

Variable risk of atopic disease due to indoor fungal exposure in NHANES 2005–2006

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Summary

Background Exposure to damp indoor environments is associated with increased risk of eczema, allergy and asthma. The role of dampness-related exposures and risk of allergic diseases are yet to be fully explored in the US population.

Objective We assess whether exposure to fungi, house dust mites and endotoxin increases the risk of eczema, allergy and asthma in children and adults participating in NHANES 2005–2006.

Methods A total of 8412 participants (2849 were children aged between 6 and 17 years) were recruited in the 2005–2006 survey. We used multiple logistic regression to investigate whether mildew/musty odour and increased concentrations of *Alternaria alternata* allergen, *Aspergillus fumigatus* antigens, house dust mite and endotoxin antigens increase the risk of eczema, allergy and asthma. We stratified models by total IgE < 170 and ≥ 170 KU/L to assess allergic and non-allergic asthma outcomes. Exposure to multiple biological agents and risk of reporting eczema, allergy and asthma were also investigated. **Results** Reporting of a mildew/musty odour was associated with increased risk of childhood asthma (OR 1.60; 95% CI 1.17–2.19), and adult eczema, allergy and asthma (OR 1.92; 95% CI 1.39–2.63, OR 1.59 95% CI 1.26–2.02 and OR 1.61 95% CI 1.00–2.57, respectively). Risk of asthma was associated with total IgE ≥ 170 KU/L in children (OR 1.81; 95% CI 1.01–3.25) and total IgE < 170 KU/L in adults (OR 1.91; 95% CI 1.07–3.42). Children and adults exposed to more than eight biological agents present in the home were at reduced risk of eczema (OR 0.17; 95% CI 0.04–0.77) and asthma (OR 0.49; 95% CI 0.25–0.97), respectively.

Conclusion Exposure to a mildew/musty odour, as a proxy for exposure to fungus, was implicated in an increased risk of atopic diseases. Sensitisation may play a different role in children and adults, and exposure to multiple allergens may reduce the risk of atopic disease.

Keywords allergy, asthma, atopy, damp, eczema, fungi, microbiome, odour

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Introduction

The as-yet-unexplained rise in diseases such as eczema, allergy and asthma is a worldwide public health concern, which not only affects people's quality of life, but also represents a considerable socio-economic burden on patients and society [1]. Eczema, allergy and asthma can be caused and/or exacerbated by both non-allergic and allergic stimuli [2]. Increased exposure to

non-allergic stimuli may lead to airway inflammation and irritation [3] of the lungs. Atopy or allergic sensitisation is a genetic predisposition to IgE antibody production in response to allergen exposures [4]. Around 44% of the US population have detectable total IgE [1], and specific IgE (sIgE) to plant, pet and fungal allergens increases the risk of allergic symptoms [5]. Total and specific IgE outside the clinical range may not necessarily mean an individual has allergic symptoms, but as

IgE (both total and specific) counts increase so too do the chances of an individual exhibiting symptoms [6, 7] of acute type I hypersensitivity.

Exposure to a variety of microbial components in the home environment is of interest because they may have divergent health effects [8] and result in different disease endotypes [9]. Increased indoor dampness can lead to increased fungal growth (with concomitant exposure of inhabitants to fungal spores, hyphae and microbial volatile organic compounds), the proliferation of house dust mites (HDM) [10] and endotoxin [11]. Living in damp indoor environments increases the risk of developing and/or exacerbating of eczema [12], allergy [13] and asthma [14], which is often assumed to be largely a result of increased exposure to fungal contamination. However, the specific causal agents for exacerbations of asthma in individuals living in damp homes have not been identified [15], and health outcomes may be a result of exposure to a myriad of physical, chemical and biological agents, consistent with a complex disease.

Few studies to date have utilised qualitative (self-reported mildew/musty odour) and quantitative assessments of fungal contamination (antigens present in house dust) and combined them with other dampness-related exposures (house dust mites and endotoxin) when assessing the risk of atopic diseases. Approximately half of current asthma cases in the United States are associated with atopy [16], and it is not clear which microbial components play a role in the development and/or exacerbation of allergic and non-allergic diseases. Assessing dampness-related exposures and health outcomes is further complicated by the variability of the indoor microbial profile (Fig. 1). Different microbial components such as fungal components may result in allergic and non-allergic pathways owing to a number of allergenic [17], toxigenic and pathogenic fungi [18]. Different components of fungi have been associated with non-allergic mechanisms via the production of microbial volatile organic compounds (mVOCs), β -glucans [3] and fungal fragments. The diversity of biological exposures may also modify health outcomes. While increased exposure to multiple indoor allergens in US homes increased the risk of asthma exacerbation in allergic individuals [19], it is also thought that increased diversity of micro-organisms is important in immune system responses [20].

Living in rural and urban environments can result in different biological, chemical and physical exposures and associated health outcomes [21]. A number of studies have shown that early exposure to a farming environment as opposed to a more 'Western lifestyle' [22], pets [23], and number of siblings [24] may provide a protective effect to the development of allergic diseases. This may be attributable to increased exposure to

infections [25], allergens, viruses, fungi and bacterial substances, such as endotoxin from gram-negative species and muramic acid, a component of peptidoglycan from the cell wall of all types of bacteria [26]. The timing and extent of exposure to microbial agents may influence allergic health outcomes [27]. For example Perzanowski et al. [28] reported that domestic endotoxin exposure was inversely associated with eczema at age 1, but positively associated with wheeze at age 2. While exposure to more rural environments appears to provide a protective effect in some studies, the evidence needs to be replicated in other populations, which is supported by Lynch and Wood [29] who found that increased allergen exposure in inner-city environments leads to a reduced risk of recurrent wheeze and sensitisation.

Household ventilation rates are important in maintaining adequate indoor air quality and regulating the diversity and concentrations of environmental exposures [10]. Household energy efficiency and/or low ventilation rates combined with the presence of a mildew/musty odour (or fungal odour) represents a risk factor for allergic symptoms [30, 31]. Fungal odour is an indication of increased dampness, visible and hidden fungal growth (in cavity walls for example) and increased mVOC concentrations [10]. The presence of odour is thought to be regulated by occupant lifestyles, behaviours, socio-economic status (SES), old housing and changes in the built environment [32]. Dampness in Europe affects around 16% of homes [33] and represents a health risk for those suffering from respiratory and allergic symptoms [34]. US housing stock, however, is thought to suffer from different dampness-related problems which may be a result of more open building envelopes leading to the ingress of water from the outdoor environment [10]. In this study, we aimed to determine whether (1) exposure to a mildew/musty odour and elevated concentrations of fungal antigens and allergens, house dust mites and endotoxin increase the risk of eczema, allergy and asthma in children and adults, and whether elevated total IgE modifies the risk of asthma; (2) whether the presence of a mildew/musty odour (proxy for the presence of dampness) is associated with increases in concentrations of *Alternaria alternata* allergen, *Aspergillus fumigatus* antigen, house dust mites and endotoxin in household dust; and (3) whether exposure to increased diversity of biological agents reduces the risk of eczema, allergy and asthma in children and adults.

Method

The following study utilises publicly released data from the National Health and Nutrition Examination Survey (NHANES), which began in the 1960s and has been

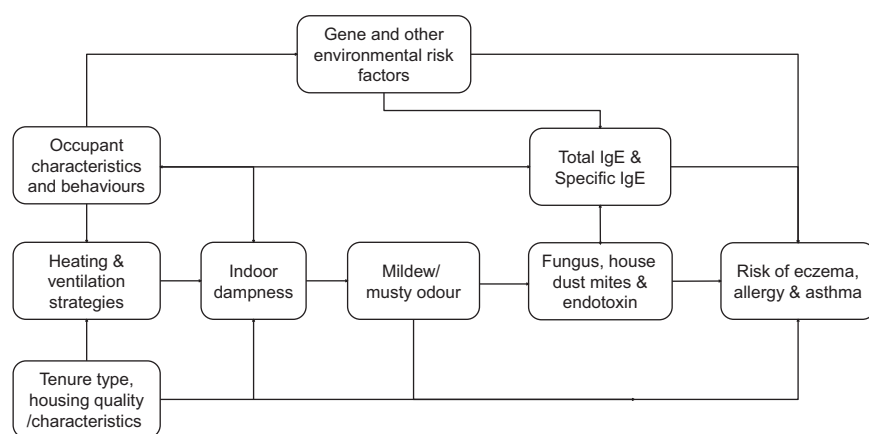


Fig. 1. Conceptual diagram of dampness-related exposures and risk of eczema, allergy & asthma.

conducted as a series of annual surveys focusing on different population groups or health topics. The following study focuses on the survey conducted during 2005–2006 (NHANES 2005–2006) because of the inclusion of housing characteristics, self-reported fungal exposure, house dust concentrations of biological agents associated with elevated dampness and health status. NHANES 2005–2006 is a cross-sectional study designed to assess the health and nutrition status of children and adults in the United States [35]. It is a stratified, multistage probability sample representative of the US civilian and non-institutionalised population [36]. About 12 000 persons per 2-year cycle are asked to participate in NHANES with an average response rate of 87.5%. Ethics approval for the survey was obtained from the NCHS Research Ethics Review Board (protocol 2005–2006). A detailed description of NHANES is provided online [35].

Participant and housing characteristics

The 2005–2006 survey includes an interview to collect demographic, socioeconomic, dietary and health-related questions on 8412 participants, which included 2849 children aged between 6 and 17 years, and adults aged 18 and 85 years. The physical examination component [carried out at the mobile examination centre (MEC)] consisted of medical, dental and physiological measurements, as well as, laboratory tests administered by a highly trained medical personal. In 2005–2006, the interview included participant housing characteristics, sampling of house dust for biological agents (combined sample taken from the bed and immediate floor area), total IgE and allergen-specific IgE (sIgE) measurements for 15 allergens taken from participants aged 6 years and older and parental questionnaires to collect demographic and health-related data (persons 16 years of age and older and emancipated minors were interviewed directly).

Five subgroups (Mexican American persons, Black persons, White and other persons at or below 130% of the federal poverty level, White and other persons aged ≥ 70 and adolescents aged 12–19) were oversampled for reliability and precision of estimates for these population subgroups [37]. Participant demographic and housing characteristic data were collected, and the ratio of family income to poverty (PIR) was calculated by dividing family income by the poverty guidelines, which is specific to family size, year and state. PIR is categorised as 0.00–0.99 (below poverty) and ≥ 1 (at or above poverty) [37]. The presence of a mildew/musty odour is used as our dichotomous variable for increased fungal contamination and our proxy for indoor dampness. Analyses include childhood and adult populations aged between 6 to 17 years and 18+ years, respectively.

Measured biological agents from house dust

House dust was collected from homes of participants attending the MEC by vacuuming each 1-square-yard marked on the bed surface and on the adjacent bedroom floor (a preference of carpeted areas) for 2 min by a trained technician, using Sanitaire™ Model 3683 vacuum cleaner and a Mitest™ Dust Collector (Indoor Biotechnologies, Inc., Charlottesville, VA, USA). Dust samples were analysed for endotoxin content (Indoor Biotechnologies, Inc) and dust allergens (Air Quality Sciences, Inc., Atlanta, GA, USA) using enzyme-linked immunosorbent assay (ELISA) and *Limulus* amoebocyte lysate (LAL) assay techniques, respectively. In this study, we assess continuous concentrations (a unit increase) of *A. alternata* (Alt a 1 allergen), *A. fumigatus* antigens, *Dermatophagoides pteronyssinus* (Der P1 allergen), *Dermatophagoides farina* (Der f1 allergen) and endotoxin (gram-negative bacteria) antigen measured from the house dust samples. In our final analyses, we include German cockroach *Blattella germanica* (Bla g1 & Bla g2 allergens), *Canis familiaris* (Can f1 allergen),

Felis domesticus (Fel d1 allergen), mouse urinary protein (Mus m1 allergen) and rat urinary protein (rat n1 allergen). These eleven biological agents were included in our final analyses as a dichotomous variables defined by their presence in house dust below and above the limit of detection (LOD), as set out by the NHANES methodology. To assess exposure to multiple biological agents, we compared exposures (present > LOD) between low (0–3 agents), medium (4–7 agents) and high (8–11 agents) diversity and risk of allergic diseases.

Immunoglobulin E and Eczema, Allergy and Asthma

Blood samples were drawn from participants at MECs, and serum was analysed for total IgE and allergen-specific IgE responses to fifteen aeroallergens using the Pharmacia Diagnostics ImmunoCAP 1000 System (Kalamazoo, MI). The lower limit of detection (LOD) for total serum IgE measurement was equal to 2.00 KU/L, and there was no upper detection limit reported. Atopy or allergic sensitisation is defined by a dichotomous outcome when total IgE exceeds the clinical cut-off of ≥ 170 KU/L. The analyses are limited to participants aged ≥ 6 years because they received the full panel of allergens.

We stratified health outcomes by age due to reported differences between childhood and adult populations [38, 39]. Due to the self-reported nature of the NHANES parental questionnaire, we combined two health-related questions (self-reported symptoms in the last 12 months and doctor diagnosis) to define dichotomous outcomes for current eczema, allergy and asthma, which are defined by participants giving a positive response to the below questions. In the following study, we use the term allergy to describe point two below because the questions refer to both hay fever and allergies:

- 1 'Have you had this itchy rash at any time in the last 12 months?' and 'Has a doctor or other health professional ever told you that you have eczema?'
- 2 'During the past 12 months, Have you had an episode of hay fever?' and 'Has a doctor or other health professional ever told you that you have allergies?'
- 3 'Has a doctor or health professional ever told you that you have asthma?' and 'Do you still have asthma?'

Statistical analysis plan

Descriptive statistics describe demographic and housing characteristics of participating children and adults. The survey weighting command in Stata 'svy' was applied to the 'least common denominator' [37]. The following analyses are based on indoor exposures (associated with

elevated dampness) and risk of eczema, allergy and asthma, which are uniquely available from the NHANES. We used the self-reported measure for the presence of a mildew/musty odour as a dichotomous variable, and then individually assess a unit increase in risk of fungi, HDM and endotoxin in house dust. Our exposure variables were assessed separately because we tested different hypotheses that are independent to each other. We log-transformed concentrations of fungus, HDM and endotoxin to account for skewed distribution in analyses where we assessed a unit increase in concentrations of fungus, HDM and endotoxin, with the exception of the final analysis where we assess exposure to multiple biological agents and risk of allergic disease. We did not log-transform the biological agents included in this analyse because we dichotomise the absence or presence of each allergen above the limit of detection.

We used Spearman's rank correlation coefficient to assess whether our exposure variables of interest (*A. alternata*, *A. fumigatus*, HDM Der f1 and Der p1 and endotoxin) correlated with one another. We considered exposure variables to be highly correlated with a Spearman's correlation coefficient > 0.3 . We found that Der f1 was highly correlated with Der p1, and for this reason restricted our analyses to Der p1, with the exception of the diversity research question addressed below. Three multivariate logistic regression models were used to assess whether exposure to mildew/musty odour, and continuous concentrations of Alt a 1, *A. fumigatus* antigens, Der p1 and endotoxin (measured from dust samples) increased the risk of (1) eczema, (2) allergy and (3) asthma. We then stratified the model assessing exposure to fungal odour and risk of asthma by total IgE < 170 and ≥ 170 KU/l to assess the role of allergic sensitisation in this population. We also used the postestimation command *chest* in Stata to further investigate whether elevated total IgE and sIgE to fungus modified the risk of asthma. Models were adjusted for age, gender, race/ethnicity, BMI, poverty income ratio, cotinine levels and the presence of any pets in the home. We also conducted additional analyses to assess other known risk factors associated with asthma.

Multivariate logistic regression was similarly used to assess whether the presence of dampness (using a mildew/musty odour as our dichotomous proxy variable) modified dust antigen concentrations of fungus (*A. alternata* Alt a1 and *A. fumigatus* antigens), HDM Der p1 and endotoxin when adjusted for household occupancy, time of survey and residency period. In our final analyses, we assessed exposure to 11 biological antigens present in house dust $\geq$ LOD, then concentrations of each being present above the weighted mean (categorised as the presence of 0–3, 4–7 and 8–11 agents) and risk of childhood and adult eczema, allergy and asthma.

We adjusted again for age, gender, race/ethnicity, BMI, poverty income ratio, cotinine levels and the presence of any pets in the home.

All statistical analyses were carried out in Stata version 13.0 (College Station, TX, USA).

Results

Of the 8412 participants in NHANES 2005–2006, 2849 were children aged between 6 and 18 years (Table 1) with a mean age of 11.6 years (SE 0.1). A total of 3.3% (SE 0.5), 3.8% (SE 1.1) and 11.6% (SE 0.6) of children had self-reported eczema, allergy and asthma, respectively, and 38.6% (SE 4.9) of children resided in a family-owned home. Sixty-six percentage of the study population were adults with a mean age of 45.6 (SE 0.8) years. Self-reported prevalence for eczema, allergy and asthma was 2.3% (SE 0.02), 8.0% (SE 0.6) and 8.1% (SE 0.5), respectively. A total of 37.2% (SE 4.6) of adults owning their home lived in poverty.

Children lived in houses where samples of dust from floor and bed were found to contain *A. alternata* (alt a1), *A. fumigatus* antigens, Der p1 and endotoxin in house dust with mean concentrations of 0.45 ng/mL (95% CI 0.40–0.50), 8613 ng/mL (95% CI 8113.52–9113.26), 107.68 ng/mL (95% CI 60.42–154.94) and 2705.20 ng/mL (95% CI 2017.82–3392.59), respectively. Adult populations were exposure to *A. alternata*, *A. fumigatus*, Der p1 and endotoxin with mean concentrations of 0.51 ng/mL (95% CI 0.43–0.58), 8768.38 ng/mL (95% CI 8241.61–9295.16), 113.35 ng/mL (95% CI 67.38–159.31) and 1695.57 ng/mL (95% CI 1384.74–2006.40), respectively.

Exposure to self-reported mildew/musty odour and risk of eczema, allergy and asthma

Childhood exposure to a mildew/musty odour increased risk of reporting current asthma (OR 1.60; 95% CI 1.17–2.19), but not eczema (OR 2.17; 95% CI 0.81–5.83) or allergy (OR 1.4; 95% CI 0.92–2.14) (Table 2). On further investigation, we found that associated risk of childhood current eczema was likely a result of insufficient power in our analyses (Table 2), but not allergy. Having elevated total IgE (≥ 170 KU/L) did not alter the risk of current asthma in children (OR 1.81; 95% CI 1.01–3.25) when exposed to odour, but the relationship may not be maintained in children with total IgE < 170 KU/L (who were 78% of the population) (Table 3).

Reporting of a mildew/musty odour was associated with increased risk of adults reporting current eczema (OR 1.92; 95% CI 1.39–2.63), allergy (OR 1.59 95% CI 1.26–2.02) and asthma (OR 1.61; 95% CI 1.00–2.57). In adults, the relationship between reporting of exposure to odour and the risk of current asthma was found to

differ on stratification by total IgE (using the clinical cut-off of 170 KU/L). No association was found (OR 0.97 95% CI 0.46–2.04) for individuals above the clinical cut-off, and those with lower total IgE < 170 KU/L, a relationship was maintained between reporting of odour and risk of self-reported current asthma (OR 1.91; 95% CI 1.07–3.42).

These results are supported by our postestimation model (Table S1), which assesses the exposure to a mouldy/mildew odour, *A. alternata*, *A. fumigatus*, HDM and endotoxin concentrations in house dust. The model suggests that children exposed to a mildew/musty odour plus having a total IgE > 170 KU/L had a 1.66% further increase in current asthma (from OR 1.59 to OR 1.61; 95% CI 1.05–2.47), but this was not evident in adults.

Exposure to Alternaria alternata, Aspergillus fumigatus, house dust mites and endotoxin in house dust, and risk of risk of eczema, allergy and asthma

No relationship was found between a range of damp environment-related measures (a unit increase in concentrations of fungi, HDM and endotoxin measured in household dust) and a range of atopic diseases apart from an association between HDM Der p1 and increased risk of childhood allergy (OR 1.37; 95% CI 1.06–1.77), and a reduction in risk of current asthma in children in households with measureable *A. alternata* (OR 0.36; 95% CI 0.13–0.95) after adjusting for a range of risk factors (Table 2). Having low total IgE < 170 KU/L did not alter the risk of current asthma in children when exposed to *A. alternata* (OR 0.25; 95% CI 0.07–0.86), but the relationship was not maintained in children with total IgE ≥ 170 KU/L. The addition of specific fungus IgE did not appear to modify the association between fungal odour and risk of asthma in children and adults in the postestimation model (Table S1).

Mildew/musty odour and levels of indoor contaminants

To assess whether participating homes suffered from elevated dampness-related exposures, we investigated the association between our proxy for dampness (presence of a mildew/musty odour) and concentrations of *A. alternata* allergen, *A. fumigatus* antigens, house dust mite and endotoxin. Variations in levels of indoor dampness (via odour) did not influence concentrations of *A. alternata*, *A. fumigatus* and endotoxin in house dust. Our proxy for dampness was associated with a unit increase in concentrations of *A. fumigatus* and Der p1 antigen (OR 1.41; 95% CI 1.07–1.85 and OR 1.14; 95% CI 1.07–1.22, respectively; Table 4). No association was observed when stratifying *A. fumigatus* and risk of asthma by the presence of dampness. But when stratifying Der p1 exposure and risk of asthma by the presence

Table 1. Childhood ($N = 2849$) and adult ($N = 5563$) demographics

Characteristic	Children aged 6–17		Adults aged 18+	
	<i>n</i>	Weighted mean/% (SE)	<i>n</i>	Weighted mean/% (SE)
Mean age (years)	2849	11.6 (0.1)	5563	45.6 (0.8)
Gender				
Male	1409	50.9 (1.3)	2675	48.2 (0.5)
Female	1440	49.1 (1.3)	2888	51.8 (0.5)
Race/ethnicity				
Non-Hispanic White	744	61.0 (3.6)	2633	71.4 (2.8)
Non-Hispanic Black	906	14.3 (2.3)	1341	11.7 (1.9)
Mexican American	948	12.7 (1.3)	1185	8.1 (1.0)
Other	251	12.0 (1.6)	404	8.8 (1.1)
BMI kg/m ²				
Underweight (0.0–18.4)	939	40.9 (1.6)	91	18.5 (0.3)
Normal (18.5–24.9)	1162	41.5 (1.5)	1593	30.8 (1.3)
Overweight (25.0–29.9)	364	9.9 (0.8)	1736	31.8 (0.8)
Obese (30.0–130)	384	7.6 (0.9)	2143	35.6 (1.4)
Family income-to-poverty ratio (FIPR)				
Below poverty (PIR < 0.99)	732	16.9 (1.9)	1026	11.1 (0.7)
At or above poverty (PIR > 1)	2117	83.0 (1.9)	4537	88.9 (0.7)
Cotinine levels (ng/mL)				
LOD < 0.015	470	17.5 (2.1)	849	15.8 (1.3)
Low (≥ 0.015 –10)	1769	62.9 (1.6)	2862	52.1 (1.7)
High (≥ 10)	610	19.6 (1.0)	1852	32.1 (1.3)
Current asthma				
No	2530	88.4 (0.6)	5104	91.9 (0.5)
Yes	314	11.6 (0.6)	436	8.1 (0.5)
Eosinophil				
Low (≤ 2.4)	1111	44.0 (2.3)	2682	51.6 (0.7)
High (> 2.4)	1295	56.0 (2.3)	2323	48.4 (0.7)
Current eczema				
No	2537	96.7 (0.5)	5237	97.7 (0.2)
Yes	87	3.3 (0.5)	106	2.3 (0.02)
Current allergy				
No	2152	96.2 (1.1)	4006	92.0 (0.6)
Yes	69	3.8 (1.1)	240	8.0 (0.6)
Total IgE				
< 170 KU/L	1746	77.74 (0.9)	3960	82.73 (0.9)
> 170 KU/L	642	22.26 (0.9)	1050	17.27 (0.9)
Current asthma with total IgE > 170				
No	925	81.9 (1.6)	1412	88.6 (1.5)
Yes	175	18.1 (1.6)	181	11.4 (1.5)
sIgE to <i>Alternaria alternata</i>				
< 0.35	2065	87.9 (1.5)	4581	92.2 (0.5)
> 0.35	321	12.1 (1.5)	417	7.8(0.05)
sIgE to <i>Aspergillus fumigatus</i>				
< 0.35	2113	91.3 (1.1)	4629	94.1 (0.3)

(continued)

Table 1 (continued)

Characteristic	Children aged 6–17		Adults aged 18+	
	<i>n</i>	Weighted mean/% (SE)	<i>n</i>	Weighted mean/% (SE)
> 0.35	268	8.7 (1.1)	366	5.9 (0.3)
sIgE to Der f1				
< 0.35	1849	81.4 (1.1)	3987	81.5 (1.0)
> 0.35	538	18.6 (1.1)	1024	18.5 (1.0)
sIgE to Der p1				
< 0.35	1834	81.2 (1.1)	3981	81.1 (1.2)
> 0.35	554	18.8 (1.1)	1030	18.9 (1.2)
Homeownership				
owned	1778	72.9 (2.2)	3560	71.3 (2.2)
Rented	1000	25.2 (2.1)	1800	25.9 (1.9)
Other arrangement	34	1.1 (0.3)	145	2.1 (0.3)
Missing, don't know or refused	37	0.8 (0.3)	58	0.1 (0.1)
Self-reported exposure to mildew or musty smell				
Yes	557	18.7 (2.1)	817	15.5 (1.1)
No	2254	81.3 (2.1)	4675	85.5 (1.1)

of dampness, we found suggestive evidence that Der p1 increased risk of adult asthma (OR 1.17; 95% CI 0.98–1.38; presence of an odour), but not in absence of dampness (OR 0.89; 95% CI 0.80–0.99). This suggests that asthma symptoms in adults may be exacerbated by Der p1 when combined with elevated indoor dampness; however, we were unable to determine whether the association was due to dampness, house dust mite or a combination of both factors.

In additional analyses, we found a number of risk factors that may contribute to the presence of a mildew/musty odour, which included buildings built before 1990 (data not shown), living in rented accommodation as opposed to homeownership (OR 1.36; 95% CI 1.73–3.13) and living in poverty (OR 1.58; 95% CI 1.19–2.09). We also found that living in poverty was associated with increased risk of dampness (OR 1.58; 95% CI 1.19–2.09), as well as a risk factor for adult asthma (OR 1.56; 95% CI 1.12–2.18), but not in children. Living in poverty also appears to be associated with non-allergic adult asthma with total IgE < 170 KU/L (OR 2.08; 95% CI 1.41–3.07), but not high total IgE ≥ 170 KU/L.

Exposure to multiple biological agents in house dust

We observed suggestive evidence that exposure to multiple allergen or antigen being present in household dust samples (measured by antigen concentrations $\geq$ LOD) was associated with reduced risk of childhood eczema (OR 0.17; 95% CI 0.04–0.77) and adult asthma (OR 0.49; 95% CI 0.25–0.97) (Tables 5 and 6). No association was found between increased exposure to multiple biological agents and risk of allergy. However,

Table 2. Childhood and adult exposure to odour, fungus, house dust mites and endotoxin, and risk of current eczema, allergy and asthma

Dampness-related exposure	Health outcome: current asthma					
	Current eczema		Current allergy		Current asthma	
	AOR	95% (CI)	AOR	95% (CI)	AOR	95% (CI)
<i>Children aged 6–17 years</i>						
Self-reported dichotomous exposure variable for fungal contamination						
Self-reported presence of a mildew/musty odour						
No	Ref		Ref		Ref	
Yes	2.17	0.81–5.83	1.4	0.92–2.14	1.6	1.17–2.19**
A unit increase in house dust concentrations of fungi, house dust mites (HDM) and endotoxin						
<i>Alternaria alternata</i>	0.92	0.17–5.10	2.07	0.54–7.91	0.36	0.13–0.95*
<i>Aspergillus fumigatus</i>	1.46	0.57–3.72	1.24	0.43–3.60	0.94	0.57–1.55
House dust mite						
Der p 1	0.92	0.69–1.21	1.37	1.06–1.77*	0.98	0.89–1.08
Endotoxin	0.78	0.57–1.07	1.32	0.91–1.92	1.17	0.88–1.55
<i>Adults aged 18+ years</i>						
Self-reported dichotomous exposure variable for fungal contamination						
Self-reported presence of a mildew/musty odour						
No	Ref		Ref		Ref	
Yes	1.92	1.39–2.63**	1.59	1.26–2.02**	1.61	1.00–2.57*
A unit increase in house dust concentrations of fungi, HDM and endotoxin						
<i>Alternaria alternata</i>	0.96	0.31–2.96	1.24	0.59–2.55	0.88	0.58–1.33
<i>Aspergillus fumigatus</i>	1.13	0.69–1.83	1.23	0.89–1.69	0.94	0.63–1.42
House dust mite						
Der p 1	1.13	0.97–1.31	1.04	0.88–1.23	0.97	0.89–1.05
Endotoxin	0.87	0.69–1.08	1.08	0.92–1.28	1.02	0.91–1.15

Presence of a mildew/musty odour is our dichotomous exposure, and fungus, house dust mites and endotoxin are continuous exposures above the limit of detection (LOD)

AOR – Adjusted model age, gender, race/ethnicity, PIR, cotinine levels, BMI and any pets in the home

*0.01 ≤ *P* < 0.05, **0.001 ≤ *P* < 0.01 and ****P* < 0.001.

there was insufficient power to further investigate variations in concentrations of multiple allergens present in dust samples and risk of eczema, allergy and asthma.

Discussion

Reported exposure to a mildew/musty odour may increase the risk of childhood allergic asthma (total IgE ≥ 170 KU/L) and non-allergic adult asthma (total IgE < 170 KU/L). Reporting odour was also found to increase risk of adults reporting current eczema and allergy. Residence in a home with increased concentrations of *A. alternata* in house dust was associated with a reduced the risk of asthma in children. Exposure to multiple biological agents (presence of an agent ≥ LOD) reduced the risks of childhood eczema and adult asthma. These findings support the notion that differences in lifestyle characteristics, communities and their interaction with the natural and built environment are important in regulating health status at the population level. These complex heterogeneous diseases are fundamentally a population-level problem of maladapted human ecology, which need to be examined through models with higher levels of complexity.

Synthesis with existing literature

Consistent with our findings, other researchers have shown that exposure to fungal odour in built environments is associated with increased risk of rhinitis [13], allergic symptoms [30] and the development [14] and/or exacerbation [40] of asthma. We add to the current body of evidence by demonstrating that the presence of reported odour was associated with allergic childhood asthma and non-allergic adult asthma. In contrast to this finding, Flamant [3] found that increased mVOC concentrations (a quantitative measure of odour) were associated with increased risk of non-allergic asthma in children, which may be due to differences in exposure definitions and health outcomes. In support of our findings, Hernberg and Sripaiboonkij [41] reported that odour was associated with lower lung function levels in healthy adults, which may be due to non-allergenic symptoms. Although non-allergic symptoms may be confounded by other risk factors such as lower SES and occupation exposures, as exemplified by our participants who had a low PIR and living in poverty (PIR ranging between 0.00 and 0.99) having an increased risk of non-allergic adult asthma.

Table 3. Exposure to odour, fungus, house dust mite and endotoxin, and allergic vs. non-allergic asthma in children and adults

Dampness-related exposure	Current asthma with atopy [†]			
	Yes		No	
	AOR	95% (CI)	AOR	95% (CI)
<i>Children aged 6–17 years</i>				
Self-reported dichotomous exposure variable for fungal contamination				
Presence of a mildew/musty odour				
No	Ref		Ref	
Yes	1.81	1.01–3.25*	1.47	0.79–2.72
A unit increase in house dust concentrations of fungi, house dust mites (HDM) and endotoxin				
<i>Alternaria alternata</i>	0.39	0.10–1.55	0.25	0.07–0.86*
<i>Aspergillus fumigatus</i>	0.84	0.41–1.72	0.94	0.51–1.77
House dust mite				
Der p 1	1.03	0.82–1.31	0.96	0.84–1.09
Endotoxin	0.99	0.79–1.26	1.3	0.92–1.84
<i>Adults aged 18+ years</i>				
Self-reported dichotomous exposure variable for fungal contamination				
Presence of a mildew/musty odour				
No	Ref		Ref	
Yes	0.97	0.46–2.04	1.91	1.07–3.42*
A unit increase in house dust concentrations of fungi, HDM and endotoxin				
<i>Alternaria alternata</i>	1.47	0.59–3.66	0.8	0.44–1.47
<i>Aspergillus fumigatus</i>	0.95	0.64–1.41	0.95	0.56–1.62
House dust mite				
Der p 1	0.84	0.68–1.03	1.01	0.92–1.12
Endotoxin	0.98	0.81–1.18	1.01	0.85–1.20

Presence of a mildew/musty odour is our dichotomous exposure, and fungus, house dust mites and endotoxin are continuous exposure above the limit of detection (LOD).

AOR – adjusted model age, gender, race/ethnicity, PIR, cotinine levels, BMI and any pets in the home.

[†]Asthma with atopy was defined as having asthma in combination with total IgE (≥ 170 KU/L).

*0.01 $\leq P < 0.05$

We also found that mildew/musty odour was associated with eczema in adults. This may have implications for the atopic march and allergen exposure through the epidermis, which can initiate systemic allergy and predispose individuals to eczema, allergic rhinitis and asthma [42]. Further research may help identify different phenotypes of the early stages of the atopic march, which may help predict the development of later atopic, comorbid chronic diseases [43]. However, despite molecular advances in the United States such as the development of the ERMI [44], which assess fungal concentrations in house dust, there is limited evidence assessing exposure to different types of fungus and risk of allergic diseases [27].

Table 4. Presence of a mildew/musty odour (proxy for dampness) and risk of increased concentrations of fungus, house dust mite and endotoxin

	n	Outcome: presence of a mildew/musty odour			
		Unadjusted		Adjusted	
		OR	95% (CI)	OR	95% (CI)
<i>Alternaria alternata</i> (unit increase > LOD)	3967	0.96	0.64–1.42	0.93	0.59–1.43
<i>Aspergillus fumigatus</i> (unit increase > LOD)	7699	1.4	1.06–1.84*	1.41	1.07–1.85*
Endotoxin (unit increase > LOD)	9353	1.05	0.93–1.19	1.04	0.92–1.19
Der p 1 (unit increase > LOD)	6151	1.14	1.07–1.22***	1.14	1.07–1.22**

Fungus, house dust mites and endotoxin are continuous exposures above the limit of detection (LOD).

Adjusted model household occupancy level, time of survey and residency period in participants current home.

*0.01 $\leq P < 0.05$, **0.001 $\leq P < 0.01$ & *** $P < 0.001$.

Table 5. Presence allergen antigens in house dust ($\geq$ LOD) and risk of eczema

Diversity of allergen antigens found in house dust at time of sampling	Current Eczema	
	Unweighted % (n/d)	Adjusted
		OR 95% (CI)
<i>Children aged 6–17 years (n = 2111)</i>		
Number of antigens present in home $\geq$ LOD		
0–3	8 (5/67)	Ref
4–7	3 (37/1172)	0.17 0.05–0.52**
8–11	4 (27/715)	0.17 0.04–0.77*
<i>Adults aged 18+ years (n = 3734)</i>		
Number of antigens present in home $\geq$ LOD		
0–3	5 (11/239)	Ref
4–7	2 (42/2199)	0.56 0.24–1.29
8–11	2 (21/1155)	0.53 0.22–1.28

Adjusted model age, gender, race/ethnicity, PIR, cotinine levels, BMI and any pets in the home.

*0.01 $\leq P < 0.05$, **0.001 $\leq P < 0.01$.

Fungal contamination is of interest because sensitisation to different fungal species (and HMD) is associated with increased risk of asthma and reduced pulmonary function [45] and is associated with atopic eczema and allergy [17]. Little is known about different fungal components associated with pathogenesis of non-allergic diseases in childhood and adult populations. Further research is required to investigate the role of different microbial exposures and risk of different disease endotypes [9], which requires a shift towards identifying and quantifying multiple indoor and outdoor agents, as well as better case definition.

Table 6. Presence allergen antigens in house dust ($\geq$ LOD) and risk of asthma

Diversity of allergen antigens found in house dust at time of sampling	Current Asthma		
	Unweighted % (n/d)	Adjusted OR	95% (CI)
Children aged 6–17 years ($n = 2111$)			
Number of antigens present in home $\geq$ LOD			
0–3	13 (10/81)	Ref	
4–7	11 (142/1269)	0.71	0.21–2.48
8–11	11 (84/757)	0.62	0.14–2.67
Adults aged 18+ years ($n = 3734$)			
Number of antigens present in home $\geq$ LOD			
0–3	8 (20/246)	Ref	
4–7	9 (197/2274)	0.83	0.41–1.65
8–11	7 (88/1198)	0.49	0.25–0.97*

Adjusted model age, gender, race/ethnicity, PIR, cotinine levels, BMI and any pets in the home.

* $0.01 \leq P < 0.05$.

Utilising NHANES also enabled us to assess exposure to concentrations of *A. fumigatus* and *A. alternata*, which allowed us to further explore the role of indoor fungal contamination and risk of allergic diseases. Despite a recent meta-analysis highlighting that *A. alternata* increased risk of exacerbating asthma symptoms [27], we observed that this fungus led to a reduced risk of asthma. This requires further investigation because having high sIgE (≥ 0.35 KU/L) to *A. fumigatus* and *A. alternata* antigens is associated with increased risk of asthma [46]. *Alternaria alternata* produces potent allergenic proteins [17], increases risk of severe asthma and elicits a robust Th2 inflammatory response exacerbating allergic airway disease [47]. *A. fumigatus* is the most commonly reported cause of severe pulmonary allergic diseases, including allergic bronchopulmonary aspergillosis [48]. Potential explanations for our finding include the geographic variation in prevalence of sensitisation [16], which we were unable to account for, and fungus in house dust has to become aerosolised in order to impact health outcomes. Air- and dustborne exposures represent different environmental exposure to residents, supporting the need to conduct both sampling techniques in future studies [49]. Also, our analyses were limited to only two species of fungi. This requires consideration because fungi are ubiquitous in outdoor and indoor environments, which includes many species of *Alternaria*, *Aspergillus*, *Penicillium* and *Cladosporium* found to increase risk of asthma [27].

Alternatively, increased concentrations of *A. fumigatus* and *A. alternata* antigens may be a surrogate for healthier and more active lifestyles in this population. For example, *A. fumigatus* and *A. alternata* are predominantly outdoor fungi, and their presence indoors relies

on human activity and the transmission of spores into the home environment (in the absence of indoor dampness and better ventilation), which may explain why *Alternaria* was inversely associated with childhood non-allergic asthma (total IgE < 170 KU/L). There are other environmental exposures that may modify our findings such as vitamin D levels that may play a role in asthma pathogenesis [50]. Vitamin D levels may provide a protective effect against wheeze and asthma exacerbations [51]. This may be due to a lack of relationship between serum 25-hydroxyvitamin D and asthma in adolescents or Vitamin D deficiency being a risk factor for allergic disorders and immune mechanisms. Other factors to consider include the roles of BMI, smoking, diet, metabolites and lifestyle [52], as well as the role of demographic and housing characteristics, which are important determinants of health [53]. It is possible that typical symptom characteristics and/or the detectable inflammatory component of disease may not be present in this population. It is also important to highlight that we were restricted to assessing concentrations of *A. fumigatus* and *A. alternata*, which are only an indication of the microbial agents that participants have come into contact within the past, as there are many other species present in households including allergenic *Cladosporium* species. The presence of a mildew/musty odour is likely to resemble other different types of fungus contamination indoor surfaces of participant homes. This may explain why our findings concerning exposure to a mildew/musty odour did not correlate with our findings for increased concentrations of fungi in house dust. Also the measurement of fungi in house dust is likely to be influenced by floor covering, vacuum capture velocity and relative humidity [54]. This is further complicated because fungi are ubiquitous and have considerable diurnal and seasonal variability in sporulation rates [27].

Another possible explanation for the lack of association between fungus and asthma may lie with the lack of association between dampness (presence of a mildew/musty odour) and concentrations of *A. alternata* or *A. fumigatus* antigens in bedding and adjacent floor dust samples. This suggests that these homes may not suffer from dampness-related problems, although we do not know the diversity and concentrations of other fungi commonly found in damp environments. Only house dust mite Der p1 antigen was associated with dampness, which has been previously reported [55], and found to increase risk of eczema [56], asthma severity and morbidity. This is important to consider because more than 50% of children and adolescents are sensitised to HDM [57]. The lack of association between *A. alternata* or *A. fumigatus*, endotoxin and the presence of dampness highlights the complexity of investigating multiple indoor exposures and impact on health.

In an attempt to further explore the role of multiple indoor exposures and health outcomes, we assessed the presence of eleven indoor biological antigens present in house dust $\geq$ LOD in an attempt to investigate the role of multiple indoor exposures and found that increased diversity reduced the risk of reporting eczema and asthma. We were unable to assess the role of varying concentrations of multiple agents due to insufficient power. This is important to consider because other authors found that six detectable allergens (Der p1, Der f1, Can f1, Fel d1, Bla g1 and mouse urinary protein) in US homes were common and associated with asthma symptoms in allergenic individuals [19]. This study differs from ours because the authors utilised cut-off points for allergen concentrations found in house dust. However, Lynch and Wood [29] found that children living in inner-city environments with the highest exposure to specific allergens and bacteria during their first year were least likely to have recurrent wheeze and allergic sensitisation. Another hypothesis suggests that the increase in prevalence of asthma and allergies may be attributable to declining exposure to environmental micro-organisms [58]. To test this hypothesis, Devereux [58] assessed exposure to increased components found in indoor soil (a major component of house dust, and a prime source of the essential elements and potentially toxic substances). The authors reported an association with adverse respiratory health (not allergic status or eczema) and postulated that this may be a result of exposure to organic components of silt. Family size, birth order [24] and living on farms where children may be exposed to a wide range of microbes [59] are also thought to be important determinants in the development of the immune system and regulation of allergic diseases. Other authors suggest that increased diversity of fungi and bacteria reduces the risk of asthma and atopy, which opens up new areas of research and further complicates the way we investigate environmental exposures [8]. It is also thought that the natural environment and increased exposure to a diverse array of micro-organisms are important in the regulation of our immune system [20] and the development of a healthy human microbiome, although the specific intrinsic (genes) and extrinsic pathways (surrounding environment, birth mode, diet or antibiotics) involved in disease pathogenesis and development [60] are yet to be fully explored at the population level.

Strengths and limitations

Strengths of NHANES 2005–2006 is that the study population is representative of the US population. It includes the largest national data set of serum IgE levels that has been collected from the US population, and allows both qualitative and quantitative assessment of

environmental exposures and health outcomes [5]. We adopted a similar methodology used by previous investigators to assess exposures to self-reported odour [14] and exposure to increased concentrations of different types of fungus in house dust [27]. We utilise mildew/musty odour, which accurately represents microbial exposures and risk of asthma in longitudinal studies [61] and has been shown to correlate with culturable fungal load [62]. Utilising ELISA techniques to quantify antigen levels in house dust overcomes some of the limitations of microscopic observations [63]. Self-reported asthma outcomes have been previously shown to correlate well with GP-recorded diagnosis [64], and we were able to allow for a large number of covariates and use the postestimation command *chest* to assess the impact of covariates. Prior to investigating NHANES data, we established a set of predefined research questions (listed in our introduction) to lower the risk of bias. We also believe that this is the first study to assess the interaction between odour and multiple biological agents, and risk of eczema, allergy and asthma in the representative US study population.

A number of limitations exist. The cross-sectional study design (questionnaire and home survey data) prevented us from assessing the extent of exposures over time or those resulting from preschool/elementary school [65] and previous occupation exposures [66]. Questionnaire data may introduce bias, for example asthmatic individuals may elicit different behaviours [67] and coping strategies [68] or include the recruitment of more healthier participants. Despite a large representative sample size stratifying by age (dividing participants into childhood and adult populations) and indoor exposures meant some analyses may suffer from a lack of power, which may be further compounded by missing data. We did not find sufficient evidence in this population to support further analyses into the interaction between different concentrations of fungi, HDM and endotoxin in house dust, such as the index methodology used by Lynch and Wood [29]. We were also unable to account for the geographic distribution of participants and conduct cluster analysis [69]. This prevented us from accounting for the heterogeneous distribution of fungi [70] and prevalence of atopy, which varies by census region [16]. Other factors we were unable to account for include housing quality/maintenance, property and occupant behaviour's regulating indoor dampness such as the provision of adequate heating and ventilation [10]. The use of self-reported odour contamination may introduce a level of uncertainty. Nevertheless, odour has been found to be an independent predictor of high allergen load [19]. The use of specific and limited sets of antigens as an indicator of fungal contamination may be a limitation as the method may not accurately reflect the representative

indoor fungal profile. In the absence of a specific disease diagnosis, we were unable to explain the lack of correlation between self-reported asthma and high eosinophil percentage, although our findings may reflect the broad spectrum of diseases and characterisation of IgE antibody, which may be modified by the use of our IgE and sIgE clinical cut-off points [46].

Conclusion

A mildew/musty odour was implicated in childhood allergic asthma and non-allergic asthma in adults. Adult exposure to odour was also associated with self-reported eczema and allergies. The lack of association between *A. alternata* and *A. fumigatus* may be due to the absence of dampness and/or interaction with multiple biological agents, which were found to reduce the risk of children and adults reporting eczema and asthma, respectively. These findings highlight the complexity of studying multiple environmental exposures and complex heterogeneous diseases at the population level. Increased exposure to micro-organisms and its possible immuno-microbiological health benefits is an increasingly important area of research.

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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:

Table S1. Fungal exposures and asthma (initial model) and inclusion of total and specific fungal IgE.