., 2008, Germany (KLAUS)	Cross-sectional (school entrance)	606	Visible mould (parental-reported)	(Q) Atopic eczema: 1.84(1.0-3.36)	Sex, maternal education, parental eczema	+	
Cuijpers et al., 1995, The Netherlands	Cross-sectional (6-12 y)	470	Mould growth (parental-reported)		Sex, age, height, education level	(Q) Wheeze, stratified **by gender** Girls: Mould growth Always: 2.69(0.48-15.21) Often: 0.79(0.06-10.66) Sometimes: 0.54(0.14-2.11) Boys: Mould growth Always: 0.95(0.16-5.46) Often: 0.46(0.05-4.47) Sometimes: 0.50(0.13-1.89)	 n.s. n.s. n.s. n.s. n.s. n.s.

Dales and Miller, 1999, Canada	Cross-sectional (~ 10 y)	403	Ever mould/mildew (parental-reported)	(Q) Physician-diagnosed asthma: 0.91(0.42-1.95)	Age, sex, parental allergy, parental education, pets in home, ETS, endotoxin, dust mites	n.s.	
Koskinen et al., 1999, Finland	Cross-sectional (≤ 7 y)	57	Mould present (parental-reported)	(Q) Rhinitis: 8.01(0.77-83.82) (Q) Atopic eczema: 3.02(0.59-16.27) (Q) Allergic eczema: 2.09(0.24-18.47)	Age, gender´, allergy, pets at home, (type of day-care), atopic predisposition	n.s. n.s. n.s.	
	(7-15 y)	147	Mould present (parental-reported)	(Q) Rhinitis: 1.77(0.69-4.53) (Q) Atopic eczema: 2.29(0.79-6.60) (Q) Allergic eczema: 3.51(0.67-18.47)		n.s. n.s. n.s.	
MOULD SPORES EXPOSURE							
Salo et al., 2006, U.S.	Cross-sectional (0-18 y)	762	Dust-borne *Alternaria alternata* **[µg/g]**	(Q) Physician-diagnosed asthma: 1.47(0.83-2.62)	Age, sex, race, education, smoking, survey season	n.s.	
Garret et al., 1998, Australia	Cross-sectional (7-14 y)	148	Air-borne *Penicillium* (100 **cfu/m^3** increase)	(Q) Current asthma: 1.43(1.03-2.00) Sensitization *Cladosporium cl*. (SPT): 1.24(0.92-1.67) Sensitization *Penicillium* mix (SPT): 1.60(1.13-2.18) Sensitization *Aspergillus* mix (SPT): 1.42(1.04-1.95) Sensitization *Alternaria* alt. (SPT): 1.18(0.88-1.57) Sensitization Mould mix A. (SPT): 1.19(0.88-1.62) Sensitization *D. pteronyssinus* (SPT): 1.22(0.89-1.67) Sensitization *D. farinae* (SPT): 1.32(0.95-1.85)	Sex, parental asthma	+ n.s. + + n.s. n.s. n.s. n.s. + + n.s. n.s. n.s.	
			Air-borne *Cladosporium* (100 *cfu/m^3* increase)	Sensitization House dust (SPT): 1.49(1.05-3.17) Sensitization Dog (SPT): 1.46(1.09-1.96) Sensitization Cat (SPT): 1.21(0.92-1.59) Sensitization Bermuda grass (SPT): 1.12(0.85-1.47) Sensitization Grass mix no. 7 (SPT): 1.12(0.85-1.47)		+ + + n.s. n.s. n.s. n.s.	
			Air-borne *Cladosporium* (500 **cfu/m^3** increase)	Sensitization *Cladosporium cl*. (SPT): 1.24(1.00-1.54) Sensitization *Penicillium* mix (SPT): 1.29(1.02-1.62) Sensitization *Aspergillus* mix (SPT): 1.37(1.07-1.76) Sensitization *Alternaria* alt. (SPT): 1.12(0.91-	Parental allergy	n.s. n.s. n.s. n.s. +	

			Aspergillus (10 **cfu/m^3** increase)	1.36) Sensitization Mould mix A. (SPT): 1.16(0.95-1.43) Sensitization *D. pteronyssinus* (SPT): 1.02(0.93-1.13) Sensitization *D. farinae* (SPT): 1.03(0.95-1.11) Sensitization House dust (SPT): 1.06(0.96-1.17) Sensitization Dog (SPT): 1.02(0.91-1.15) Sensitization Cat (SPT): 1.08(0.98-1.19) Sensitization Bermuda grass (SPT): 1.04(0.94-1.15) Sensitization Grass mix no. 7 (SPT): 1.03(0.95-1.11) (Q) Current asthma: 1.92(0.96-3.80) (Q) Current wheeze: 1.58(1.00-2.50) Sensitization inhalant allergens (SPT): 1.48(1.10-1.99)	Sex, parental allergy		+
MOULD COMPONENTS EXPOSURE							
Karadag et al, 2007, Austria, Germany, the Netherlands, Switzerland (PARSIFAL)	Cross-sectional (5-13 y)	933	(1,3)-*ß*-D-glucan from children's mattresses (settled house dust) **[µg/g]**	(Q) Symptoms of atopic eczema: 0.75(0.53-1.06) (Q) Physician-diagnosed atopic eczema: 0.69(0.53-0.90)	Centre, study group	n.s. -	
			EPS from children's mattresses (settled house dust) **[EPSU/g]**	(Q) Symptoms of atopic eczema: 0.76(0.65-0.90) (Q) Physician-diagnosed atopic eczema: 0.91(0.75-1.11)		- n.s.	
Ege et al., 2007, Germany, Austria, Switzerland, the Netherlands (PARSIFAL)	Cross-sectional (5-13 y)	440	(1,3)-*ß*-D-glucan from children's mattresses (settled house dust, self sampling) **[µg/g]**	(Q) Ever asthma: + (Q) Current wheeze: +	Sex, study centre, group, parental asthma, EPS or (1,3)-*ß*-D-glucan	n.s. n.s.	
			EPS from children's mattresses (settled house dust, self sampling) **[EPSU/g]**	(Q) Ever asthma: - (Q) Current wheeze: -		- -	
			(1,3)-*ß*-D-glucan from children's mattresses (settled house dust, self sampling) **[µg/g]**	Sensitization inhalant and food allergens: +	Sex, study centre, group, parental atopy, maternal exposure to animal sheds during		n.s.

	EPS from children's mattresses (settled house dust, self sampling) **[EPSU/g]**	Sensitization inhalant and food allergens: +	pregnancy, EPS or (1,3)-ß-D-glucan	n.s.

PR = prevalence ratio
ETS = Environmental Tobacco Smoke
SES = Socioeconomic Status
cOR = Crude Odds Ratios
cPR = Crude Prevalence Ratios
pReg = Poisson Regression
IgE = Immunoglobulin E, immune reaction in the serum
SPT = Skin Prick Test Reaction

SUPPLEMENTARY MATERIAL

3) Evaluation of evidence of causation according to the Bradford Hill Criteria

(1) Strength

We observed a statistically significant increased risk of asthma (**1.49** (**1.28-1.72**), wheeze (**1.68** (**1.48-1.90**) and allergic rhinitis (**1.39** (**1.28-1.51**) in children when exposed to visible mould in studies over the past 30 years. According to Hill's criteria, inadequacy could derive from systematic errors such as reverse causation, without affecting the effect estimates. We were not able to look into every study included in the meta-analysis, however, numerous studies validated self-reported visible mould questions against inspector reported observations (1-6) and did not find any evidence for over- or underreporting of dampness and mould by occupants.

(2) Consistency

Has the association between visible mould exposure and allergic health outcomes been repeatedly observed by different persons, in different places, circumstances and times? This review was comprised of a number of publications on adverse health effects of mould and mould derived components exposure over the past 30 years. Visible mould exposure was reported to increase the risk for allergic health outcomes in every epidemiological study design described in our study. The same was found for mould spore exposure; however, the number of studies was limited. In contrast, we observed a protective tendency of mould derived components on allergic diseases in prospective and retrospective studies. Therefore, mould derived components such as (1,3)-ß-D-glucan or EPS might be an inadequate surrogate of visible mould exposure.

(3) Specificity

Multi-causation is generally more likely in epidemiological studies. Although we observed strong associations between mould exposure and allergic health outcomes, there were other risk factors such as second hand smoke (SHS), hidden microbial pollution or subject related risk factors. In order to account for specificity in the meta-analysis we included only studies with clearly defined exposure ("visible mould at home"), well-defined health outcomes and similarly adjusted effect estimates.

(4) Temporality

Temporality refers to the temporal relationship of the association. In almost all birth cohort and cohort studies not recruited at birth there is at minimum one year between exposure and health outcome assessment. For case-control and cross-sectional based

studies, exposure was determined retrospectively. However, for the meta-analysis we had to combine studies regardless of their study design due to the limited number of prospective (birth) cohort studies.

(5) Biological gradient

There were only 8 publications included in this review that looked at the dose-response relationship between exposure and health outcomes assessment. One birth cohort study and one cross-sectional study reported that the number of mould sites at home significantly increased the risk for wheeze in 1 year old children, which was confirmed at the age of three years within the U.S. birth cohort study. Two U.S. cohort studies and one case-control study from Germany found that higher levels of Penicillium and Cladosporium increased the risk for allergic disorders. In contrast, two birth cohort studies from the U.S. and Europe found that higher levels of mould (1,3)-ß-D-glucan and EPS were associated with a decreased risk for physician-diagnosed asthma and (recurrent) wheeze.

(6) Plausibility and (7) Coherence

The inflammatory and allergenic potential of fungal allergens was investigated in a number of experimental studies (7, 8). Fungal exposure was associated with type 1 allergies, with a focus on proteins produced by the respective species as suggested by IgE-inducing allergens (9-11). Several fungi and isolated mycotoxins were linked with inflammatory responses in vitro (12-14). Mould derived components such as (1,3)-ß-D-glucans originate among others from fungi and have the capacity to initiate a variety of inflammatory reactions in vertebrates (15).

(8) Experiment

There are some intervention studies which investigated the effect of remediation of mould or microbial exposure in affected home or school environments in follow-up studies. After remediation, symptoms of allergic respiratory health outcomes and medication use decreased (16-20).

(9) Analogy

The indoor environment consists of a complex mixture of viable and non-viable organisms and a clear assignment to the observed health effect is difficult (21). Home dampness and visible mould, as an apparent exposure, might be only partly responsible for the observed health effects. However, most of the studies included in the meta-analysis were adjusted for important risk factors which are thought to have an effect on the health effects.

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6 Meta-Analysis of Mould and Allergy in 8 European Birth Cohorts

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Meta-analysis of mould and dampness exposure on asthma and allergy in eight European birth cohorts: an ENRIECO initiative

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Abstract

Background: Several cross-sectional studies during the past 10 years have observed an increased risk of allergic outcomes for children living in damp or mouldy environments.

Objective: The objective of this study was to investigate whether reported mould or dampness exposure in early life is associated with the development of allergic disorders in children from eight European birth cohorts.

Methods: We analysed data from 31 742 children from eight ongoing European birth cohorts. Exposure to mould and allergic health outcomes were assessed by parental questionnaires at different time points. Meta-analyses with fixed- and random-effect models were applied. The number of the studies included in each analysis varied based on the outcome data available for each cohort.

Results: Exposure to visible mould and/or dampness during first 2 years of life was associated with an increased risk of developing asthma: there was a significant association with early asthma symptoms in meta-analyses of four cohorts [0–2 years: adjusted odds ratios (aOR), 1.39 (95%CI, 1.05–1.84)] and with asthma later in childhood in six cohorts [6–8 years: aOR, 1.09(95%CI, 0.90–1.32) and 3–10 years: aOR, 1.10 (95%CI, 0.90–1.34)]. A statistically significant association was observed in six cohorts with symptoms of allergic rhinitis at school age [6–8 years: aOR, 1.12 (1.02–1.23)] and at any time point between 3 and 10 years [aOR, 1.18 (1.09–1.28)].

Conclusion: These findings suggest that a mouldy home environment in early life is associated with an increased risk of asthma particularly in young children and allergic rhinitis symptoms in school-age children.

Reviews conducted in the past 10 years have found an increased risk of respiratory and allergic health outcomes in children with a parent-reported damp and mouldy home environment (1–5). Although a small number of collaborative investigations have consistently reported increased risks of asthma, wheeze and allergic rhinitis in children exposed to visible mould (6–8), these previous studies have been primarily cross-sectional, comprised different definitions of exposure and health outcome and assessed the health outcome at a single time point. The Environmental Health Risks in European Birth Cohorts (ENRIECO) initiative included only population-based prospective birth cohort studies and allows for investigation into allergic health outcomes from birth to 10 years of age in a large, comprehensive, longitudinal data set from eight European studies. The objective of this investigation was to assess whether early residential exposure to mould/dampness up to 2 years is associated with the development of asthma, symptoms of allergic rhinitis and sensitization in children at different time points between birth and 10 years.

Methods

Birth cohort characteristics

ENRIECO is a project conducted within the European Union's Seventh Framework Programme [Theme 6, Environment (Including Climate Change)] focusing on the potential health effects of environmental exposures. For this investigation, eight birth cohort studies with suitable information on

Table 1 Descriptive overview of the eight European birth cohorts

Acronym and key reference	LEICESTER (9)	ALSPAC (10)	BAMSE (11)	GINIplus (12)	PIAMA-NHS (13)	LISAplus (14)	DARC (15)	CO.N.ER (16)	*P*-value
Country	UK	UK	Sweden	Germany	The Netherlands	Germany	Denmark	Italy	
First year of recruitment	1985	1991	1994	1995	1996	1997	1998	2004	
N (birth)	330	14 057	4089	5991	3182	3097	562	434	
Sex (female)	48%	48%	49%	48%	48%	49%	49%	51%	0.777
Early mould and/or dampness (0–2 years)	19%	67%	28%	26%	55%	37%	36%	13%	<0.001
Early asthma (0–2 years)*	No data	No data	14%	3%	4%	0.5%	24%	13%	<0.001
School-age asthma (6–8 years)	16%	10%	9%	5%	6%	5%	4%	No data	<0.001
Ever asthma (3–10 years)	16%	10%	18%	5%	9%	4%	4%	No data	<0.001
Symptoms of allergic rhinitis at school-age (6–8 years)†	36%	19%	14%	25%	33%	24%	3%	No data	<0.001
Ever symptoms of allergic rhinitis (3–10 years)	36%	19%	19%	27%	42%	27%	3%	No data	<0.001
Sensitization to aero-allergens at early school age (6 –8 years)‡	No data	No data	25% (8)	28% (6)	30% (8)	27% (6)	17% (6)	No data	<0.001
Sensitization to mould at early school age (6–8 years)	No data	No data	2% ()	2% (6)	2% (8)	0.8% (6)	3% (6)	No data	<0.001
Parental allergy§	61%	72%	61%	50%	40%	55%	59%	51%	<0.001
Parental education									
Low	57%	–	20%	13%	13%	6%	19%	–	<0.001
Medium	26%	47%	27%	29%	37%	37%	65%	54%	
High	17%	53%	53%	57%	50%	57%	16%	46%	
Maternal smoking (pregnancy)	No data	30%	13%	15%	27%	18%	33%	12%	<0.001
Early SHS¶ exposure (0–2 years)	29%	25%	6%	15%	13%	12%	21%	3%	<0.001
Early day care (1–2 years)	No data	10%	84%	53%	56%	25%	94%	No data	<0.001
Breastfeeding (≥4 months)**	27%	20%	80%	51%	32%	58%	20%	91%	<0.001

IgE, Immunoglobulin E.

*DARC: High prevalence of early asthma because of high medication intake (medication prescribed for asthma or bronchitis).

†DARC: Low prevalence of school-age symptoms of allergic rhinitis partly because of different assessment methods.

‡Sensitization to aero-allergens (6–8 years): IgE >0.35 ku/l for at least one of the measured aero-allergens in each birth cohort (cat dander, dog dander, mite, mould, grass and tree pollen).

§Parental allergy: mother or father having at least one of the following allergic dispositions: asthma, hay fever, atopic eczema, pet allergy and house dust mite allergy (ever).

¶SHS: second-hand smoke (maternal smoking).

**Breastfeeding (at least 4 months).

exposure and health outcomes were included. The cohorts recruited subjects between 1985 and 2004 with a sample size between 330 and 14 057 children (Table 1). Most studies were single-centre studies, except two German birth cohorts (LISAplus and GINIplus) and a Dutch birth cohort (PIAMA-NHS). All cohorts were population-based except the PIAMA-NHS cohort that over-sampled nonallergic pregnant women in the NHS component. All cohorts obtained ethical approval from their local review boards.

Definition of exposure and health outcomes

Exposure was defined as parent-reported mould and/or dampness in any room of the home during the first 2 years of life (Data S1).

We defined seven health end points, based on the comparability across the birth cohort studies (Data S2): 'Early childhood asthma' (0–2 years), 'school-age asthma' (6–8 years), and 'ever asthma' at any time between 3 and 10 years of life. The asthma definition was based on the ISAAC-related questions (17) and satisfied two of three conditions: physician-diagnosed asthma ever, parent-reported wheezing (last 12 months) and asthma medication (last 12 months). If there was no comprehensive information on 'physician-diagnosed asthma ever', we used 'physician-diagnosed asthma in the past 12 months' for the assessment. School-age and childhood 'symptoms of allergic rhinitis' (6–8 years) were defined as sneezing attacks, runny, blocked and itchy nose without having a cold.

Sensitization against aero-allergens and mould allergens was available for five of the eight cohorts. Sensitization was defined as having specific Immunoglobulin E (IgE) of at least 0.35 kU/l to at least one of the measured aero-allergens (cat dander, dog dander, mite, mould allergens, grass or tree pollen) between 6 and 8 years.

Definition of potential confounders

Individual cohort analyses were adjusted for the following potential confounders: gender, parental atopy, parental educational level at birth (proxy for socio-economic status), maternal smoking during pregnancy, environmental tobacco smoke during the first 2 years of life, breastfeeding (at least 4 months) and early day care attendance. A confounder was considered a risk factor in the adjusted model if it was associated (χ^2-test, $P < 0.1$) with the respective health outcome in at least two birth cohorts.

Statistical analysis

Logistic regression was used to calculate crude odds ratios (OR) and adjusted odds ratios (aOR) to assess the effect of early exposure to mould and/or dampness on the development of allergic disorders including asthma, symptoms of allergic rhinitis and sensitization to aero-allergens individually for each cohort and combined. Meta-analyses with fixed- and random-effect models were applied to account for the heterogeneity between the cohorts. The number of the studies included varies according to the data available from each individual cohort. The results of the meta-analysis are presented as forest plots with central point estimates and 95% confidence intervals (CI). We further stratified the analyses by atopic sensitization status and parental allergy.

Statistical analyses were performed using the statistical software R, version R 2.12.2 (The R Foundation for Statistical Computing).

Results

Study population

There were considerable variations among the European birth cohorts regarding the distribution of most exposure variables and potential confounders. During the first 2 years of life, exposure to residential mould and/or dampness ranged from 13% in Bologna, Italy (CO.N.ER), to 67% in Bristol, UK (ALSPAC). Further, in the Netherlands (PIAMA-NHS), Denmark (DARC) and one German birth cohort (LISAplus), more than one-third of the parents reported exposure to mould and/or dampness at home within the first 2 years of life (Table 1).

Early exposure to visible mould and/or dampness in relation to allergic health outcomes

The results from the crude and adjusted logistic regression models are presented in Table 2 and Fig. 1.

Among the Danish children, early asthma was reported in 24% when compared to a lower prevalence in the remaining cohorts. In DARC, the question regarding medication intake referred to any respiratory medication, not only asthma medication, which might partly explain the increased prevalence. Further, the prevalence of symptoms of allergic rhinitis at school age was reported for only 3% among the Danish children who were assessed during clinical visits by physicians and not by parent-reported symptoms. Owing to these differences in the assessment, the DARC cohort was excluded from the adjusted analysis of asthma and symptoms of allergic rhinitis to minimize classification bias. However, we performed a sensitivity analysis including the DARC cohort and observed no major changes in the summary effects for asthma [(0–2 years): aOR, 1.33 (95% CI, 1.05–1.69), (6–8 years): aOR, 1.39 (95% CI, 0.90–1.29), (3–10 years): aOR, 1.08 (95% CI, 0.89–1.32) and symptoms of allergic rhinitis (6–8 years): aOR, 1.12 (95% CI, 1.03–1.23) and (3–10 years): aOR, 1.17 (95% CI, 1.08–1.26)]. Because of a similar prevalence of allergic sensitization at school age among the cohorts, the DARC cohort was included in the analysis of early exposure to mould and allergic sensitization status at school age.

Asthma

There was a statistically significant association between early exposure to mould and asthma (< 3 years) in adjusted analysis of four cohorts (aOR, 1.39 (95% CI, 1.05–1.84), Fig. 1A). The Leicester cohort showed a reduced risk of school-age

Table 2 Crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) of asthma, symptoms of allergic rhinitis and sensitization to aero-allergens, respectively, in relation to early exposure to mould and/or dampness (0–2 years), from random-effect meta-analyses (combined effect) and separately by each cohort

OR (95% CI)	Summary estimate	LEICESTER	ALSPAC	BAMSE	GINIplus	PIAMA-NHS	LISAplus	DARC	CO.N.ER	Test for homogeneity (*P*)
Asthma										
Early (0–2 years)										
Crude	**1.44 (1.07–1.95)**	n.a.	n.a.	1.58 (1.29–1.94)	1.27 (0.86–1.87)	1.04 (0.72–1.51)	DEM	n.i.	2.86 (1.39–5.89)	0.0526
Adjusted*	**1.39 (1.05–1.84)**	n.a.	n.a.	1.52 (1.24–1.87)	1.26 (0.85–1.87)	1.03 (0.71–1.49)	DEM	n.i.	2.73 (1.26–5.95)	0.0929
School age (6–8 years)										
Crude	1.16 (0.99–1.36)	0.50 (0.16–1.50)	1.29 (1.05–1.57)	1.30 (1.00–1.70)	1.16 (0.76–1.76)	0.99 (0.66–1.49)	0.93 (0.49–1.40)	n.i.	n.a.	0.3048
Adjusted†	1.09 (0.90–1.32)	0.23 (0.06–0.86)	1.19 (0.95–1.48)	1.20 (0.92–1.58)	1.17 (0.76–1.80)	1.01 (0.68–1.52)	0.83 (0.47–1.47)	n.i.	n.a.	0.196
Ever (3–10 years)										
Crude	1.16 (0.97–1.39)	0.50 (0.16–1.50)	1.32 (1.12–1.55)	1.44 (1.19–1.74)	1.24 (0.92–1.67)	0.94 (0.73–1.21)	0.92 (0.62–1.37)	n.i.	n.a.	0.0310
Adjusted‡	1.10 (0.90–1.34)	0.23 (0.06–0.86)	1.19 (0.95–1.49)	1.35 (1.11–1.65)	1.23 (0.90–1.68)	0.91 (0.70–1.17)	0.95 (0.61–1.47)	n.i.	n.a.	0.0275
Symptoms of allergic rhinitis										
School age (6–8 years)										
Crude	**1.16 (1.06–1.26)**	2.07 (1.10–3.89)	1.15 (0.99–1.33)	1.24 (0.99–1.56)	1.12 (0.92–1.37)	1.08 (0.89–1.32)	1.14 (0.89–1.46)	n.i.	n.a.	0.5183
Adjusted§	**1.12 (1.02–1.23)**	1.81 (0.91–3.58)	1.12 (0.95–1.32)	1.14 (0.91–1.44)	1.09 (0.89–1.33)	1.09 (0.89–1.33)	1.14 (0.88–1.48)	n.i.	n.a.	0.8362
Ever (3–10 years)										
Crude	**1.22 (1.11–1.34)**	2.07 (1.10–3.89)	1.19 (1.07–1.33)	1.41 (1.17–1.70)	1.29 (1.11–1.50)	1.13 (0.98–1.31)	1.05 (0.87–1.26)	n.i.	n.a.	0.0932
Adjusted¶	**1.18 (1.09–1.28)**	1.81 (0.91–3.58)	1.14 (1.00–1.29)	1.33 (1.11–1.60)	1.28 (1.08–1.50)	1.13 (0.97–1.31)	1.04 (0.84–1.28)	n.i.	n.a.	0.3184
Sensitization against										
Aero-allergens (6–8 years)										
Crude	1.04 (0.92–1.18)	n.a.	n.a.	0.88 (0.70–1.09)	1.14 (0.91–1.42)	1.00 (0.78–1.27)	1.21 (0.93–1.57)	1.16 (0.64–2.10)	n.a.	0.3291
Adjusted**	1.05 (0.89–1.24)	n.a.	n.a.	0.85 (0.68–1.06)	1.10 (0.88–1.37)	0.97 (0.76–1.24)	1.33 (1.01–1.74)	1.33 (0.72–2.44)	n.a.	0.1215

Table 2 Continued

OR (95% CI)	Summary estimate	LEICESTER	ALSPAC	BAMSE	GINIplus	PIAMA-NHS	LISAplus	DARC	CO.N.ER	Test for homogeneity (*P*)
Mould allergens (6–8 years)										
Crude	1.02	n.a.	n.a.	0.68	1.01	1.13	3.22	1.11	n.a.	0.4061
	(0.70–1.49)			(0.34–1.37)	(0.49–2.12)	(0.54–2.34)	(0.80–12.94)	(0.32–3.88)		
Adjusted††	1.01	n.a.	n.a.	0.66	1.00	1.09	3.11	1.30	n.a.	0.3926
	(0.69–1.49)			(0.32–1.33)	(0.48–2.09)	(0.52–2.27)	(0.77–12.51)	(0.36–4.70)		

DEM, did not enter the model (number too small).
n.a., no early exposure and/or respective health outcome information available.
n.i., DARC: data not included for asthma and symptoms of allergic rhinitis because of different assessment methods.
*Adjusted for sex, parental allergy, early exposure to second-hand smoke (SHS).
†Adjusted for sex, parental allergy, parental education, early exposure to second-hand smoke (SHS).
‡Adjusted for sex, parental allergy, smoking during pregnancy, early exposure to second-hand smoke (SHS), breastfeeding.
§Adjusted for sex, parental allergy, parental education, early day care.
¶Adjusted for sex, parental allergy, parental education, early day care, early ETS exposure, smoking during pregnancy.
**Adjusted for sex, parental allergy.
††Adjusted for parental allergy.
Significance of bold values:
1. Early asthma: cOR: 1.44 (1.07–1.95) $P = 0.02$; aOR: 1.39 (1.05–1.84) $P = 0.02$.
2. School age allergic rhinitis: cOR: 1.16 (1.06–1.26) $P < 0.001$; aOR: 1.12 (1.02–1.23) $P = 0.01$.
3. Ever allergic rhinitis: cOR: 1.22 (1.11–1.34) $P < 0.001$; aOR: 1.18 (1.09–1.28) $P < 0.001$.

asthma and asthma between age 3 and 10 in children who were exposed to mould/dampness; however, this result should be interpreted with caution because of the small sample size of the included data set from the Leicester cohort. There was statistically significant heterogeneity between the cohorts in the relationship between visible mould and/or dampness and childhood asthma (3–10 years, $P < 0.05$).

Symptoms of allergic rhinitis

The combined OR between early exposure to mould and symptoms of allergic rhinitis during early school age (6–8 years) and childhood (3–10 years) were significantly increased in adjusted analyses [aOR, 1.12 (95% CI, 1.02–1.23) and 1.18 (95% CI, 1.09–1.28)], respectively, Fig. 1B). There was no significant heterogeneity between the cohorts observed.

Sensitization to aero-allergens and mould allergens

It was possible to model the relationship between early exposure to mould and sensitization (assessed by specific IgE) against aero-allergens in general and specifically against mould allergens at 6–8 years of age for five cohorts. We observed no association between early exposure to visible mould and/or dampness and sensitization against aero-allergens including mould at early school age in adjusted analyses [aOR, 1.05 (95% CI, 0.89–1.24) and 1.01 (95% CI, 0.69–1.49)], respectively (Fig. 1C). Further, we observed no significant heterogeneity between the cohorts.

To improve the comparison of the association between early exposure to mould and asthma outcomes over time, we restricted analyses to those birth cohorts with information at each age category (BAMSE, GINIplus and PIAMA-NHS). The subanalysis revealed similar results – the strongest effect was found for early asthma [0–2 years: aOR, 1.31 (95%CI, 1.03–1.66), 6–8 years: aOR, 1.15 (95%CI, 0.94–1.40) and 3–10 years: aOR, 1.15 (95%CI, 0.90–1.48), respectively].

Stratified analysis

We further stratified the analysis for sensitization against aero-allergens and parental allergy. The estimates showed a positive but not statistically significant association between early exposure to mould and/or dampness and school-age asthma in children without sensitization to aero-allergens [OR 1.31 (95% CI, 0.92–1.86), Data S3].

Children with parental allergy and exposure to mould had an increased risk of early asthma symptoms [aOR, 1.49 (95%CI, 1.00–2.22)], school-age asthma [aOR, 1.14 (95%CI, 0.97–1.35)] and asthma diagnoses between 3 and 10 years of age [aOR, 1.28 (95%CI, 1.12–1.47)]. Similar effects were observed for school-age and ever symptoms of allergic rhinitis [aOR, 1.14 (95%CI, 1.02–1.28) and aOR, 1.22 (95%CI, 1.11–1.33), respectively], but there was no association between early exposure to mould in relation to sensitization against aero-allergens in children with parental allergy. For children without parental allergy, no association with aller-

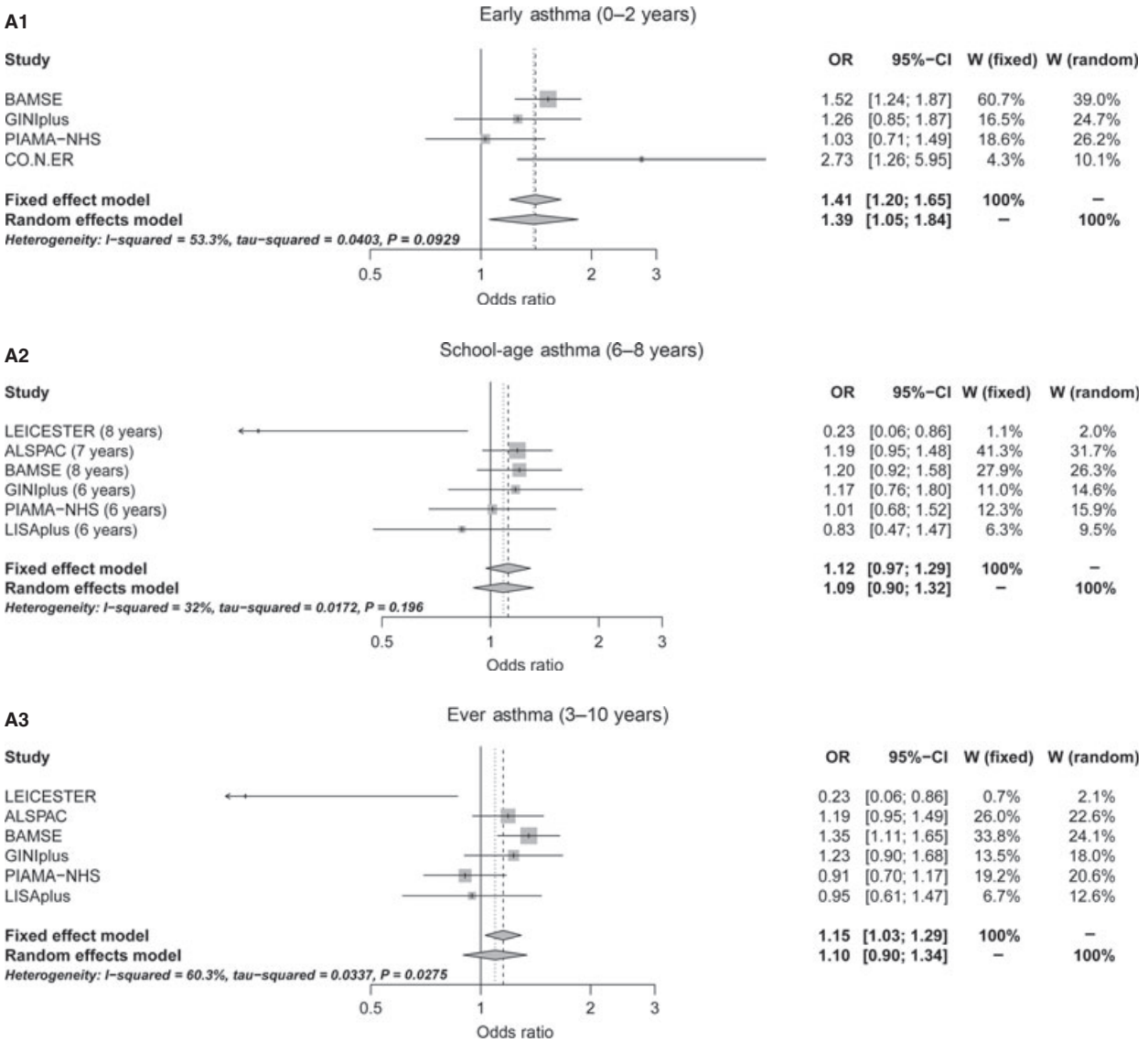

Figure 1 (A) Asthma, (B) Symptoms of Allergic Rhinitis, (C) Allergic Sensitization: Adjusted odds ratios and 95% confidence intervals (95% CI) of asthma, symptoms of allergic rhinitis and allergic sensitization in relation to early exposure to mould and/or dampness (0–2 years), from random-effect meta-analyses (combined effect) and separately by each cohort. For each study, the size of the box represents the variance, the horizontal line the confidence interval of each individual cohort. W (fixed) and W (random) indicate the percentage weight of each cohort contributing to the combined summary estimate.

gic, respiratory symptoms was observed; however, there was a significant decreased risk of sensitization against mould allergens at school age [aOR, 0.41 (95%CI, 0.17–0.98), Data S3].

Discussion

Our main findings of the meta-analysis of European birth cohorts indicated that early-life exposure to visible mould and/or dampness significantly increased the risk of allergic rhinitis symptoms up to 10 years of age. We also found a modest and significantly increased risk of asthma (< 3 years) and a nonsignificantly increased risk of later asthma outcomes (6–8 and 3–10 years). No association was observed for sensitization against aero-allergens or mould allergens at school age (Fig. 1A–C).

Our results are in agreement with recent studies on mould exposure and respiratory diseases in children (18–20). Collaborative studies observed statistically significant increased risks of asthma for children exposed to mould and dampness at home ranging from adjusted ORs of 1.35–1.56 (6–8). Similar results were reported for wheeze and allergic rhinitis.

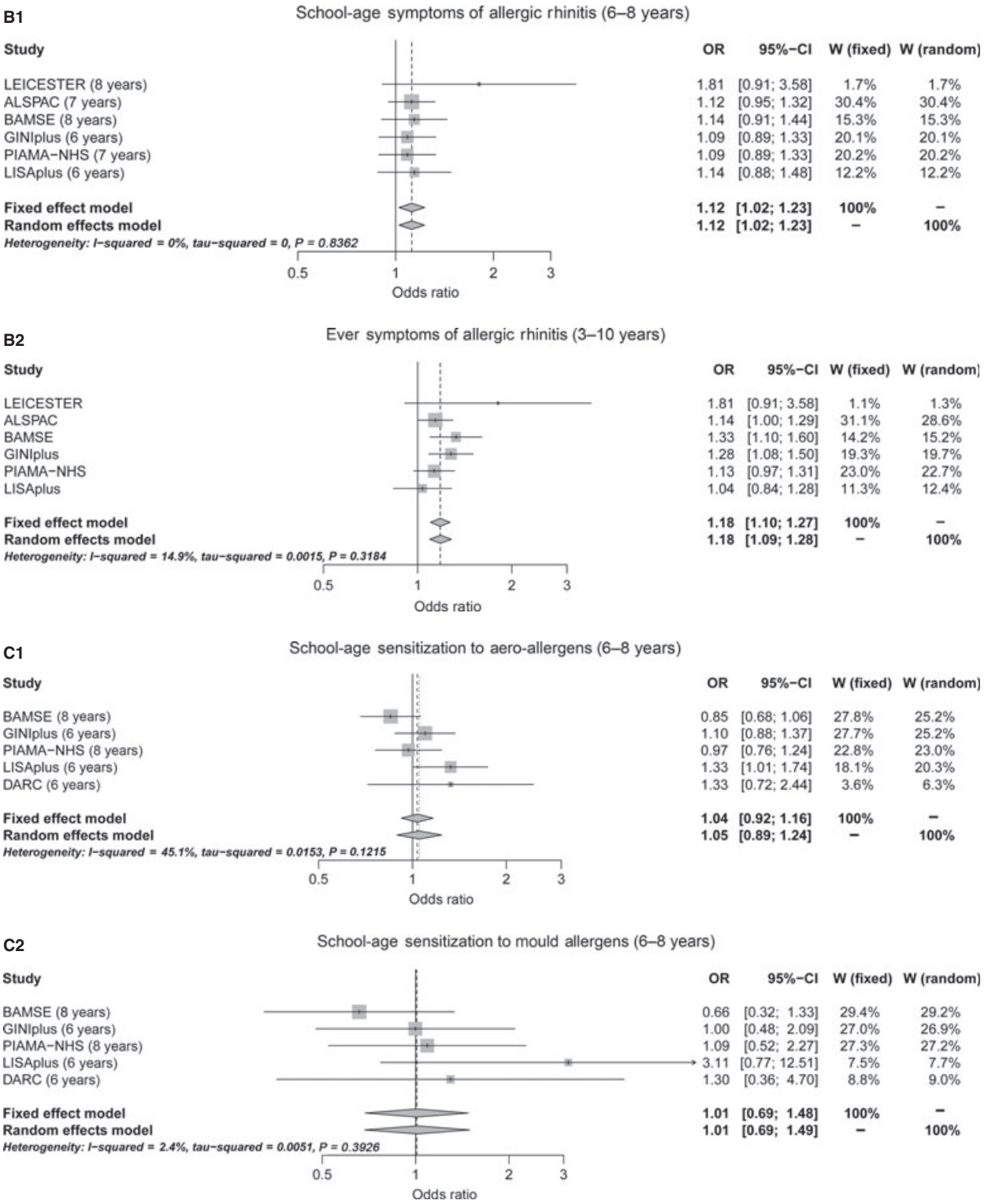

Figure 1 Continued

This is the largest investigation made into the association of visible mould exposure and allergic disorders, using individual participant data from birth cohorts. The prospective design of these cohort studies is the best approach to assess the temporal sequence between early childhood exposure and health outcomes. Exposure assessment before health outcome

observation strengthens the independence of both measurements. Crude variables from each of the eight birth cohorts were harmonized. A cohort was eligible for the analysis if there was information available for at least one of the seven defined health outcomes. As a result, not all cohorts contributed to all analysis.

Although visible mould has been consistently associated with allergic outcomes, the causal agents have not been identified (5). 'The enormous diversity of the Fungal Kingdom is well recognized': in 'healthy' indoor environments, the predominant part of fungi is presented by the outdoor air genera *Cladosporium*, followed by the genera mainly found indoors including *Penicillium* and *Aspergillus* (19, 21). Once there is moisture or mould, the composition of the fungal profile is shifting mainly to *Penicillium* and *Aspergillus*. Species such as *Penicillium chrysogenum*, *Penicillium expansum*, *Aspergillus versicolor*, *Aspergillus penicillioides* and also *Stachybotris chartarum* were reported to be detected typically in moisture damaged environments (22, 23). It has been shown that fungal species induce inflammatory processes (5, 24) and that sensitization to mould allergens is linked to severe asthma (25). In several studies, associations between exposure to spores of *Penicillium* indoors and an increased risk of respiratory disorders were described (26, 27). However, the epidemiological evidence for the association between exposure to specific fungal spores and asthma and allergy remains inconclusive (5, 28).

In this investigation, mould exposure was defined as parent-reported visible mould and/or dampness within the homes. This common approach in epidemiological studies might lead to misclassification if moulds were not reported in spite of being present (e.g. hidden behind furniture) or not aerosolized and therefore not relevant to inhaled exposure. As the appearance of visible mould might not be sensitive enough, some studies measured mould exposure via settled house dust or air sampling to reflect the potential inhaled amount (29–31). This objective method is often not realizable in large cohort populations. However, the correlation between visible mould and the airborne concentration of fungal spores seemed to be good (32), and slightly higher indoor spore concentrations were reported for buildings with apparent mould problems (33, 34). In summary, the presence of visible mould serves as a good indicator that the indoor environment is out of balance (19).

Our meta-analysis showed that early exposure to mould was significantly associated with asthma, especially in young children. However, this positive effect could be masked by uncertainties of the diagnosis in the first 2 years of life and therefore might be of a transient character. Nevertheless, owing to the asthma definition used in our investigation, satisfying at least two of three allergic conditions implies a more serious health disorder rather than transient symptoms arising from a common cold, and adverse effects of mould exposure on asthma were also observed at later ages.

Although the risk of allergic respiratory disorders was increased in children exposed to mould/dampness, we found no association with allergic sensitization at the same age – which is considered to be a major risk factor (35, 36). Moreover, after stratifying children by their atopic status, there was a positive, not-statistically-significant association for non-IgE-mediated asthma compared with the nonallergic and nonsymptomatic children. In approximately 80% of childhood asthma cases, allergy seems to be mediated by IgE antibodies (37), but it is unclear whether the tested aero-allergens are causal agents. The mechanisms of nonallergic asthma result from similar inflammatory changes, but are suggested to be driven by the production of antibodies mainly of the IgG, IgA and IgM isotype after inhalation of large amounts of protein as in mould (37, 38). We further observed an increased risk of visible mould exposure in relation to asthma and symptoms of allergic rhinitis in children with parental allergy, but not in children without this hereditary component. In addition, early exposure to mould was observed to decrease the risk of sensitization to mould allergens in children without parental allergy; however, because of the small sample size, these findings should be interpreted with caution. Nevertheless, allergic parents are well advised to ensure healthy indoor air quality and to both remove visible mould or signs of moisture and actively prevent its formation in the first place.

Conclusion

The results of the first collaborative effort of European birth cohorts with regard to mould and/or dampness indicated an increased risk of subsequent allergic respiratory symptoms in children spending their first years of life in homes with visible mould and/or dampness. To draw a causal relationship is hindered by the variability of microbial components in indoor air. Assessment techniques such as molecular methods and measurements of airborne enzyme activity are considered both highly sensitive and specific and may help to identify patterns of causal agents in relation to asthma and allergy.

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Author contribution

Christina G. Tischer (first author) carried out statistical analysis, manuscript preparation and revision; Joachim Heinrich designed the study and provided data and commented on the draft; Cynthia Hohmann prepared data (common data base) and commented on the draft; Thomas Keil (MAS), Elisabeth Thiering (LISAplus), Olf Herbarth (LISAplus), Andrea Müller (LISAplus), John Henderson (ALSPAC), Raquel Granell (ALSPAC), Maria Pia Fantini (CO.N.ER), Lorenza Luciano (CO.N.ER), Anna Bergström (BAMSE), Inger Kull (BAMSE), Elke Link (GINIplus), Andrea von Berg (GINIplus), Claudia E. Kühni (LEICESTER), Marie-Pierre F. Strippoli (LEICESTER), Ulrike Gehring (PIAMA-NHS), Alet Wijga (PIAMA-NHS), Esben Eller (DARC) and Carsten Bindslev-Jensen (DARC) provided data, commented on

the manuscript and participated in the critical revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article found at: www.wileyonlinelibrary.com

Data S1. Definition of early mould and/or dampness exposure in each participating birth cohort.

Data S2. Definition of health outcomes in each participating birth cohort.

Data S3. Mould and/or dampness exposure in relation to asthma and allergy, stratified by sensitization and parental allergy.

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SUPPLEMENTARY MATERIAL

1) Definition of early mould and/or dampness exposure in each participating birth cohort

Birth Cohort	Early exposure to visible mould and/or dampness (birth to 2y)
LEICESTER	• In your child's bedroom, during the winter months, are there patches of mould or fungus? (birth, 2y) • Please list other rooms in your house affected by mould or fungus. (birth, 2y) • In your child's bedroom, during the winter months, does condensation ever form on the walls? (birth, 2y) • Please list other rooms in your house affected by condensation or damp. (birth, 2y)
ALSPAC	• Is there ever any damp, condensation or mould in your home? (birth to 2y) • Mould on walls? (birth to 1y)
BAMSE	• Has there been any visible mould/mildew in the home in the past year? (parental reported, birth) • Is there, or has there ever been, any type of moisture damage (spots and the like) in the home? (parental reported, birth) • Is there damage from dampness, mould or rot in wet areas, or is there a suspected (hidden) damage? (inspector reported, 2y)
GINIplus	• (Were or) Are there damp stains or visible mould somewhere in the dwelling (except on food)? (birth) • (Were or) Are there damp stains or visible mould somewhere in the dwelling (except on food)? (2y)
PIAMA-NHS	• Did you see any damp stains or mould spots in the bathroom/living room/parents' bedroom, child's bedroom in the past 12 months? (birth to 2y)
LISAplus	• (Were or) Are there damp stains or visible mould somewhere in the dwelling (except on food)? (birth to 2y)
DARC	• Is there mould in child's sleeping room (birth to 1y)? • Are there visible wet spots or mould spots on walls/ceilings in the bathroom (birth to 1y)?
CO.N.ER	• Have you noticed dampness in the child's bedrooms? (birth to 1y) • Have you noticed mould on walls? (kitchen, dining room, parents bedroom, bathrooms, other rooms) (birth)

2) Definition of health outcomes in each participating birth cohort

Definition of "Early Asthma" in each participating birth cohort
based on the ISAAC questions (Asher et al., 1995) defined as satisfying 2 out of 3 conditions

Birth Cohort	Early Asthma (birth to 2y)
LEICESTER	*No early asthma*
ALSPAC	*No early asthma*
BAMSE	• Has your child ever had problems involving: wheezy or raspy breathing? (1y) • Has your child, after the age of one year, ever had problems involving: Wheezy or raspy breathing? (2y) • Has your child ever/after the age of one year been prescribed any of the medicines listed below for treatment of asthma or breathing problems characterized by wheezing, heavy or difficult breathing? (1y, 2y) • Has a doctor diagnosed your child as having asthma after the age of one year? (1y, 2y
GINIplus	• Did your child suffer from wheezing in the chest while breathing in the last 12 months? (1y, 2y)? • Has a doctor diagnosed asthma on your child in the last 12 months? (2y)?
PIAMA-NHS	• Has your child had wheezing or whistling in the chest during the past 12 months? (2y) • Has your child had asthma medication prescribed by a doctor during the last 12 months? (2y) • Has a doctor ever diagnosed asthma in your child? (2y)
LISAplus	• Did your child have chest wheezing in the last 12 months/last 6 months? (6 months, 1y, 2y)? • Doctor diagnosed asthma in the last 12 months/last 6 months (6 months, 1y, 2y)?
DARC	• Has or has had the child occasionally suffered from wheezing since last follow-up? (birth to 2y) • Has the child received any prescriptive medication since the last follow-up?* (birth to 2y) • Do you think your child has asthma? (physician-diagnosed) (birth to 2y)
CO.N.ER	• Has your children ever had breath difficulty with wheezing symptoms at least once? (birth) • In the last 12 months has your children had breath difficulty with wheezing symptoms? (2y) • Asthma medication intake ever? (2y) • Has a doctor ever diagnosed asthma on your child? (2y)

**DARC: high prevalence of early asthma due to high medication intake (medication prescribed for asthma OR bronchitis!)*

Definition of "School age Asthma" in each participating birth cohort (6-8y)
based on the ISAAC questions (Asher et al., 1995) defined as satisfying 2 out of 3 conditions

Birth Cohort	School age Asthma (6-8y)
LEICESTER	• Has your child had wheezing or whistling in the chest in the last 12 months? (8y) • Asthma medication intake in the last 12 months? (8y)
ALSPAC	• Does he/she have wheezing with whistling on the chest when she/he breathes? (6y) • Child had asthma medication in past 12 months? (6y)
BAMSE	• Has your child had trouble with wheezing or raspy breathing since the age of 4? (8y) • Has your child received treatment for breathing difficulties in the last 12 months? (8y) • Has a doctor diagnosed your child as having asthma? (8y
GINIplus	• Did your child suffer from wheezing in the chest while breathing in the last 12 months? (6y) • Has a doctor diagnosed asthma on your child in the last 12 months? (6y) • Was your child treated for asthma in the last 24 months? (6y)
PIAMA-NHS	• Has your child had wheezing or whistling in the chest during the past 12 months? (6y) • Has your child had asthma medication prescribed by a doctor during the last 12 months? (6y) • Has a doctor ever diagnosed asthma in your child? (6y)
LISAplus	• Did your child have chest wheezing in the last 12 months? (6y) • Doctor diagnosed asthma in the last 12 months? (6y) • Was your child treated for asthma in the 5th or 6th year of life?
DARC	• Has or has had the child occasionally suffered from wheezing since last follow-up? (6y) • Has the child received any prescriptive medication since the last follow-up? (6y) • Do you think your child has asthma? (physician-diagnosed) (6y)
CO.N.ER	*no school age asthma*

Definition of "School age Symptoms of Allergic Rhinitis" in each participating birth cohort (6-8y)

Birth Cohort	School age Symptoms of Allergic Rhinitis (6-8y)
LEICESTER	• In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu? (8y)
ALSPAC	• In the past 12 months, the child had sneezing or runny, blocked nose without having a cold or flu? (6y)
BAMSE	• Has your child been afflicted with sneezing, runny nose, or stuffy nose without having a cold in the last 12 months? (8y)
GINIplus	• Did your child have sneezing attacks or a blocked, runny or itchy nose in the last 12 months, without having a cold? (6y)
PIAMA-NHS	• Hast the child had sneezed, runny or congested nose without having a cold during past 12 months? (6y)
LISAplus	• Did your child have sneezing attacks or a blocked, runny or itchy nose in the last 12 months, without having a cold? (6y)
DARC	• Has the child had blocked nose (without cold) in the past 12 months? (6y)
CO.N.ER	*no school age symptoms of allergic rhinitis*

3) Mould and/or dampness exposure in relation to asthma and allergy, stratified by sensitization and parental allergy

Crude odds ratios and 95% confidence intervals of **school age asthma (6-8y)** in relation to early exposure to mould and/or dampness (0-2 years), **stratified by sensitisation status against aero-allergens**

	Summary estimate	BAMSE	GINIplus	PIAMA-NHS	LISAplus	Test for homogeneity (p)
Asthma (6-10y)						
Children with sensitisation to inhalant allergens (IgE positive)						
	1.02 (0.78 – 1.35)	1.18 (0.81 – 1.71)	0.90 (0.46 – 1.74)	0.86 (0.42 – 1.74)	0.79 (0.34 – 1.86)	p = 0.7343
Children without sensitisation to inhalant allergens (IgE negative)						
	1.31 (0.92 – 1.86)	1.37 (0.87 – 2.17)	1.45 (0.67 – 3.16)	0.86 (0.32 – 2.31)	1.41 (0.43 – 4.65)	p = 0.8458

Adjusted Odds ratios (OR) and 95% confidence intervals (95% CI) of asthma, symptoms of allergic rhinitis and sensitisation to aero-allergens, respectively, in relation to early exposure to mould and/or dampness (0-2 years), **stratified by parental allergy**

CHILDREN WITH PARENTAL ALLERGY										
OR (95% CI))	**Summary estimate**	**LEICESTER**	**ALSPAC**	**BAMSE**	**GINIplus**	**PIAMA-NHS**	**LISAplus**	**DARC**	**CO.N.ER**	**Test for homogeneity (p)**
Asthma										
Early (0-2y)	**1.49 (1.00 – 2.22)**	n.a.	n.a.	1.28 (1.43 – 2.32)	0.97 (0.58 – 1.61)	1.18 (0.71 – 1.97)	DEM	n.i.	3.00 (1.22 – 7.42)	p = 0.0449
School age (6-8y)	1.14 (0.97 – 1.35)	0.28 (0.06 – 1.22)	1.13 (0.88 – 1.44)	1.23 (0.90 – 1.67)	1.18 (0.70 – 1.98)	1.19 (0.66 – 2.14)	1.09 (0.57 – 2.09)	n.i.	n.a.	p = 0.5796
Ever (3-10y)	**1.28 (1.12 – 1.47)**	DEM	1.15 (0.90 – 1.47)	1.55 (1.23 – 1.94)	1.26 (0.88 – 1.80)	1.07 (0.74 – 1.54)	1.15 (0.68 – 1.93)	n.i.	n.a.	p = 0.4811
Symptoms of allergic rhinitis										
School age (6-8y)	**1.14 (1.02 – 1.28)**	DEM	1.10 (0:90 – 1.33)	1.18 (0.91 – 1.54)	1.19 (0:93 – 1.54)	1.08 (0.81 – 1.45)	1.18 (0.85 – 1.63)	n.i.	n.a.	p = 0.9923
Ever (3-10y)	**1.22 (1.11 – 1.33)**	DEM	1.15 (0.99 – 1.33)	1.38 (1.11 – 1.73)	1.31 (1.07 – 1.61)	1.08 (0.86 – 1.37)	1.25 (0.96 – 1.63)	n.i.	n.a.	p = 0.6364
Sensitisation against										
Aero-allergens (6-8y)	1.11 (0.95 – 1.30)	n.a.	n.a.	0.92 (0.71 – 1.19)	1.21 (0.93 – 1.59)	1.03 (0.72 – 1.48)	1.28 (0.91 – 1.80)	1.74 (0.79 – 3.80)	n.a.	p = 0.3353
Mould allergens (6-8y)	1.35 (0.86 – 2.11)	n.a.	n.a.	0.78 (0.36 – 1.67)	1.55 (0.66 – 3.61)	1.73 (0.65 – 4.62)	2.57 (0.61 – 10.83)	2.21 (0.43 – 11.29)	n.a.	p = 0.4824

CHILDREN WITHOUT PARENTAL ALLERGY										
OR (95% CI))	**Summary estimate**	**LEICESTER**	**ALSPAC**	**BAMSE**	**GINIplus**	**PIAMA-NHS**	**LISAplus**	**DARC**	**CO.N.ER**	**Test for homogeneity (p)**
Asthma										
Early (0-2y)	1.15 (0.77 – 1.73)	n.a.	n.a.	0.95 (0.63 – 1.43)	1.93 (1.02 – 3.64)	0.87 (0.50 – 1.50)	DEM	n.i.	2.11 (0.42 – 10.68)	p = 0.1815
School age (6-8y)	1.06 (0.80 – 1.41)	DEM	1.46 (0.87 – 1.47)	1.08 (0.61 – 1.93)	1.14 (0.53 – 2.47)	0.83 (0.47 – 1.47)	0.37 (0.10 – 1.33)	n.i.	n.a.	p = 0.4408
Ever (3-10y)	0.92 (0.74 – 1.14)	DEM	1.35 (0.78 – 2.33)	0.92 (0.62 – 1.37)	1.14 (0.62 – 2.07)	0.76 (0.53 – 1.10)	0.63 (0.27 – 1.44)	n.i.	n.a.	p = 0.5196
Symptoms of allergic rhinitis										
School age (6-8y)	1.09 (0.93 – 1.28)	DEM	1.08 (0:78 – 1.51)	1.04 (0.63 – 1.70)	1.05 (0:73 – 1.52)	1.08 (0.82 – 1.43)	1.27 (0.76 – 2.10)	n.i.	n.a.	p = 0.9949
Ever (3-10y)	1.09 (0.95 – 1.26)	DEM	1.12 (0.86 – 1.46)	1.16 (0.80 – 1.69)	1.23 (0.94 – 1.61)	1.15 (0.94 – 1.39)	0.72 (0.49 – 1.04)	n.i.	n.a.	p = 0.3018
Sensitisation against										
Aero-allergens (6-8y)	0.94 (0.74 – 1.18)	n.a.	n.a.	0.69 (0.45 – 1.07)	0.90 (0.60 – 1.34)	0.92 (0.66 – 1.29)	1.43 (0.90 – 2.27)	0.87 (0.32 – 2.34)	n.a.	p = 0.2744
Mould allergens (6-8y)	**0.41 (0.17 – 0.98)**	n.a.	n.a.	0,29 (0.04 – 2.24)	0.25 (0.03 – 1.93)	0.52 (0.15 – 1.79)	DEM	0.54 (0.05 – 5.31)	n.a.	p = 0.9695

7 Microbial Components in Relation to Asthma and Allergy

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

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ABSTRACT: For a long time, exposure to mould and dampness-derived microbial components was considered a 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)-β-D-glucan and extracellular polysaccharides (EPSs), may protect children from developing allergy. We investigated the association of exposure to (1,3)-β-D-glucan, EPS and endotoxin with asthma and allergies in 6-yr-old children.

This investigation was the follow-up to a 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)-β-D-glucan, EPS and endotoxin were measured in settled house dust sampled from children's mattresses and living-room floors when the children were, on average, 5 yrs of age. At the age of 6 yrs, health outcome information was available for 678 children.

In the two German subsets, domestic EPS and endotoxin exposure from children's mattresses were significantly negatively associated with physician-diagnosed asthma (OR per interquartile range increase 0.60 (95% CI 0.39–0.92) and 0.55 (95% CI 0.31–0.97), respectively). In addition, EPS exposure was inversely related to physician-diagnosed allergic rhinitis (OR 0.50, 95% CI 0.31–0.81). For the Dutch population, no associations were observed between exposure to microbial agents and respiratory health outcomes.

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.

KEYWORDS: Allergy, asthma, childhood, endotoxin, rhinitis

The effect of visible mould and mould components in indoor environments on asthma and allergic diseases in children has been widely discussed in 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 USA concluded that 1-yr-old children of asthmatic and allergic mothers who were exposed to high levels of *Penicillium*, a common genus of mould, were at significantly higher risk for wheeze and persistent cough [7]. Another US study showed that exposure to dust-borne *Aspergillus*, *Alternaria* and *Aureobasidium* at 3 months of age was associated with the development of physician-diagnosed allergic rhinitis within the first 5 yrs of life [8].

Few studies measured biological components of mould, such as (1,3)-β-D-glucan and extracellular polysaccharides (EPSs), as surrogates for mould exposure [3, 9]. (1,3)-β-D-glucans are nonallergenic, water-insoluble, structural cell wall components of most fungi. This biologically active polyglucose molecule may account for ⩽60% of the dry weight of the fungal cell wall [10]. However, (1,3)-β-D-glucans are also part of the

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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)-β-D-glucan as a surrogate. Fungal EPSs are stable carbohydrates secreted or shed during fungal growth and have antigenic specificity at the genus level. In contrast with the findings on visible mould and measured specific mould species, longitudinal studies showed that exposure to (1,3)-β-D-glucan and EPS was inversely associated with wheezing symptoms and parentally reported physician-diagnosed asthma in children [3, 5, 11]. In addition, one case–control study reported that elevated levels of (1,3)-β-D-glucan and EPS exposure from mattress dust were associated with a lower prevalence of allergic sensitisation in 2–4-yr-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.* [12] reported that visible mould growth was significantly correlated with the concentration of fungal spores. As opposed this, a US cohort study did not observe a correlation between (1,3)-β-D-glucan exposure and visible mould [3, 5].

Early exposure to mould components compared with exposure later in life also showed a different impact on allergic health outcomes [13]. The immune response of newborns is dominated by T-helper (Th)2-cells and a shift to Th1-mediated immune response takes place during early childhood. It has been hypothesised that exposure to (1,3)-β-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 hypothesised that microbial products such as endotoxin could affect the development of children's immune systems early in life and play a crucial role in the development of tolerance to allergens ubiquitous in natural surroundings [21, 22].

We prospectively investigated the associations between exposure to mould components and endotoxin in settled house dust with respiratory and allergic health outcomes in 6-yr-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 from the 6-yr follow-up were 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 (Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood) and 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 3,097 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 5,991 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 to 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 to the noninterventional arm. Detailed descriptions of the LISA [24] and GINI [25] studies were published elsewhere. For the PIAMA study, a total of 4,146 pregnant females 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. Nonallergic pregnant females were invited to participate in a ''natural history'' study arm. Pregnant females 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 yrs in the Netherlands and at ages 2 and 3 yrs in Germany (fig. 1 and supplementary material 1). The target population size was ~180 sensitised children and 180 nonsensitised children as controls in each country. The controls were not matched by any criteria. Based on serum immunoglobulin (Ig)E determination, cases were defined as children who were sensitised to common aeroallergens. The number of children sensitised to aeroallergens was not reached in Germany and the Netherlands; the cases were supplemented with children sensitised to food. Allergen panels differed between the cohorts, but specific IgE to egg white, milk, house dust mites, cat, and 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 present investigation, 317 sensitised and 379 nonsensitised children were selected from the GINI, LISA and PIAMA birth cohort studies. At the age of 6 yrs, health endpoint data was available from 346 and 332 of the German and Dutch participants of the AirAllerg study, respectively.

Questionnaire data

In the German and Dutch populations, information on respiratory and allergic disorders, history of moving home, and visible mould in the child's home was collected at age 6 yrs, using self-administered questionnaires. An online supplement is provided to display the exact health outcome definitions within the 6-yr follow-up period of both subsets (see supplementary material 2). Information on parental educational level, family history of allergic diseases, smoking

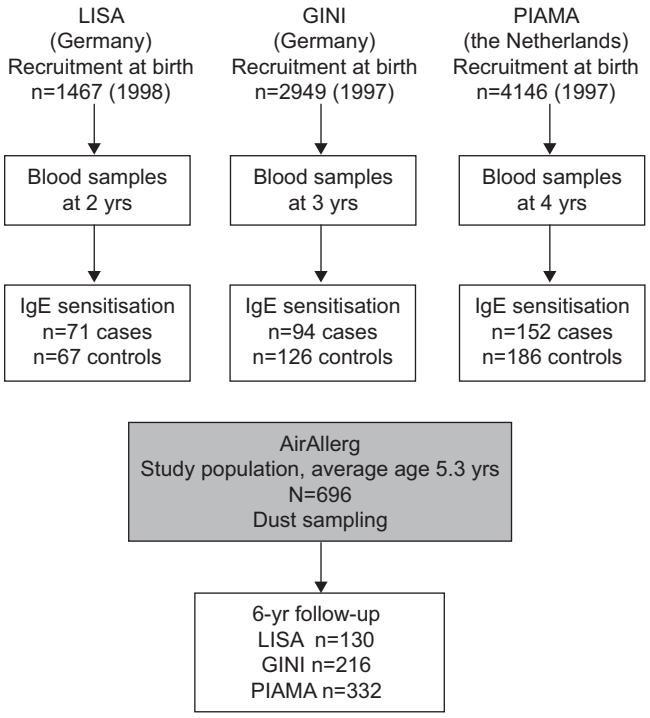

FIGURE 1. Study design and population. LISA: Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood; GINI: German Infant Nutritional Intervention; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; Ig: immunoglobulin.

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 yrs (LISA and PIAMA) and 6 yrs (GINI) of age. A detailed description of the analysis and collection of the house-dust samples is 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)-β-D-glucan was measured in the third supernatant with a (1,3)-β-D-glucan-specific inhibition enzyme immunoassay [28]. The detection limits of the assay were 0.05 endotoxin units·mL^{-1}, 3.3 μg·mL^{-1} and 0.9 EPS units·mL^{-1} for endotoxin, (1,3)-β-D-glucan and EPS of *Aspergillus* and *Penicillium* spp., respectively. Exposures were expressed as both per gram of sampled dust (concentration) and per square metre of sampling surface area (load). Samples of (1,3)-β-D-glucan and EPS below the limit of detection (LOD) were assigned a value of two-thirds of the respective LOD [11].

Statistical analysis

Distributions of the biocontaminant levels in house-dust samples were highly skewed and, therefore, were described using median (interquartile range (IQR)). Spearman's rank correlation coefficient was used to calculate the correlations. The skewed variables were log-transformed for further analysis. Generalised additive models using a local regression smoothing operation were fitted to assess the relationship of the associations between continuous indoor biological 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 the literature. For the German subset, confounders included in all models were sex, parental allergy, parental education, current pet ownership, breastfeeding, 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 ETS, current pet ownership, breastfeeding, 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 affected the health outcomes in terms of allergic diseases and symptoms, but also the exposure and is, therefore, a confounder that we took into account for the current investigation.

The results are presented as OR (95% CI) for an IQR increase in microbial exposure. We focused on exposure from children's mattresses due to a considerable amount of nondetectable values from living-room floor dust samples. The analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population

A total 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 yrs are presented in table 1. There were some significant differences between the German and Dutch subsets. A higher percentage of the Dutch children were exposed to visible mould and were reported to have a pet at home compared with the German cohort. A considerable number of

TABLE 1 Description of the German and Dutch AirAllerg study population at age 6 yrs

	LISA and GINI	PIAMA	p-value
Subjects n	358	332	
Cohort type			
LISA	138 (39)		
GINI	220 (61)		
Males	204/358 (57)	186/332 (56)	0.859
Parental allergy[#]	294/358 (82)	260/332 (78)	0.246
Parental education[¶]			
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 at 6 yrs of age	56/323 (17)	108/329 (33)	**<0.001**
Dwelling considered damp at 6 yrs of age	10/339 (3)	NA	NA
Any pets in child's home at 6 yrs of age	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[+]	179/333 (54)	201/328 (61)	0.060
Smoking in child's home at 6 yrs of age	72/344 (21)	89/331 (27)	0.084
Maternal smoking during pregnancy	50/357 (14)	45/328 (14)	0.985
Moving home[§] **at 6 yrs of age**	39/346 (11)	13/330 (4)	**<0.001**
Physician-diagnosed asthma[f]	17/343 (5)	27/328 (8)	0.119
Physician-diagnosed allergic rhinitis[f,##]	47/342 (14)	24/327 (7)	**0.010**
Allergic respiratory symptoms[f]			
Rhinoconjunctivitis	48/343 (14)	28/327 (7)	**0.036**
Wheezing	43/341 (13)	48/331 (15)	0.546
Dry cough[¶¶]	56/343 (16)	80/330 (24)	**0.014**
Physician-diagnosed infections of the upper airways[f]	275/342 (80)	47/329 (14)	**<0.001**
Dust sampling season[++]			
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 (%) or n/N (%), unless otherwise stated. Bold indicates statistically significant p-values. LISA: Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood; GINI: German Infant Nutritional Intervention; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; NA: not available. [#]: defined as asthma and/or hay fever and/or eczema (at least one parent) for LISA and GINI and as asthma and/or allergy to house dust (mite) or pets, and/or hay fever in at least one parent for PIAMA; [¶]: categorised according to the German educational system as less than, equal to and more than grade 10 for low, medium and high, respectively, for LISA and GINI and as the highest attained educational level of mother and father, where low is primary school, lower vocational or lower secondary education, medium is intermediate vocational education or intermediate/ higher secondary education, and high is higher vocational education and university for PIAMA; [+]: defined as exclusive breastfeeding during the first 4 months of life for LISA and GINI and as any breastfeeding at the age of 3 months for PIAMA; [§]: defined as moving home in the previous 24 months for GINI, and as moving home in the previous 12 months for LISA and PIAMA; [f]: in the previous 12 months; [##]: defined as hayfever and/or allergic rhinitis (all seasons) for LISA and GINI, and as hayfever ever for PIAMA; [¶¶]: defined as nocturnal dry cough for PIAMA; [++]: autumn defined as October–November, winter as December–February and spring as March–April.

the Dutch subjects, but only a small number of the German children, had visited day-care within the first year of life. The prevalence of physician-diagnosed respiratory infections in the previous 12 months at the age of 6 yrs was five times higher among the German compared with the Dutch population. The German children often reported more physician-diagnosed allergic rhinitis and rhinoconjunctivitis, whereas the Dutch children showed a higher prevalence of nocturnal dry cough. The season of dust sampling differed considerably between Germany and the Netherlands.

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

The number of samples below the LOD, the median (IQR) 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)-β-D-glucan loads, and (1,3)-β-D-glucan concentrations from children's mattresses in Germany were significantly than the Dutch sample. There were weak correlations between the

TABLE 2 Biocontaminant levels measured from children's mattress and living-room floors

	LISA and GINI	PIAMA	Wilcoxon test p-value
Subjects n	358#	332	
Child's mattress dust load			
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)-β-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 concentration			
Endotoxin EU·g^{-1}	2/12222 (7379–21337)	0/10608 (6550–17366)	0.021
(1,3)-β-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 load			
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)-β-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 concentration			
Endotoxin EU·g^{-1}	14/19400 (10104–32678)	23/18196 (9522–32106)	0.451
(1,3)-β-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**

Data are presented as n/median (interquartile range), where n is the number of values below the limit of detection, unless otherwise stated. Bold indicates statistically significant p-values. LISA: Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood; GINI: German Infant Nutritional Intervention; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; EU: endotoxin units; EPS: extracellular polysaccharide; EPSU: EPS units. #: two subjects more than in [9] (n=356).

biocontaminant levels from children's mattresses and living-room floors both for surface load and per gram of dust (Spearman's correlation coefficient: GINI and LISA <0.25, PIAMA <0.13). The correlations between (1,3)-β-D-glucan, EPS and endotoxin from mattress dust samples were weak when these were expressed as units per gram of collected dust; however, the correlations were stronger when they were defined as surface loads (table 3).

Associations between mould components and endotoxin, and respiratory diseases and symptoms

Adjusted logistic regression models showed inconsistent results in the German and Dutch subsets. In the German population, EPS and endotoxin exposure from children's mattresses was significantly negatively associated with physician-diagnosed asthma (OR per IQR 0.60 (95% CI 0.39–0.92) and OR 0.55 (95% CI 0.31–0.97), respectively). EPS exposure was also

TABLE 3 Correlation between the measured microbial components

	LISA and GINI				PIAMA			
	Dust	**(1,3)-β-D-glucan**	**EPS**	**Endotoxin**	**Dust**	**(1,3)-β-D-glucan**	**EPS**	**Endotoxin**
Children's mattresses								
Dust	1.00				1.00			
(1,3)-β-D-glucan	***0.86***	1.00	**0.04**	**0.24**	***0.78***	1.00	**0.13**	**0.15**
EPS	***0.76***	***0.67***	1.00	**0.22**	***0.70***	***0.63***	1.00	**0.07**
Endotoxin	***0.63***	***0.66***	***0.60***	1.00	***0.59***	***0.54***	***0.51***	1.00
Living-room floor								
Dust	1.00				1.00			
(1,3)-β-D-glucan	***0.94***	1.00	**0.21**	**0.26**	***0.95***	1.00	**0.36**	**0.42**
EPS	***0.87***	***0.89***	1.00	**0.24**	***0.90***	***0.89***	1.00	**0.49**
Endotoxin	***0.87***	***0.88***	***0.82***	1.00	***0.93***	***0.93***	***0.87***	1.00

Data are presented as Spearman's ρ. Amount of dust sampled is per square metre of surface area. Endotoxin, (1,3)-β-D-glucan and extracellular polysaccharide (EPS) levels are per gram of dust (concentration; bold only) or per square metre of surface area (load; bold and italic). LISA: Lifestyle Related Factors on the Immune System and the Development of Allergies in Childhood; GINI: German Infant Nutritional Intervention; PIAMA: Prevention and Incidence of Asthma and Mite Allergy.

inversely related to physician-diagnosed allergic rhinitis (OR 0.67, 95% CI 0.49–0.92) (table 4). 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 5). The associations between exposure from living-room floor dust and assessed health outcomes were similar to exposure from children's mattresses, but were not significant (data not shown); this may be due to a higher number of nondetectable values for living-room floor dust samples compared with mattress dust samples.

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

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 (17%) homes in Germany and 108 (33%) homes in the Netherlands were reported as 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 6).

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 ages of 5 and 6 yrs. However, in contrast with 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 yrs, the German and Dutch birth cohorts had different time points of follow-up (*i.e.* 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 standardised exposure and health outcome measurement, we determined the 6-yr 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)-β-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)-β-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)-β-D-glucan and EPS, may have immune stimulatory properties [9, 11, 14]. A US birth cohort study observed that exposure to high levels of (1,3)-β-D-glucan from settled house

TABLE 4 Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and (1,3)-β-D-glucan, extracellular polysaccharide (EPS), endotoxin loads, and total amount of mattress dust at 6 yrs of age in the German birth cohorts

	All subjects					Children with parental allergy				
	Subjects N	(1,3)-β-D-glucan[#,¶] µg·m^{-2}	EPS[#,¶] EPSU·m^{-2}	Endotoxin[#,+] EU·m^{-2}	Dust[#,+] mg·m^{-2}	Subjects n	(1,3)-β-D-glucan[#,¶] µg·m^{-2}	EPS[#,¶] EPSU·m^{-2}	Endotoxin[#,+] EU·m^{-2}	Dust[#,+] mg·m^{-2}
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										
Rhinoconjunctivitis	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)

Data are presented as OR (95% CI), unless otherwise stated. Bold indicates significant associations. EPSU: EPS units; EU: endotoxin units. [#]: per interquartile range increase in ln-transformed exposure; [¶]: adjusted for sex, parental allergy, parental education, current pet ownership, breastfeeding, AirAllerg case status and season of dust-sampling; [+]: adjusted for sex, parental allergy, parental education, current environmental tobacco smoke exposure at home, current pet ownership, breastfeeding, AirAllerg case status and season of dust-sampling.

TABLE 5 Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and mattress dust (1,3)-β-D-glucan, extracellular polysaccharide (EPS) and endotoxin loads at 6 yrs of age in the Dutch birth cohort

	All subjects					Children with parental allergy				
	Subjects N	(1,3)-β-D-glucan[#,¶] µg·m^{-2}	EPS[#,¶] EPSU·m^{-2}	Endotoxin[#,¶] EU·m^{-2}	Dust[#,¶] mg·m^{-2}	Subjects n	(1,3)-β-D-glucan[#,¶] µg·m^{-2}	EPS[#,¶] EPSU·m^{-2}	Endotoxin[#,¶] EU·m^{-2}	Dust[#,¶] mg·m^{-2}
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)
Hayfever	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										
Rhinoconjunctivitis	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)

Data are presented as OR (95% CI), unless otherwise stated. EPSU: EPS units; EU: endotoxin units. #: per interquartile range increase in ln-transformed exposure; ¶: adjusted for sex, parental allergy, parental education, current environmental tobacco smoke exposure at home, current pet ownership, breastfeeding, AirAllerg case status and season of dust-sampling.

TABLE 6 Adjusted logistic regression results describing the association between allergic health outcomes and symptoms and visible mould in any room at home at 6 yrs of age

	Visible mould	
	Germany[#]	The Netherlands[¶]
Subjects n	56	108
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)

Data are presented as OR (95% CI). #: adjusted for sex, parental allergy, parental education level, outdoor activity (in hours), breastfeeding, maternal smoking during pregnancy and AirAllerg case-status; ¶: adjusted for sex, parental allergy, parental education, current environmental tobacco smoke exposure at home, current pet ownership, breastfeeding, study arm and AirAllerg case-status; +: defined as physician-diagnosed allergic rhinitis in Germany and as hayfever in the Netherlands.

dust in the first year of life was associated with a persistent decreased risk for recurrent wheezing among genetically predisposed children up to the age of 3 yrs [3, 5]. DOUWES *et al.* [11] observed a statistically significant protective effect of *Penicillium* and *Aspergillus* EPS exposure from living-room floor dust at the age of 3 months on persistent wheeze in the first 4 yrs of life in the whole Dutch PIAMA study population. In the previous AirAllerg case–control investigation, higher amounts of mattress dust were reported to decrease the risk of allergic sensitisation to inhalant allergens in 2–4-yr-old children [9]. Compared with 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 populations. 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 USA, which did not observe a correlation between (1,3)-β-D-glucan and EPS mould components and visible mould [3, 5]. Furthermore, it is known that (1,3)-β-D-glucan also derives from many other sources than mould, such as pollen or plants, which may explain the differences. Since the 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 postulates an inverse effect of household size and siblings on the risk of hayfever [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 large amounts of microbial products, including endotoxin [30]. Endotoxin has been suggested to have strong immune-stimulatory properties. It may therefore be capable of enhancing the Th1-dominated immune response and suppress the Th2-dominated allergic response in newborns and infants [16, 17]. Being born and growing up on a farm was protective against the risk of developing hayfever 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 nonfarming environments. Children who were exposed to a 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 US birth cohort showed that exposure to the 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 standardised exposure measurement from two different countries. We observed that endotoxin and (1,3)-β-D-glucan loads, and (1,3)-β-D-glucan concentrations from children's mattresses in Germany were significantly higher compared with the Dutch sample. Furthermore, 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 those measured here. 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.* [33] presented the number of addresses per square kilometre as a proxy for the degree of urbanisation. As for our study population, 87% of the Dutch children and 94 % of the German children lived in an area with more than 1,500 addresses in a circular buffer with a 1,000-m 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 1,000-m radius.

A limitation of the present study is that it had only a single dust sampling over a period of 6 yrs. 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)-β-D-glucan and EPS measurements was small compared with the between-home variance [34]. However, some investigations looked at variations over time and performed repeatability analyses within and between homes. HEINRICH *et al.* [35] concluded that a single dust sampling and analysis of endotoxin is representative of the exposure to these components for at least a period $\leqslant$1 yr. To take into account the importance of early-life exposure to biocontaminants on the developing immune system, we restricted the 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)-β-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 observed a significant inverse effect of exposure to domestic endotoxin from children's mattresses with the risk of physician-diagnosed hayfever. 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 differed considerably between the German and the Dutch samples: 2% of the German children but 25% of the Dutch children had visited a large scale day-care institution within the first year of life. This difference is persistent up to the age of 4 yrs. A number of studies observed a higher infection rate among children with early day-care [36, 37], which was 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 yrs [33]. At the age of 6 yrs, 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 yrs 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 proportion of the German and Dutch parents (82% and 78%, respectively) had allergic diseases, and they may therefore more frequently remove mould or dust, especially when having children diagnosed with allergic disorders. However, there is 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)-β-D-glucan, EPS and the total amount of dust from children's mattresses were not different between allergic and nonallergic parents, except that there was 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–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)-β-D-glucan levels per gram of dust, both from the children's matresses, were significantly associated with the month of dust collection. However, given the large overall variability in exposure levels between the homes, the seasonal variation can be neglected as a reason for the biased results.

Considering all of the potential reasons for the inconsistent findings in the German and Dutch population discussed here, 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 samples. 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.

STATEMENT OF INTEREST

None declared.

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SUPPLEMENTARY MATERIAL

1) Definition of allergic sensitization within three cohorts

	Germany		The Netherlands
	GINI	**LISA**	**PIAMA**
Inhalant allergens			
Animals			
Cat	**x**	**x**	**x**
Dog			**x**
House dust			
D. farinae	**x**	**x**	
D. pteronyssinus	**x**	**x**	**x**
House dust mix*		**x**	
Tree, grass and weed pollens			
Birch	**x**		**x**
Cocksfoot			**x**
Timothy grass	**x**		
Tree, grass and weed pollen mix$		**x**	
Moulds			
Alternaria alternate			**x**
Mould mix§		**x**	
Food allergens			
Cow's milk	**x**	**x**	**x**
Egg white	**x**	**x**	**x**
Food mix**		**x**	
Soy bean	**x**		

*House dust mix: Hollister-Stier Labs, D. pteronyssinus, D. farinae, German cockroach
$Tree, grass and weed pollen mix: timothy grass, mugwort, ribwort, wall pellitory, birch
§Mould mix: *Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Alternaria alternate*
**Food mix: egg white, milk, fish, wheat, peanut, soy bean

SUPPLEMENTARY MATERIAL

2) Definition of respiratory diseases and symptoms

	GINI & LISA	PIAMA
Physician-diagnosed respiratory diseases		
Asthma	Physician-diagnosed asthma in the last 6 months between the 5th and the 6th year?: Yes/No	Did a doctor ever diagnose asthma in your child? If yes, has your child had asthma in the past 12 months?
Allergic Rhinitis	Physician-diagnosed hay-fever **AND/OR** physician-diagnosed all-season allergic rhinitis, e.g. mite allergy between the 5th and the 6th year: Yes/No	Has your child ever had hay fever?
Respiratory symptoms		
Rhinoconjunctivitis	Did your child have sneezing attacks or runny, blocked nose without having a cold in the past 6 months? Yes/No **AND** Did your child have itchy, watery eyes at the same time with nose complaints in the last 12 months? Yes/No	Has your child been sneezing or did he/she have a runny/blocked nose accompanied by itchy, watering eyes when he/she did not have a cold during the past 12 months?
Wheezing	Did your child have wheezing or whistling sounds in the chest in the last 12 months? Yes/No	Has your child had wheezing or whistling in the chest in the last 12 months?
Dry cough	Did your child ever have nocturnal chesty cough without having a cold or bronchitis? Yes/No	Has your child ever had cough during the night, when she/he did not have a cold or an infection in the chest in the last 12 months?

SUPPLEMENTARY MATERIAL

3) Adjusted logistic regression analysis (results displayed for all covariates)

3.1) Adjusted logistic regression results describing the association between **Physician-diagnosed asthma** 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.

(1,3)-ß-D-GLUCAN

GINI/LISA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²) **OR_{adj}+**	**(1,3)-ß-D-glucan*** (µg/m²) **OR_{adj}+**
Asthma (n=17)	0.76 (0.40 – 1.45)	0.59 (0.30 – 1.19)
Sex	1.13 (0.40 – 3.18)	1.02 (0.34 – 3.06)
Parental allergy	1.71 (0.36 – 8.11)	-
Parental education		
Medium vs. low	0.53 (0.12 – 2.42)	0.51 (0.09 – 3.07)
High vs. low	0.73 (0.19 – 2.84)	1.18 (0.26 – 5.28)
Current pets at home	1.28 (0.41 – 4.00)	1.82 (0.54 – 6.16)
Breastfeeding	1.41 (0.48 – 4.14)	1.11 (0.35 – 3.50)
Case status	3.68 (1.15 – 11.84)	4.47 (1.19 – 16.75)
Season of dust sampling:		
Autumn	Could not be converged	Could not be converged
Winter	0.23 (0.03 – 1.81)	0.28 (0.03 – 2.30)

PIAMA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²) **OR_{adj}+**	**(1,3)-ß-D-glucan*** (µg/m²) **OR_{adj}+**
Asthma (n=27)	1.28 (0.72-2.29)	1.28 (0.67-2.43)
Sex	1.64 (0.67-4.03)	1.70 (0.61-4.68)
Parental allergy	0.79 (0.23-2.75)	-
Parental education		
Low/medium vs. high	0.74 (0.31-1.78)	0.93 (0.34-2.51)
Current ETS at home	0.89 (0.37-2.18)	1.45 (0.55-3.83)
Current pets at home	0.47 (0.20-1.08)	0.51 (0.20-1.28)
Breastfeeding	1.84 (0.68-4.95)	1.92 (0.72-5.17)
Case status	6.77 (2.41-19.02)	4.74 (1.62-13.82)
Season of dust sampling:		
Autumn	0.93 (0.35-2.49)	1.06 (0.34-3.25)
Winter	0.45 (0.15-1.36)	0.48 (0.14-1.69)

EPS

GINI/LISA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Asthma (n=17)	**0.60 (0.39 – 0.92)**	**0.50 (0.31 – 0.81)**
Sex	1.17 (0.41 – 3.36)	1.07 (0.35 – 3.31)
Parental allergy	1.78 (0.34 – 9.18)	-
Parental education		
Medium vs. low	0.53 (0.11 – 2.54)	0.42 (0.06 – 2.71)
High vs. low	0.79 (0.20 – 3.15)	1.04 (0.22 – 4.79)
Current pets at home	1.24 (0.39 – 3.96)	1.92 (0.55 – 6.70)
Breastfeeding	1.36 (0.45 – 4.06)	1.12 (0.34 – 3.70)
Case status	3.74 (1.15 – 12.17)	4.90 (1.25 – 19.26)
Season of dust sampling:		
Autumn	Could not be converged	Could not be converged
Winter	0.24 (0.03 – 1.94)	0.27 (0.03 – 2.31)

PIAMA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Asthma (n=27)	1.24 (0.78-1.96)	1.29 (0.77-2.15
Sex	1.63 (0.67-3.97)	1.71 (0.62-4.70)
Parental allergy	0.82 (0.23-2.85)	-
Parental education Low/medium vs. high	0.73 (0.30-1.76)	0.91 (0.34-2.48)
Current ETS at home	0.91 (0.38-2.22)	1.49 (0.56-3.93)
Current pets at home	0.45 (0.20-1.06)	0.49 (0.19-1.24)
Breastfeeding	1.77 (0.66-4.77)	1.85 (0.69-4.98)
Case status	6.60 (2.36-18.47)	4.57 (1.57-13.24)
Season of dust sampling:		
Autumn	0.90 (0.33-2.40)	1.01 (0.33-3.12)
Winter	0.41 (0.13-1.25)	0.43 (0.12-1.53)

ENDOTOXIN

GINI/LISA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Asthma (n=17)	**0.55 (0.31 – 0.97)**	**0.46 (0.25 – 0.85)**
Sex	1.22 (0.42 – 3.56)	1.66 (0.36 – 3.71)
Parental allergy	1.44 (0.30 – 6.99)	-
Parental education		
Medium vs. low	0.43 (0.09 – 2.07)	0.42 (0.07 – 2.68)
High vs. low	0.59 (0.14 – 2.52)	0.97 (0.16 – 4.96)
Current ETS at home	0.62 (0.15 – 2.59)	0.8 (0.18 – 3.64)
Current pets at home	1.56 (0.48 – 5.04)	2.16 (0.61 – 7.57)
Breastfeeding	1.33 (0.44 – 3.99)	0.94 (0.29 – 3.05)
Case status	3.56 (1.09 – 11.56)	4.61 (1.2 – 17.71)
Season of dust sampling:		
Autumn	Could not be converged	Could not be converged
Winter	0.18 (0.02 – 1.51)	0.22 (0.03 – 1.92)

PIAMA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Asthma (n=27)	1.51 (0.94-2.42)	1.49 (0.89-2.50)
Sex	1.59 (0.65-3.91)	1.68 (0.61-4.65)
Parental allergy	0.73 (0.21-2.58)	-
Parental education		
Low/medium vs. high	0.75 (0.31-1.79)	0.92 (0.34-2.50)
Current ETS at home	0.88 (0.36-2.13)	1.46 (0.56-3.85)
Current pets at home	0.49 (0.21-1.13)	0.54 (0.21-1.39)
Breastfeeding	1.96 (0.72-5.32)	2.04 (0.75-5.52)
Case status	6.95 (2.46-19.66)	4.75 (1.62-13.93)
Season of dust sampling:		
Autumn	0.96 (0.36-2.61)	1.08 (0.35-3.35)
Winter	0.44 (0.14-1.34)	0.46 (0.13-1.61)

TOTAL AMOUNT OF MATTRESS DUST

GINI/LISA	**Amount of dust*** (mg/m^2) **$OR_{adj.}$**++	**Parental allergy** **Amount of dust*** (mg/m^2) **$OR_{adj.}$**++
Asthma (n=17)	0.65 (0.35 – 1.21)	0.54 (0.27 – 1.08)
Sex	1.04 (0.36 – 2.95)	0.94 (0.31 – 2.90)
Parental allergy	1.73 (0.36 – 8.37)	-
Parental education		
Medium vs. low	0.48 (0.10 – 2.30)	0.48 (0.08 – 2.95)
High vs. low	0.63 (0.15 – 2.66)	1.03 (0.21 – 5.12)
Current ETS at home	0.58 (0.14 – 2.44)	0.8 (0.18 – 3.59)
Current pets at home	1.38 (0.44 – 4.38)	1.89 (0.56 – 6.34)
Breastfeeding	1.36 (0.46 – 4.01)	1.05 (0.33 – 3.33)
Case status	3.7 (1.15 – 11.92)	4.53 (1.2 – 17.08)
Season of dust sampling:		
Autumn	Could not be converged	Could not be converged
Winter	0.22 (0.03 – 1.76)	0.26 (0.03 – 2.11)

PIAMA	**Amount of dust*** (mg/m^2) **$OR_{adj.}$**++	**Parental allergy** **Amount of dust*** (mg/m^2) **$OR_{adj.}$**++
Asthma (n=27)	1.28 (0.76-2.17)	1.25 (0.70-2.23)
Sex	1.61 (0.66-3.93)	1.67 (0.61-4.58)
Parental allergy	0.79 (0.23-2.75)	-
Parental education		
Low/medium vs. high	0.75 (0.31-1.79)	0.94 (0.35-2.54)
Current ETS at home	0.89 (0.36-2.16)	1.44 (0.55-3.82)
Current pets at home	0.47 (0.20-1.08)	0.51 (0.20-1.28)
Breastfeeding	1.83 (0.68-4.93)	1.92 (0.72-5.14)
Case status	6.80 (2.42-19.10)	4.71 (1.62-13.70)
Season of dust sampling:		
Autumn	0.97 (0.36-2.63)	1.10 (0.35-3.41)
Winter	0.44 (0.15-1.33)	0.47 (0.13-1.64)

3.2) Adjusted logistic regression results describing the association between **Physician-diagnosed allergic rhinitis** 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.

(1,3)-ß-D-GLUCAN

GINI/LISA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Allergic Rhinitis (n=47)	0.69 (0.45 – 1.05)	**0.58 (0.37 – 0.91)**
Sex	1.40 (0.71 – 2.77)	1.23 (0.60 – 2.52)
Parental allergy	2.06 (0.74 – 5.73)	-
Parental education		
Medium vs. low	0.63 (0.23 – 1.69)	0.69 (0.23 – 2.12)
High vs. low	0.61 (0.24 – 1.54)	0.79 (0.29 – 2.19)
Current pets at home	1.22 (0.58 – 2.59)	1.07 (0.46 – 2.48)
Breastfeeding	1.76 (0.87 – 3.55)	1.54 (0.73 – 3.26)
Case status	3.28 (1.62 – 6.64)	3.61 (1.68 – 7.76)
Season of dust sampling:		
Autumn	1.10 (0.41 – 2.97)	1.27 (0.46 – 3.55)
Winter	0.53 (0.19 – 1.49)	0.70 (0.24 – 2.04)

PIAMA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Hay fever (n=23)	0.83 (0.42-1.63)	0.79 (0.39-1.60)
Sex	3.62 (1.19-10.99)	3.32 (1.07-10.31)
Parental allergy	6.94 (0.77-62.32)	-
Parental education		
Low/medium vs. high	0.44 (0.17-1.14)	0.46 (0.17-1.25)
Current ETS at home	0.25 (0.08-0.83)	0.18 (0.05-0.69)
Current pets at home	2.45 (0.83-7.25)	2.24 (0.74-6.74)
Breastfeeding	1.32 (0.49-3.54)	1.30 (0.48-3.51)
Case status	3.33 (1.22-9.08)	3.95 (1.36-11.45)
Season of dust sampling:		
Autumn	0.29 (0.09-0.94)	0.29 (0.09-0.97)
Winter	0.21 (0.06-0.69)	0.20 (0.06-0.70)

EPS

GINI/LISA		Parental allergy
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Allergic Rhinitis (n=47)	**0.67 (0.49 – 0.92)**	**0.66 (0.47 – 0.93)**
Sex	1.41 (0.71 – 2.80)	1.23 (0.60 – 2.51)
Parental allergy	2.02 (0.71 – 5.74)	-
Parental education		
Medium vs. low	0.63 (0.23 – 1.72)	0.69 (0.23 – 2.09)
High vs. low	0.60 (0.24 – 1.52)	0.71 (0.26 – 1.95)
Current pets at home	1.17 (0.55 – 2.46)	0.98 (0.43 – 2.23)
Breastfeeding	1.65 (0.81 – 3.35)	1.43 (0.68 – 3.02)
Case status	3.45 (1.70 – 7.01)	3.81 (1.77 – 8.19)
Season of dust sampling:		
Autumn	1.16 (0.42 – 3.16)	1.31 (0.47 – 3.67)
Winter	0.54 (0.19 – 1.52)	0.70 (0.24 – 2.03)

PIAMA		Parental allergy
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Hay fever (n=23)	1.00 (0.61-1.65)	1.00 (0.59-1.70)
Sex	3.74 (1.23-11.34)	3.42 (1.10-10.62)
Parental allergy	6.60 (0.74-58.55)	-
Parental education		
Low/medium vs. high	0.43 (0.16-1.12)	0.45 (0.17-1.23)
Current ETS at home	0.24 (0.07-0.80)	0.17 (0.05-0.67)
Current pets at home	2.42 (0.81-7.22)	2.21 (0.73-6.73)
Breastfeeding	1.35 (0.50-3.62)	1.34 (0.50-3.60)
Case status	3.42 (1.26-9.27)	4.06 (1.41-11.69)
Season of dust sampling:		
Autumn	0.30 (0.09-0.97)	0.30 (0.09-1.01)
Winter	0.21 (0.06-0.71)	0.21 (0.06-0.73)

ENDOTOXIN

GINI/LISA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Allergic Rhinitis (n=47)	0.71 (0.48 – 1.04)	**0.60 (0.40 – 0.92)**
Sex	1.44 (0.72 – 2.86)	1.28 (0.62 – 2.63)
Parental allergy	1.86 (0.67 – 5.21)	-
Parental education		
Medium vs. low	0.58 (0.21 – 1.59)	0.63 (0.21 – 1.94)
High vs. low	0.55 (0.21 – 1.41)	0.65 (0.23 – 1.85)
Current ETS at home	0.92 (0.38 – 2.19)	0.80 (0.30 – 2.13)
Current pets at home	1.24 (0.59 – 2.63)	1.06 (0.46 – 2.44)
Breastfeeding	1.63 (0.81 – 3.28)	1.37 (0.65 – 2.89)
Case status	3.27 (1.62 – 6.63)	3.66 (1.71 – 7.84)
Season of dust sampling:		
Autumn	1.14 (0.42 – 3.07)	1.32 (0.47 – 3.70)
Winter	0.47 (0.17 – 1.34)	0.62 (0.21 – 1.80)

PIAMA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Hay fever (n=23)	0.61 (0.35-1.07)	0.59 (0.33-1.05)
Sex	3.66 (1.21-11.13)	3.37 (1.08-10.52)
Parental allergy	7.11 (0.80-63.10)	-
Parental education		
Low/medium vs. high	0.44 (0.17-1.16)	0.47 (0.17-1.27)
Current ETS at home	0.24 (0.07-0.81)	0.17 (0.04-0.66)
Current pets at home	2.31 (0.78-6.82)	2.07 (0.68-6.27)
Breastfeeding	1.27 (0.47-3.45)	1.26 (0.46-3.43)
Case status	3.38 (1.23-9.28)	4.01 (1.37-11.72)
Season of dust sampling:		
Autumn	0.28 (0.09-0.91)	0.28 (0.08-0.94)
Winter	0.19 (0.06-0.64)	0.18 (0.05-0.64)

TOTAL AMOUNT OF MATTRESS DUST

GINI/LISA		**Parental allergy**
	Amount of dust* (mg/m^2)	**Amount of dust*** (mg/m^2)
	$OR_{adj.}$ ++	**$OR_{adj.}$ ++**
Allergic Rhinitis (n=47)	0.71 (0.47 – 1.08)	**0.63 (0.40 – 0.99)**
Sex	1.35 (0.68 – 2.67)	1.16 (0.57 – 2.37)
Parental allergy	2.06 (0.74 – 5.76)	-
Parental education		
Medium vs. low	0.62 (0.23 – 1.67)	0.66 (0.22 – 2.03)
High vs. low	0.58 (0.22 – 1.49)	0.68 (0.24 – 1.95)
Current ETS at home	0.87 (0.36 – 2.09)	0.78 (0.29 – 2.08)
Current pets at home	1.19 (0.57 – 2.51)	1.01 (0.45 – 2.30)
Breastfeeding	1.66 (0.82 – 3.34)	1.44 (0.69 – 3.05)
Case status	3.33 (1.65 – 6.74)	3.66 (1.71 – 7.83)
Season of dust sampling:		
Autumn	1.10 (0.41 – 2.98)	1.25 (0.45 – 3.48)
Winter	0.52 (0.18 – 1.45)	0.65 (0.23 – 1.89)

PIAMA		**Parental allergy**
	Amount of dust* (mg/m^2)	**Amount of dust*** (mg/m^2)
	$OR_{adj.}$ ++	**$OR_{adj.}$ ++**
Hay fever (n=23)	0.88 (0.49-1.60)	0.86 (0.47-1.59)
Sex	3.70 (1.22-11.20)	3.39 (1.09-10.51)
Parental allergy	6.72 (0.76-59.62)	-
Parental education		
Low/medium vs. high	0.44 (0.17-1.14)	0.46 (0.17-1.25)
Current ETS at home	0.25 (0.08-0.82)	0.18 (0.05-0.69)
Current pets at home	2.46 (0.83-7.26)	2.25 (0.75-6.78)
Breastfeeding	1.34 (0.50-3.59)	1.32 (0.49-3.56)
Case status	3.37 (1.24-9.16)	3.99 (1.38-11.52)
Season of dust sampling:		
Autumn	0.29 (0.09-0.95)	0.29 (0.08-0.98)
Winter	0.21 (0.06-0.70)	0.21 (0.06-0.71)

3.3) Adjusted logistic regression results describing the association between **Rhinoconjunctivitis** 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.

(1,3)-ß-D-GLUCAN

GINI/LISA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²) **OR_{adj}+**	**(1,3)-ß-D-glucan*** (µg/m²) **OR_{adj}+**
Rhinoconjuntivitis (n=48)	0.74 (0.49 – 1.12)	0.71 (0.46 – 1.09)
Sex	1.66 (0.83 – 3.30)	1.53 (0.75 – 3.10)
Parental allergy	4.01 (1.15 – 13.96)	-
Parental education		
Medium vs. low	1.64 (0.57 – 4.74)	2.00 (0.64 – 6.29)
High vs. low	1.50 (0.55 – 4.08)	1.78 (0.61 – 5.18)
Current pets at home	1.27 (0.60 – 2.67)	1.15 (0.52 – 2.54)
Breastfeeding	0.81 (0.41 – 1.60)	0.74 (0.36 – 1.50)
Case status	4.55 (2.18 – 9.50)	4.86 (2.25 – 10.51)
Season of dust sampling:		
Autumn	1.12 (0.41 – 3.05)	1.2 (0.43 – 3.36)
Winter	0.89 (0.34 – 2.27)	1.1 (0.41 – 2.92)

PIAMA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²) **OR_{adj}+**	**(1,3)-ß-D-glucan*** (µg/m²) **OR_{adj}+**
Rhinoconjunctivitis (n=28)	1.11 (0.62-1.97)	1.07 (0.59-1.95)
Sex	1.48 (0.63-3.50)	1.68 (0.69-4.09)
Parental allergy	9.90 (1.21-80.67)	-
Parental education		
Low/medium vs. high	0.66 (0.28-1.54)	0.73 (0.30-1.75)
Current ETS at home	0.19 (0.06-0.60)	0.20 (0.06-0.64)
Current pets at home	0.76 (0.33-1.75)	0.82 (0.35-1.94)
Breastfeeding	0.88 (0.38-2.04)	0.90 (0.39-2.09)
Case status	2.46 (1.04-5.80)	2.26 (0.94-5.39)
Season of dust sampling:		
Autumn	0.78 (0.30-2.04)	0.67 (0.25-1.81)
Winter	0.32 (0.11-0.97)	0.31 (0.10-0.94)

EPS

GINI/LISA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Rhinoconjuntivitis (n=48)	0.77 (0.56 – 1.07)	0.81 (0.58 – 1.15)
Sex	1.66 (0.83 – 3.32)	1.52 (0.75 – 3.07)
Parental allergy	3.99 (1.14 – 13.96)	-
Parental education		
Medium vs. low	1.64 (0.57 – 4.73)	1.97 (0.63 – 6.16)
High vs. low	1.43 (0.53 – 3.89)	1.62 (0.56 – 4.67)
Current pets at home	1.21 (0.58 – 2.52)	1.07 (0.49 – 2.33)
Breastfeeding	0.77 (0.39 – 1.52)	0.70 (0.35 – 1.43)
Case status	4.71 (2.25 – 9.84)	5.00 (2.31 – 10.80)
Season of dust sampling:		
Autumn	1.13 (0.41 – 3.11)	1.21 (0.43 – 3.36)
Winter	0.89 (0.35 – 2.28)	1.09 (0.41 – 2.88)

PIAMA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}+**	**EPS*** (EPSU/m^2) **OR_{adj}+**
Rhinoconjunctivitis (n=28)	1.19 (0.75-1.90)	1.17 (0.73-1.87)
Sex	1.48 (0.63-3.50)	1.68 (0.69-4.09)
Parental allergy	10.07 (1.24-82.12)	-
Parental education		
Low/medium vs. high	0.64 (0.27-1.50)	0.71 (0.29-1.71)
Current ETS at home	0.19 (0.06-0.60)	0.20 (0.06-0.64)
Current pets at home	0.73 (0.31-1.69)	0.79 (0.33-1.88)
Breastfeeding	0.87 (0.37-2.02)	0.89 (0.38-2.07)
Case status	2.46 (1.05-5.79)	2.26 (0.95-5.38)
Season of dust sampling:		
Autumn	0.74 (0.28-1.95)	0.64 (0.24-1.74)
Winter	0.31 (0.10-0.92)	0.30 (0.10-0.90)

ENDOTOXIN

GINI/LISA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**$^{++}$	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**$^{++}$
Rhinoconjuntivitis (n=48)	0.78 (0.53 – 1.15)	0.70 (0.47 – 1.06)
Sex	1.74 (0.86 – 3.50)	1.60 (0.78 – 3.29)
Parental allergy	3.80 (1.09 – 13.27)	-
Parental education		
Medium vs. low	1.63 (0.56 – 4.73)	1.90 (0.60 – 6.01)
High vs. low	1.46 (0.53 – 4.06)	1.60 (0.54 – 4.79)
Current ETS at home	1.22 (0.52 – 2.85)	1.16 (0.52 – 2.56)
Current pets at home	1.24 (0.59 – 2.60)	1.16 (0.52 – 2.56)
Breastfeeding	0.76 (0.39 – 1.51)	0.68 (0.33 – 1.39)
Case status	4.62 (2.21 – 9.64)	4.97 (2.30 – 10.77)
Season of dust sampling:		
Autumn	1.17 (0.43 – 3.21)	1.25 (0.45 – 3.52)
Winter	0.83 (0.32 – 2.13)	1.01 (0.38 – 2.69)

PIAMA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**$^{++}$	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**$^{++}$
Rhinoconjunctivitis (n=28)	0.96 (0.61-1.51)	0.93 (0.59-1.48)
Sex	1.47 (0.62-3.46)	1.67 (0.69-4.05)
Parental allergy	10.14 (1.24-82.87)	-
Parental education		
Low/medium vs. high	0.67 (0.28-1.56)	0.74 (0.31-1.77)
Current ETS at home	0.19 (0.06-0.61)	0.20 (0.06-0.65)
Current pets at home	0.76 (0.33-1.74)	0.82 (0.35-1.92)
Breastfeeding	0.87 (0.37-2.03)	0.89 (0.38-2.07)
Case status	2.40 (1.02-5.63)	2.20 (0.93-5.23)
Season of dust sampling:		
Autumn	0.77 (0.30-2.01)	0.67 (0.25-1.78)
Winter	0.32 (0.11-0.95)	0.31 (0.10-0.92)

TOTAL AMOUNT OF MATTRESS DUST

GINI/LISA		**Parental allergy**
	Amount of dust* (mg/m^2) **$OR_{adj.}^{++}$**	**Amount of dust*** (mg/m^2) **$OR_{adj.}^{++}$**
Rhinoconjuntivitis (n=48)	0.81 (0.52 – 1.24)	0.83 (0.52 – 1.31)
Sex	1.65 (0.83 – 3.30)	1.48 (0.73 – 3.00)
Parental allergy	3.99 (1.15 – 13.86)	-
Parental education		
Medium vs. low	1.68 (0.58 – 4.90)	1.96 (0.62 – 6.16)
High vs. low	1.49 (0.53 – 4.18)	1.61 (0.54 – 4.78)
Current ETS at home	1.20 (0.52 – 2.79)	0.95 (0.38 – 2.38)
Current pets at home	1.20 (0.58 – 2.51)	1.08 (0.49 – 2.35)
Breastfeeding	0.78 (0.40 – 1.54)	0.71 (0.35 – 1.44)
Case status	4.61 (2.21 – 9.62)	4.89 (2.26 – 10.54)
Season of dust sampling:		
Autumn	1.14 (0.42 – 3.11)	1.19 (0.43 – 3.31)
Winter	0.87 (0.34 – 2.22)	1.05 (0.40 – 2.76)

PIAMA		**Parental allergy**
	Amount of dust* (mg/m^2) **$OR_{adj.}^{++}$**	**Amount of dust*** (mg/m^2) **$OR_{adj.}^{++}$**
Rhinoconjunctivitis (n=28)	1.01 (0.62-1.65)	0.99 (0.60-1.65)
Sex	1.47 (0.62-3.47)	1.67 (0.69-4.06)
Parental allergy	10.01 (1.23-81.60)	-
Parental education		
Low/medium vs. high	0.66 (0.28-1.56)	0.74 (0.31-1.77)
Current ETS at home	0.19 (0.06-0.61)	0.20 (0.06-0.65)
Current pets at home	0.76 (0.33-1.75)	0.82 (0.35-1.94)
Breastfeeding	0.88 (0.38-2.04)	0.90 (0.39-2.09)
Case status	2.42 (1.03-5.69)	2.22 (0.93-5.30)
Season of dust sampling:		
Autumn	0.78 (0.30-2.03)	0.67 (0.25-1.80)
Winter	0.32 (0.11-0.96)	0.31 (0.10-0.93)

3.4) Adjusted logistic regression results describing the association between **Wheezing** 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.

(1,3)-ß-D-GLUCAN

GINI/LISA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Wheezing (n=43)	0.78 (2.35 – 11.54)	0.68 (0.42 – 1.09)
Sex	1.10 (0.54 – 2.22)	1.02 (0.48 – 2.16)
Parental allergy	1.11 (0.44 – 2.80)	-
Parental education		
Medium vs. low	0.88 (0.33 – 2.31)	0.82 (0.27 – 2.52)
High vs. low	0.47 (0.18 – 1.21)	0.63 (0.22 – 1.78)
Current pets at home	0.74 (0.33 – 1.66)	0.99 (0.41 – 2.38)
Breastfeeding	1.59 (0.77 – 3.28)	1.38 (0.63 – 3.03)
Case status	5.21 (2.35 – 11-54)	5.43 (2.24 – 13.15)
Season of dust sampling:		
Autumn	1.3 (0.47 – 3.59)	1.24 (0.41 – 3.75)
Winter	0.61 (0.21 – 1.74)	0.86 (0.29 – 2.59)

PIAMA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Wheezing (n=47)	0.82 (0.53-1.28)	0.92 (0.56-1.50)
Sex	1.40 (0.71-2.75)	1.36 (0.64-2.87)
Parental allergy	1.19 (0.44-3.22)	-
Parental education		
Low/medium vs. high	0.72 (0.37-1.41)	0.83 (0.40-1.75)
Current ETS at home	0.90 (0.45-1.79)	1.04 (0.49-2.20)
Current pets at home	0.81 (0.42-1.57)	0.93 (0.45-1.91)
Breastfeeding	1.69 (0.81-3.52)	1.77 (0.84-3.69)
Case status	3.54 (1.77-7.08)	3.29 (1.55-6.98)
Season of dust sampling:		
Autumn	0.69 (0.30-1.58)	0.74 (0.29-1.89)
Winter	0.76 (0.34-1.68)	1.07 (0.45-2.56)

EPS

GINI/LISA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}^+**	**EPS*** (EPSU/m^2) **OR_{adj}^+**
Wheezing (n=43)	1.02 (0.71 – 1.48)	0.92 (0.62 – 1.38)
Sex	1.08 (0.54 – 2.18)	0.99 (0.47 – 2.09)
Parental allergy	1.14 (0.45 – 2.86)	-
Parental education		
Medium vs. low	0.85 (0.32 – 2.22)	0.83 (0.27 – 2.50)
High vs. low	0.43 (0.16 – 1.10)	0.56(0.20 – 1.57)
Current pets at home	0.70 (0.31 – 1.56)	0.88 (0.37 – 2.08)
Breastfeeding	1.56 (0.76 – 3.21)	1.30 (0.60 – 2.84)
Case status	5.44 (2.46 – 12.01)	5.68 (2.35 – 13.71)
Season of dust sampling:		
Autumn	1.31 (0.48 – 3.59)	1.24 (0.41 – 3.69)
Winter	0.58 (0.20 – 1.66)	0.82 (0.27 – 2.43)

PIAMA		**Parental allergy**
	EPS* (EPSU/m^2) **OR_{adj}^+**	**EPS*** (EPSU/m^2) **OR_{adj}^+**
Wheezing (n=47)	1.02 (0.74-1.42)	1.12 (0.77-1.64)
Sex	1.43 (0.73-2.82)	1.39 (0.66-2.94)
Parental allergy	1.18 (0.44-3.20)	-
Parental education		
Low/medium vs. high	0.71 (0.36-1.39)	0.81 (0.38-1.70)
Current ETS at home	0.87 (0.44-1.73)	1.03 (0.49-2.18)
Current pets at home	0.80 (0.41-1.54)	0.91 (0.44-1.88)
Breastfeeding	1.68 (0.80-3.51)	1.74 (0.83-3.65)
Case status	3.62 (1.81-7.22)	3.34 (1.58-7.07)
Season of dust sampling:		
Autumn	0.71 (0.31-1.63)	0.75 (0.29-1.92)
Winter	0.79 (0.36-1.73)	1.08 (0.45-2.58)

ENDOTOXIN

GINI/LISA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Wheezing (n=43)	0.82 (0.54 – 1.24)	0.69 (0.43 – 1.1)
Sex	1.11 (0.55 – 2.26)	1.09 (0.51 – 2.35)
Parental allergy	1.06 (0.41 – 2.69)	-
Parental education		
Medium vs. low	0.83 (0.31 – 2.21)	0.82 (0.26 – 2.55)
High vs. low	0.43 (0.17 – 1.14)	0.62 (0.21 – 1.81)
Current ETS at home	0.94 (0.39 – 2.27)	1.34 (0.52 – 3.43)
Current pets at home	0.75 (0.33 – 1.68)	0.96 (0.40 – 2.30)
Breastfeeding	1.52 (0.74 – 3.13)	1.28 (0.58 – 2.80)
Case status	5.24 (2.37 – 11.61)	5.52 (2.28 – 13.36)
Season of dust sampling:		
Autumn	1.33 (0.48 – 3.69)	1.39 (0.46 – 4.25)
Winter	0.57 (0.20 – 1.64)	0.83 (0.28 – 2.47)

PIAMA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**[++]	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**[++]
Wheezing (n=47)	1.11 (0.77-1.59)	1.21 (0.82-1.80)
Sex	1.43 (0.73-2.82)	1.38 (0.65-2.93)
Parental allergy	1.17 (0.43-3.15)	-
Parental education		
Low/medium vs. high	0.71 (0.36-1.38)	0.81 (0.39-1.70)
Current ETS at home	0.86 (0.44-1.71)	1.02 (0.48-2.15)
Current pets at home	0.81 (0.42-1.55)	0.95 (0.46-1.97)
Breastfeeding	1.71 (0.82-3.57)	1.82 (0.87-3.82)
Case status	3.66 (1.83-7.31)	3.41 (1.61-7.24)
Season of dust sampling:		
Autumn	0.73 (0.32-1.66)	0.77 (0.30-1.99)
Winter	0.80 (0.36-1.76)	1.12 (0.47-2.68)

TOTAL AMOUNT OF MATTRESS DUST

GINI/LISA		**Parental allergy**
	Amount of dust* (mg/m^2)	**Amount of dust*** (mg/m^2)
	$OR_{adj.}$ ++	**$OR_{adj.}$ ++**
Wheezing (n=43)	0.81 (0.52 – 1.27)	0.76 (0.46 – 1.25)
Sex	1.07 (0.53 – 2.17)	1.02 (0.48 – 2.17)
Parental allergy	1.11 (0.44 – 2.81)	-
Parental education		
Medium vs. low	0.86 (0.32 – 2.29)	0.85 (0.28 – 2.64)
High vs. low	0.45 (0.17 – 1.19)	0.64 (0.22 – 1.86)
Current ETS at home	0.91 (0.38 – 2.21)	1.33 (0.52 – 3.39)
Current pets at home	0.73 (0.33 – 1.64)	0.91 (0.38 – 2.18)
Breastfeeding	1.54 (0.75 – 3.16)	1.33 (0.61 – 2.91)
Case status	5.32 (2.40 – 11.76)	5.58 (2.31 – 13.5)
Season of dust sampling:		
Autumn	1.3 (0.47 – 3.62)	1.32 (0.44 – 4.00)
Winter	0.60 (0.21 – 1.72)	0.85 (0.28 – 2.53)

PIAMA		**Parental allergy**
	Amount of dust* (mg/m^2)	**Amount of dust*** (mg/m^2)
	$OR_{adj.}$ ++	**$OR_{adj.}$ ++**
Wheezing (n=47)	1.00 (0.68-1.49)	1.14 (0.73-1.77)
Sex	1.43 (0.73-2.81)	1.39 (0.66-2.93)
Parental allergy	1.18 (0.44-3.19)	-
Parental education		
Low/medium vs. high	0.71 (0.36-1.39)	0.82 (0.39-1.71)
Current ETS at home	0.88 (0.44-1.74)	1.02 (0.48-2.15)
Current pets at home	0.80 (0.42-1.54)	0.92 (0.45-1.89)
Breastfeeding	1.69 (0.81-3.52)	1.77 (0.85-3.70)
Case status	3.62 (1.81-7.24)	3.40 (1.60-7.22)
Season of dust sampling:		
Autumn	0.72 (0.31-1.64)	0.79 (0.30-2.04)
Winter	0.79 (0.36-1.74)	1.11 (0.47-2.66)

3.5) Adjusted logistic regression results describing the association between **Dry cough** 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.

(1,3)-ß-D-GLUCAN

GINI/LISA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Dry cough (n=56)	0.78 (0.53 – 1.13)	**0.65 (0.43 – 0.98)**
Sex	1.18 (0.64 – 2.17)	0.89 (0.46 – 1.71)
Parental allergy	1.07 (0.50 – 2.33)	-
Parental education		
Medium vs. low	0.74 (0.31 – 1.76)	0.81 (0.30 – 2.21)
High vs. low	0.57 (0.25 – 1.31)	0.61 (0.24 – 1.56)
Current pets at home	1.45 (0.75 – 2.78)	1.33 (0.63 – 2.82)
Breastfeeding	1.14 (0.62 – 2.11)	1.11 (0.56 – 2.20)
Case status	1.23 (0.68 – 2.25)	0.99 (0.51 – 1.93)
Season of dust sampling:		
Autumn	0.85 (0.33 – 2.18)	0.77 (0.27 – 2.17)
Winter	0.66 (0.27 – 1.61)	0.78 (0.29 – 2.07)

PIAMA		**Parental allergy**
	(1,3)-ß-D-glucan* (µg/m²)	**(1,3)-ß-D-glucan*** (µg/m²)
	OR_{adj}+	**OR_{adj}+**
Dry cough (n=79)	0.88 (0.61-1.25)	0.93 (0.63-1.38)
Sex	1.36 (0.79-2.35)	1.12 (0.62-2.04)
Parental allergy	2.23 (0.99-5.02)	-
Parental education		
Low/medium vs. high	0.84 (0.49-1.46)	0.99 (0.54-1.82)
Current ETS at home	1.21 (0.70-2.10)	1.64 (0.90-2.99)
Current pets at home	0.63 (0.37-1.07)	0.55 (0.30-0.99)
Breastfeeding	1.04 (0.58-1.85)	1.10 (0.61-1.98)
Case status	1.71 (1.00-2.91)	0.90 (0.42-1.95)
Season of dust sampling:		
Autumn	0.65 (0.33-1.29)	0.90 (0.42-1.95)
Winter	0.84 (0.44-1.59)	1.35 (0.66-2.77)

EPS

GINI/LISA	**EPS*** (EPSU/m^2) **OR_{adj}+**	**Parental allergy** **EPS*** (EPSU/m^2) **OR_{adj}+**
Dry cough (n=56)	0.93 (0.68 – 1.27)	0.84 (0.58 – 2.55)
Sex	1.17 (0.64 – 2.15)	0.88 (0.46 – 1.69)
Parental allergy	1.08 (0.50 – 2.34)	-
Parental education		
Medium vs. low	0.73 (0.31 – 1.73)	0.80 (0.30 – 2.17)
High vs. low	0.55 (0.24 – 1.25)	0.57 (0.22 – 1.43)
Current pets at home	1.38 (0.73 – 2.64)	1.22 (0.58 – 2.55)
Breastfeeding	1.10 (0.60 – 2.03)	1.04 (0.53 – 2.05)
Case status	1.28 (0.70 – 2.33)	1.05 (0.54 – 2.03)
Season of dust sampling:		
Autumn	0.84 (0.33 – 2.15)	0.75 (0.27-2.11)
Winter	0.64 (0.26 – 1.57)	0.74 (0.28 – 1.97)

PIAMA	**EPS*** (EPSU/m^2) **OR_{adj}+**	**Parental allergy** **EPS*** (EPSU/m^2) **OR_{adj}+**
Dry cough (n=79)	1.03 (0.79-1.34)	0.94 (0.68-1.29)
Sex	1.38 (0.80-2.39)	1.15 (0.63-2.11)
Parental allergy	2.22 (0.99-5.00)	-
Parental education		
Low/medium vs. high	0.84 (0.48-1.45)	0.96 (0.52-1.78)
Current ETS at home	1.19 (0.69-2.06)	1.64 (0.90-3.01)
Current pets at home	0.62 (0.36-1.06)	0.53 (0.29-0.96)
Breastfeeding	1.03 (0.58-1.84)	1.07 (0.59-1.94)
Case status	1.73 (1.01-2.95)	2.02 (1.12-3.63)
Season of dust sampling:		
Autumn	0.66 (0.34-1.31)	0.91 (0.42-1.96)
Winter	0.86 (0.45-1.62)	1.35 (0.66-2.76)

ENDOTOXIN

GINI/LISA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**$^{++}$	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**$^{++}$
Dry cough (n=56)	0.89 (0.63 – 1.26)	0.82 (0.55 – 1.22)
Sex	1.18 (0.64 – 2.17)	0.90 (0.47 – 1.75)
Parental allergy	1.04 (0.47 – 2.27)	-
Parental education		
Medium vs. low	0.70 (0.29 – 1.68)	0.82 (0.30 – 2.26)
High vs. low	0.53 (0.23 – 1.23)	0.59 (0.22 – 1.55)
Current ETS at home	0.93 (0.44 – 1.97)	1.17 (0.52 – 2.64)
Current pets at home	1.43 (0.74 – 2.76)	1.23 (0.58 – 2.60)
Breastfeeding	1.10 (0.59 – 2.02)	1.04 (0.53 – 1.05)
Case status	1.26 (0.69 – 2.29)	1.04 (0.54 – 2.01)
Season of dust sampling:		
Autumn	0.84 (0.33 – 2.16)	0.78 (0.28 – 2.2)
Winter	0.61 (0.25 – 1.50)	0.72 (0.27 – 1.91)

PIAMA		**Parental allergy**
	Endotoxin* (EU/m^2) **$OR_{adj.}$**$^{++}$	**Endotoxin*** (EU/m^2) **$OR_{adj.}$**$^{++}$
Dry cough (n=79)	1.05 (0.78-1.41)	1.10 (0.79-1.52)
Sex	1.38 (0.80-2.38)	1.13 (0.62-2.06)
Parental allergy	2.21 (0.98-4.96)	-
Parental education		
Low/medium vs. high	0.84 (0.48-1.45)	0.98 (0.53-1.81)
Current ETS at home	1.19 (0.69-2.05)	1.63 (0.89-2.97)
Current pets at home	0.62 (0.36-1.06)	0.55 (0.31-0.99)
Breastfeeding	1.04 (0.58-1.86)	1.11 (0.62-2.00)
Case status	1.74 (1.02-2.96)	2.04 (1.13-3.66)
Season of dust sampling:		
Autumn	0.67 (0.34-1.32)	0.93 (0.43-2.00)
Winter	0.87 (0.46-1.63)	1.40 (0.69-2.85)

TOTAL AMOUNT OF MATTRESS DUST

GINI/LISA		**Parental allergy**
	Amount of dust* (mg/m^2) **$OR_{adj.}^{++}$**	**Amount of dust*** (mg/m^2) **$OR_{adj.}^{++}$**
Dry cough (n=56)	0.92 (0.63 – 1.34)	0.76 (0.50 – 1.16)
Sex	1.16 (0.63 – 2.13)	0.88 (0.46 – 1.70)
Parental allergy	1.08 (0.50 – 2.34)	-
Parental education		
Medium vs. low	0.72 (0.30 – 1.72)	0.84 (0.30 – 2.31)
High vs. low	0.54 (0.23 – 1.25)	0.61 (0.23 – 1.60)
Current ETS at home	0.91 (0.43 – 1.92)	1.17 (0.52 – 2.63)
Current pets at home	1.40 (0.73 – 2.69)	1.22 (0.58 – 2.56)
Breastfeeding	1.10 (0.60 – 2.03)	1.05 (0.54 – 2.06)
Case status	1.27 (0.7 – 2.31)	1.03 (0.53 – 1.99)
Season of dust sampling:		
Autumn	0.83 (0.32 – 2.14)	0.77 (0.27 – 2.17)
Winter	0.63 (0.26 – 1.55)	0.74 (0.28 – 1.97)

PIAMA		**Parental allergy**
	Amount of dust* (mg/m^2) **$OR_{adj.}^{++}$**	**Amount of dust*** (mg/m^2) **$OR_{adj.}^{++}$**
Dry cough (n=79)	0.88 (0.61-1.25)	0.93 (0.63-1.38)
Sex	1.37 (0.80-2.36)	1.12 (0.62-2.05)
Parental allergy	2.22 (0.99-5.00)	-
Parental education		
Low/medium vs. high	0.84 (0.49-1.46)	0.98 (0.53-1.82)
Current ETS at home	1.20 (0.69-2.07)	1.64 (0.90-2.99)
Current pets at home	0.62 (0.36-1.06)	0.55 (0.30-0.99)
Breastfeeding	1.04 (0.58-1.85)	1.10 (0.61-1.98)
Case status	1.72 (1.01-2.93)	2.02 (1.12-3.64)
Season of dust sampling:		
Autumn	0.65 (0.33-1.29)	0.75 (0.29-1.92)
Winter	0.85 (0.45-1.61)	1.08 (0.45-2.58)

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Hier mit erkläre ich, Christina Tischer, dass ich die vorliegende Dissertation selbstständig angefertigt habe. Ich habe mich außer der angegebenen keiner weiterer Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen. Ich habe bisher noch keinen Promotionsversuch unternommen, und die vorliegende Dissertation wurde nicht in gleicher oder ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht.

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(Christina Tischer)