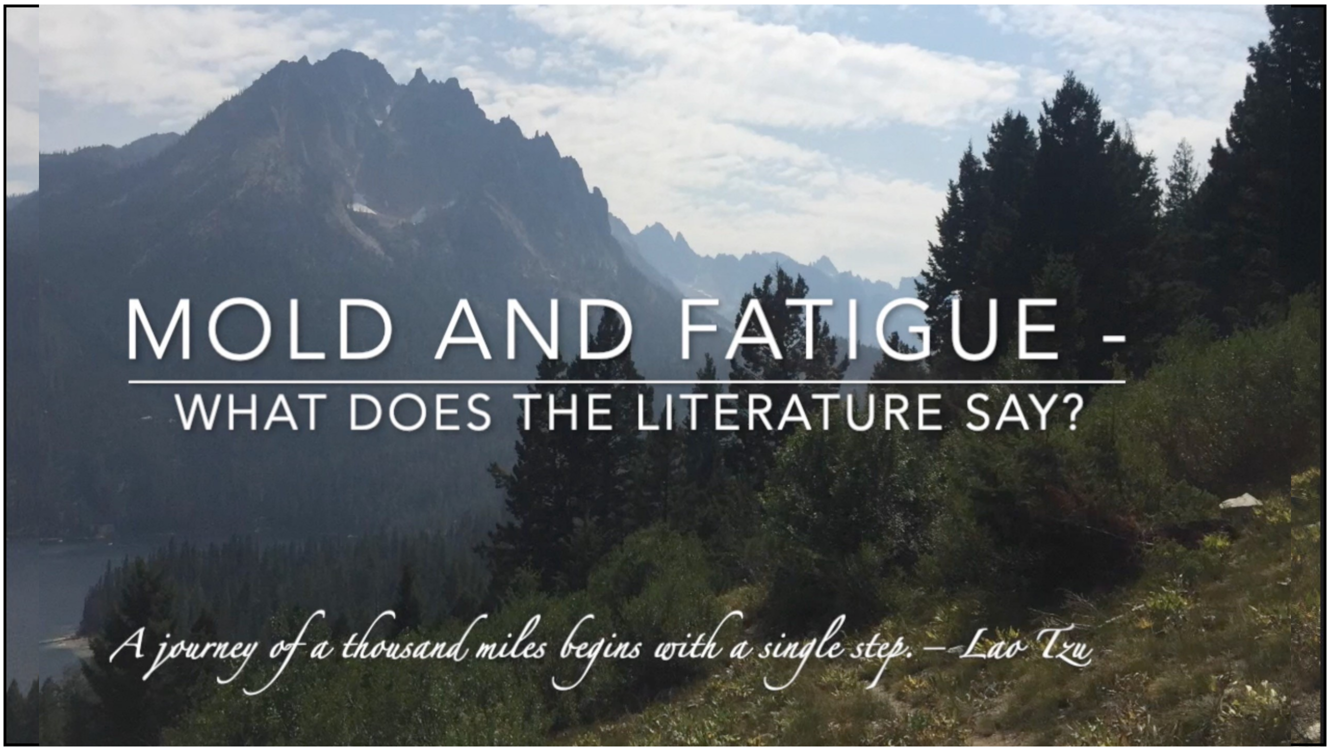


MOLD AND FATIGUE - WHAT DOES THE LITERATURE SAY?

A journey of a thousand miles begins with a single step. – Lao Tzu

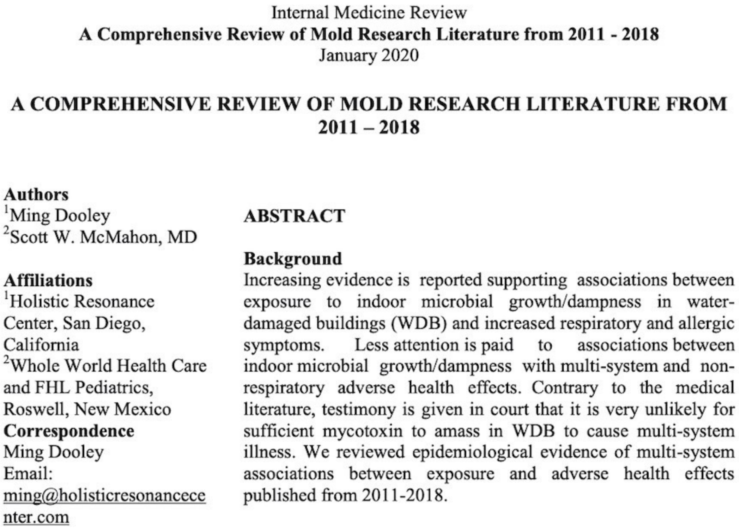
2022 CIRSX ANNUAL CONFERENCE
APRIL 29TH, 2022
DR MING DOOLEY

A journey of a thousand miles begins with a single step. – Lao Tzu

CONFLICTS OF INTEREST

MING DOOLEY HAS NO CONFLICTS OF INTEREST TO REPORT

Builds on Previous Review



Internal Medicine Review
A Comprehensive Review of Mold Research Literature from 2011 - 2018
January 2020

A COMPREHENSIVE REVIEW OF MOLD RESEARCH LITERATURE FROM 2011 – 2018

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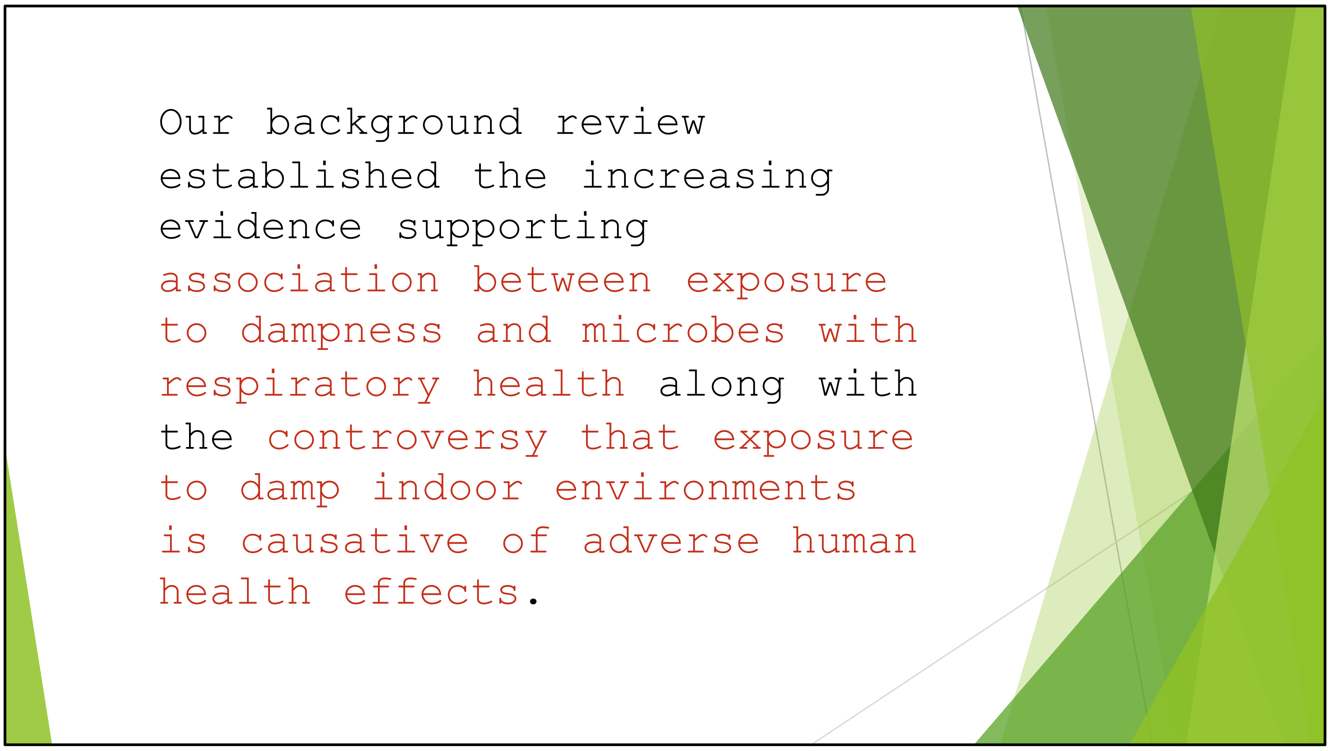
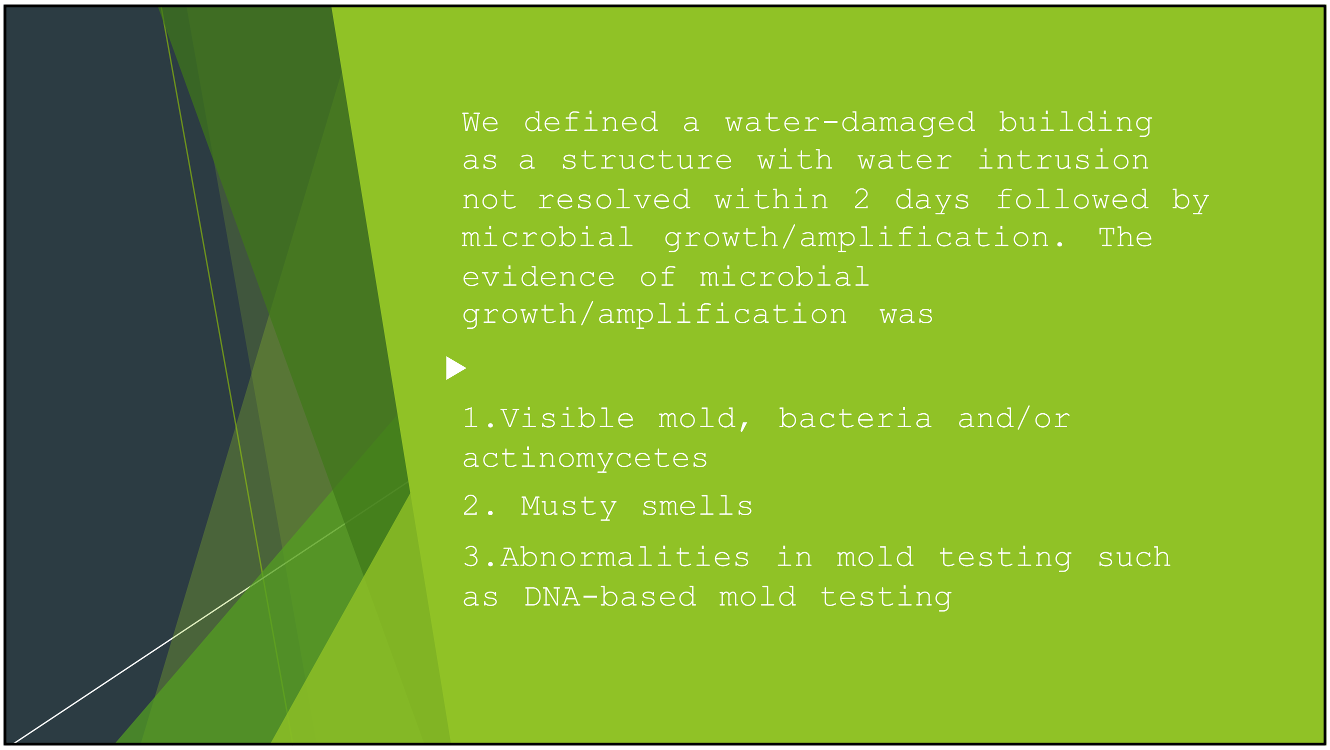
ABSTRACT

Background
Increasing evidence is reported supporting associations between exposure to indoor microbial growth/dampness in water-damaged buildings (WDB) and increased respiratory and allergic symptoms. Less attention is paid to associations between indoor microbial growth/dampness with multi-system and non-respiratory adverse health effects. Contrary to the medical literature, testimony is given in court that it is very unlikely for sufficient mycotoxin to amass in WDB to cause multi-system illness. We reviewed epidemiological evidence of multi-system associations between exposure and adverse health effects published from 2011-2018.



Our background review established the increasing evidence supporting association between exposure to dampness and microbes with respiratory health along with the controversy that exposure to damp indoor environments is causative of adverse human health effects.



We defined a water-damaged building as a structure with water intrusion not resolved within 2 days followed by microbial growth/amplification. The evidence of microbial growth/amplification was

-

1. Visible mold, bacteria and/or actinomycetes
2. Musty smells
3. Abnormalities in mold testing such as DNA-based mold testing

2018 review by Caillaud et al, concluding for the first time that there was both sufficient evidence of a casual relationship for asthma development and exacerbation as well as sufficient evidence of an association with allergic rhinitis

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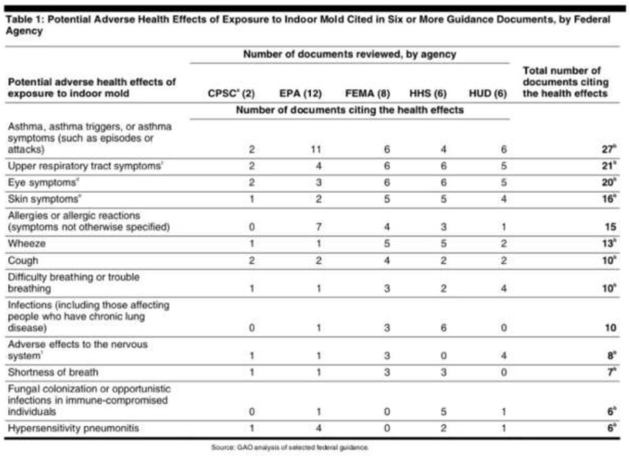
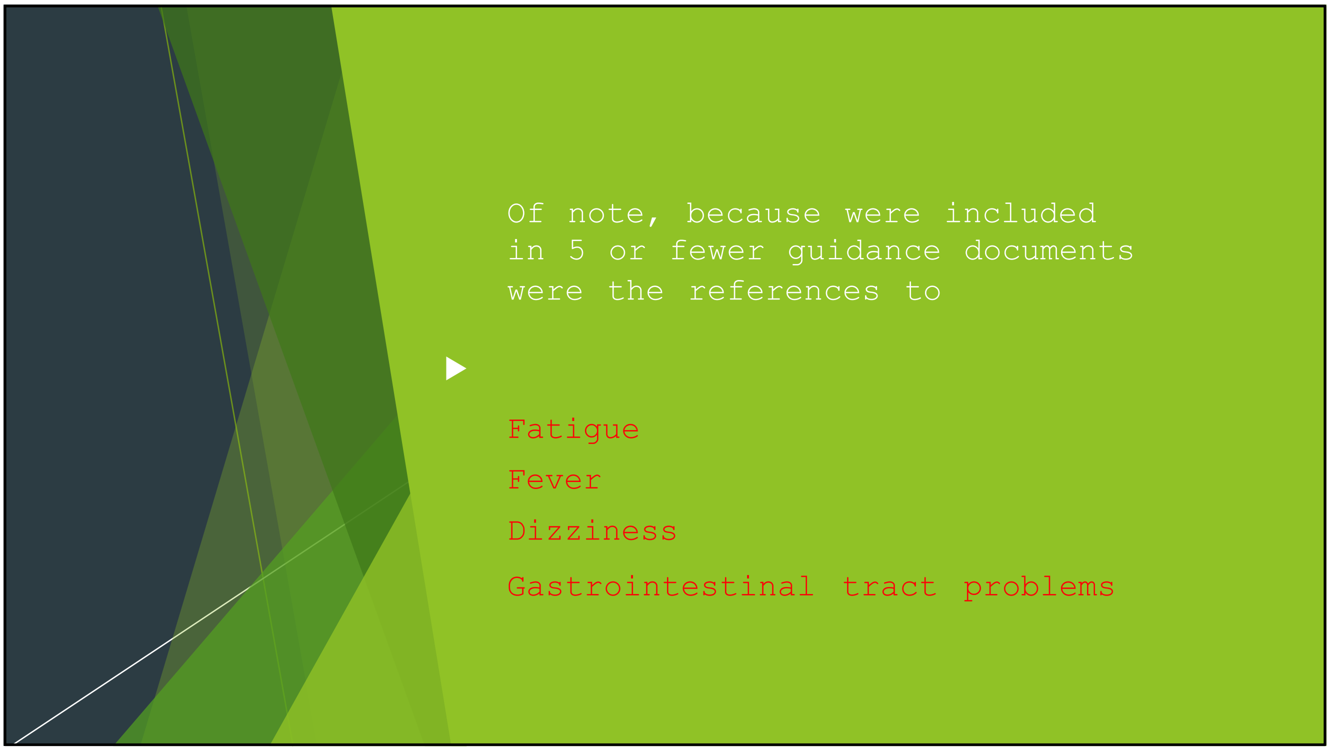


Table 1: Potential Adverse Health Effects of Exposure to Indoor Mold Cited in Six or More Guidance Documents, by Federal Agency

Potential adverse health effects of exposure to indoor mold	Number of documents reviewed, by agency					Total number of documents citing the health effects
	CPSC[a] (2)	EPA (12)	FEMA (8)	HHS (6)	HUD (6)	
	Number of documents citing the health effects					
Asthma, asthma triggers, or asthma symptoms (such as episodes or attacks)	2	11	6	4	6	**27[b]**
Upper respiratory tract symptoms[c]	2	4	6	6	5	**21[b]**
Eye symptoms[d]	2	3	6	6	5	**20[b]**
Skin symptoms[e]	1	2	5	5	4	**16[b]**
Allergies or allergic reactions (symptoms not otherwise specified)	0	7	4	3	1	**15**
Wheeze	1	1	5	5	2	**13[b]**
Cough	2	2	4	2	2	**10[b]**
Difficulty breathing or trouble breathing	1	1	3	2	4	**10[b]**
Infections (including those affecting people who have chronic lung disease)	0	1	3	6	0	**10**
Adverse effects to the nervous system[f]	1	1	3	0	4	**8[b]**
Shortness of breath	1	1	3	3	0	**7[b]**
Fungal colonization or opportunistic infections in immune-compromised individuals	0	1	0	5	1	**6[b]**
Hypersensitivity pneumonitis	1	4	0	2	1	**6[b]**

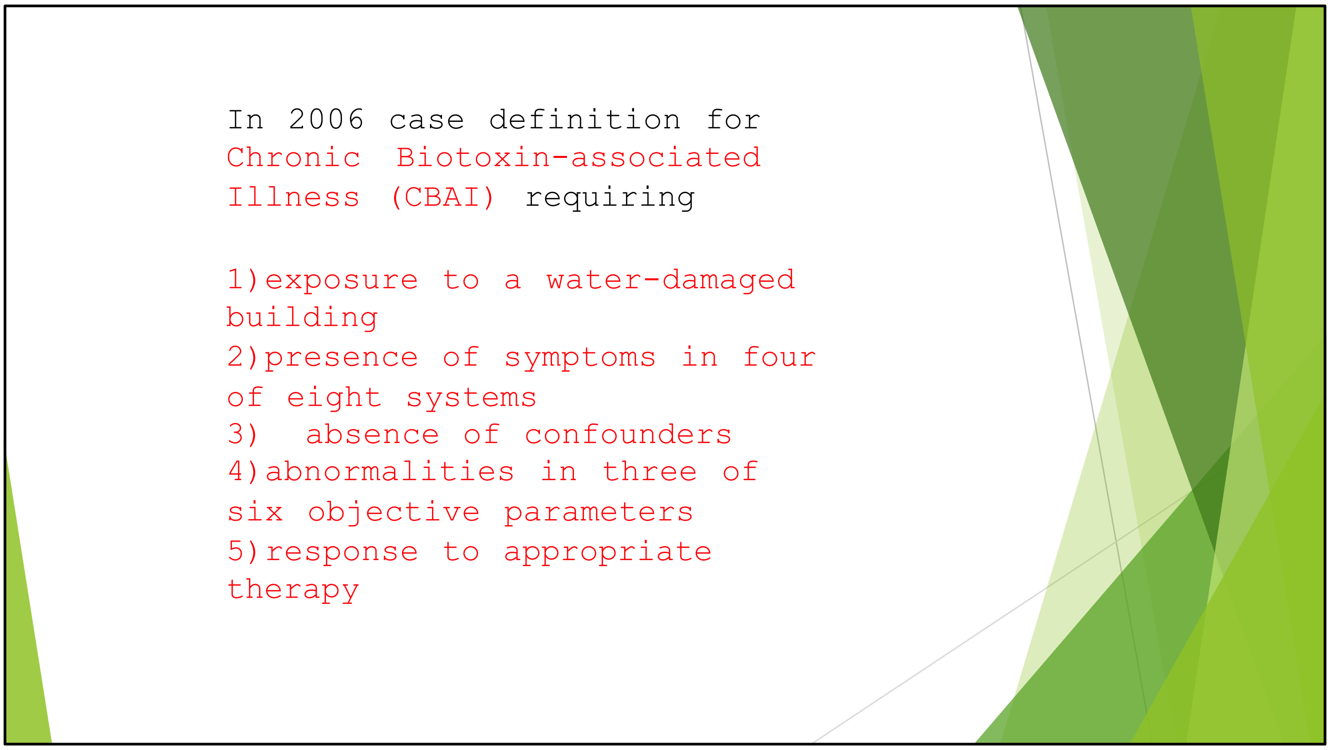
Source: GAO analysis of selected federal guidance.

Figure 1. GAO Table of Potential Adverse Health Effects of Exposure to Indoor Mold



Of note, because were included in 5 or fewer guidance documents were the references to

- Fatigue
- Fever
- Dizziness
- Gastrointestinal tract problems



In 2006 case definition for Chronic Biotoxin-associated Illness (CBAI) requiring

1) exposure to a water-damaged building
2) presence of symptoms in four of eight systems
3) absence of confounders
4) abnormalities in three of six objective parameters
5) response to appropriate therapy

In 2008 the GAO report also offered a case definition which paralleled the 2006 case definition of CBAI

1. There must be the potential for exposure to a damp indoor space.
2. There must be laboratory testing results similar to those seen in peer-reviewed, published studies.
3. There must be documentation of response to therapy.
4. There must be a multisystem, multi-symptom illness present with symptoms similar to those seen in peer-reviewed publications.

It was in the 2010 Policy Holder of America's publication that this syndrome first became known as Chronic Inflammatory Response Syndrome.

-

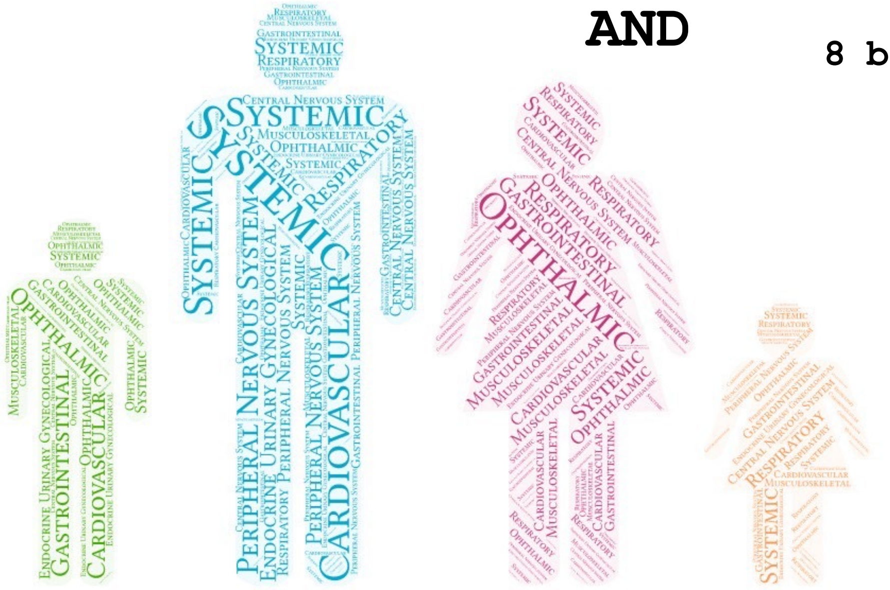
The mechanism of illness proposed was dysregulated chronic innate immune activation.

As early as 2006 Shoemaker and Maizel presented experimental evidence for causality for CIRS through prospective exposure studies in patients who had previously improved with CIRS treatment.

Mold/Mold/Dampness

AND

Photo courtesy of David Lark



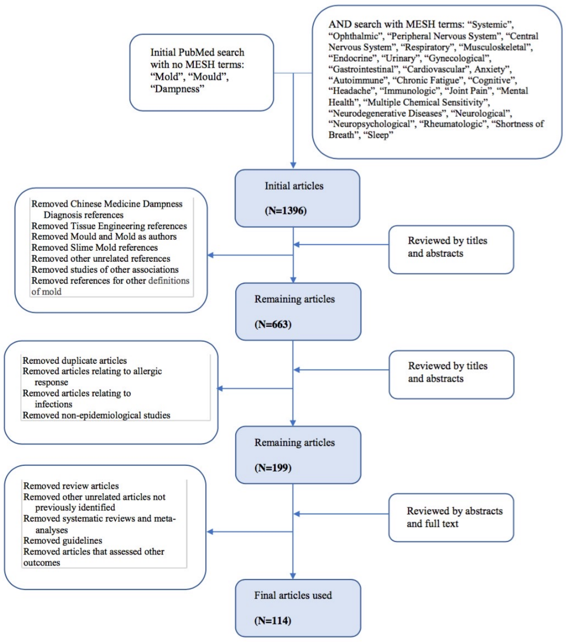
AND

8 body Systems:

Systemic
Ophthalmic
Peripheral Nervous System
Central Nervous System
Respiratory
Musculoskeletal
Endocrine/Urinary/Gynecological
Gastrointestinal
Cardiovascular

AND

Anxiety
Autoimmune
Chronic Fatigue
Cognitive
Headache
Immunologic
Joint Pain
Mental Health
Multiple chemical sensitivity
Neurodegenerative diseases
Neurological
Neuropsychological
Rheumatologic
Shortness of Breath
Sleep


Initial PubMed search with no MESH terms: “Mold”, “Mould”, “Dampness”

AND search with MESH terms: “Systemic”, “Ophthalmic”, “Peripheral Nervous System”, “Central Nervous System”, “Respiratory”, “Musculoskeletal”, “Endocrine”, “Urinary”, “Gynecological”, “Gastrointestinal”, “Cardiovascular”, Anxiety”, “Autoimmune”, “Chronic Fatigue”, “Cognitive”, “Headache”, “Immunologic”, “Joint Pain”, “Mental Health”, “Multiple Chemical Sensitivity”, “Neurodegenerative Diseases”, “Neurological”, “Neuropsychological”, “Rheumatologic”, “Shortness of Breath”, “Sleep”

Initial articles

(N=1396)

Reviewed by titles and abstracts

Removed Chinese Medicine Dampness Diagnosis references
Removed Tissue Engineering references
Removed Mould and Mold as authors
Removed Slime Mold references
Removed other unrelated references
Removed studies of other associations
Removed references for other definitions of mold

Remaining articles

(N=663)

Reviewed by titles and abstracts

Removed duplicate articles
Removed articles relating to allergic response
Removed articles relating to infections
Removed non-epidemiological studies

Remaining articles

(N=199)

Reviewed by abstracts and full text

Removed review articles
Removed other unrelated articles not previously identified
Removed systematic reviews and meta-analyses
Removed guidelines
Removed articles that assessed other outcomes

Final articles used

(N=114)

Figure 2. Methods for Article Selection

The criteria used for inclusion: study was

1. an original study
2. a cohort/longitudinal, cross-sectional, case/control or case series/history
3. reported on the relationship between mold or dampness with any adverse human health effect without regard to method for quantifying exposure
4. was published in peer reviewed literature between 2011 and November of 2018

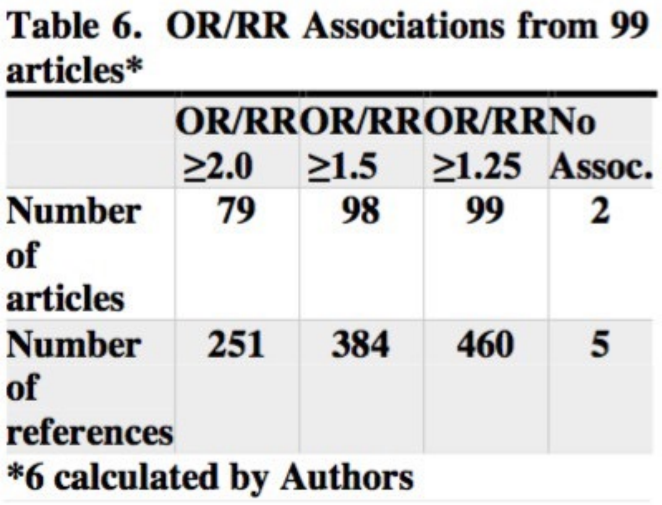


Table 6. OR/RR Associations from 99 articles*

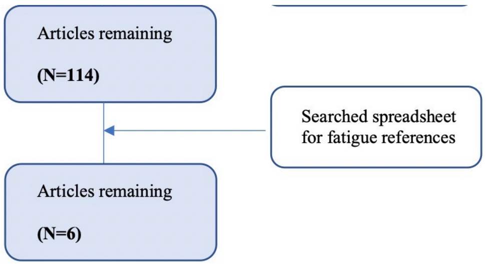
	OR/RR ≥2.0	OR/RR ≥1.5	OR/RR ≥1.25	No Assoc.
Number of articles	79	98	99	2
Number of references	251	384	460	5

*6 calculated by Authors

- The proposition that inhaled mold, mold fragments, toxins and inflammagens, or other components of the air in WDB, cause single and multi-system illness, is supported by **112 of 114 (98.2%)** epidemiological articles published between 2011 and 2018.

It should also be noted that one hundred (87.7%) of the entire 114 reports further supported previous works regarding respiratory symptoms along with 60 (52.6%) supporting immunological results. This was followed by 24 (21.1%) with reference to general symptoms. Sixteen (14.0%) studies supported cognitive decline. Varying numbers of articles supported neurologic, gastrointestinal, musculoskeletal, dermatologic, and other systems.

Searching and compiling the results from the previous data extraction worksheet for statistically significant references to fatigue


Articles remaining

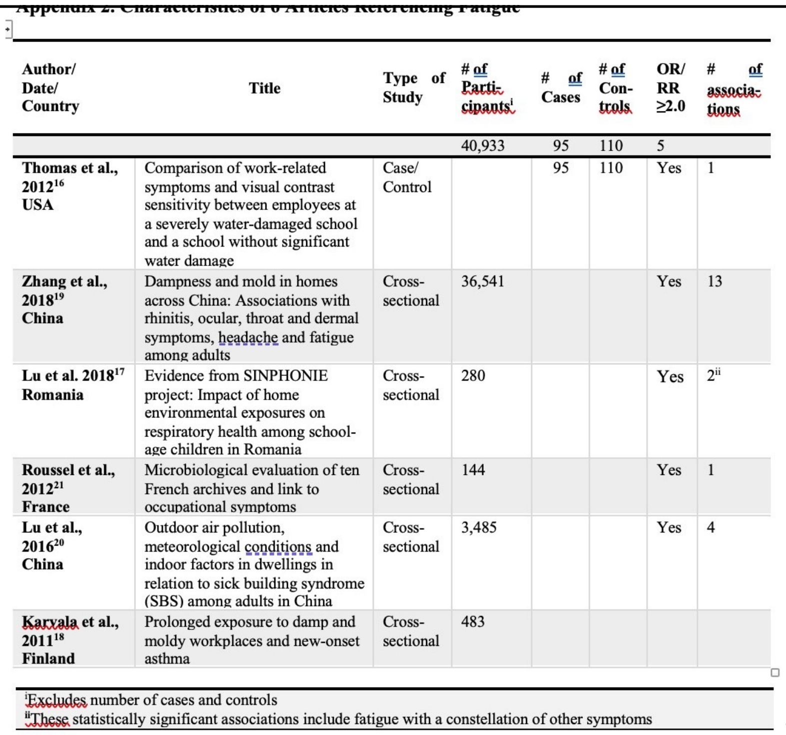
(N=114)

Searched spreadsheet for fatigue references

Articles remaining

(N=6)

Characteristics of Six Articles Referencing Fatigue



Appendix 2: Characteristics of 6 Articles Referencing Fatigue

Author/ Date/ Country	Title	Type of Study	# of Parti-cipants[i]	# of Cases	# of Con-trols	OR/ RR ≥2.0	# of associa-tions
			40,933	95	110	5	
Thomas et al., 2012[16] **USA**	Comparison of work-related symptoms and visual contrast sensitivity between employees at a severely water-damaged school and a school without significant water damage	Case/ Control		95	110	Yes	1
Zhang et al., 2018[19] **China**	Dampness and mold in homes across China: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue among adults	Cross-sectional	36,541			Yes	13
Lu et al. 2018[17] **Romania**	Evidence from SINPHONIE project: Impact of home environmental exposures on respiratory health among school-age children in Romania	Cross-sectional	280			Yes	2[ii]
Roussel et al., 2012[21] **France**	Microbiological evaluation of ten French archives and link to occupational symptoms	Cross-sectional	144			Yes	1
Lu et al., 2016[20] **China**	Outdoor air pollution, meteorological conditions and indoor factors in dwellings in relation to sick building syndrome (SBS) among adults in China	Cross-sectional	3,485			Yes	4
Karvala et al., 2011[18] **Finland**	Prolonged exposure to damp and moldy workplaces and new-onset asthma	Cross-sectional	483				

[i] Excludes number of cases and controls

[ii] These statistically significant associations include fatigue with a constellation of other symptoms

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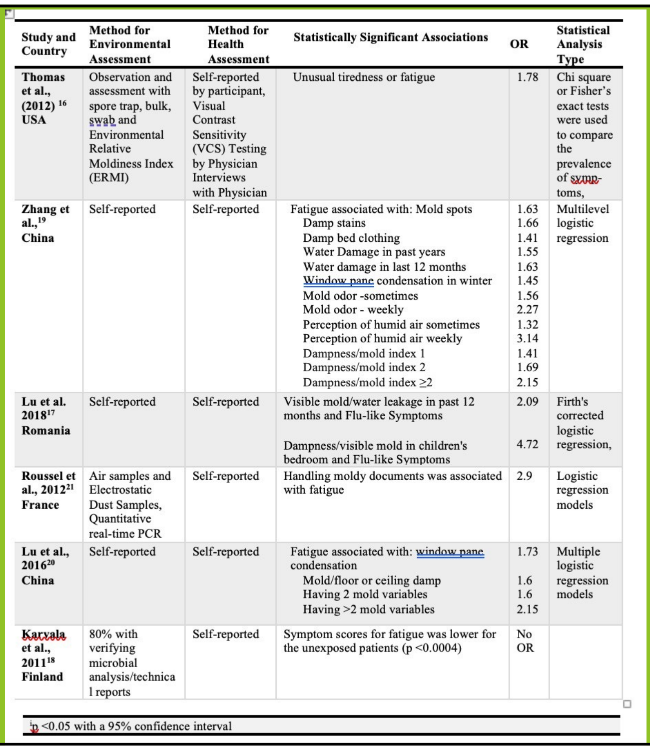
The studies were ranked according to the following parameters:

- *statistically significant results for direct associations with fatigue;*
  1 point
- *statistically significant OR ≥ 2.0;*
  1 point
- *>1 statistically significant reference for fatigue; 1 point*
- *physician assessment of participants;*
  2 points
- *environmental assessment by testing;*
  2 points
- *number of participants >1000; 1 point*

Assessment Methods and Statistically Significant Associations



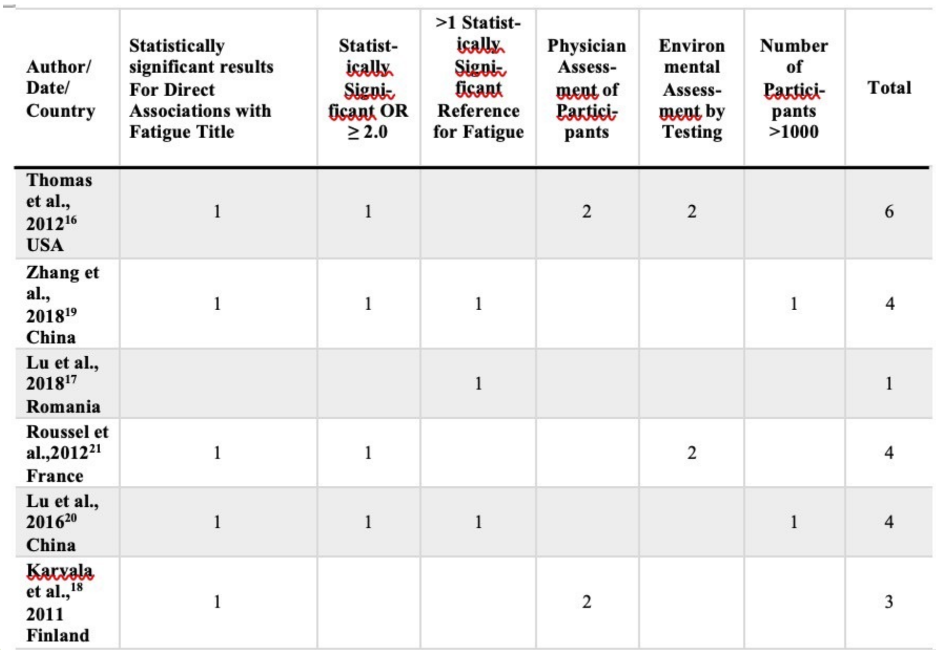
Study and Country	Method for Environmental Assessment	Method for Health Assessment	Statistically Significant Associations	OR	Statistical Analysis Type
Thomas et al., (2012) [16] USA	Observation and assessment with spore trap, bulk, swab and Environmental Relative Moldiness Index (ERMI)	Self-reported by participant, Visual Contrast Sensitivity (VCS) Testing by Physician Interviews with Physician	Unusual tiredness or fatigue	1.78	Chi square or Fisher's exact tests were used to compare the prevalence of symp-toms,
Zhang et al.,[19] China	Self-reported	Self-reported	Fatigue associated with: Mold spots Damp stains Damp bed clothing Water Damage in past years Water damage in last 12 months Window pane condensation in winter Mold odor -sometimes Mold odor - weekly Perception of humid air sometimes Perception of humid air weekly Dampness/mold index 1 Dampness/mold index 2 Dampness/mold index ≥2	1.63 1.66 1.41 1.55 1.63 1.45 1.56 2.27 1.32 3.14 1.41 1.69 2.15	Multilevel logistic regression
Lu et al. 2018[17] Romania	Self-reported	Self-reported	Visible mold/water leakage in past 12 months and Flu-like Symptoms Dampness/visible mold in children's bedroom and Flu-like Symptoms	2.09 4.72	Firth's corrected logistic regression,
Roussel et al., 2012[21] France	Air samples and Electrostatic Dust Samples, Quantitative real-time PCR	Self-reported	Handling moldy documents was associated with fatigue	2.9	Logistic regression models
Lu et al., 2016[20] China	Self-reported	Self-reported	Fatigue associated with: window pane condensation Mold/floor or ceiling damp Having 2 mold variables Having >2 mold variables	1.73 1.6 1.6 2.15	Multiple logistic regression models
Karvala et al., 2011[18] Finland	80% with verifying microbial analysis/technical reports	Self-reported	Symptom scores for fatigue was lower for the unexposed patients ($p < 0.0004$)	No OR	

[i]p <0.05 with a 95% confidence interval

Levels of Support by Total Ranking Points

Total Ranking Points	Level of Support
1	Very Low Support
2	Low Support
3	Moderate Support
4	Moderately High Support
5	High Support
6	Very High Support

Ranking Points Assigned



Author/ Date/ Country	Statistically significant results For Direct Associations with Fatigue Title	Statist-ically Signi-ficant OR ≥ 2.0	>1 Statist-ically Signi-ficant Reference for Fatigue	Physician Assess-ment of Partici-pants	Environ mental Assess-ment by Testing	Number of Partici-pants >1000	Total
Thomas et al., 2012[16] USA	1	1		2	2		6
Zhang et al., 2018[19] China	1	1	1			1	4
Lu et al., 2018[17] Romania			1				1
Roussel et al.,2012[21] France	1	1			2		4
Lu et al., 2016[20] China	1	1	1			1	4
Karvala et al.,[18] 2011 Finland	1			2			3

Number of Studies by Level of Support

Support Ranking	Number of Studies
Reference Only	2
Very Low Support	1
Low Support	0
Moderate Support	0
Moderate Support	1
Moderately High Support	3
Very High Support	1

Studies by Support Rating

Support Ranking	Study
Reference Only	Tuuminen and Rinne Brewer, et al.
Very Low Support	Lu et al. from Romania
Low Support	0
Moderate Support	0
Moderate Support	Karvala et al.
Moderately High Support	Zhang et al. Lu et al. from China Roussel et al.
Very High Support	Thomas et al.

Peer-reviewed publications document that approximately 25% of the population have a genetic susceptibility for developing CIRS, an estimated 50% of the buildings in the U.S. are water-damaged, and the prevalence of CIRS is conservatively calculated at ≥ 7.01% in children and likely higher in adults due to the progressive nature of CIRS.

- Based on these figures, a conservative calculation of the rate of exposure of predisposed persons in the US is 52.1 million persons. This can be contrasted with the 2017 CDC figures of 30.3 million people with diagnosed and undiagnosed diabetes in the United States.

FUNDING

THIS STUDY WAS COMPLETELY FUNDED BY HOLISTIC RESONANCE CENTER

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frontiers in Immunology

Case RepoRt
published: 03 April 2017
doi: 10.3389/fimmu.2017.00382

severe sequelae to Mold-Related Illness as Demonstrated in two Finnish Cohorts

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The presence of toxic indoor molds with accompanying bacterial growth is clearly detrimental to human health. The pathophysiological and toxicological effects of toxins and structural components of molds and bacteria have been clarified in experiments conducted in tissue culture and animals, and there is convincing epidemiologic evidence; nonetheless their implications for human health are either ignored or denied, at least in Finland. In this communication, we describe two cohorts suffering severe sequelae to mold-related illness. One cohort is a nine-member family with pets that moved into a new house, which soon proved to be infested with pathogenic molds. The other cohort consists of 30 teachers and 50 students from a mold-infested school building. The first cohort experienced a plethora of mucosal irritation, neurological, skin, allergic, and other symptoms, with all family members ultimately developing a multiple chemical syndrome. In the second cohort, we detected a greatly elevated prevalence of autoimmune conditions and malignancies. We claim that mold-related illness exists in multiple facets; if not simply a transient mucosal irritation or even an increased risk of asthma onset or its exacerbation. We propose a scheme to explain the natural course of the mold-related illness. We recommend that future studies should combine data from, e.g., cancer, autoimmune, and endocrine disorder registers and neurological and mental health or neuropsychological registers with mold-exposed individuals being monitored for prolonged follow-up times.

Keywords: sick building syndrome, autoimmune conditions, malignancies, multiple chemical syndrome, environmental molds, hypothyroidism, indoor air, mold-related illness

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Specialty section:
This article was submitted to Microbial Immunology, a section of the journal Frontiers in Immunology

Received: *11 January 2017*
Accepted: *17 March 2017*
Published: *03 April 2017*

Citation:
Tuuminen T and Rinne KS (2017) Severe Sequelae to Mold-Related Illness as Demonstrated in Two Finnish Cohorts. Front. Immunol. 8:382. doi: 10.3389/fimmu.2017.00382

BaCKGRoUND

The recent publication of the Audit Committee of the Finnish Parliament (1) indicated that approximately 7–9% of terraced houses; 6–9% of apartment buildings; 12–18% of schools and kindergartens; 20–26% of nursing homes, hospitals, and outpatient departments; and 2.5–5% of offices have been significantly damaged with dampness and are infested with indoor molds. It has been estimated that approximately 800,000 or every seventh Finnish citizen (1) has been exposed to some extent and become sensitized to compounds present in poor quality indoor air. However, since there is no ICD-10 coding system for mold-related illness, its exact incidence is unknown. If one extrapolates from the above presented figures (1), one could argue that the incidence of mold-related illness may be much higher than the incidences for cardiovascular conditions, cancers, and accident-induced traumas. Despite (or perhaps due to its ubiquitousness and its all-too-frequent involvement in highly publicized litigation issues) there is no consensus by the medical authorities on how this disease should be recognized. Marginalization of patients (2) with this disorder results inevitably in serious social welfare problems. The very recently (11/2016) issued, and in our opinion totally biased, Current Care Recommendations for treating patients suffering from moisture-damaged buildings (3) only aggravate this injustice. These "recommendations" are inconsistent with the constitutional rights guaranteeing to all Finnish citizens that *"Anyone who cannot obtain decent livelihood has a right to receive appropriate subsistence and care"* (4). The official rhetoric of denial of the mold-related illness (5–7) can be summarized into three main points: (1) asthma is the only clear disease that can be associated with moisture-damaged buildings; (2) there is not sufficient evidence that dampness and mold overgrowth are associated with adverse health conditions; and (3) the mechanisms causing dampness-related illness are still unknown.

In this publication, we describe two Finnish cohorts; our goal is to raise awareness that indoor dampness associated with toxic molds and bacteria overgrowth can cause a plethora of serious sequelae including neurological, autoimmune diseases, e.g., hypothyroidism as well as cancer and multiple chemical syndrome (MCS), and even higher mortality.

The term multiple chemical syndrome has been defined (8–11); it is already accepted as a distinct clinical entity in several EU countries. The criteria for MCS definition have been set (8, 9) as (1) the condition is chronic; (2) with symptoms recur reproducibly; (3) in multiple organ systems; (4) in response to low levels of exposure; (5) to multiple unrelated chemicals and which; (6) improve or are resolved when incitants are removed. Although mold-related illness has not been associated with the development of MCS, we will convincingly demonstrate that MCS illness can indeed be a consequence of mold-related disease when the exposure to toxic molds has been prolonged and the symptoms have become chronic.

There have been suspicions that mold-exposed individuals experience a higher prevalence of hypothyroidism, and therefore we started to collect evidence to investigate this association. We reviewed the medical records of the personnel in one school, which had been identified as a mold-infected building. We will present novel data that the presence of toxic molds in a building may indeed associate with a higher prevalence of hypothyroidism in its users/inhabitants than in the general population. This prevalence was calculated using statistics of thyroid hormone substitution therapy in 2015 provided by the Social Insurance Institute of Finland, KELA. We document also that mold-related disease can include malignancies. The incidences of breast cancer and lymphomas were calculated in the personnel from the school and compared to the register data detailing the corresponding incidence of the region (9). The overall prevalence of autoimmune disease was compared to our best estimate of the corresponding value in the general Finnish population.

The evidence that there is an association for the development of malignancies in individuals chronically exposed to toxic molds is an even more challenging task because this requires a lengthy follow-up and access to several cancer registers. In this report, we present data that should raise concern about the potential carcinogenic properties of mycotoxins and other toxic products present in moldy buildings.

ethics statement

This research is IRB exempt because it is a retrospective chart review study. Informed written consent was obtained from the mother (Cohort 1) and all the patients (Cohort 2) whose medical records were reviewed.

Cohort 1

The evidence that toxic indoor molds can cause chronic respiratory symptoms, cognitive disorders, neurological symptoms such as insomnia and migraine, failure to thrive in a newborn, and MCS in occupants of a moisture-damaged house was collected by careful inspection of medical histories and interviews of the affected individuals.

A family of nine with seven children, three cats, and two dogs moved into a brand new house in November 2011. The seventh child was born in this problem house in November 2013. None of the family members had required any long-term medication prior to November 2011. During their infancy, two of the family's daughters had suffered from milk or soy allergy, but these symptoms declined as the girls grew older such that before their move into the new house, they were asymptomatic. The mother had been sensitized to molds in her workplaces during the years 2006–2009 and 2010–2011. At that time, she experienced nose and ear itchiness, which later changed to ear infections and respiratory symptoms, but by November 2011 she was asymptomatic because she changed her place of employment. Soon after moving into their new house, the parents smelled a strong odor of sewage, which the building contractors attributed to inadequate ventilation. The contractors made several attempts to correct the defect in the sewerage; however, the odor remained.

Approximately 1 month later, all members of the family were experiencing many symptoms, e.g., intense mucosal irritation of the eyes, coughing, pain in the throat region, throat infections, shortness of breath, sinus infections, congestion, etc. These symptoms usually appeared whenever they were present in the house. These mucous membrane irritations led to a cycle of infections, which resulted in many medical consultations. Soon afterward, the symptoms experienced by the family members also developed in various organs (**Figure 1**). Many had headaches, all of them experienced some kind of skin symptoms, 6/9 had functional abdominal symptoms, at least 3/9 had either fever or low body temperature, and at least 4/9 suffered muscle and joint pains. Four of the children developed asthma, which required medical treatment. With time, seven members had developed food and pollen allergies. It is significant that the mother's aunt, who stayed in the house between October and November 2013, suffered a migraine attack sufficiently severe to require hospital care. She had also experienced severe cough during her stay in the house.

Even the family's pets became unwell; the dogs had throat infections, cats had "flu" and eye infections, one of the cats displayed asthma-like symptoms, especially in spring and autumn 2011–2013 (in 2013, the pets were given away).

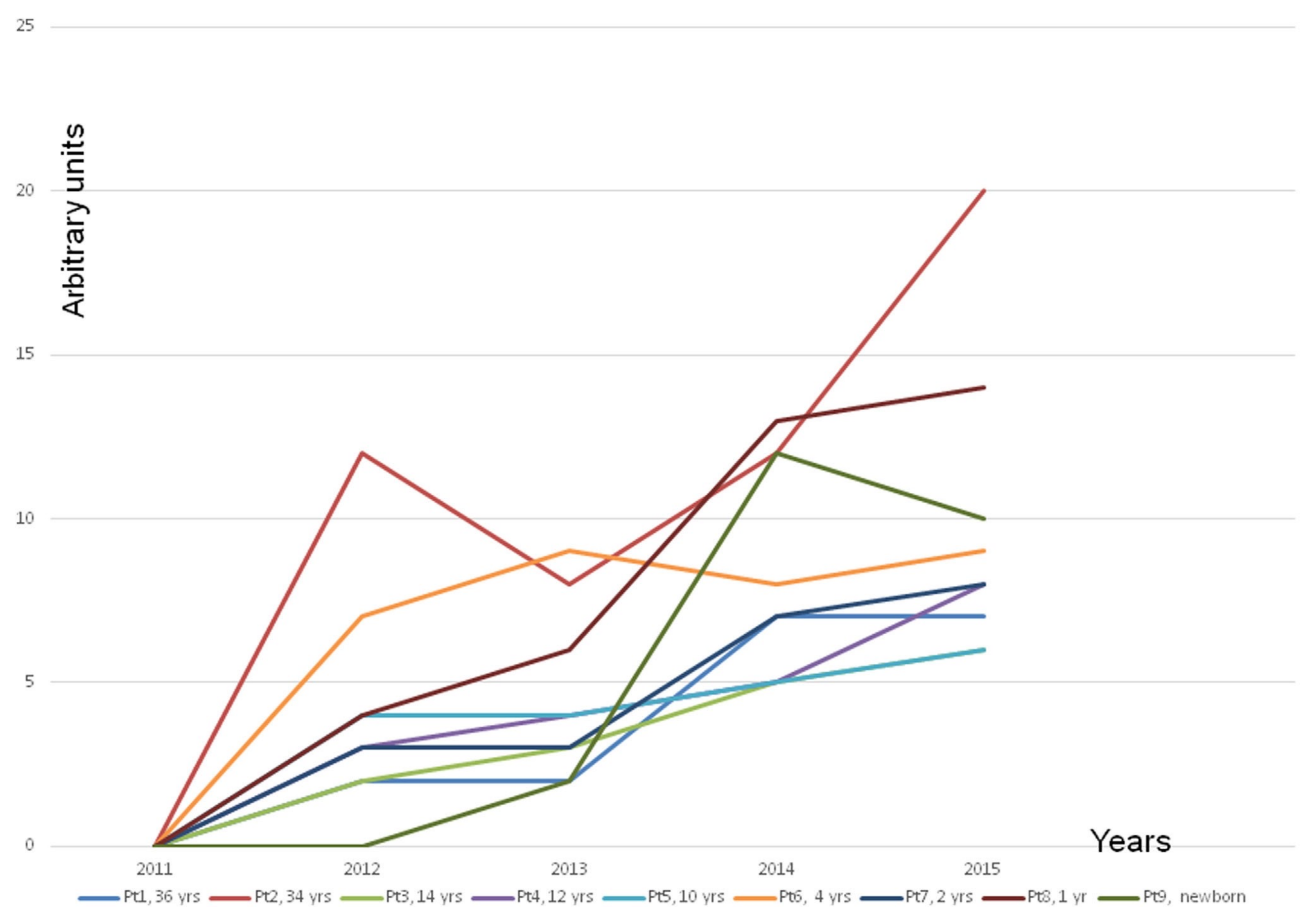

FIGURe 1 | the frequency of symptoms other than infections. The other symptoms were insomnia, migraine, motor and sensory peripheral neuropathy, headache, tremor, twitching, tic of the body and in the eyes; tiredness, exhaustion; increased blood pressure, increased heart rate; muscle pain, joint pain; numbness in the hands and feet; weakness; feeling thirsty; feeling cold, shivers; balance problems; muscle weakness; slightly cyanotic limbs; irritability, melancholy, nervousness, memory disorders; facial flushing, skin rash, full-body rash, urticaria of the whole body; intestinal disorders, e.g., diarrhea or constipation; increased allergic reactions; excessive sweating or no sweat at all; crusted skin behind the ears or in the scalp of children; increased secretion of ear wax, jamming in matters in children, restlessness, difficulty falling asleep; retardation in growth; gastrointestinal reflux; too high or too low acidity of the stomach; weight loss without deliberate dieting; swelling of the face and the abdomen region; increased frequency of urination; nightly horror scenes in children; unexplained fever spikes; pallor, dark under the eyes; prolonged jaundice in a newborn; hypothermia; bedwetting in a child who was for many years dry at nights; child's rage; jaundice of skin; night waking in children; significant hair thinning; dermatitis of the face skin and acne-like symptoms also in adults; unexplained vomiting in children; easy bruising; hypersensitivity to noise in children; dark urine in children although well hydrated.

The family's seventh child was born in the problem house and suffered from a prolonged bout of jaundice (3 months), his excrements were strangely dark, and had a horrible smell instead of the usual milky feces. He failed to thrive during the first few months.

The next dramatic turn experienced by all family members was the development of MCS or MCS illness as it is alternatively described. The severity of the disease was variable (**Figure 2**). In Finland, there is no recognition of the MCS, only the national R68.81 code, which according to official policy is nihilistically called "a feature," not a disease. It is noteworthy that the children even reacted to new toys, which are clearly at odds with the officially propagated explanation that MCS illness is a conditional reflex (12).

In 2015, the presence of *Penicillium* and *Aspergillus* was confirmed. For the latter species, the cell equivalent (CE, which is defined as any particle of mold or spore containing DNA) value was 13,000 CE/g, compared to the maximum permitted cutoff value of 2,400 CE/g. Furthermore, various bacterial species were also detected from the material taken from internal surfaces in the majority of the living space in quantities greatly exceeding the reference values. The family moved to a rented apartment in February 2016, and gradually their symptoms have started to resolve.

Cohort 2

Our evidence of a cluster of very rare diseases, a high incidence of oncologic diseases, and autoimmune conditions associated with a moisture-damaged school has been obtained through a lengthy, personally conducted follow-up of all the patients described below.

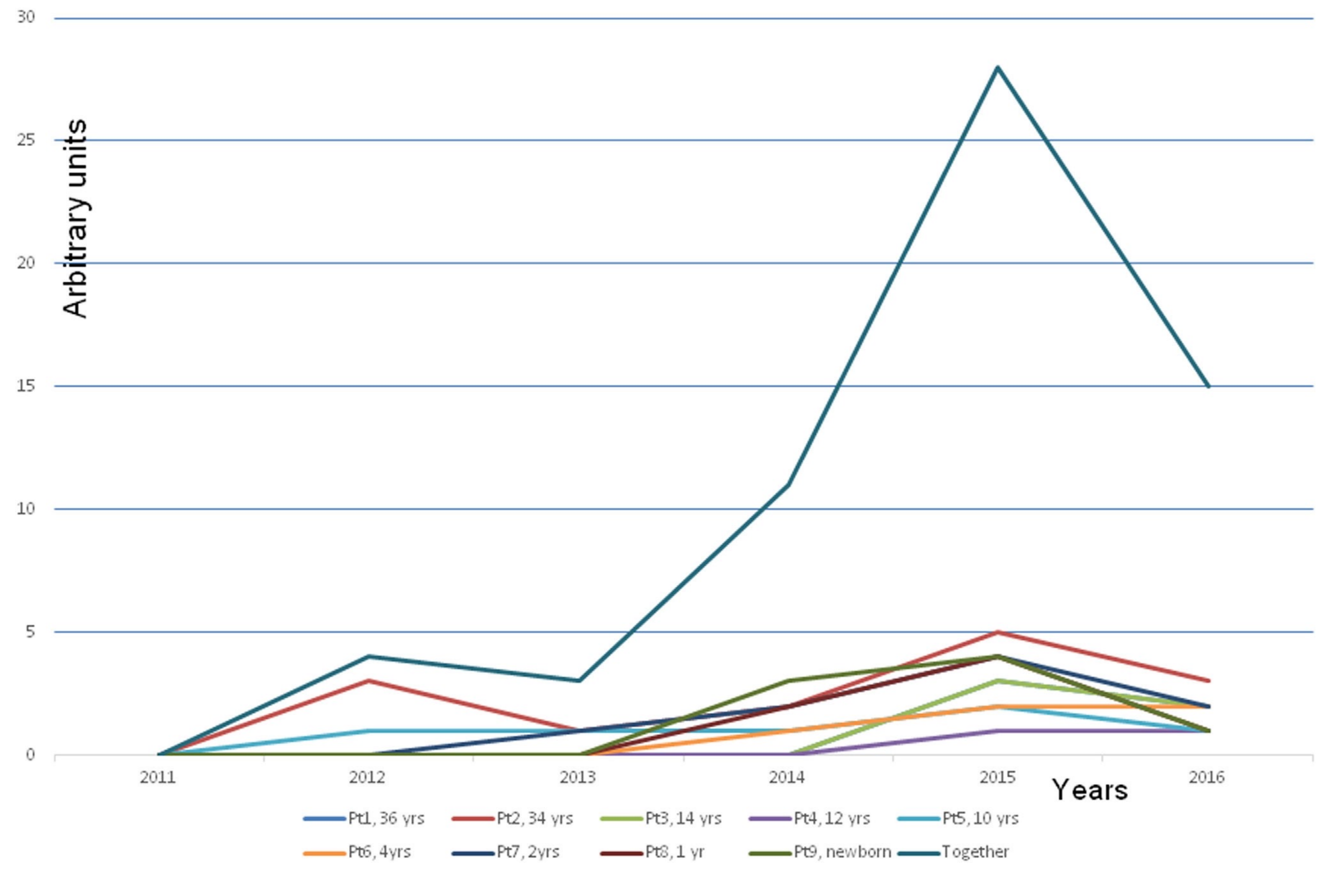

FIGURe 2 | Development of a multiple chemical syndrome (MCs illness) in every family member. Note that the symptoms developed gradually along with increased time of staying in the problem house. The presentation of the MCS illness was estimated by an arbitrary score. The blue line is the cumulative score of all the members. *The explanation of the arbitrary score*: +, hypersensitivity to strong detergents, softeners, and perfumed hygiene products. A child reacts to the odor by avoiding the perfume or the smell of a person or an object. ++, a clear hypersensitivity to detergents and perfumed products. A child avoids entering several departments in a department store with a strong smell burden (e.g., bags, shoes, textiles, toys, and detergents) and avoids contacts with people who have strong smell from their clothing, etc. +++, a clear hypersensitivity to perfumes and flagrancies, the same as above but in addition hypersensitivity to gasoline, exhaust, and windshield washing fluid, new fabrics, and new furniture. ++++, reacts strongly by a production of mucous excreta and itching feeling on the mucosa. The symptoms as above but in addition the hypersensitivity to adhesive surfaces, printing ink, plastic, rubber, markers, ballpoint pens, new toys, books, games, and textiles. Symptoms appear also in various dusty environment, e.g., to road dust. +++++, as above but in addition the hypersensitivity to perfume-free detergents and even to natural scents of flowers, trees, grass, soil, etc.

A wooden school was built in a small city in the late 1880s. Over the years, this building has undergone several reconstructions. During the last 20 years, there have been approximately 30 occupants working in this building for variable periods of time (3–20 years; min–max). Medical records collection at the school was supervised by a medical doctor employed at the school since 1985. The records reveal an astonishingly high incidence of severe morbidity and a high mortality rate associated with even a short period of working or studying in this building (median 12 years). All of the available demographic and clinical data collated from the medical records are presented in **Table 1**.

In addition to individuals suffering from serious symptoms (**Table 1**), many other employees suffered from chronic eye and ear irritation, sinusitis, bronchitis, fever, skin problems, fatigue, and joint pains, and some experienced exacerbation of some underlying disease. Some occupants, whose exposure to the school's poor indoor air was short, became asymptomatic when they moved to another school that did not seem to have any indoor air problems. For those whose symptoms and illnesses became chronic, the median exposure time was approximately 12 years. The majority of students in the school experienced "flu-like" symptoms and fatigue.

Table 1 reveals extremely alarming statistics: 2 out of 30 occupants developed very rare autoimmune diseases: 1 teacher suffered from inclusion body myositis (the average incidence in Finland is 1:1,000,000), and the other suffered neurosarcoidosis (the average incidence in Finland is 1:500,000). Altogether, more than every third building occupant (11/30 = 36.6%) experienced different types of autoimmune conditions. Depending on how one calculates the value, the average prevalence of autoimmune diseases in Finland is in a range of 5–8%, i.e., the observed prevalence is at least fourfold higher than the average. Hypothyroidism or goiter was diagnosed in every fifth occupant (6/30 = 20%), whereas in Finland, the average calculated prevalence is known

TABLE 1 | The morbidities and mortalities diagnosed in a personnel working in the problem school (Cohort 2).

Person	Years of exposure	Gender	Profession	Morbidities	Employment status
A	25–30	Male	School rector	Inclusion body myositis Exacerbation of allergy Irritation of mucosa Voice problems	Retired due to age
B	20	Female	Instructor	Asthma Sjögren syndrome Thyroiditis Dystonia Migraine	Partly employed
C	12	Male	Instructor	Vasculitis with purpura	Working
D	13	Female	Instructor	Goiter with hypothyroidism Diabetes Sleep apnea Skin symptoms	Working
E	5	Female	Instructor	Chronic flu-like illness Cough Sneezing Voice problems Eye irritation that led to iritis	Working half time
F	20	Female	Special teacher	Breast cancer	Retired due to age
G	12	Female	Teacher	Asthma exacerbation Chronic sinusitis Chronic otitis Allergy Eosinophilia Hypothyroidism Nasal polyps (st post polypectomy) Difficulties to concentrate Memory problems Chronic fatigue Depression Altogether, history of sickness that lasted for 35 years	Retired due to disability
H	20	Female	Special teacher	Breast cancer	Working
I	5	Female	Instructor	Chronic sinusitis Allergic rhinitis Partial hearing loss	Working
J	10	Female	Special teacher	Neurosarcoidosis Hypothyroidism (severe) Intestinal stoma	To be retired due to disability
K	3	Female	Instructor	Chronic sinusitis Chronic otitis Chronic eye irritation Joint pains	Working
L	20	Female	Instructor	Asthma Allergy Breast cancer	Retired due to age
M	5	Female	Special teacher	Underlying disease: Cilium dysfunction The following symptoms exacerbated: Chronic sinusitis Bronchiectasias Chronic otitis Pneumonia Joint pains Fybromyalgy	Retired due to disability

(*Continued*)

TABLE 1 | Continued

Person	Years of exposure	Gender	Profession	Morbidities	Employment status
				Asthma Hypothyroidism In the beginning during the holiday period, the symptoms relieved, but later they became chronic, and now her morbidity is severe	
N	8	Female	Instructor	Non-smoker, previously healthy Chronic rhinitis Flu-like symptoms Chronic bronchitis After the prolonged period of flu illness died from sepsis	Dead
O	10	Female	Special teacher	Lung cancer (non-smoker)	Dead
P	10	Female	Instructor	Asthma Chronic sinusitis Multiple chemical syndrome illness Severe sleep apnea Memory problems At the time of this communication her thyroid function and neurological disorders are being investigated High sensitivity to poor indoor air	Partly employed
R	10	Female	Instructor	Chronic sinusitis Psoriasis-like skin problems Eye irritation Chronic otitis Voice problems Memory problems Problems to concentrate Chronic fatigue that led to depression	Working
S	4	Female	Special teacher	Goiter	Retired due to age

To make the assessment of the disease category more illustrative we use colors: blue for autoimmune diseases; violet for oncology; red for nervous disorder; brown for asthma, and green for upper and lower respiratory symptoms, eye and skin irritation.

to be 5.78%. Therefore, in our cohort the prevalence of thyroid dysfunction was elevated by 3.4-fold.

The incidence of oncologic diseases was also greatly elevated; lymphoma was diagnosed in 2 out of 50 students. One student had attended the school for 1.5 years and the other for 4.5 years. Therefore, the calculated incidence is 666:100,000 (which is not an exact estimate due to the small size of the cohort). From the register of lymphomas for the same region, we estimate an average incidence of 14:100,000. Therefore, in our small cohort, the incidence was 47.5-fold higher. We documented 3 breast cancers among 25 female teachers who worked in the school during the 20-year observation period. The calculated incidence is therefore 600:100,000, whereas the average incidence for the region is 101.5:100,000. Thus, the incidence of breast cancer in our cohort was elevated by approximately sixfold. In addition, we documented also an extremely high mortality rate among what should be a potentially healthy population (i.e., schoolchildren and teachers) during the period 1980–2011: one student died from pneumonia; two students died from lymphoma; one non-smoker teacher died from lung cancer; and one young and previously healthy female instructor succumbed to sepsis, which is very rare in Finland.

From **Table 1**, we can also see that 2 Teachers retired prematurely since they had been diagnosed with a work disability; 2 teachers were forced to work only part time because of their disability. In general, the employees of the school were frequently absent from work due to sickness. High employee morbidity is expensive, i.e., each day of sick leave costs approximately 250–300€.

Microbiological investigations performed in 2011 revealed an excessive growth of *Paecilomyces*, *Exophiala*, *Penicillium*, *Aspergillus peniccilloides/restrictus, Aspergillus fumigates, Tritira chium,* and Paecilomces species (all above the cutoff values of 96–194 cfu/g) in mineral wool and other types of insulation material. After appraisal of these microbiological data, this school was closed, but it was reopened again as a primary school without adequate consultations with the community. Subsequently, it was closed again according to our (KR) recommendations.

THE BUILDINGS

In both cohorts, the evidence that the microbiota was related to moisture in the building was substantiated by indoor air studies and validated culture techniques performed by certified environmental experts.

DISCUSSION

Here, we present convincing evidence that toxic indoor molds can indeed cause not only chronic respiratory symptoms or irritation of mucous membranes but also cognitive and neurological disorders such as insomnia, migraine, motor, and sensory peripheral neuropathy; failure to thrive in a newborn baby. Furthermore, all occupants of the moisture-damaged house suffered from MCS (Cohort 1). We claim that toxic molds can cause severe morbidity and mortality in adults and children, even domestic pets; and a relatively short stay in a damaged building is a potential hazard to health and life. We also present evidence of a cluster of very rare autoimmune and oncologic diseases associated with a moisture-damaged school. For example, in Cohort 2 we documented that 36.6% of the teachers suffered from autoimmune condition, whereas in the general population the prevalence is much lower—approximately 5–8%—depending on which source is used in the calculation. The incidence of cancer was also alarmingly high. These diseases can be hardly overrepresented by chance. Although some may be skeptical of our data, we can confirm that both of the buildings were mold infested as proven by certified professional bodies, and that the clinical data were collected by careful evaluation of the medical records.

As far as we are aware, this is the first time that MCS illness has been demonstrated to occur after prolonged exposure to indoor molds when the disease turns chronic. This conclusion is justified when we consider the symptoms experienced by all the occupants investigated in Cohort 1 (**Figure 2**). Even very small children whose reactions cannot possibly be attributed to conditioning or an exaggerated fear of putative environmental threats (i.e., young children are always eager to play with new toys and are not old enough to understand the concept of an environmental threat) developed nausea, headaches, and respiratory symptoms when they were confronted with strong perfumes or detergents from new toys, textiles, and furniture. Unlike the situation in some other EU countries, regrettably in Finland MCS illness has not yet gained any official recognition. Instead, individuals suffering from this disorder are referred to psychiatrists, their symptoms labeled as psychosomatic, they themselves may be considered psychotic. All of the cases from Cohort 1 were not in need of psychiatric help; they had truly developed a MCS illness related to indoor molds (13).

Mold-related illness should not be viewed as a so-called medically unexplained syndrome, as has been claimed (14, 15). In our opinion, providing these patients with cognitive or behavioral therapy (14–16) is medically unethical—it represents a denial that mold-exposed individuals are suffering from a somatic illness. Moreover, cognitive/behavioral therapy is not effective (14). We can assume that providing the mold-exposed patient with only psychotherapy (14, 15) in combination with high dosages of corticosteroids while he/she continues to live or work in a hazardous environment is inappropriate "medication"; in fact, it will aggravate their risks of suffering severe morbidity and even dying. On the basis of the present data, we think that it is irresponsible to claim that indoor molds cause only transient irritation symptoms and pose only a 1.5-fold risk for the development of asthma (3, 5–7). Even though more and more knowledge is available on the mechanisms underpinning the health hazards associated with moldy environments, mold-related disease is still called a "non-disease," or "somatoform disorder," with some physicians trying to label it as a "fashionable" disorder, or stating that its sufferers are exhibiting hysteria (17). Mold-related illness is a somatic disorder; the symptoms are physical, not psychosocial problems, although this has been claimed for almost 20 years (17). In most cases, later it can become a psychosocial problem as patients suffer mental distress from their failure to convince physicians that they are ill. Our data show that occupying an infested building for even 2–3 years (either a home or a school) can seriously impair the well-being of potentially healthy individuals, even to the extent of loss of life. Therefore, any attempt by governmental/medical authorities to deny the serious effects of toxic molds on human health should be combatted.

Mold toxins may impair the immune system or other organs in many ways (18–29). They can either cause an overwhelming immune activation (autoimmunity) or an immune deficiency (e.g., leading to an inability to combat clones of malignant cells) making the individual liable to suffer infections that may be fatal, such as in our sepsis-induced death of a young, previously healthy, female instructor. Mold toxins and structural components of bacteria and fungi present in moisture-damaged buildings can exert synergic pro-inflammatory interactions (20) and trigger cellular autophagocytosis (25). Some peptide toxins from moisture-damaged surfaces may trigger immunotoxic and exert growth inhibitory effects in mammalian cells (19–21, 23). The anti-immune strategies mounted by pathogenic fungi including species also detected in moisture-damaged buildings have been described in a recent comprehensive review (24). The fungi have developed many complex strategies to evade attack by the host's immune systems. One example of this complexity is the fact that some components of the molds and bacteria can activate a structure called the inflammasome (i.e., a pro-inflammatory action), whereas other components may hamper immune cell activation or even destroy immune competent cells, such as NK or T lymphocytes (21). It has been shown that *Aspergillus* species can inhibit the function of dendritic cells (25); these are crucial cells in immune defense as they first recognize and then present foreign molecules to the host's secondary immune defense system. This is simply one of the many mechanisms of action of mold toxins; it is not the aim of the present communication to systematically review all the possible pathophysiological pathways. Instead, our goal is to highlight the evidence that toxic molds can be responsible for serious morbidity, even mortality. One characteristic feature of the mold-related illness is severe fatigue; this has been attributed to a toxicosis resulting from mitochondrial deprivation and low energy production (21).

The very high prevalence of hypothyroidism in mold-related illness is evident when reviewing the data from Cohort 2. The thyroid, the pancreas, and the heart are organs that require high energy production in order to function properly (28). Mycotoxins are cytotoxic and disrupt mitochondrial enzymatic functions, depriving tissues of energy (21). Thus, it is not illogical to argue that mold toxins would impair the metabolic activity of the thyroid gland leading to hypothyroidism.

A worrying signal emerges from the data from Cohort 2, i.e., a building with toxic mold overgrowth can cause a higher prevalence of malignancies. We appreciate that actually proving this association is a very challenging task. Nonetheless, our report has revealed a cluster of malignancies with a much higher prevalence than the prevalence of these malignancies in the

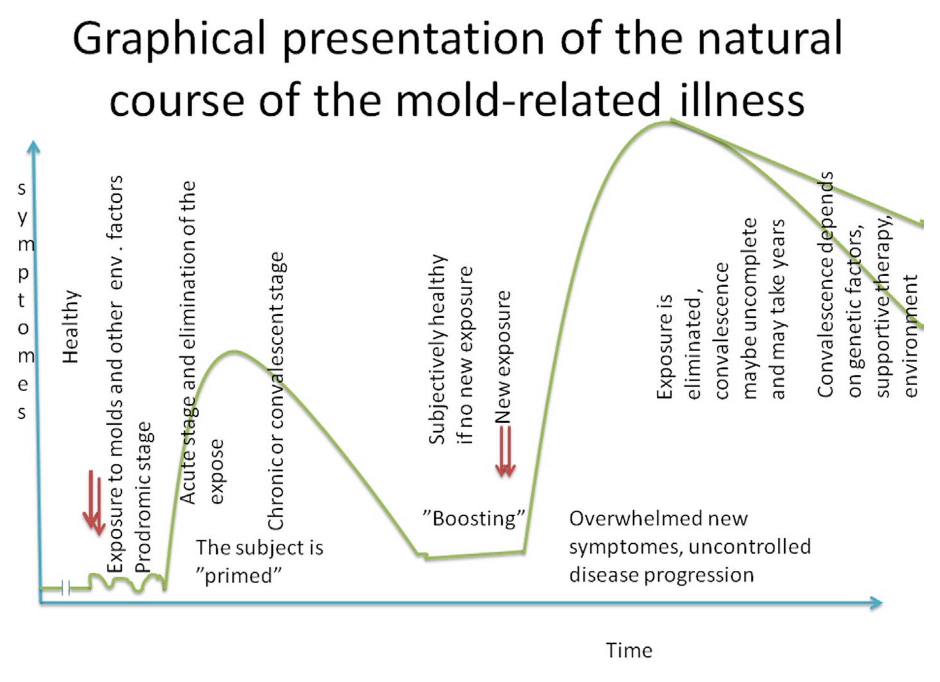

FIGURE 3 | Graphical presentation of the natural course of the mold-related illness.

general population. This association should be taken seriously. For example, although nowadays no one doubts the link between tobacco smoking and lung cancer, it should be recalled that it took several decades before this association was accepted by the medical profession. The recognition of the asbestos hazards also took a long time. Today, we should be concerned about the possible carcinogenic effects of indoor mold toxins and structural components hosting toxic microbes, especially compounds derived from materials pretreated with putative anti-mold substances (29). Cytotoxic and modulatory effects of mycotoxins on human breast cells have been recently documented (27).

The limitation of our study is that we present descriptive evidence based on the observation of only two cohorts. The strength of our publication is its novelty; we hope that it will encourage a change in attitudes toward patients with mold-related disorders. We also hope that it may initiate extensive, well-designed studies combining lengthy follow-up with data extracted from various disease registers.

On the basis of our experience, we will present an empiric graphical reconstruction of the natural course of the mold-related illness (**Figure 3**). We believe that once a person has become sensitized to indoor molds, he/she will be sensitized for the rest of their life span. However, a subjectively asymptomatic healthy state can be achieved but that requires the avoidance of indoor molds, an appropriately controlled detoxification strategy, and a restoration of immune homeostasis with well-designed dietary interventions and other supportive therapies (30). If the patient becomes exposed again to mold-infested indoor air impurities, his/her symptoms may reappear almost immediately. The defense symptoms can be overwhelmed, and the disease may manifest itself with new symptoms, such as autoimmune diseases, neurologic disorders, etc. (**Figure 3**). A full recovery after the first encounter with mold-related toxins may take some time, but if these encounters continue for a prolonged period of time, full recovery may be unlikely; the disease can become chronic and spread to new organs.

CONCLUDING REMARKS

In conclusion, we present clinical evidence that poor indoor air due to mold infestation can cause severe morbidity not restricted to asthma. These sequelae are oncological (31), neurological, autoimmune diseases, and even death. We emphasize that scientific discussion based on facts should be pursued without intervention from biased "opinion leaders." What is not yet known should be studied with an open mind. In conclusion, the absence of the evidence should not be construed as evidence of absence.

AUTHOR CONTRIBUTIONS

TT and KR collected data and wrote the article together.

ACKNOWLEDGMENTS

The authors thank the mother from the Cohort 1 family and the teacher from Cohort 2 for helping them collecting the data.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Toxins **2014**, *6*, 66-80; doi:10.3390/toxins6010066

OPEN ACCESS

toxins

ISSN 2072-6651

www.mdpi.com/journal/toxins

Article

Chronic Illness Associated with Mold and Mycotoxins: Is Naso-Sinus Fungal Biofilm the Culprit?

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Received: 2 December 2013; in revised form: 16 December 2013 / Accepted: 17 December 2013 / Published: 24 December 2013

Abstract: It has recently been demonstrated that patients who develop chronic illness after prior exposure to water damaged buildings (WDB) and mold have the presence of mycotoxins, which can be detected in the urine. We hypothesized that the mold may be harbored internally and continue to release and/or produce mycotoxins which contribute to ongoing chronic illness. The sinuses are the most likely candidate as a site for the internal mold and mycotoxin production. In this paper, we review the literature supporting this concept.

Keywords: mycotoxin; biofilm; rhinosinusitis; chronic fatigue syndrome

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1. Introduction

Exposure to water damaged buildings (WDB) have been associated with numerous health problems that include fungal sinusitis, abnormalities in T and B cells, central and peripheral neuropathy, asthma, sarcoidosis, respiratory infections and chronic fatigue [1–14]. It has been well established that mold and mycotoxins are important constituents of the milieu in WDB that can lead to illness [15–22]. Using a sensitive and specific assay developed by RealTime Laboratories (RTL), we recently published a study linking the presence of aflatoxins (AT), ochratoxin A (OTA) and/or macrocyclic trichothecenes (MT) to chronic fatigue syndrome (CFS) [14]. The specific methods for these assays have been previously published [14]. A significant number of these chronically ill patients were ill for many years, with an average duration of more than seven years (range 2–36). Furthermore, over 90% of the patients gave a history of exposure to a WDB, mold or both. Exposure histories often indicated the WDB/mold exposure occurred many years prior to the mycotoxin testing. Many of these patients have not had recent or current exposure to a WDB or moldy environment. Despite the remote history of exposure, these patients had chronic symptoms and the presence of significantly elevated concentrations of AT, OTA and MT in their urine specimens. The persistence of mycotoxins suggests that there may be an internal source of mold that represents a reservoir for ongoing mold toxins that are excreted in the urine. Otherwise, one would anticipate that the toxins would have cleared over time. Herein, we discuss the concept that the nose and sinuses may be major internal reservoirs where the mold is harbored in biofilm communities and generates "internal" mycotoxins.

2. Example Case Studies

1. Case One

A 71 year old (y.o.) female was first seen in 1989 with a long standing chronic illness that was subsequently diagnosed as CFS. She had been symptomatic since approximately 1970. She met the Fukuda criteria for CFS as published in 1994 [23]. She has remained chronically ill over the years with minimal variation or improvement in symptoms. The patient had reported long standing sinus problems dating back to childhood. She was diagnosed with chronic sinusitis by the mid-1980s. She underwent two nasal/sinus surgeries, the first in 1988 which entailed nasal reconstruction and the second in 2003 with creation of antral windows. This patient continued with chronic sinus symptoms and required nasal/sinus "clean out" by her Ear, Nose and Throat (ENT) physician about every three months. In 1999, she underwent endoscopy by her ENT physician at which time fungal cultures were obtained. These cultures grew a pure growth of *Aspergillus niger*. The environmental history obtained in 2012, indicated remote exposure to WDB and moldy environments in a home in which she previously lived as well as a work building. These exposures would have occurred in the 1960s. In 2012, a urine mycotoxin assay was sent to RTL which came back positive for OTA at a level of 5.9 parts per billion (ppb). *Aspergillus niger* is one of the fungal species known to produce OTA [15,24].

2. Cases Two and Three

These cases involve a father (41 y.o.) and daughter (8 y.o.) exposed to mold in a water damaged home as previously reported [25]. They developed numerous health problems following exposure including chronic fungal sinusitis that required surgery [25,26].

Father: The endoscopic sinus surgery performed on the father involved turbinate septoplasty, surgical removal of polyps and debridement of affected sinuses. MRI and CT scans revealed mucosal thickening of all sinuses, particularly the frontal, ethmoid and sphenoid sinuses. The right maxillary sinus had nodular opacities. Surgical specimens were sent to RTL to assay for mycotoxins in the specimens. AT was detected at 1.1 ppb. Culture from the sinus tissue grew *Penicillium* species.

Daughter: The endoscopic exam revealed that maxillary, ethmoid, sphenoid and frontal sinuses were edematous, there were enlarged turbinates (4+) and deviated septum to the left. The endoscopic surgery performed on the daughter involved left sphenoidotomy, ethmoidotomy and maxillary sinusotomy. Surgical specimens sent to RTL demonstrated AT level of 1.2 ppb. A culture obtained from the sinuses was positive for *Aspergillus fumigatus* (*A. fumigatus*).

As previously reported both the father and daughter were positive for mycotoxins in the urine and nasal secretions. The father's specimens showed the following values: urine OTA 18.2 ppb; nasal secretions AT 11.2 ppb and OTA 13 ppb. The daughter's results were as follows: urine OTA 28 ppb and MT 0.23 ppb; nasal secretions OTA 3.8 ppb and MT 4.68 ppb. Mycotoxin results for both father and daughter are summarized in Table 1.

Table 1. Mycotoxin detection in two cases following exposure in WDB.

Patient: Source	AT [a]	OTA [a]	MT [a]
Father: Sinus Tissue	1.1	NF [b]	NF
Father: Nasal Secretions	11.2	13	NF
Father: Urine	NF	18.2	NF
Daughter: Sinus Tissue	1.2	NF	NF
Daughter: Nasal Secretions	NF	3.8	4.68
Daughter: Urine	NF	28	0.23

Notes: [a]: ppb; [b]: Not Found.

3. Chronic Rhinosinusitis (CRS)

The nose and paranasal sinuses virtually always harbor numerous fungal species. In a study done at the Mayo Clinic by Ponikau *et al.*, numerous types of fungi were recovered from the sinuses of CRS and normal control patients [27]. Amongst the species recovered, many have the potential to produce mycotoxins including *Aspergillus* (*flavus*, *niger*, *fumigatus*, *versicolor*), *Chaetomium*, *Fusarium*, *Penicillium* and *Trichoderma*. This group also found "fungal elements (hyphae, destroyed hyphae, conidiae and spores)" in 82 of 101 (81%) of the surgical specimens from the sinuses. Similarly, Braun *et al.* studied 92 CRS patients and 23 healthy control subjects. Positive cultures for fungi from nasal mucous were found in 91% of CRS patients and 91% of the controls [28]. Fungi and eosinophilic mucin were the markers of sinus involvement in the CRS patients. The species of fungi were very similar to the Mayo study, including potential toxin producing fungi (*Aspergillus*, *Penicillium*, *Chaetomium*, *Trichoderma*). Additionally, of 37 surgical cases, 75% had fungal elements (hyphae and spores) on histological examination. In this paper, the authors state "we conclude that nearly everybody has fungi in his or her nose." Between the two studies, the total number of different fungal genera identified was 66. Fungal DNA in the sinuses has been identified by quantitative polymerase chain reaction (Q-PCR) of nasal brushings [29]. Similar to the studies noted above, potential mycotoxin producing fungal species were found in the nasal brushings with this method of testing. The species present in the nasal brushings were similar to species found by Q-PCR testing of dust samples in their homes. In another study of CRS, fungal DNA was present in tissue specimens taken from patients with polyploid CRS who underwent surgery [30]. Two PCR primer sets were utilized; one was panfungal and the other specific for *Alternaria*. Fungal DNA was found in all 27 of the CRS patients with both primers. In surgical specimens from healthy controls, the panfungal DNA was positive in 10 of 15 cases but all were negative for the *Alternaria* DNA. Studies have also shown that pre-digestion of tissue slides with trypsin before staining dramatically improves identification of fungi by immunofluorescence as does as PCR-DNA analysis [30,31].

4. Detection of Mycotoxins in Invasive Aspergillosis: Humans and Animals

Gliotoxin was detected in the sera of cancer patients with invasive aspergillosis (IA) ranging from 65 to 785 ng/mL. It was also detected in the lungs (3976 ± 1662 μg/g) and in sera (36.5 ± 30.28 ng/mL) of mice with experimentally induced IA [32]. Wild and domestic animals have been reported with IA. Gliotoxin was detected in the lungs of wild birds at 0.1–0.45 mg/kg; an infected bovine udder at 9.2 mg/kg; and turkey poults exceeding 6 ppm in infected tissues [33–35]. Moreover, aflatoxin B_1, B2 and M were detected in the lungs and skin of a patient who died from an invasive infection of *A. flavus* [36]. Aflatoxin B, ranging from 2.0 to 170 μg/g, was recovered in silkworms infected with *A. flavus* [37]. These observations demonstrate that *Aspergillus* species produced mycotoxins in the infectious state in humans and animals. Biofilms may play an important role in that there are up regulated secondary metabolite enzyme pathways in the production of mycotoxins in IA and other mycoses [38,39]. This is discussed further in Section 9.

5. Urine Mycotoxins in CRS Patients

In a study of CRS patients (*n* = 79) by Dennis *et al.*, eight patients underwent urine mycotoxin testing for MT that were sent to RTL [2]. Of the eight specimens tested for MT, seven (87%) were positive. Lieberman *et al.* studied 18 patients with CRS. Mycotoxins were detected in urine assays in four of 18 (22%) at 2X the standard deviation above the limit of detection (all were ochratoxin) [40].

6. Detection of Mycotoxins from Nasal Washings, Sera and Tissues

Hooper *et al.* found mycotoxins in nasal washings and other tissues of mold exposure cases [41]. The most frequently recovered mycotoxins were MT, found in 44% of the nasal washing specimens, whereas AT were present in 17% of these cases. All nasal washings were negative for mycotoxins in the healthy controls (*n* = 27). In a study of a family exposed to mold in a water damaged home with AT, OTA and MT in environmental samples, nasal washings were positive for mycotoxins (AT, OTA, MT) in three of three family members in which nasal washings were tested [25]. All three cases had positive urine mycotoxins, as well. The specifics of the father and daughter are discussed above in Section 2. Interestingly, in two of the cases, the MT levels recovered from the nasal washings were higher than the urine levels. Between the two studies cited above, AT, OTA and MT have all been demonstrated in nasal washings of patients with clinical illnesses and exposure to a WDB and/or mold. However, mycotoxins were not found in nasal washings of a healthy control population. The results from studies of direct fungal isolation and mycotoxins are summarized in Table 2.

Other positive findings for the presence of mycotoxins in various tissues include the following: MT in sera of individuals exposed in a WDB; breast milk, placenta, umbilical cord and tissues (sinus) from family members exposed to a water damaged home [25,42]. Goats that had *Stachybotrys chartarum* (*S. chartarum*) spores instilled into their trachea were also positive for MT. Although MT cleared from the sera in 24 h, mycotoxins were present at 72 h post installation in the lungs, spleen and lymph nodes [43]. Since *Stachybotrys* is not considered a human pathogen, the uptake of the MT probably occurs from the lysis of spores and/or from other particulate matter. In addition, the detection of MT in lung, spleen and lymph nodes indicates peripheral organ storage has occurred.

Table 2. Presence of fungi and mycotoxins in healthy individuals, Chronic Rhinosinusitis (CRS) patients and mold exposure cases.

Study	Type of patients	Fungi present sinuses	Potential mycotoxin producing fungi in sinuses	Urine mycotoxins present	Nasal washing mycotoxins present
Ponikau [27]	Normal	Yes	Yes	ND [b]	ND
Ponikau	CRS [a]	Yes	Yes	ND	ND
Braun [28]	Normal	Yes	Yes	ND	ND
Braun	CRS	Yes	Yes	ND	ND
Murr [29]	CRS	Yes	Yes	ND	ND
Dennis [2]	CRS	ND	ND	Yes	ND
Lieberman [40]	CRS	ND	ND	Yes	ND
Hooper [41]	Normal	ND	ND	No	No
Hooper	Mold exposure	ND	ND	Yes	Yes
Thrasher [25]	Mold exposure	Yes	Yes	Yes	Yes

Notes: a: Chronic rhinosinusitis; b: Not done.

7. Indoor Microbes and Their Fragments

Mycotoxins (AT, OTA, MT) produced by several species of mold have been identified in water-damaged indoor environments [15–19,21,25]. They have been detected in the sera, urine and tissues of individuals with illness associated with exposure to microbes in these contaminated environments [2,3,14,25,40–42]. Whereas species of *Aspergillus* and *Penicillium* have been demonstrated in the nasal cavity and sinuses of individuals with CRS, accounting for the probable source of AT and OTA, the detection of MT appears to be somewhat of an enigma. *Trichoderma* has been found in the sinuses and does produce MT [27,28]. However, *S. chartarum* does not germinate and grow in animal tissues [22]. Furthermore, *S. chartarum* has not been recovered from patients with CRS either by culture or Q-PCR, although it is present in the dust of homes with affected occupants [15–22]. Since, *S. chartarum* does not readily shed its spores, what are the possible explanations for the detection of its mycotoxins in humans exposed to damp-indoor environments? We will briefly review the literature regarding the release of ultrafine particles (nanoparticles) by colonies of mold commonly present in damp-indoor spaces.

S. chartarum, other molds and bacteria produce large quantities of fine (nano range) fragments (0.03 to 0.3 microns) when compared to airborne spore counts [44–49]. The number of fine fragments is at least 500 times greater than the spore counts [46–48]. The respiratory deposition of these fine fungal fragments is 230 times that of spores including the anterior nasal region [46]. Furthermore, the fragments (small particulates) produced by *Stachybotrys* contain MT while other mold fragments (e.g., *Aspergillus* and *Penicillium*) contain antigens and toxins as determined by ELISA testing [19,42,44,45]. Thus, fungal fragments, which contain MT, as well as other mycotoxins and antigens, are inhaled and most likely deposited in the nasal cavity and sinuses. The fungal fragments are not detected by spore counts, in culture or even Q-PCR [44–49]. It has been recommended that the role of the fine particulates shed by mold and bacteria needs to be evaluated for contribution to the health problems of the exposed, rather than relying upon airborne mold spore counts [44–49]. These fine particulates may contribute to the colonization of the nose and sinuses which may be a particularly significant issue with *S. chartarum*.

8. Antifungal Therapy Directed at the Sinuses

In a study of treatment of patients with an intranasal antifungal agent (amphotericin B solution), Ponikau *et al*. showed significant improvements in several clinical parameters (symptoms, endoscopic findings and CT scanning of the sinuses) in CRS patients [50–53]. The authors concluded that reducing the amount of fungal antigen with the antifungal therapy led to clinical improvements. Varied results from studies of CRS treatments may be due to the fact that CRS can result from infection by bacteria, invasive mold, mold colonization in the presence of biofilms, the extent of sinus involvement (e.g., sphenoid sinuses) or a combination of factors [38,39,52,53]. Surgical debridement is also a common treatment in CRS [1,2,53]. Identifying specific fungal organisms in CRS caused by mold requires specific fungal staining methods to identify hyphae in sinus specimens or identification of mold by Q-PCR [30,31,54,55]. Treatment of fungal CRS may require the use of oral antifungals, as well as intranasal sprays with antifungal activity, depending upon the improvement of individual patient condition [56]. In addition, biofilms, antifungal shelf-life and antifungal resistance must be considered as other variables in effectiveness of treatment [57–64].

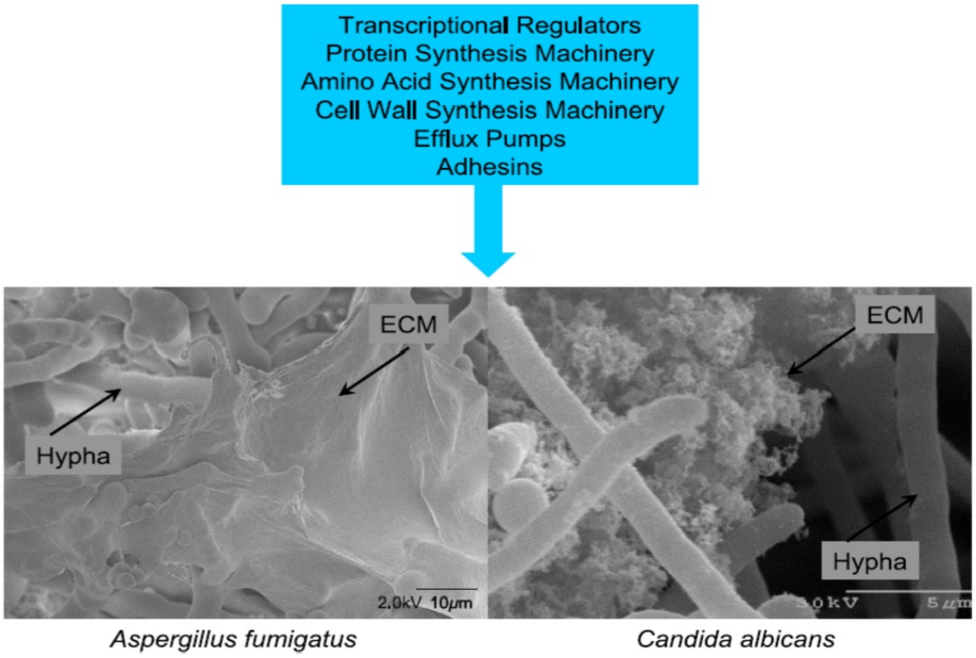
A recent study of mold exposed patients (n = 25) with a variety of systemic symptoms was presented [63]. The vast majority of the patients were positive for mycotoxins in the urine. The patients were treated with intranasal amphotericin B with or without systemic antifungals which represented biofilm focused therapy. The patients were monitored before and after treatment. Ninety per cent of the patients had a dramatic decrease in their systemic symptoms, including neurological conditions of tremor, ataxia and vertigo, among others [63].

9. Role of Biofilm

Biofilms are produced by bacteria and molds and are present in CRS. We will briefly review the key aspects of biofilms and their role in resistance of the microorganisms to antifungal treatment. Often the failure of such treatments lead to surgical intervention [1,2,53,61,64].

Briefly, biofilms are complex surface-associated populations of microorganisms embedded in an extracellular matrix (ECM) that possess distinct phenotypes compared to planktonic (free living) organisms. *In vitro* and *in vivo* observations have revealed the morphology and matrix of fungal biofilms [60–62,64]. Epithelial cells isolated from sinuses of CRS patients and controls were grown to a confluent monolayer *in vitro* and then infected with *A. fumigatus* under static and flow conditions [62]. The formation of the biofilm occurred in five stages: (1) conidial attachment to epithelial cells; (2) hyphal proliferation; (3) extracellular matrix (ECM); (4) hyphal parallel packing and cross linking; and (5) channel/pore formation. Biomass of the film was greater in flow *versus* static conditions [62]. The architecture of the biofilm was similar to that reported from *in vivo* CRS conditions as shown in Figure 1 [39]. The fungal ECM consists of polysaccharides (galactomannan, β-D-glucans, lipopolysaccharides), among other extracellular proteins, exotoxins, melanin, hydrophobins, exotoxins, monosaccharides and probably mycotoxins [39,59,64–68]. In this regard, biofilm cells have phenotypes and gene expressions distinct from the planktonic cells. Gene expression of a variety of pathways can be up or down regulated in the biofilm cells when compared to the planktonic phenotypes [38,39,69]. For example, over 3,000 differentially regulated genes have been identified under the two conditions [70]. Some of the genes impart antifungal resistance or up regulation of secondary metabolite pathways [38,39].

Figure 1. Common features of fungal biofilms. Gene expression has been compared between planktonic and biofilm cells of both *A. fumigatus* and *Candida albicans.* The major categories of genes up regulated in biofilms are summarize in the blue box. The photos depict the biofilm of *A. fumigatus* and *C. albicans*. The missing ingredient of the blue box is the up regulation of secondary metabolite pathways as demonstrated *in vitro* by Bruns *et al*. [38]. Permission to publish this figure was given by Dr. Fanning and Mitchell [39].



Aspergillus fumigatus *Candida albicans*

Gliotoxin produced by *A. fumigatus* was detected in an *in vitro* biofilm model. The proteins of the gliotoxin secondary metabolite pathway were up regulated in the biofilm cultures [38]. The ability of *A. fumigatus* to form biofilms is considered an important factor in invasive disease [67,68,70–73]. Thus, the presence of mycotoxins in human tissues and body fluids with invasive mycoses probably occurs. The gliotoxin detected in the sera of cancer patients and in various animals with invasive IA was reviewed in Section 4. Moreover, the detection of aflatoxin B_1, B_2 and M were detected in the lungs and skin of a patient who died from an IA was also reviewed in Section 4. These observations demonstrate that *Aspergillus* species produced mycotoxins in the infectious state in humans and animals. Biofilm may be a factor in up regulated secondary metabolite enzyme pathways in the production of mycotoxins in IA and other mycoses.

There is an apparent interaction and possible synergy between bacteria and fungi in biofilm development and survival. In a sheep model, bacteria appear to induce epithelial damage that promotes fungal biofilm formation by *A. fumigatus.* Co-inoculation of *Staphylococcus aureus* (*S. aureus*) and *A. fumigatus* into sheep sinuses resulted in an 80% formation of biofilms *versus* 10% with *A. fumigatus* inoculation alone [74,75]. Such interaction may provide better surface adherence and ECM formation. In a study by Foreman *et al*., the microbiology of biofilms was studied in CRS patients using a sensitive fluorescent *in situ* hybridization (FISH) assay [60]. 36 of 50 CRS patients had biofilms compared to 0 of 10 controls. *S. aureus* was the most common bacterial isolate found. Fungi (using a panfungal probe) were found in 11 of 50 cases. Of these 11 fungal biofilms, seven also demonstrated *S. aureus* biofilms. In another publication, *Haemophilus influenzae* produced less severe disease than *S. aureus* [65]. *S. aureus*, coagulase negative staphylococci (CNS) and other bacteria are frequently found in the sinuses, both in controls and CRS patients [76–82]. CNS has clearly been demonstrated to produce biofilm which represents a major pathogenic mechanism for these bacteria in certain clinical settings [80,81]. Since *S. aureus*, CNS and other bacteria frequently occur in the sinuses and commonly form biofilm, this may potentially represent another significant co-pathogen for fungal biofilm formation.

The biofilm confers considerable protection for the organisms including resistance to host defenses and antifungal treatments [38,39,64,83,84]. ECM acts as a physical barrier between the embedded fungal cells and clinically useful antifungal agents, thus leading to ongoing colonization of fungi in the sinuses despite maximal treatment [39,64,83]. Biofilm may allow for chronic persistence of fungi in the nose and sinuses and make treatments more difficult. Although the efficacy of antifungal treatments has been questioned in biofilm, amphotericin B has worked reasonably well in clinical settings and in biofilm models [50,63,84,85]. This may be especially the case for higher concentrations of amphotericin B, which can be used in sinus irrigation since there is no systemic absorption [50–52,84]. A combination of amphotericin B with voriconazole and caspofungin was tested on *A. fumigatus* from early to late stages of colony growth. The combination was effective during early growth, while amphotericin B alone was most effective in the later stages of mycelial growth [84,85].

Given the role of bacterial pathogens in fungal sinus biofilm (e.g., *S. aureus*), antibacterial therapy may be a helpful adjunct. For example, mupirocin was shown to be effective in the post surgical treatment of recalcitrant CRS [82].

Other agents such as N-acetyl cysteine (NAC) and EDTA may assist with disruption of biofilm and enhance the activity of antifungal and antibacterial drugs [86].

Therapies directed at the fungal biofilm may be promising potential interventions for patients with chronic illness secondary to mycotoxins. Examples of such therapies could include agents to disrupt biofilm (e.g., intranasal EDTA) and intranasal antifungal administration (e.g., amphotericin B).

10. Conclusions

(1) Indoor water-damaged environments contain a variety of mold and bacterial species that produce mycotoxins, volatile organic compounds, exotoxins and other metabolites that are present in the dust, furnishings and air [15–22];

(2) The occupants of these environments experience chronic adverse health effects that range from upper and lower respiratory disease, central and peripheral neurological deficits, chronic fatigue type illness, among others [1–14];

(3) Patients that remain chronically ill (e.g., CFS) after exposure to WDB and/or mold, very commonly demonstrate mycotoxins in the urine [14,25,40,41]. Many of these patients have remained chronically ill despite leaving the moldy environment several years previous to the urine testing [14]. This suggested to us that there may well be an internal presence of toxin producing mold. We raised the question, where was the mold located in the body? Herein, we have reviewed the medical literature as it relates to the presence of fungi/mold in the nose and sinuses;

(4) We reviewed data for three patients with chronic illness who required surgery for chronic fungal rhinosinusitis. Mycotoxin testing revealed the presence of AT, OTA, and MT in nasal secretions, urine and tissues samples (Tables 1 and 2) as reported herein and by others [2,3,14,25,40–42]. Additionally, fungal organisms were recovered in cultures from the sinuses in these three cases including *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium*;

(5) Humans and animals with IA have gliotoxin and aflatoxins in their sera and tissues [32–37]. These observations suggest that *Aspergillus* species produce mycotoxins during IA. In addition, after intratracheal administration of *Stachybotrys* spores, animals were found to have MT in their lungs, spleen and lymph nodes at 72 h after treatment [42]. Also, storage of mycotoxins occurs in variety of tissues [36,41,42];

(6) Fungal species can be found in the sinuses of normal, healthy individuals, as well as CRS patients [27,28,55]. Species that have been recovered include those that have the capacity to produce mycotoxins. Additionally, mycotoxins (AT, OTA and MT) have been recovered from nasal washings in patients exposed to a moldy environment, however they were not found in nasal washings of healthy individuals [41];

(7) The fungi that are present in the sinuses are in biofilm communities which allows for chronic persistence [39,60,61,65–68]. This would explain the chronic nature of the fungi/mold in the sinuses and explain the difficulty in treatment [39,64,83]. However, despite that, studies have demonstrated success with treating patients with intranasal amphotericin B. This was shown in both CRS patients and those with chronic illness following mold exposure [50,51,63]. Amphotericin B has been shown to have superior activity in biofilm models as opposed to other antifungal agents [50,51,84];

(8) Fungal fragments from 0.03 to 0.3 microns are shed from fungal colonies known to contain antigens and toxins [44–48]. Fine particulates shed by *Stachybotrys* contain MT [18,19]. The fragments are readily deposited in the nasal cavity [46]. MT have been detected in the sera of occupants exposed to *Stachybotrys* [42];

(9) Prior exposure to toxic mold and mycotoxins may represent an important feature of chronically ill patients such as CFS as well as those with CRS. An internal reservoir of toxin producing mold (e.g., sinuses) that persists in biofilms could produce and release mycotoxins. This model of fungal persistence may help explain these chronic illnesses and represent a potential new understanding of mechanisms of disease that can be treated and/or lessened.

Conflicts of Interest

Joseph Brewer declares no conflict of interest. Dennis Hooper and Jack Thrasher have served as expert witnesses in mold and mycotoxin exposure litigation.

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Science of the Total Environment 621 (2018) 75–84

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Evidence from SINPHONIE project: Impact of home environmental exposures on respiratory health among school-age children in Romania

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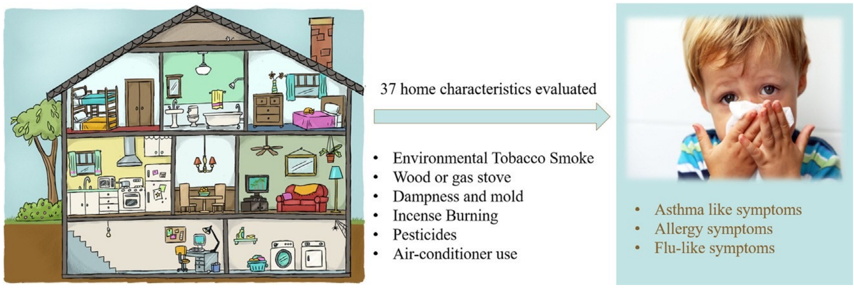
HIGHLIGHTS

- We described the characteristics of Romanian homes and compared them with U.S. homes.
- ETS, dampness/mold, wood/gas stoves use were associated with respiratory symptoms.
- We found a high prevalence of indoor smoking and wide use of wood stove.

GRAPHICAL ABSTRACT



article info

Article history:
Received 28 June 2017
Received in revised form 14 November 2017
Accepted 14 November 2017
Available online 22 November 2017

Editor: Scott Sheridan

Keywords:
Indoor environment
Respiratory symptoms
School-age children
Romania
Housing characteristic
Environmental tobacco smoke

abstract

Background: Exposure to indoor air pollutants at home was found to be associated with respiratory diseases. As lifestyle changes with rapid economic growth in Romania, the aim of our study is to describe the characteristics of Romanian homes and their impact on children's respiratory health.

Methods: Self-reported information on respiratory symptoms was collected from 280 Romanian elementary school students in 2011, and the symptoms were categorized into allergy, asthma-like, and flu-like symptoms. Home characteristics and demographic information were collected from questionnaires answered by parents. The association between home characteristics and respiratory health was assessed through multivariate logistic regression controlling for school indoor exposure.

Results: As compared to U.S. households, Romanian homes have a higher percentage of smokers, limited use of indoor climate control, and higher use of iron stoves. Exposure to environmental tobacco smoke was associated with both asthma and allergy symptoms. Additional risk factors identified for allergy symptoms include living in apartments, near pesticide sprayed areas, and the use of incense sticks. The significantly higher risk of flu-like symptoms was associated with mold and dampness issues, the use of air conditioner, gas heater/iron stove in children's bedroom.

Conclusion: Our findings suggest that an increase in respiratory symptoms among Romanian school-age children can be partly related to their environmental exposure at home. Since most of the identified risk factors are preventable, our results provide critical information and evidence for policymakers, to develop target intervention and education strategies.

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https://doi.org/10.1016/j.scitotenv.2017.11.157
0048-9697/

1. Introduction

School-age children spend on average, more than 16 h a day at home (Wiley et al., 1991). Previous studies have shown that poor housing conditions are associated with poor respiratory health outcomes, especially in children (Breysse et al., 2004; Kanchongkittiphon et al., 2015). Sources of indoor pollutants in homes such as tobacco smoke and unvented gas heater were found to be related to an increase in respiratory symptoms and diseases in children (Li et al., 1999; Pilotto et al., 2004). School-age children who suffer from respiratory diseases are more prone to school absenteeism (Hsu et al., 2016; Simons et al., 2010) and poorer academic performance (Diette et al., 2000; Mendell and Heath, 2005; Vir et al., 1997) and this is often reflected in lower perceived educational attainment and career success (Restuccia and Urrutia, 2004). A report by the World Health Organization (WHO) identified Romania as having one of the highest prevalence of asthma and allergy symptoms among school-age children in the European Region (Wirl and Puklová, 2007). A study conducted in Cluj Napoca, Romania reported that the prevalence of asthma and allergic rhinitis-related symptoms has increased substantially over a six-year period (Chereches-Panta et al., 2011). Nevertheless, except for a few studies on passive smoking (Arghir et al., 2013; Mitchell and Stewart, 2001), there remains no prior research evaluating home environment exposures, as well as the influence of lifestyle and socioeconomic status on student's respiratory health in Romania. Differences in indoor environment and sources of indoor air pollution between developed and developing countries have been shown in previous studies (Bruce et al., 2000; Górny and Dutkiewicz, 2002). Therefore, it is necessary to understand the unique characteristics of homes in Romania before implementing intervention programs and policies that were predominantly designed in developed countries (e.g. Western Europe).

We hypothesize the rapid increase in asthma and allergy symptoms among children in Romania, is partly due to changes in the residential environment. To the best of our knowledge, there are no prior published articles using data from the “Schools Indoor Pollution and Health: Observatory Network in Europe” (SINPHONIE) project to assess the influence of home indoor environment on student's health while controlling for school environment, and fewer studies explored the influence of indoor environment exposures on multiple health symptoms among the Eastern European population. In this study, we intend to (1) describe comprehensive housing characteristics in Romanian homes and compare these characteristics with homes in the U.S. and Western European countries; and (2) assess the association between home exposure and respiratory symptoms (asthma-like symptoms, allergy symptoms, and flu-like symptoms) among school-age children in Romania, controlling for exposure in the school environment.

2. Material and methods

2.1. Overview of SINPHONIE project

SINPHONIE is a Europe-wide *cross sectional* study, funded by European Parliament, focusing on assessing student's exposure to indoor and outdoor air quality in school and other settings, and its impact on student's health. The project was conducted from 2010 to 2012, in 25 European countries (Csobod et al., 2014; Kephalopoulos et al., 2014). Five questionnaire surveys were conducted among school administrators, teachers, students and their parents, to collect information on school environment and policy, home environment, school occupants' health condition, and demographic information. One-time measurement of indoor air pollutant levels including particulate matter with a diameter smaller than 2.5 μm ($PM_{2.5}$), nitrogen dioxide (NO_2), carbon dioxide (CO_2), carbon monoxide (CO), and volatile organic compounds (VOCs) was performed in all participating classrooms. Microclimate conditions (temperature and humidity) and ventilation rate were also measured at the same occasion. This study will only focus on Romanian data collected between October and December in 2011, following the SINPHONIE project protocols.

2.2. Study population

Schools and classrooms in this study were selected following detailed selection criteria (Csobod et al., 2014; Regional Environmental Center, 2014). Five primary schools in Alba County attended by students in grades I to IV were included in the study. In each school, indoor air pollutants and microclimate conditions were monitored in three selected classrooms. A representative sample of classrooms in each school was chosen based on the following selection criteria: (1) classrooms located on different floors and in different areas of the building (towards the street or schoolyard); and (2) occupied by the same class for most of the academic year. Overall, 280 students and their parents agreed to participate in the study and completed the questionnaires, with a response rate of 89.7%.

2.3. Exposure measurement

Information on student's home environment characteristics was gathered from 37 related questions extracted from the self-administered questionnaire answered by their parent(s). Questions asked about housing characteristics including (1) general building information (e.g. construction year, building type); (2) indoor environment characteristics in the dwelling (e.g. heating type, type of cooker); (3) indoor environment characteristics in children's bedroom (e.g. heating type, floor material); (4) exposure to environmental tobacco smoke (ETS); (5) dampness and mold issues; and (6) other environmental concerns (e.g. pets, use of air freshener).

2.4. Outcome measurement

An interview-based survey was conducted with students by a trained interviewer. During the interview, students were asked about whether they ever had an asthma attack and twenty-nine other questions pertaining to health symptoms in the past week, as well as the location where those symptoms occurred (home, school, and other). Survey questions were developed based on standardized questionnaires from the International Study of Asthma and Allergies in Childhood (ISAAC) (Pearce et al., 2007) and the Health Effects of School Environment study (HESE) (Health Effects of School Environment (HESE) Final Scientific Report, 2006). Health symptoms reported by students were categorized into three dichotomous outcomes (see Table A.1): asthma-like symptoms (Yes/No); allergy symptoms (Yes/No); and flu-like symptoms (Yes/No), based on a systematic review (Sá-Sousa et al., 2014) and clinical diagnosis criteria (Quillen and Feller, 2006; Rapid Reference to Influenza Resource Center, 2006). Asthma-like symptoms were defined as either: 1) ever had an asthma attack or wheezing at school, or 2) having any of the following symptoms in the past week: dry cough, difficulty in breathing, wheezing, and difficulty in breathing with wheezing. Fifteen symptoms (e.g. sneezing, skin rash, itching skin or eyes) were defined as allergy-related symptoms and ten symptoms (e.g. running nose, sore throat, fever) were defined as flu-like symptoms.

2.5. Covariates

Demographic and socioeconomic characteristics of households were reported by students' parents and included parental information such as mother's educational levels, employment status, and whether the family received other government benefits (e.g. tax exemptions or government subside for electricity and natural gas). Information on family history of any allergic disorders among core family members (siblings and parents), was also collected. To control for student's exposure to indoor air pollutants at school, CO_2 levels for all 15 classrooms were measured during the school day (from 8 AM to 1 or until 4 PM, depending on the school schedule) at the same time with the questionnaire survey. A multi-parameter direct reading device (IAQ-Calc model 7545, TSI Inc., Aachen, Germany) was used and the 5 or 8 hour-average (depending on the school program) was calculated. As indicated by a previous study in the U.K. (Chatzidiakou et al., 2015), classroom CO_2 level can serve as a proxy for indoor air quality (IAQ) due to its strong correlation with ventilation rate and some major indoor air pollutants (e.g. PMs and VOCs).

2.6. Statistical analysis

Univariate analyses were conducted for all home characteristics and covariates, and means or medians were reported for continuous variables according to their distributions. Firth's corrected logistic regression, a preferred method for studies with small sample size and separation issues, was used to estimate all odds ratios (ORs) in this study. Adjusted OR (aOR) for each home characteristic was calculated in a single-pollutant model, controlling for potential confounders. Directed Acyclic Graph (DAG) was constructed to identify confounders including student's age, gender, family history of allergic conditions, maternal education, and receiving government benefits. To construct a reduced multi-pollutant model with multiple home exposures, the backward stepwise variable selection and variance inflation factor (VIF) were applied to select important home exposures and control for high collinearity between variables. The final reduced model was constructed including home exposures with $P < 0.25$ and all identified confounders and no VIF of any independent variable exceeded 10. We also conducted a sensitivity analysis where classroom average CO_2 concentration was included as a potential confounder in the final model. Percentage change in OR was calculated to assess the magnitude of the potential confounding related to school indoor environment. All analyzes were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Health condition among students & socio-economic status

Health condition and demographic information of all participants were shown in Tables 1 and A.1. Most participating students were between 8 and 9 years old, and our sample was balanced between genders. Among all participating families, 16% reported at least one direct family member having a family history of allergic conditions. Approximately one-third of the children reported having at least one health symptom in the past week (see Table A.1). The flu-like symptoms were the most common among students, leaded by runny nose (20%), stuffy nose (19%), and sore throat (12%). Consistent with the low prevalence across participating countries in the SINPHONIE study (1.6%) (Csobod et al., 2014), only two students reported that they ever had an asthma attack at school (0.7%) in our study. However, asthma-like symptom such as dry cough was commonly reported among students (20%). Among allergy symptoms, sneeze (11%) is the most common, followed by skin rash (2%) and itchy hands or forearms (2%). When health symptoms were stratified by location of occurrence, the ranks of frequencies of symptoms were similar across different locations. Most of the health symptoms were reported as occurring at home, and happened less often at school or in other locations, such as gyms.

We found that most of the children lived in households with stable financial support and relatively well-educated parents. The employment rate of mothers in our sample was 56%, similar to the 57% reported among all Romanian women (European Platform for Investing in Children, 2016). About 83% of the mothers in the study had a high school or higher degree, while 25% of mothers had a college degree. About 14% of families received state benefits, of which, most reported having disabled and/or unemployed family members.

3.2. Housing characteristics in Romanian homes

As shown in Table 2, most students lived in a house that was constructed after 1970 (with the oldest being built in 1896), and averaged three rooms. Approximately 39% of our participants reported renovating their house within the last 12 months. Soluble and water-resistant paint were commonly used for wall decorations during the renovation. Additionally, most homes were located near some traffic, but only half of the participants reported living within 200 m from heavy traffic (major roads with peak traffic counts > 1 car/min). Exposure to ETS at home was very common and at high frequency among the participating students. About 48% of students in our study were exposed to ETS at home, which is much higher than the 17.4%–25% reported in U.S. homes (Centers for Disease Control and Prevention, 2007; Sobotova et al., 2011). Visible mold issue was more common somewhere else in the house (18%) than in children's bedroom (6%). Low use of indoor environment control tools such as air conditioner (AC) (6%), mechanical ventilation (8%), and humidifier (13%) was reported, as compared to

Table 1
Description of students' demographic status.

Variable	N (%)	Asthma like symptoms (n = 55)	Allergy symptoms (n = 49)	Flu-like symptoms (n = 85)
Gender				
Female	141 (50.36)	Ref	Ref	Ref
Male	139 (49.64)	0.89 (0.49, 1.60)	0.82 (0.43, 1.56)	0.78 (0.47, 1.30)
Age (years)[a]				
6-7	31 (11.07)	0.91 (0.67, 1.23)	1.11 (0.80, 1.54)	1.13 (0.88, 1.47)
8-9	188 (67.14)			
10-11	61 (21.79)			
Mother's education				
Primary school or less	47 (16.79)	1.08 (0.38, 2.98)	1.22 (0.42, 3.43)	0.48 (0.19, 1.11)
Secondary school	71 (25.36)	1.61 (0.68, 3.90)	1.66 (0.69, 4.17)	0.88 (0.44, 1.77)
Vocational school	80 (28.57)	2.12 (0.95, 4.96)	1.43 (0.60, 3.59)	1.11 (0.57, 2.17)
College	71 (25.36)	Ref	Ref	Ref
Missing	11 (3.93)	–	–	–
Mother's employment				
Full-time employee	158 (56.43)	Ref	Ref	Ref
Part time/unemployed/disabled/pensioner	73 (26.07)	1.07 (0.53, 2.10)	1.12 (0.52, 2.32)	0.91 (0.50, 1.64)
Missing	49 (17.50)			
Receiving state benefit	39 (13.93)	1.34 (0.58, 2.90)	1.49 (0.61, 3.32)	0.76 (0.34, 1.58)
Family history of any allergic conditions	44 (15.71)	0.42 (0.13, 1.06)	1.16 (0.46, 2.65)	1.40 (0.71, 2.73)
	Mean (ppm)			
Classroom CO_2 level[a]	1995	0.96 (0.90, 1.03)	1.01 (0.94, 1.09)	0.98 (0.92, 1.04)

[a] Age and classroom average CO_2 level (per 100 ppm increase) were included as continuous variable when calculating their crude ORs.

Table 2
Description of home exposures and their association with health outcomes among students in single-pollutant model.
The bolded OR in the table represents an estimated OR with a corresponding p-value less than 0.05.

Home characteristics	N (%)	Adjusted OR[a] in single-pollutant model (95%CI)		
		Asthma-like symptoms	Allergy symptoms	Flu-like symptoms
Building general information				
Housing type				
Single family house	163 (60.59)	Ref	Ref	Ref
Apartment	78 (29.00)	1.30 (0.65, 2.57)	2.08 (1.02, 4.21)	1.05 (0.57, 1.90)
Semi-detached house/other	28 (10.41)	0.90 (0.26, 2.55)	0.36 (0.04, 1.54)	0.52 (0.17, 1.37)
Location to traffic				
Away from traffic	49 (18.01)	Ref	Ref	Ref
Near heavy or light traffic	223 (81.99)	1.02 (0.46, 2.49)	0.56 (0.26, 1.30)	0.88 (0.45, 1.80)
b200 m from heavy traffic				
No	131 (48.88)	Ref	Ref	Ref
Yes	137 (51.12)	0.45 (0.23, 0.86)	0.91 (0.46, 1.81)	0.62 (0.36, 1.09)
	Median			
# of rooms	3	0.95 (0.72, 1.23)	0.78 (0.55, 1.07)	1.03 (0.83, 1.29)
# of people in dwelling	4	0.93 (0.72, 1.21)	1.02 (0.80, 1.29)	1.01 (0.83, 1.23)
Construction year	1979	Not calculated	Not calculated	Not calculated
Renovated in 12 months				
No	168 (61.31)	Ref	Ref	Ref
Yes	106 (38.69)	1.02 (0.53, 1.94)	0.86 (0.42, 1.73)	0.98 (0.55, 1.71)
Indoor environmental characteristics in dwelling				
Fireplaces				
No	207 (76.38)	Ref	Ref	Ref
Yes	64 (23.62)	1.46 (0.71, 2.91)	0.97 (0.42, 2.10)	0.88 (0.46, 1.64)
Mechanical ventilation				
No	247 (92.16)	Ref	Ref	Ref
Yes	21 (7.84)	1.19 (0.35, 3.40)	1.46 (0.42, 4.20)	1.73 (0.67, 4.32)
Air conditioner				
No	255 (93.75)	Ref	Ref	Ref
Yes	17 (6.25)	1.53 (0.44, 4.48)	1.47 (0.37, 4.60)	2.21 (0.80, 6.03)
Humidifier				
No	232 (86.89)	Ref	Ref	Ref
Yes	35 (13.11)	0.80 (0.27, 2.01)	0.78 (0.24, 2.09)	0.87 (0.38, 1.88)
Gas heater				
No	88 (33.59)	Ref	Ref	Ref
Yes	174 (66.41)	1.07 (0.53, 2.26)	0.35 (0.16, 0.76)	0.85 (0.46, 1.60)
Type of cooker in kitchen				
Coal/wood fired oven	11 (4.01)	0.86 (0.09, 4.53)	1.91 (0.31, 8.81)	0.68 (0.07, 3.51)
Gas/electronic cooker	263 (95.99)	Ref	Ref	Ref
Functioning extractor fan above cooker				
No	102 (37.50)	Ref	Ref	Ref
Yes	170 (62.50)	0.90 (0.47, 1.77)	0.94 (0.46, 1.99)	1.63 (0.90, 3.01)
Gas boiler in bathroom				
No	248 (93.58)	Ref	Ref	Ref
Yes	17 (6.42)	2.07 (0.66, 5.86)	0.78 (0.15, 2.70)	1.41 (0.48, 3.80)
Exists garage communicating with dwelling				
No	249 (91.54)	Ref	Ref	Ref
Yes	23 (8.46)	1.43 (0.41, 4.19)	1.83 (0.53, 5.34)	1.41 (0.51, 3.70)
Indoor environmental characteristic in children's bedroom				
Air conditioner				
No	267 (97.80)	Ref	Ref	Ref
Yes	6 (2.20)	0.38 (0.00, 3.62)	0.49 (0.00, 4.71)	2.07 (0.32, 11.11)
Bedroom floor material				
Linoleum/plastic/tile	18 (6.50)	0.13 (0.00, 1.09)	0.81 (0.15, 3.12)	0.65 (0.15, 2.18)
Wall to wall carpet	57 (20.58)	0.85 (0.36, 1.84)	0.74 (0.27, 1.77)	1.03 (0.51, 2.03)
Wood/parquet	202 (72.92)	Ref	Ref	Ref
Carpets				
No	24 (8.89)	Ref	Ref	Ref
Yes	246 (91.11)	1.26 (0.42, 4.94)	1.13 (0.38, 4.45)	1.24 (0.49, 3.50)
Heating type				
Electric/radiator/floor heating	35 (12.96)	Ref	Ref	Ref
Gas heater/gas stove	161 (59.63)	1.09 (0.41, 3.29)	2.17 (0.64, 11.23)	1.76 (0.69, 5.23)
Tiled clay/iron stove	74 (27.41)	1.19 (0.41, 3.88)	2.23 (0.61, 11.95)	2.13 (0.77, 6.72)
Environmental tobacco smoke				
Frequency of children's exposure to ETS				
Never	167 (61.17)	Ref	Ref	Ref
Daily	30 (10.99)	2.87 (1.09, 7.32)	5.18 (1.95, 13.71)	1.32 (0.53, 3.18)
Often	40 (14.65)	1.90 (0.77, 4.53)	1.98 (0.69, 5.35)	1.32 (0.58, 2.92)
Sometimes	36 (13.19)	1.68 (0.64, 4.13)	2.51 (0.90, 6.57)	1.30 (0.57, 2.84)
# of cigarettes per day				
None	175 (63.87)			
1–2 cigarettes/day	22 (8.03)	5.00 (1.86, 13.28)	1.69 (0.41, 5.42)	3.07 (1.23, 7.82)
3–4 cigarettes/day	29 (10.58)	1.67 (0.57, 4.39)	3.83 (1.34, 10.50)	2.08 (0.87, 4.91)

Table 2 (*continued*)

Home characteristics	N (%)	Adjusted OR[a] in single-pollutant model (95%CI)		
		Asthma-like symptoms	Allergy symptoms	Flu-like symptoms
5–10 cigarettes/day	30 (10.95)	2.52 (0.95, 6.39)	5.07 (1.96, 13.04)	0.80 (0.29, 2.00)
N10 cigarettes/day	18 (6.57)	2.58 (0.69, 8.57)	3.96 (1.02, 13.71)	1.40 (0.38, 4.46)
	Mean			
# of smokers in dwelling	0.66	1.62 (1.10, 2.39)	1.86 (1.23, 2.82)	1.35 (0.96, 1.90)
Dampness and mold				
Visible mold/water leakage in past 12 months				
No	216 (81.82)	Ref	Ref	Ref
Yes	48 (18.18)	0.74 (0.27, 1.75)	0.30 (0.06, 0.95)	2.09 (1.04, 4.20)
Condensation on windows in winter				
No	193 (71.22)	Ref	Ref	Ref
Yes	78 (28.78)	0.90 (0.42, 1.83)	1.47 (0.69, 3.04)	1.70 (0.94, 3.08)
Dampness/visible mold in children's bedroom				
No	258 (93.82)	Ref	Ref	Ref
Yes	17 (6.18)	0.99 (0.18, 3.74)	0.56 (0.06, 2.51)	4.72 (1.55, 15.71)
Dampness/mold issue in past 5 years				
No	223 (81.68)	Ref	Ref	Ref
Yes	50 (18.32)	0.80 (0.30, 1.88)	0.91 (0.33, 2.18)	1.91 (0.95, 3.81)
Allergen and chemical products				
Pets				
No	240 (87.91)	Ref	Ref	Ref
Yes	33 (12.09)	1.16 (0.42, 2.82)	0.25 (0.03, 0.99)	1.04 (0.43, 2.34)
Seen cockroaches in the house				
Never	220 (80.29)	Ref	Ref	Ref
Rarely	34 (12.41)	0.92 (0.34, 2.22)	1.54 (0.59, 3.64)	1.26 (0.56, 2.71)
Sometimes	20 (7.30)	0.51 (0.10, 1.78)	1.38 (0.34, 4.43)	1.19 (0.40, 3.26)
House is located near area sprayed with pesticides				
No	201 (74.44)	Ref	Ref	Ref
Yes	69 (25.56)	1.11 (0.51, 2.29)	1.47 (0.66, 3.12)	1.06 (0.55, 2.00)
Use of air fresheners				
No	126 (46.67)	Ref	Ref	Ref
Yes	144 (53.33)	0.87 (0.46, 1.68)	1.62 (0.80, 3.39)	1.03 (0.59, 1.79)
Use of incense sticks				
No	240 (87.91)	Ref	Ref	Ref
Yes	33 (12.09)	1.51 (0.61, 3.45)	2.06 (0.83, 4.72)	2.13 (1.00, 4.49)
Use of glues solvents & industrial products				
No	260 (95.94)	Ref	Ref	Ref
Yes	11 (4.06)	0.89 (0.16, 3.58)	3.88 (0.95, 14.84)	1.01 (0.23, 3.78)

[a] Adjusted for age, gender, family history of allergic conditions, maternal education, receiving state benefit or not.

the U.S. homes, where 87% of them own AC system (U.S. Energy Information Administration, 2011). Heating appliances with higher emissions of indoor pollutants such as gas heaters (66%) and tile clay or iron stoves (27%) were widely used in participating households. Higher use of coal or wood for cooking was also reported in Romanian homes (4.1%) compared to U.S. homes (2.1%) (U.S. Energy Information Administration, 2014). Wood flooring was used in the majority of Romanian households compared to the higher use of wall-to-wall carpet in the U.S. home (Crain et al., 2002). Noteworthy, for 91% of the households was reported the use of carpets in children's bedroom, which can serve as a reservoir of VOCs and indoor allergens. Pets are less common in Romanian families (12%) compared to U.S. families (56%), indicating less exposure to pets' fur, a known allergen (American Humane Association, 2012). However, cockroach, a known source of allergen that triggers allergic sensitization, was reported in 20% of the households. Air fresheners were used in half of the households, a slightly lower rate compared to U.S. homes (75%) (National Resources Defence Council, 2007), while incense stick use seems to be more common in Romanian homes (12%).

3.3. Association between home characteristics and student's asthma-like symptoms

Exposure to ETS at home was strongly associated with asthma-like symptoms. Asthma-like symptoms were significantly associated with all three ETS exposure indicators (frequency of exposure, the number of smokers, and the number of cigarettes smoked per day), in the single-pollutant model with estimated ORs range from 1.62 to 5.00 (Table 2). We also saw a possible dose-response effect when using frequency of exposure. Daily exposure was associated with the highest risk (aOR = 2.87, 95%CI: 1.09, 7.32) followed by "often" exposure category (aOR = 1.90, 95%CI: 0.77, 4.53), and then "sometimes" exposure category (OR = 1.68, 95%CI: 0.64, 4.13) (Table 2). Surprisingly, living within 200 m of traffic was associated with reduced asthma-like symptoms (aOR = 0.45, 95%CI: 0.23, 0.86).

After backward selection, six home characteristics were maintained in the final reduced model (Table 3.1). The number of cigarettes smoked per day was chosen in the reduced model as a representative for ETS exposure. Smoking 1–2 cigarette(s) per day (aOR = 5.13, 95%CI: 1.84, 14.28) was significantly associated with asthma-like symptoms among children, while other categories were marginally significant. In the sensitivity analysis, these significant associations were also observed. The changes in estimated aORs for exposures were relatively small after controlling for classroom average CO_2 level (0%–10%) (data not shown).

3.4. Association between home characteristics and student's allergy symptoms

In the single-pollutant model, exposure to ETS and housing type were associated with an increased risk of allergy symptoms among children. Similar to the results for asthma-like symptoms, the ETS exposure indicators were also significantly associated with 1.7 to 5.2-fold increased risk of allergy symptoms (Table 2). However, a dose-response effect was not seen. A two-fold increased risk of allergy symptoms was also shown among children living in an apartment as compared

Table 3.1
Adjusted ORs of association between selected home exposures and student's self-reported asthma-like symptoms in the multi-pollutant model (N = 231).
The bolded OR in the table represents an estimated OR with a corresponding p-value less than 0.05.

Home characteristics	Adjusted OR[a] (95%CI)	
	Final reduced model	Final reduced model control for classroom CO_2 level
Housing type		
Single family house	Ref	Ref
Apartment	1.49 (0.68, 3.26)	1.46 (0.66, 3.19)
Semi-detached house/others	0.30 (0.05, 1.22)	0.31 (0.05, 1.28)
# of cigarettes smoked per day		
None	Ref	Ref
1–2 cigarettes/day	5.13 (1.84, 14.28)	4.89 (1.74, 13.75)
3–4 cigarettes/day	2.48 (0.79, 7.25)	2.65 (0.84, 7.79)
5–10 cigarettes/day	2.42 (0.82, 6.74)	2.39 (0.81, 6.69)
N10 cigarettes/day	3.47 (0.86, 12.86)	3.81 (0.93, 14.53)
# of rooms	1.23 (0.84, 1.80)	1.20 (0.82, 1.77)
Having gas boiler in the bathroom	2.55 (0.70, 8.78)	2.62 (0.72, 8.93)
Having carpet in children's bedroom	1.53 (0.46, 6.60)	1.65 (0.49, 7.18)
Reported visible mold/water leakage in past 12 months	0.58 (0.20, 1.46)	0.58 (0.20, 1.47)

[a] Both models adjusted for age, gender, family history of allergic conditions, maternal education, receiving state benefit or not.

to a single house. Using a gas heater in the house was negatively related to allergy symptoms among children (aOR = 0.35, 95%CI: 0.16, 0.76).

Eleven home characteristics were included in the final reduced model (Table 3.2). Consistent with the single-pollutant model, the number of cigarettes smoked per day and housing type were positively associated with allergy symptoms. An increased risk of allergy symptoms was also shown among families that used incense stick frequently and for the households near an area where pesticides are sprayed. Pets' ownership was positively related to the risk of allergy symptoms, but with wide confidence interval. Surprisingly, visible mold or water leakage in the house was associated with a reduced risk of allergy symptoms among children (aOR = 0.1, 95%CI: 0.01–0.44).

3.5. Association between home characteristics and student's flu-like symptoms

Increased risk of flu-like symptoms was observed among children living in the households with reported dampness and mold issues, in the single-pollutant model. Among the four indicators of dampness and mold issues, both mold growth/water leakage in the past 12 months (aOR = 2.09, 95%CI: 1.04, 4.20) and mold/dampness issues in child's bedroom (aOR = 4.72, 95%CI: 1.55, 15.71) were significantly associated with flu-like symptoms, while another two indicators were marginally significant (Table 2). Flu-like symptoms were also more common among children who lived in households with a smoker who smoked 1–2 cigarette(s) per day (aOR = 3.07, 95%CI: 1.23, 7.82) or households with frequent use of incense stick (aOR = 2.13, 95%CI: 1.00, 4.49).

In the final model, seven home characteristics were selected (Table 3.3). As expected, elevated risk of flu-like symptoms was associated with mold/dampness issues in child's bedroom, with a similar magnitude of the effect. Type of heating used in child's bedroom was another important risk factor for flu-like symptoms. Elevated risks of flu-like symptoms were seen among families using tile clay/iron stove (aOR = 4.80, 95%CI: 1.44, 20.13) and families using gas heater/gas stove (aOR = 3.92, 95%CI: 1.26, 15.62), as compared to families using electric/radiator heater. AC use in the house was also associated with a 4.2 times higher risk of flu-like symptoms. After including multiple home characteristics, the association between the number of cigarettes smoked per day and flu-like symptoms was attenuated and marginally significant. Consistent results were shown in the sensitivity analysis.

Table 3.2
Adjusted ORs of association between selected home exposure and student's self-reported allergy symptoms in the multi-pollutant model (N = 230).
The bolded OR in the table represents an estimated OR with a corresponding p-value less than 0.05.

Home characteristics	Adjusted OR[a] (95%CI)	
	Final reduced model	Final reduced model control for classroom CO_2 level
Housing type		
Single family house	Ref	Ref
Apartment	3.32 (1.32, 8.91)	3.56 (1.37, 10.10)
Semi-detached house/others	0.41 (0.04, 2.01)	0.44 (0.04, 2.22)
# of cigarettes smoked per day		
None	Ref	Ref
1–2 cigarettes/day	2.24 (0.45, 9.25)	1.89 (0.37, 8.03)
3–4 cigarettes/day	12.25 (3.02, 56.20)	12.94 (3.11, 62.00)
5–10 cigarettes/day	7.92 (2.16, 31.12)	8.24 (2.26, 32.41)
N10 cigarettes/day	3.89 (0.70, 21.28)	3.97 (0.70, 22.83)
Children's bedroom floor material		
Wood/parquet	Ref	Ref
Linoleum/plastic/tile	0.36 (0.02, 3.20)	0.27 (0.01, 2.48)
Wall-to-wall carpet	1.56 (0.41, 5.55)	1.55 (0.42, 5.35)
Children's bedroom heating type		
Electric/radiator/floor heating	Ref	Ref
Gas heater/gas stove	3.29 (0.61, 40.39)	2.65 (0.50, 29.53)
Tiled clay/iron stove	2.50 (0.41, 32.23)	2.91 (0.49, 33.97)
Having gas boiler in the bathroom	0.70 (0.09, 3.79)	0.73 (0.10, 3.92)
Having pets in the dwelling	24.81 (3.25, 396.50)	29.70 (3.76, 541.11)
Reported mold/water leakage in past 12 months	0.10 (0.01, 0.44)	0.09 (0.01, 0.40)
Reported dampness/condensation on windows in winter	1.81 (0.64, 4.99)	1.96 (0.69, 5.51)
Frequent use of air fresheners	0.68 (0.28, 1.62)	0.61 (0.24, 1.48)
Frequent use of incense stick	3.25 (1.03, 10.26)	4.66 (1.37, 16.52)
Close to cultivation sprayed by pesticides	3.53 (1.27, 10.29)	4.17 (1.43, 13.26)

[a] Both models adjusted for age, gender, family history of allergic conditions, maternal education, receiving state benefit or not.

4. Discussion

In this study, we provided an overview of comprehensive housing characteristics in Romanian homes, which were unique as compared to the U.S. or Western European countries. Indoor smoking was very common in Romanian homes and served as an important risk factor for triggering asthma and allergy-related symptoms, and was marginally significant for flu-like symptoms among children in Romania. Sources of VOCs such as pesticide and incense sticks use were found to be contributing factors to allergy related symptoms among children. Dampness/mold were the strongest risk factors related to flu-like symptoms, followed by iron or gas stove use and AC use.

4.1. Characteristics of Romanian homes

When compared to the U.S. and Western European countries, Romanian homes face unique challenges. Exposure to ETS was one of the most common health risk factor identified in Romanian homes. Children were also exposed to a very high level of ETS at home, since 50% of the smokers in our study reported consuming more than five cigarettes per day (data not shown). Romanian homes may also have higher levels of pollutants such as PMs and NO_2 related to indoor combustion due to the higher prevalence of gas heaters and solid fuel use. While having extractor fans in the house may help, 88% of fans in Romanian homes did not have an outlet outdoors, which indicated poor emission control. Less indoor climate control was used in Romanian homes compared to developed countries, indicating less control over indoor temperature and humidity. This may pose a challenge in managing dampness and mold issues, which were common among participating families. Unlike homes in developed countries, Romanian homes were

Table 3.3
Adjusted ORs of association between selected home exposure and student's self-reported flu-like symptoms in the multi-pollutant model (N = 242).
The bolded OR in the table represents an estimated OR with a corresponding p-value less than 0.05.

Home characteristics	Adjusted OR[a] (95%CI)	
	Final reduced model	Final reduced model control for classroom CO_2 level
# of cigarettes smoked/day		
None	Ref	Ref
1–2 cigarettes/day	2.45 (0.88, 6.87)	2.43 (0.87, 6.84)
3–4 cigarettes/day	1.99 (0.76, 5.15)	1.99 (0.76, 5.16)
5–10 cigarettes/day	0.94 (0.32, 2.47)	0.93 (0.32, 2.44)
N10 cigarettes/day	1.84 (0.48, 6.37)	1.91 (0.49, 6.70)
Children's bedroom heating type		
Electric/radiator/floor heating	Ref	Ref
Gas heater/gas stove	3.92 (1.26, 15.62)	3.73 (1.20, 14.95)
Tiled clay/iron stove	4.80 (1.44, 20.13)	4.92 (1.48, 20.71)
Having air conditioner in the dwelling	4.21 (1.34, 13.97)	3.85 (1.22, 12.80)
Having functioning extractor fan above cooker	1.76 (0.92, 3.48)	1.77 (0.93, 3.50)
Dwelling renovated in the past 12 months	0.82 (0.44, 1.51)	0.87 (0.46, 1.62)
Reported dampness/condensation on windows in winter	1.73 (0.90, 3.31)	1.81 (0.94, 3.51)
Reported dampness/visible mold in children's bedroom	4.23 (1.21, 16.78)	3.89 (1.10, 15.56)

[a] Both models adjusted for age, gender, family history of allergic conditions, maternal education, receiving state benefit or not.

less prone to the allergen from pets, but more from pests. As compared to the U.S., where air fresheners and wall-to-wall carpet served as important emission sources and reservoir for VOCs, Romanian homes were more likely to have VOCs emitted from incense sticks burning and pesticide spray nearby.

4.2. Environmental tobacco smoke

Exposure to ETS was consistently shown to be the strongest risk factor for both asthma-like and allergy symptoms in the current study. Smoking indoors is a major source of multiple indoor air pollutants including PMs, VOCs, and heavy metals like cadmium. Toxicological experiments have shown that exposure to cigarette smoke can reduce lung function and increase IgE levels in both laboratory animals and humans (Ferrante et al., 2014). Our study observed stronger effects as compared to pooled estimates in a recent review, where passive smoking inside households was associated with the incidence of wheezing (pooled OR = 1.32) and incidence of asthma (pooled OR = 1.30) among children ages 5–18 years (Randolph, 2012). This difference may be partly explained by the larger percentage of children exposed to ETS more frequently and at a higher level, in our study. Romanian children are also more likely to be exposed to ETS in other public places. Although Romania banned smoking in most indoor public/work spaces (e.g. school, theaters, and restaurants) by 2015, lower public support was reported among Romanians as compared to other Eastern European countries (Muilenburg et al., 2010). Moreover, even though mothers in our sample were overall well-educated, the prevalence of exposure to ETS in households observed in our study (39%) was higher than the prevalence of smoking among Romanian adults reported by the European Commission Eurobarometer survey (31%) in 2006 (Bogdanovica et al., 2011). In summary, exposure to ETS may pose a substantial health risk to school-age children in Romania, due to its high prevalence.

4.3. Moisture and mold

Our study also indicated a four-fold increase in the risk of flu-like symptoms among school-age children due to dampness/mold issue in their bedroom, after controlling for exposure to ETS. Similar findings were reported by LARES study where visible mold in the house was associated with cold among children (OR = 1.4, 95%CI: 1.2, 1.7) (World Health Organization Regional Office for Europe, 2007). Although no direct measurement of mold was taken in our study, a significant association between self-reported visible mold and mold detected in air samples was reported in previous studies (Polyzois et al., 2014). The potential mechanisms suggested by previous experimental studies include: 1) specific microorganisms can lead to airway inflammation and immunosuppressive reaction and, 2) the dampness in the house can result in the excess emission of irritating air pollutants from building materials (e.g. formaldehyde) (Fisk et al., 2010). Although Romania has a moderate precipitation (637 mm per year), dampness and mold issues were more common in Romanian homes than in U.S. homes (American Housing Survey for the United States: 2009, 2011), or other nearby European countries (World Health Organization Regional Office for Europe, 2007). While use of mechanical ventilation was rare in Romanian homes, increasing frequency of natural ventilation and cleaning may help reduce the indoor humidity and prevent mold growth.

4.4. Indoor VOCs sources

We found that children living in homes where incense sticks were frequently used had a 3-fold increased risk of allergy symptoms. One study conducted among Taiwanese school-age children also found a significant association between incense sticks burning at home and coughing, but not with asthma or allergic rhinitis (Yang et al., 1997). However, there were conflicting results regarding the health impact of incense sticks use, since no harmful health effects were shown in two other studies conducted among school-age children (Koo et al., 1995; Lee et al., 2003). This may be partly explained by the diverse chemical compositions of incense sticks smoke. While burning of incense sticks generally leads to the emission of particulate matters, the emission of VOCs varies among incense sticks made of different materials or used for different purposes (Manoukian et al., 2013).

Living near a pesticide sprayed area, a potential outdoor source of VOCs, was also associated with a 3.5-fold increased risk of allergy symptoms among children. Since our study had a good mixture of households from rural and urban areas, the pesticide sprayed areas consisted of both farm and non-farm setting. While most studies focused on pesticide exposure in an occupational setting or agriculture community, there is an increasing body of literature that associates chronic residential exposure to pesticides with respiratory symptoms among children (Eskenazi et al., 1999; Salam et al., 2003; Salameh et al., 2003). Children are more vulnerable and more likely to be exposed to higher level of pesticide compared to adults, due to their hand-to-mouth behavior and fast metabolism (Salam et al., 2003).

4.5. Indoor environment control

The use of AC in the house was associated with a 4.2-fold increased risk of flu-like symptoms. This finding is consistent with a 4.1-fold increased risk of influenza-like illness due to AC use reported in a study conducted in Brazil (Silva et al., 2014). The use of AC can help create an environment with low humidity, which helps airborne microorganisms to survive and transmit. Furthermore, microorganisms that are allergens or pathogenic have been found to grow on AC filters, which were rarely changed or cleaned in homes (Ager and Tickner, 1983).

Children with gas heaters or iron stoves in their bedrooms had 4 times and 5 times higher risk of flu-like symptoms, respectively, compared to other heating systems. Low efficient heating systems, especially when using wood or coal, were well documented as indoor sources of

PMs, NO_2, and SO_2 (Bernstein et al., 2008). One study conducted in inner-city homes in Baltimore, showed a strong association between indoor NO_2 level and gas heater/heating stove use, after adjusting for other home characteristics (Hansel et al., 2008). A community randomized trial in New Zealand also found that when replaced with a non-polluting and efficient heating system, lower respiratory tract symptoms in children were significantly reduced (Howden-Chapman et al., 2008).

Unexpectedly, a negative relationship was found between the use of the gas heater in the dwelling and allergy symptoms. A possible explanation was that tile clay/iron stoves were more common in child's bedroom in households that did not have gas heaters (64%), compared to those with gas heaters (10%).

4.6. Housing type and location

After adjusting for socioeconomic factors, children living in an apartment still had a significantly higher risk of allergy symptoms compared to children living in a single house. As shown in LARES report, housing type may be associated with a wide range of factors such as ownership (owner/rental), building age and style (e.g. size of the window), ventilation system (natural/mechanical), infestation, and cleaning practices (e.g. centralized garbage storage) (World Health Organization Regional Office for Europe, 2007). While housing type may have limited impact on IAQ directly, it might have a large indirect effect on IAQ through the factors mentioned above.

Unexpectedly, living within 200 m of heavy traffic was associated with lower risk of asthma-like symptoms. One possible explanation was that this variable was not a good indicator for household proximity to traffic. When we compared this variable with the other variable on household proximity to traffic (house location towards the traffic), little agreement was found between these two variables. Half of the people who reported to live within 200 m of heavy traffic chose the option of "lived near light traffic", and another 9% of them chose the option "live away from traffic" in the other question. The association between the variable house location towards the traffic and asthma-like symptoms was not protective, with an estimated OR of 1.02.

4.7. Strengths and limitations

To our knowledge, this is the first study focusing on the association between a wide variety of home characteristics and children's respiratory health in Romania. Based on data collected by the SINPHONIE project, we evaluated multiple home characteristics in the same model and adjusted for important confounders such as socioeconomic status and family disease history. Using the information on both school and home environment collected simultaneously, we were able to adjust for potential confounding due to school environment exposure, to improve the study validity. In contrast with most of the previous studies, which only focused on one specific disease, we evaluated the impact of home environment on multiple outcomes, including asthma-like, allergy, and flu-like symptoms.

One concern in our study was the accuracy of our outcome definition. As discussed by Sá-Sousa et al., there was no unified definition of asthma in the current literature (Sá-Sousa et al., 2014). According to a national survey in 2013, the prevalence of asthma among Romanians varied widely when defined by symptoms instead of doctor diagnosis, which indicated a severe under-diagnosis of asthma and allergy (Bumbacea et al., 2013). Therefore, we decided to define our outcome on related health symptoms instead of doctor diagnosis, to secure the sensitivity and statistical power in this study. Additionally, due to limited knowledge and attention to personal health, health symptoms could be underreported by students as compared to parents' observation. However, self-reported symptoms by students were also more likely to capture symptoms that occurred in locations outside the house, and less biased by the relationship between children and parents. This also reduced the potential reporting bias, since we collected our exposure and outcomes data from two different sources. In our future study, we also plan to assess the consistency between symptoms reported by student or parent and student's spirometry test result.

Another concern was the limited statistical power and considerable uncertainty in our study. Although we had a moderate sample size, some of the estimates had wide confidence intervals due to uncommon home characteristics (e.g. pets in the dwelling) or strata (e.g. smoke N10 cigarettes/day). To improve precision, we combined strata that may have a similar effect (e.g. floor heating and radiator) and used Firth's logistic regression model to calculate ORs. We kept more detailed strata for important factors such as smoking, to assess the potential dose-response effect. Our estimates were also less vulnerable to bias due to missing values since only two variables included had N5% missing.

The final limitation was the cross-sectional design of the SINPHONIE project. Because of the absence of temporality, our findings should be interpreted with caution and cannot indicate a causal relationship. However, this cross-sectional study was based on high-quality data and valid design, and can be very helpful by providing baseline knowledge for future research and policy development. Home characteristics were reported by parents which are considered to be a reliable source of information for the indoor environment studies (Naydenov et al., 2008). Also, multiple important confounders such as family socioeconomic status, history of allergy, and school environment were controlled for in the analysis. As there was no other comprehensive survey of home characteristics conducted in Romania, our study may be the first to provide an overview of home characteristics that may be associated with potential health risks for the occupants. Although housing characteristics were unlikely to cause health effect on their own, they provide valuable information for future development of effective intervention strategies.

5. Conclusion

For Romanian homes, we reported a higher frequency of indoor smoking, more common use of gas heater and iron stove, and low use of mechanical ventilation and AC. In our study, exposure to ETS at home was found to be strongly associated with elevated risk of self-reported asthma-like and allergy symptoms among school-age children. Other factors that were associated with allergy symptoms in children included house-building type, use of incense stick, and living near a pesticide sprayed area. Potential risk factors for flu-like symptoms were dampness and mold issues, type of heating, use of AC, and use of incense sticks. Most of the home characteristics identified in this study are easy to remediate and can be used as indicators for developing specific intervention programs such as education programs to reduce indoor smoking and increase natural/mechanical ventilation.

Acknowledgements

This work was supported by SINPHONIE (*Schools Indoor Pollution and Health – Observatory Network in Europe*) a project carried out under contract (contract SANCO/2009/c4/04) for the European Commission, and funded by the European Parliament. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders. The funders played no role in the study design, data collection, analysis, and interpretation, in the writing of the report, or in the decision to submit this article for publication.

We would also like to thank the participants, whose generous time and effort made this study possible.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Appendix A

Table A.1
Definition of self-reported health outcomes and break-down of outcomes by occurrence location.

	Any location	Home	School	Other
	N (%)	N (%)	N (%)	N (%)
Asthma like symptoms				
Ever had an asthma attack (or wheezing or whistling in the chest) while at school?	2 (0.7)		2 (0.7)	
Dry cough	55 (19.6)	54 (19.3)	32 (11.4)	0 (0.0)
Difficult breathing	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Wheezing in the chest	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Difficult breathing with wheezing in the chest	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Allergy symptoms				
Skin rash on hands or forearms	6 (2.1)	5 (1.8)	1 (0.4)	0 (0.0)
Skin rash on face or neck	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)
Eczema	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Itching hands or forearms	6 (2.1)	5 (1.8)	1 (0.4)	0 (0.0)
Itching face or neck	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)
Burning eyes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Itching eyes	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)
Dry eyes	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sensation of “sand in the eyes”	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)
Red eyes	2 (0.7)	2 (0.7)	1 (0.4)	0 (0.0)
Swollen eyes	3 (1.1)	3 (1.1)	2 (0.7)	0 (0.0)
Itching or irritated nose	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sneezes	31 (11.1)	30 (10.7)	23 (8.2)	0 (0.0)
Bleeding nose	2 (0.7)	2 (0.7)	1 (0.4)	0 (0.0)
Dry throat	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)
Flu-like symptoms				
Runny nose	57 (20.4)	54 (19.3)	36 (12.9)	1 (0.4)
Stuffy or blocked nose	52 (18.6)	51 (18.2)	32 (11.4)	1 (0.4)
Sore throat	34 (12.1)	34 (12.1)	18 (6.4)	0 (0.0)
Feeling like getting a cold	7 (2.5)	6 (2.1)	4 (1.4)	1 (0.4)
Fatigue	1 (0.4)	1 (0.4)	0 (0.0)	0 (0.0)
Having a cold	11 (3.9)	9 (3.2)	5 (1.8)	1 (0.4)
Influenza or fever	7 (2.5)	7 (2.5)	0 (0.0)	0 (0.0)
Muscle pain				
Headache	8 (2.9)	7 (2.5)	4 (1.4)	0 (0.0)
Malaise	1 (0.4)	1 (0.4)	1 (0.4)	0 (0.0)

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Science of the Total Environment 560–561 (2016) 186–196

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Outdoor air pollution, meteorological conditions and indoor factors in dwellings in relation to sick building syndrome (SBS) among adults in China

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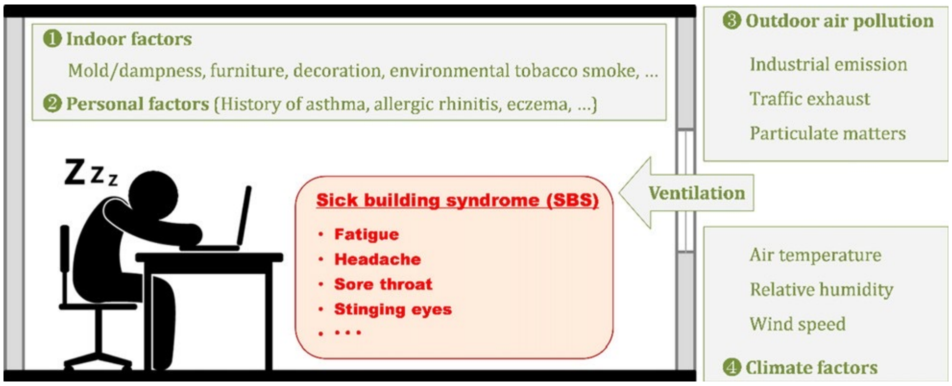
HIGHLIGHTS

- Mold/dampness and redecoration are important risk factors for SBS symptoms in adults.
- Improving indoor ventilation could reduce the risk of SBS symptoms.
- Association between indoor factors and SBS shows difference between genders.

GRAPHICAL ABSTRACT



article info

Article history:
Received 8 January 2016
Received in revised form 5 April 2016
Accepted 6 April 2016
Available online 19 April 2016

Editor: D. Barcelo

Keywords:
Outdoor air pollution
Climate change
Atopy
Mold and dampness

abstract

Indoor environment is associated with the sick building syndrome (SBS), but little is known about the contribution of outdoor air pollution and meteorological conditions to SBS. We studied associations between outdoor air pollution, meteorological parameters and selected indoor exposure and building characteristics at home and weekly SBS symptoms in a standardized questionnaire study among 3485 randomly selected adults in China. Outdoor factors included particulate matters with diameter b 10 μm (PM_{10}), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), outdoor temperature (T), relative air humidity (RH), and wind speed (WS) during last three months. Multiple logistic regression was applied calculating odds ratios (OR) with 95% confidence interval (95% CI). Asthma or allergic rhinitis (atopy) was associated with all types of SBS symptoms except fatigue. Indoor factors played a major role in SBS symptoms. Mold/dampness on the floor/ceiling was associated with fatigue OR = 1.60 (1.11–2.30) and headache OR = 1.80 (1.07–3.04). Moldy odor was associated with fatigue OR = 1.59 (1.07–2.37) and dermal symptoms OR = 1.91 (1.21–3.02). Window pane condensation in winter was associated with fatigue OR = 1.73 (1.30–2.31) and throat symptoms OR = 1.53 (1.01–2.31). Damp bed clothing was related with throat symptom OR = 1.62 (1.09–2.40). Home redecoration was associated with fatigue OR = 1.49 (1.07–2.06).

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http://dx.doi.org/10.1016/j.scitotenv.2016.04.033

Building ventilation
Redecoration

Frequent window opening was associated with less nose symptoms OR = 0.54 (0.36–0.82) and mechanical ventilation in the bathroom reduced dermal symptoms OR = 0.66 (0.44–0.99). Females were more susceptible to redecoration and window pane condensation than men. No associations with SBS were observed for outdoor air pollutants or meteorological parameters in the final models combining indoor and outdoor factors, although SO_2, T, and RH were associated with some SBS symptoms (fatigue, eyes and nose symptoms) in the separate outdoor models. In conclusion, indoor mold/dampness, air pollution from redecoration and poorer ventilation conditions in dwellings can be risk factors for SBS symptoms in an adult Chinese population, especially among females.

1. Introduction

Sick building syndrome (SBS) was defined as certain medical symptoms experienced by occupants in specific indoor environments, which includes symptoms of headache, fatigue and irritation in upper respiratory tract, nose throat, eyes, hand and or facial skin (WHO, 1983; Redlich et al., 1997). People in industrialized countries spend about 90% of their life indoors, mostly at home, which may result in a high risk of SBS in general population. SBS is suggested to be associated with personal and environmental factors (Norback, 2009). Although some personal factors, such as female gender, history of atopy/allergic disorders, and smoking status, have been widely reported for more SBS due to higher sensitivity (Björnsson et al., 1998; Brasche et al., 2001; Lim et al., 2015; Molina et al., 1993; Runeson et al., 2006), the prevalent complains of SBS from general populations (i.e. residences) are more likely to be related with the environmental issues.

Indoor environmental factors in relation to SBS have been largely investigated, especially for office environment. A number of studies have found some important indoor factors that are closely associated with SBS, such as building dampness, ventilation flow, volatile organic compounds (VOCs), mold or microbial contaminations, and indoor climate factors (room temperature and relative humidity) (Bakke et al., 2008; Brinke et al., 1998; Fang et al., 2004; Jaakkola et al., 1991; Molhave et al., 1993; Norback and Nordstrom, 2008; Reinikainen and Jaakkola, 2001; Sundell et al., 2011; Teeuw et al., 1994; Wolkoff, 2008; Zhang et al., 2012). Most previous studies on sick building syndrome (SBS) have dealt with symptoms among office workers (Apter et al., 1994), but only a few studies have addressed SBS in relation to the home environment. (Araki et al., 2010; Bornehag et al., 2003; Kanazawa et al., 2010; Kishi et al., 2009; Sahlberg et al., 2010; Sahlberg et al., 2013; Saijo et al., 2009; Takigawa et al., 2010). In the office environment, SBS may have important implications affecting productivity. However, since we spend most of our time at home the influence of the home environment on SBS deserves further attention (Norback, 2009). A few home environment studies with respect to SBS have been recently performed in China (Wang et al., 2013a; Wang et al., 2013b), but more investigations is needed since there is a big difference in home environments and climate among different areas in China.

Most previous studies on SBS have focused on indoor environment without considering outdoor air pollution or climate factors. However, with the rapid development of economy and urbanization during past decades, urban air pollution has been more and more serious and prevailed in many Chinese cities. In addition, extreme climate (i.e. heat waves) can be more prevalent due to global warming (Luber and McGeehin, 2008), which may cause a heavy burden and serious health risk in general population (Epstein, 2000; McMichael et al., 2006; Patz et al., 2005). Since the outdoor exposure may influence the indoor environment, the role of outdoor air pollution and meteorological conditions for SBS in dwellings should not be neglected. However, a few studies from China have examined associations between outdoor factors and SBS. A longitudinal study in China investigated associations between outdoor air pollution in schools (SO_2, NO_2, O_3, and PM_{10}) and SBS among pupils, and found that outdoor air pollution could increase the prevalence and incidence of SBS and decrease the remission rate (Zhang et al., 2014). However, there is no SBS study in relation to domestic exposure to outdoor air pollution in China, which warrants to be investigated.

In the present study, we hypothesized that there are associations between outdoor air pollution (PM_{10}, SO_2, and NO_2), climate factors (T, RH, and WS) and indoor exposures (mold/dampness, renovations, and ventilation conditions) and adult's SBS symptoms. This was investigated in a part of a large national multi-centre study "China, Children, Homes, Health (CCHH)" (Zhang et al., 2013). Our aim was to investigate: (1) Whether outdoor air pollution and meteorological parameters exposure during past three months is associated with weekly SBS symptoms in adults (2) Which indoor exposures in dwellings are associated with SBS symptoms? (3) Whether there is interaction between gender and environmental exposures in relation to SBS symptoms.

2. Material and methods

2.1. Ethics statement

We sent an information letter to the parents' children's kindergarten addresses, along with the questionnaire, stating that if they answered and returned the questionnaire, they were thereby given their informed consent. The nature and possible consequences of the study were explained to the subjects before the study began. The questionnaire study and the exposure measurements in the kindergartens had been granted permission by the principal of each kindergarten and the teachers of each kindergarten involved in the study. The study protocol and the informed consent procedure were reviewed and approved by the Ethical Committee of the Medical Faculty of Central South University, Changsha, China which did not require a written consent since the study did not include any clinical tests. In addition, the study was approved by the health department and school board of each kindergarten.

2.2. Study population

The present study is a part of the multi-cities project (CCHH) study on asthma and allergies among preschool children in relation to the home environment in China. The participants in this study are the parents who filled in the questionnaire about their own health and the child's health (one person per family). The questionnaires asked about sick building syndrome (SBS) symptoms for the person who filled the questionnaire, as well as their gender, smoking habits and asthma/allergy (atopy). In this publication we do not use any data about the child. The questionnaire was mostly answered by the child's parents, but in a few cases by grandparents, or others persons. We have restricted our study to the parents.

The study was conducted between September 2011 and January 2012 in 36 kindergartens in Changsha, the capital city of Hunan Province in south-central China, having a population of 7.22 million inhabitants and covering an area of 1,909 km^2. Changsha has a humid subtropical climate with an annual mean temperature of 17 °C, and a monthly mean of 4.6 °C in January and 29 °C in July. The four seasons are distinct. The summers are very hot and winter is chilly. Autumn is the driest season. There are five main districts in Changsha (Furong, Kaifu, Tianxin, Yuhua, and Yuelu). We randomly selected 36 kindergartens from all five districts, which would like to participate in our survey, and these kindergartens were almost evenly distributed in Changsha. Then we randomly selected children from each kindergarten. The number of the questionnaires sent to the kindergartens was based on the total number of children in each kindergarten, China. The percentage of distributed questionnaires were 20% for Furing district, 28% for Kaifu, 14% for Tianxin, 16% for Yuhua and 21% for Yuelu. Detailed information and map of the location of the 36 kindergartens has been previously published (Deng et al., 2015).

A Chinese version of the standard questionnaire designed by the International Study of Asthma and Allergies in Childhood (Asher et al., 2006), with some changes to address housing and cultural characteristics in China, was administered to collect information on health status of children and family members. Each participant was asked to write the date when the questionnaire was answered. A total of 4,988 questionnaires were randomly distributed to the parents whose children were in 36 participating kindergartens (Deng et al., 2015). Parents or other guardians were instructed to complete the questionnaire and to return it to kindergartens within one week. We received 3,897 completed questionnaires and the overall response rate was 78%. We first excluded the questionnaires filled by grandmothers (n = 104), grandfathers (n = 89), others (n = 23), and those without information on who answered the questionnaire (n = 110), and thus selected 3571 questionnaires filled by the child's father (n = 831) or mother (n = 2740). Then, we excluded 96 subjects who did not answer the questions on their children's sex (n = 10) and age (n = 86) since they were not considered as reliable. Finally, 3485 parents (one parent per child aged 1–8 years old) were included in this study.

2.3. Assessment of sick building syndrome (SBS) symptoms

The symptoms of sick building syndrome (SBS) during past 3 months included three groups: general symptoms (fatigue, and headache); mucosal symptoms (itching, burning or irritation of the eyes, irritating, stuffy or runny nose, and hoarse, dry throat); dermal symptoms (any symptom among dry or flushed facial skin, scaling/itching scalp or ears, and hands dry, itching, red skin). There were three options to choose for each symptom: weekly/sometimes/never. Any SBS symptom was defined as the subjects who reported an occurrence of weekly symptom.

2.4. Assessment of personal factors

Potential confounding variables obtained from the parent administered questionnaires included the parent's gender, current smoking, and history of atopy. Asthma and rhino-conjunctivitis are related to an atopic background, and thus the question asking "Have you ever been diagnosed as having allergic asthma, or nose/eyes allergy by a doctor?" was used to define history of atopy in the present study.

2.5. Assessment of exposure to outdoor air pollutants and meteorological factors

We examined three pollutants, sulfur dioxide (SO_2), nitrogen dioxide (NO_2) and particles b 10 μm (PM_{10}) to represent ambient air pollution in Changsha where SO_2 was used as an indicator of industry-related air pollution, NO_2 as traffic-related air pollution, and PM_{10} as a surrogate of complex mixture of air pollutants (Kan et al., 2012). Daily 24-hour average ambient concentrations of PM_{10}, SO_2 and NO_2 were obtained from 7 municipal air pollution monitoring stations during the period from June to December 2011, that is, a period that encompassed the periods for all the subjects in the present study. Measurements at the monitoring stations strictly followed the standard methods set by the State Environmental Protection Agency of China: PM_{10} by a tapered element oscillating microbalance (TEOM1400, Rupprecht & Patashnick, USA), SO_2 by ultraviolet fluorescent method (ML/EC9850, Ecotech, Australia) and NO_2 by the chemiluminescent method (ML/EC9841B, Ecotech, Australia). The detailed information of the monitoring stations and surveyed kindergartens was provided in our previous study (Deng et al., 2015).

We used an inverse distance weighted (IDW) method (Bell, 2006; Marshall et al., 2008) to estimate the air pollution concentrations at the kindergartens based on the data at the monitoring stations. The concentration at each kindergarten was calculated by the concentrations at the closest four monitoring stations, with an inverse of the squared distance ($1 / d^2$) used as the weighting function. At first, we used the available daily (24-h) average concentrations at 7 monitoring stations to obtain the daily mean concentrations at 36 kindergartens. Then the individual three months mean concentration of PM_{10}, SO_2 and NO_2 for each person was calculated, based on the date when the questionnaire was filled in (counting daily means 3 months back) for the particular kindergarten. The exposure of the subjects was calculated in terms of the air pollutant concentrations at their children's kindergartens, because parents usually enrolled their children in the kindergarten nearest to their home and hence exposure at home was considered to be the same as that at the kindergarten.

We selected three meteorological parameters, temperature (T), relative humidity (RH), and wind speed (WS) to present the climate factors in this city. Daily meteorological data were obtained from the website of the Weather Underground (http://wunderground.com). There was only one monitoring station for meteorological parameters in the city. Individual mean values for T, RH and WS were calculated based on the date when the questionnaire was filled in (counting daily means 3 months back).

2.6. Assessment of exposure to indoor factors in the home environment

Indoor factors included four groups as follows, and the question regarding each factor is shown in Table S1:

- Building characteristics: building age and house size.
- Mold/dampness factors: mold/damp stains on floor/ceiling, moldy odor, dampness on bed/clothing, window pane condensation in winter, and water damage.

Furthermore, to address the dose-response relationships between symptoms and exposure to mold/dampness factors, a building dampness score (0–5) was constructed. Each "yes" or "frequently" responses to each factor was coded as "1", and each "no" or "never/sometimes" response was coded as "0". The total score was obtained by adding the score of above 5 factors together for each subject, and divided into four categories: 0 (no factor), 1 (1 factor), 2 (2 factors) and N2 (3, 4, and 5 factors).

In addition, another mold/dampness factor was also considered defined by "putting bedding in sunshine". Putting bedding into sunshine is a Chinese habit aiming to reduce humidity in the bed. Not putting the bedding to sunshine was considered an indoor dampness factor but is not primarily a building factor.

- Indoor air pollution: new furniture, redecoration, and environmental tobacco smoke (ETS) at home.
- Ventilation factors: window opening habits, mechanical ventilation in kitchen, and exhaust fan in bathroom.

2.7. Statistical analysis

Firstly, we used multiple logistic regression models to evaluate the associations between SBS symptoms and outdoor air pollutants (PM_{10}, SO_2, and NO_2) and meteorological parameters (T, RH, and WS) by adjusting for personal factors (gender, current smoking and a history of atopy). We used both normal multiple logistic regression and multi-level multiple logistic regression. Two levels were used, individual level and kindergarten level. Secondly, we used multiple logistic regression models to examine the associations between SBS symptoms and indoor exposures including building characteristics (building age, and house size), indoor mold/dampness (mold/dampness stains on floor/ceiling, moldy odor, dampness on bed/clothing, putting bedding in sunshine, window pane condensation in winter, and water damage) and dampness score (score = 0, 1, 2, and >2), indoor air pollution (new furniture, and redecoration at home), and indoor ventilation condition (window opening habits, mechanical ventilation in kitchen, and exhaust fan in bathroom). Thirdly, we used stepwise regression model to investigate the associations between SBS symptoms and all the outdoor and indoor factors to rank the effects of these factors. Then, we performed the final multiple regression models (final model) by using the combined personal factors and the significant factors analyzed in the stepwise model. In addition Wald chi-square was calculated. Since results concerning outdoor factors were almost identical between logistic regression and multi-level logistic regression, we used normal logistic regression in the final models including indoor and outdoor factors. Finally, interactions between gender index and exposures to outdoor and indoor factors and were investigated by including an interaction term into the final model (Melén et al., 2004), which represents the differences in the estimated effects of exposure between the male and female gender.

We assess the association between each SBS symptom and air pollutants and meteorological parameters by per IQR increase in the exposure level. The group of dampness score = 0 was applied to reference, and the odds ratios of the groups of 1, 2, and >2 (2–5) dampness scores were analyzed. Results of the regression analysis were interpreted by odds ratio (OR) with 95% confidence interval (95% CI) where p value < 0.05 was considered statistically significant. p values of interaction < 0.1 was considered in the present study. All statistical analyses were performed by SPSS software (version 16.0) except multi-level logistic regression which was performed with STATA 13.0 statistical package.

3. Results

3.1. Personal characteristics and symptoms

A total of 76.8% of the participants were females, 12.8% were current smokers and 6.2% had a history of atopy (allergic asthma or allergic rhino-conjunctivitis). Most of the current smokers were males, 50.7% of the males but only 1.2% of the females were smokers ($p < 0.001$). Of 3485 respondents, 489 (14.6%) reported weekly fatigue and 132 (4.2%) weekly headache. Mucosal and dermal symptoms were less common than general symptoms, with 102 (3.2%) reported for weekly eyes symptoms, 106 (3.4%) for weekly nose symptoms, 154 (4.8%) for weekly throat symptoms, and 137 (4.3%) for any weekly dermal symptoms. Table 1 shows the prevalence of each SBS symptom stratified by the personal factors. Females had more headache and eyes symptoms than males. Subjects with a history of atopy had higher prevalence of all the SBS symptoms as compared to those without atopy. However, no significant associations between subject's smoking habit and SBS symptoms were observed except for a borderline significance for eyes symptom.

3.2. Descriptive data on outdoor air pollution and meteorological data

Table 2 summarizes the statistics of exposure levels of outdoor air pollution and meteorological parameters during past three months. The average individual exposures (mean ± SD) to PM_{10}, SO_2 and NO_2 were 80 ± 13 μg/m^3, 31 ± 5 μg/m^3, and 40 ± 5 μg/m^3 μg/m^3, respectively. Interquartile range (IQR) of PM_{10} was the largest, but the values for the other two pollutants were similar during the exposure period. The average individual exposures to T, RH and WS were 25.2 ± 2.9 °C, 75.6 ± 1.3%, and 2.4 ± 1.5 m/s, and the IQR were 5 °C, 2%, and 0.83 m/s, respectively.

3.3. Descriptive data on indoor environment

Table 3 presents the statistics of exposure to indoor factors. Less than half of the participants lived in an older house with building age larger than 10 years; and one third of people lived in a home with an area ≥ 75 m^2. For indoor mold/dampness exposure, only few homes had mold/damp stains on floor/ceiling, moldy odor, or water damage; less than half observed dampness on bed/clothing; half found window pane condensation in winter; and most of the subjects put bedding in sunshine frequently to reduce mold/dampness. About one third did not observe any signs of mold/dampness in their home; slight more than one third reported one sign and slightly less than one third reported more than one mold/dampness sign. For indoor air pollution exposure, slightly more than one third of the families had installed new furniture since their children older than 1 year; only a small part had

Table 1
Number and prevalence of sick building symptoms stratified by demographic information on participating parents (n = 3485).

SBS symptoms	Frequency	Total n = 3485 (%)	Gender			History of atopy			Current smoking		
			Male (n = 808) (%)	Female (n = 2677) (%)	p	No (n = 3268) (%)	Yes (n = 217) (%)	p	No (n = 3010) (%)	Yes (n = 440) (%)	p
General symptoms											
Fatigue	Never/sometimes	2862 (85.4)	86.0	85.2	0.589	86.0	75.9	<0.001	85.4	85.4	0.982
	Weekly	489 (14.6)	14.0	14.8		14.0	24.1		14.6	14.6	
Headache	Never/sometimes	3047 (95.8)	97.8	95.3	0.003	96.1	92.0	0.005	95.7	97.1	0.175
	Weekly	132 (4.2)	2.2	4.7		3.9	8.0		4.3	2.9	
Mucosal symptoms											
Itching, burning or irritation of eyes	Never/sometimes	3045 (96.8)	98.2	96.3	0.014	97.4	87.1	<0.001	96.5	98.4	0.048
	Weekly	102 (3.2)	1.8	3.7		2.6	12.9		3.5	1.6	
Irritating, stuffy or runny nose	Never/sometimes	3035 (96.6)	97.8	96.3	0.054	98.1	76.0	<0.001	96.5	97.7	0.248
	Weekly	106 (3.4)	2.2	3.7		1.9	24.0		3.5	2.3	
Hoarse, dry throat	Never/sometimes	3024 (95.2)	95.9	94.9	0.308	95.5	90.4	0.001	95.2	94.6	0.613
	Weekly	154 (4.8)	4.1	5.1		4.5	9.6		4.8	5.4	
Any dermal symptom	Never/sometimes	3042 (95.7)	96.6	95.4	0.157	96.1	90.1	<0.001	95.6	95.8	0.858
	Weekly	137 (4.3)	3.4	4.6		3.9	9.9		4.4	4.2	

1) N and %(within the parentheses) of responses on reported diseases and symptoms in different stratified group were calculated when the missing data was excluded.
2) p-value was calculated by chi-squared test on prevalence of diseases and symptoms between different stratified groups.
3) Bold p-values indicate p.

Table 2
Descriptive statistics of calculated individual air pollution exposure levels and meteorological parameters during last three months (n = 3.485).

	Mean ± SD	Minimum	25th percentile	50th percentile	75th percentile	Maximum
Air pollution exposure						
PM_{10}	80 ± 13	53	73	79	85	121
SO_2	31 ± 5	22	28	30	33	51
NO_2	40 ± 5	31	36	40	42	63
Meteorological parameters exposure						
T	25.2 ± 2.9	13.9	22.6	26.3	27.7	28.0
RH	75.6 ± 1.3	74.1	74.5	75.4	76.9	78.3
WS	2.4 ± 1.5	0.83	1.4	1.7	2.2	6.4

Abbreviations: SD, standard deviation; PM_{10} (μg/m^3), particulate matter ≤ 10 μm in aerodynamic diameter, SO_2 (μg/m^3), sulfur dioxide; NO_2 (μg/m^3), nitrogen dioxide; T (°C), temperature; RH (%), relative humidity; WS (m/s), wind speed.

redecorated their house; and two thirds had residences who smoked at home. For indoor ventilation conditions, nearly two thirds of the subjects opened windows frequently; and most homes had mechanical ventilation in the kitchen and exhaust fan in the bathroom.

3.4. Correlation between outdoor air pollution and meteorological parameters

Table 4 showed Pearson correlations between air pollutants and meteorological parameters exposures during past three months calculated from the date when answered the questionnaire. Due to high correlation between T and RH, we adjusted for T and WS but not RH when investigating associations between air pollutants and SBS symptoms. Furthermore, in the regression models for meteorological conditions, we did not adjusted for air pollutants because of high correlations between air pollutants.

3.5. SBS symptoms in relation to outdoor factors

Table 5 provides effect estimates of SBS symptoms by per IQR increase of PM_{10}, SO_2, and NO_2 after adjusting for personal factors. Significant association was observed between outdoor SO_2 exposure and nose symptoms, with OR (95% CI) of 1.32 (1.01, 1.72). Outdoor T was positively associated with eyes symptoms, whereas RH was negatively related with fatigue symptom.

3.6. Correlation between outdoor and indoor factors

Table 6 showed Pearson correlations between outdoor and indoor factors. SO_2 and T were selected to indicate outdoor air pollution and meteorological factors due to their relatively stronger associations with SBS symptoms. Due to low correlations of each factor among or between outdoor and indoor factors, we could include both indoor and outdoor factors in the models.

3.7. SBS-symptoms in relation to indoor factors

Table 7 provides the associations between indoor factors exposure and SBS symptoms. Subjects living in a house with older age or smaller size had lower risk of weekly fatigue than those living in a house with younger age or larger size. For mold/dampness factors, mold/dampness on floor/ceiling, moldy odor, and dampness on bed/clothing were positively associated with most of the symptoms; window pane condensation was related with fatigue and throat symptoms; and water damage was associated with eyes symptoms. We found that the habit of putting bedding in sunshine significantly decreased the risk of fatigue and nose symptoms. Moreover, people who lived in a home with more than one sign of mold/dampness (dampness score ≥ 2) were more likely to have SBS symptoms than those with one sign (dampness score = 1) as compared to homes with no mold/dampness. For indoor air pollution, new furniture and redecoration was associated with increased risk of fatigue, and ETS exposure at home was mainly related with nose and throat symptoms. For ventilation conditions, frequent opening windows significantly decreased the risk of fatigue, eyes, and nose symptoms compered to never/sometimes opening windows. The use of exhaust fan in bathroom significantly reduced the risk of dermal symptoms. However, no associations were observed between mechanical ventilation in kitchen and SBS symptoms.

Table 3
Descriptive statistics of indoor factors (n = 3.485).

Indoor factors	Variable	Number	Percentage (%)
Building characteristics			
Building age (year)	b10	1867	55.3
	≥10	1508	44.7
House size (m^2)	b75	1051	30.6
	≥75	2354	69.4
Mold/dampness			
Mold/damp stains on floor or ceiling	No	2870	87.6
	Yes	408	12.4
Moldy odor	No	2887	88.2
	Yes	386	11.8
Dampness on bed/clothing	No	1935	56.3
	Yes	1504	43.7
Window pane condensation in winter	No	1110	48.5
	Yes	1181	51.5
Water damage	No	2914	89.5
	Yes	343	10.5
Putting bedding in sunshine	Never/sometimes	1368	39.7
	Frequently	2074	60.3
Dampness score[a]			
0	No variable	1167	33.6
1	1 variable	1267	36.5
2	2 variables	664	19.1
N2	N2 variables	377	10.8
Indoor air pollution			
New furniture (after the child N 1 year)	No	1870	61.4
	Yes	1175	38.6
Redecoration (after the child N 1 year)	No	2382	80.8
	Yes	567	19.2
ETS at home[b]	No	1146	33.2
	Yes	2304	66.8
Ventilation			
Window opening habits	Never/sometimes	1250	36.5
	Frequently	2173	63.5
Mechanical ventilation in kitchen	No/only fan	1140	33.4
	Smoke exhaust ventilator	2277	66.6
Exhaust fan in bathroom	No	685	20.2
	Yes	2711	79.8

[a] Dampness score was defined by the cumulated number of presence of the mold/dampness variables among mold/damp on floor/ceiling, moldy odor, dampness on bed/clothing, window pane condensation in winter, and water damage.

[b] Any people among the child's mother, father, grandmother, grandfather, brothers or sisters of the child, and others living at home smokes.

Table 4
Correlation between calculated individual levels of air pollutants and meteorological parameters during last three months (n = 3685).

	PM_{10}	SO_2	NO_2	T	RH	WS
PM_{10}	1	0.597**	0.824**	−0.601**	0.546**	−0.103**
SO_2		1	0.470**	−0.319**	0.192**	−0.164**
NO_2			1	−0.693**	0.550**	−0.035*
T				1	−0.921**	0.209**
RH					1	−0.220**
WS						1

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Table 5
Associations of exposure to outdoor air pollution and meteorological parameters during last three months with weekly SBS symptoms by 2-level hierarchic (child, kindergarten) multiple logistic regression models (n = 3.485).

	IQR	General symptoms		Mucosal symptoms			Dermal symptoms[a]
		Fatigue	Headache	Itching, burning or irritation of eyes	Irritating, stuffy or runny nose	Hoarse, dry throat	Any weekly dermal symptom
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Three months exposure to air pollution[b]							
PM_{10}	12 ($\mu g/m^3$)	0.92 (0.80, 1.06)	0.92 (0.75, 1.13)	0.94 (0.73, 1.19)	1.18 (0.92, 1.51)	1.00 (0.82, 1.21)	0.98 (0.79, 1.21)
SO_2	5 ($\mu g/m^3$)	0.97 (0.86, 1.11)	0.84 (0.69, 1.02)	0.91 (0.71, 1.17)	1.32 (1.01, 1.72)*	1.13 (0.93, 1.38)	1.07 (0.87, 1.32)
NO_2	6 ($\mu g/m^3$)	1.05 (0.87, 1.27)	0.90 (0.68, 1.19)	0.99 (0.72, 1.38)	1.21 (0.87, 1.69)	1.13 (0.87, 1.47)	1.12 (0.84, 1.51)
Three months exposure to meteorological parameters[c]							
T	5 (°C)	1.20 (0.96, 1.53)	1.04 (0.76, 1.42)	1.51 (1.02, 2.24)*	0.96 (0.67, 1.37)	1.15 (0.86, 1.54)	0.99 (0.72, 1.35)
RH	2 (%)	0.82 (0.68, 0.99)*	1.06 (0.80, 1.40)	0.72 (0.52, 1.01)	1.02 (0.74, 1.41)	0.81 (0.62, 1.06)	0.95 (0.71, 1.26)
WS	0.83 (m/s)	1.02 (0.97, 1.08)	0.99 (0.90, 1.09)	1.01 (0.90, 1.13)	0.99 (0.88, 1.12)	0.94 (0.85, 1.03)	1.07 (0.98, 1.17)

ORs are calculated per IQR increase of calculated individual exposure.

[a] Dermal symptoms is defined as any weekly dermal symptom among dry/flushed facial skin, scaling/itching scalp or ears, and hands dry/itching/red skin.

[b] Adjusted for gender, current smoking, history of atopy, T, and WS during last three months.

[c] Adjusted for gender, current smoking, and history of atopy.

* $p < 0.05$.

3.8. *SBS-symptoms in relation to outdoor and indoor factor in final models*

Table 8 provides the associations between both outdoor and indoor factors and SBS symptoms. We observed associations mainly for indoor factors and SBS symptoms in the final models. Atopy was significantly associated with all SBS symptoms except fatigue, while no significant association was observed for gender and current smoking with any symptoms. In addition, history of atopy ranked as the top factor with an especially high Wald value for nose symptom (170.10). Mold/dampness on floor/ceiling was associated with general SBS symptoms including fatigue and headache (p = 0.012 and 0.028, respectively); moldy odor was related with fatigue and dermal symptoms (p = 0.021 and 0.006, respectively); window pane condensation in winter was associated with fatigue and throat symptoms ($p < 0.001$ and 0.045, respectively); and dampness on bed/clothing was related with throat symptom (p = 0.018). Among indoor air pollution, redecoration exposure was associated with fatigue (p = 0.017). Among indoor ventilation factors, frequent opening windows and the use of exhaust fan in the bathroom was negatively associated with nose symptoms (p = 0.004) and dermal symptoms (p = 0.045). However, we did not find any associations for outdoor air pollutants or meteorological parameters.

4. Discussion

The present study regarding associations between SBS symptoms in adults and both outdoor and indoor factors in dwellings showed that indoor factors, such as indoor mold/dampness, air pollution emitted from renovations, and ventilation, were associated with weekly SBS symptoms in general populations. A history of atopy had the strongest association with most SBS symptoms. Mold/dampness factors were associated with most SBS symptoms including fatigue, headache, throat, and dermal symptoms. Redecoration was associated with fatigue only. Ventilation factors including window opening habits and exhaust fan in bathroom were associated with nose and dermal symptoms. However, no significant associations with SBS symptoms were detected for outdoor air pollution or meteorological conditions. Moreover, our study indicates that females can be more sensitive than males to the effects of indoor redecoration and window pane condensation in winter with respect to fatigue and headache.

In our study, general symptoms were more common than mucosal and skin symptoms, which is consistent with another CCHH study from Chongqing, China (Wang et al., 2013a). The prevalence of general symptoms including weekly fatigue and headache in our study were about 1.7 and 2.2 times higher than those in their study. Prevalence of mucosal symptoms (itching/burning/irritation of eyes, irritating/study/runny nose, and hoarse/dry throat) in our study was about 1.5 times higher than their study. In contrast, the prevalence of weekly dermal symptoms in our study (4.3%) was similar as in the Chongqing study (4.4%). In the present study, females did not have significantly more headache, eyes, and nose symptoms than males. This result is consistent with other Chinese studies of both adults and high school pupils' SBS symptoms which showed only a small difference between genders (Wang et al., 2013a; Zhang et al., 2012).

This study, to our knowledge, is one of few studies investigating association between SBS symptoms and outdoor air pollution and climate factors. A strength of our study is that outdoor exposure levels, mean values for the 3 months recall period of the SBS questions, was calculated for each participant, based on information on when the questionnaire was answered as well as calculated air pollution levels for the particular kindergarten. A recent Chinese study regarding association between SBS and both outdoor air pollution and climate among pupils have found that outdoor SO_2 was positively associated with new onset of skin symptoms, while NO_2 and PM_{10} were positively associated with new onset of skin, general and mucosal symptoms. We found positive associations between nose symptoms and SO_2 but the association was not significant in the final models adjusting for indoor factors.

Our finding that a history of asthma and allergies was strongly associated with most SBS symptoms is consistent with previous studies (Björnsson et al., 1998; Lim et al., 2015; Menzies et al., 1998; Molina et al., 1993; Runeson et al., 2006; Sahlberg et al., 2012). However, some studies did not find any association (Bakke et al., 2008; Gomzi et al., 2008). Significantly higher prevalence of SBS symptoms among subjects with atopy than those without atopy may be due to active allergic inflammation (Lim et al., 2015). We found gender differences for headache, eye symptoms and nose symptoms in the initial analysis but none of these gender differences were statistically significant in the final models. A Swedish study found that females had a higher prevalence of SBS symptoms than men but these differences were not statistically significant when adjusting for differences in allergy to nickel, non-specific airway hyperactivity and proneness to infections (Norbäck and Edling, 1991). However, many studies have found that females have more SBS symptoms than men (e.g. Runeson et al., 2006; Björnsson et al., 1998; Engvall et al., 2010; Jaakkola et al., 1991). It has been suggested that women suffer more SBS than men independent of personal, but most related to work and building factors (Brasche et al., 2001). However, the interaction analysis in our study indicated that females could be more susceptible to the effects of indoor redecoration and window pane condensation in winter on fatigue and headache symptoms respectively as compared to males, with ORs (95% CI) =

Table 6
Pearson correlations between outdoor and indoor factors (n = 3.485).

	SO2	T	Building age	House size	Window opening	Mold/ dampness	Mold odor	Dampness on clothing/bed	Put bedding in sunshine	Window pane condensation	Water damage	New furniture	Redecoration	ETS
SO2	1	−0.319**	0.175**	0.111**	−0.053**	−0.012	−0.008	−0.028	−0.002	−0.076**	−0.005	−0.041*	−0.031	0.017
T		1	−0.050**	−0.096**	0.072**	0.011	0.019	0.055**	0.024	0.051**	−0.002	−0.013	−0.039*	−0.062**
Building age			1	0.420**	0.009	0.073**	0.103**	0.030	0.022	−0.096**	0.013	−0.170**	−0.182**	0.078**
House size				1	−0.016	0.069**	0.103**	0.039*	−0.007	−0.098**	0.028	−0.128**	−0.113**	0.045**
Window opening					1	−0.016	−0.018	−0.002	0.080**	−0.038*	−0.001	0.024	0.024	−0.038*
Mold/dampness						1	0.241**	0.208**	−0.028	0.103**	0.134**	−0.070**	−0.053**	0.007
Mold odor							1	0.231**	−0.065**	0.091**	0.123**	−0.027	−0.028	0.033
Dampness on bed/clothing								1	−0.035*	0.203**	0.086**	−0.016	−0.042*	0.002
Put bedding in sunshine									1	−0.035*	−0.084**	−0.057**	−0.023	−0.015
Window pane condensation										1	0.101**	0.060**	0.017	−0.025
Water damage											1	0.025	−0.013	0.024
New furniture												1	0.499**	−0.026
Redecoration													1	−0.028
ETS														1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

1.85 (1.28, 2.69) vs. 0.74 (0.35, 1.55) (interaction p = 0.03) and 1.75 (1.07, 2.85) vs. 0.69 (0.22, 2.14) (interaction p = 0.09) (data not shown in the tables).

About half of the homes were new, constructed the last 10 years, and most had an area of b 75 m^2. Indoor mold and dampness was very common, 66% had at least one such sign. Dampness on bed clothing (56.3%) and window pane condensation in winter (51.5%) were most common while visible mold/damp stains (12.4%), moldy odor (11.8%) and water damage (10.5%) were less common. The habit to frequently put bed clothing in the sunshine was very common (60.3%). Putting bedding into sunshine is a Chinese habit aiming to reduce the humidity in the bed. Environmental tobacco smoke at home was very common, 66.8% reported that tobacco smoking occurred indoors. In addition new furniture, which can emit formaldehyde, was very common (38.6%) as well as recent redecoration of the home (19.2%). The most common method to increase the ventilation was frequent window opening (63.5%) but exhaust fan in the bathroom was very common as well (79.8%).

Our findings that that indoor mold/dampness factors, including mold/dampness on floor/ceiling, moldy odor, window pane condensation in winter, dampness on bed/clothing, and putting bed in sunshine, were associated with most SBS symptoms is consistent with previous studies regarding associations between dampness factors and SBS symptoms. A number of studies found that an increased risk of SBS was associated with visible mold, mold/damp spots, and moisture problem in floors in dwellings (Bornehag et al., 2003; Kishi et al., 2009; Saijo et al., 2009; Wang et al., 2013a; Wang et al., 2013b). A home study from Japan found that an increased concentration of "Microbial Volatile Organic Compounds" (MVOC) in house was significantly associated with mucosal symptoms (Araki et al., 2010). Some studies also found an association between moldy odor and SBS symptoms in home environment (Engvall et al., 2001; Engvall et al., 2002; Kishi et al., 2009; Wang et al., 2013b). A number of studies found that condensation on windows panes and/or walls in residential homes was associated with SBS symptoms (Bornehag et al., 2003; Engvall et al., 2001; Kishi et al., 2009; Saijo et al., 2009; Wang et al., 2013a; Wang et al., 2013b). In the present study, we observed an association between dampness on bed/clothing and hoarse/dry throat symptom, which has not been found in other studies. Some other CCHH studies in Chongqing, China have found that frequent putting bed in sunshine could significantly reduce the risk of SBS symptoms (Wang et al., 2013a; Wang et al., 2013b), which is confirmed by the present study. Moreover, some studies also found an association between dampness index/score and SBS symptoms (Engvall et al., 2001; Engvall et al., 2002; Kishi et al., 2009; Wang et al., 2013b), and these investigations indicate that the increased dampness index could enhance the risk of SBS symptoms. In addition, some Swedish studies found that dampness or indoor molds in the dwellings were associated with increased incidence of SBS symptoms (Sahlberg et al., 2010; Sahlberg et al., 2012). Microbial contaminations including mold, yeasts, wood-rooting fungi, and bacteria due to dampness or moisture damage have been commonly reported in home environments (Nevalainen et al., 1991), which could affect human health by a variety of biological mechanisms, including infections, allergic or hypersensitivity reactions, and irritant reactions. Some important allergens, such as house dust mites, cockroaches, cats or dogs commonly appeared in damp buildings have been suggested to be associated with allergic asthma, rhinitis eczema, and SBS symptoms in China (Li et al., 2009; Wang et al., 2013a; Zhang et al., 2011). Biological plausibility for associations between these bioaerosols and SBS symptoms have been provided by toxicological studies (Laumbach and Kipen, 2005). However, the extent to which bioaerosol exposure may explain the nonspecific symptoms of the condition is still unclear and warrants further investigations.

Our finding that home redecoration was associated with weekly general SBS symptom (fatigue) is agreement with other studies. Some recent Chinese studies found associations between home renovations including new furniture and redecoration and SBS symptoms (Wang

Table 7
Associations between exposure to indoor factors and weekly SBS symptoms (n = 3485).

Indoor factors	Variable	General symptoms		Mucosal symptoms			Dermal symptoms[a]
		Fatigue	Headache	Itching, burning or irritation of eyes	Irritating, stuffy or runny nose	Hoarse, dry throat	Any weekly dermal symptom
		OR[b] (95% CI)	OR[b] (95% CI)	OR[b] (95% CI)	OR[b] (95% CI)	OR[b] (95% CI)	OR[b] (95% CI)
Building characteristics							
Building age (year)	≥ 10 vs. b 10	0.80 (0.66, 0.98)*	1.07 (0.74, 1.54)	1.02 (0.67, 1.55)	0.96 (0.63, 1.48)	0.73 (0.52, 1.03)	0.82 (0.57, 1.17)
House size (m^2)	b 75 vs. ≥ 75	0.79 (0.63, 0.98)*	0.91 (0.61, 1.36)	0.76 (0.46, 1.24)	0.86 (0.53, 1.39)	0.92 (0.64, 1.33)	1.03 (0.71, 1.51)
Mold/dampness							
Mold/damp on floor/ceiling	Yes vs. no	1.64 (1.26, 2.14)***	1.89 (1.21, 2.94)**	1.64 (0.98, 2.75)	1.05 (0.57, 1.94)	1.65 (1.06, 2.55)*	1.78 (1.14, 2.78)*
Moldy odor	Yes vs. no	1.84 (1.41, 2.42)***	1.77 (1.10, 2.86)*	1.92 (1.14, 3.24)*	1.64 (0.90, 2.99)	1.91 (1.23, 2.95)**	2.08 (1.33, 3.25)(***)
Dampness on bed/clothing	Yes vs. no	1.47 (1.21, 1.79)***	1.43 (1.00, 2.04)*	1.65 (1.09, 2.50)*	0.94 (0.62, 1.43)	1.61 (1.16, 2.24)**	1.19 (0.84, 1.69)
Window pane condensation in winter	Yes vs. no	1.82 (1.41, 2.33)***	1.47 (0.95, 2.26)	1.65 (0.98, 2.77)	1.33 (0.79, 2.24)	1.72 (1.15, 2.59)**	1.39 (0.90, 2.14)
Water damage	Yes vs. no	1.12 (0.82, 1.53)	1.26 (0.74, 2.13)	2.14 (1.26, 3.62)**	1.36 (0.74, 2.52)	0.73 (0.40, 1.33)	1.41 (0.85, 2.34)
Putting bedding under sunshine	Yes vs. no	0.74 (0.61, 0.90)**	0.88 (0.62, 1.25)	0.81 (0.54, 1.22)	0.53 (0.35, 0.81)**	0.73 (0.53, 1.02)	0.74 (0.52, 1.05)
Dampness score							
0	No variable	1.00	1.00	1.00	1.00	1.00	1.00
1	1 variable	1.42 (1.10, 1.82)**	1.41 (0.87, 2.28)	1.61 (0.90, 2.89)	1.77 (1.02, 3.08)*	1.58 (1.00, 2.50)*	1.10 (0.70, 1.73)
2	2 variables	1.60 (1.20, 2.13)(***)	2.12 (1.29, 3.50)**	2.29 (1.25, 4.21)**	1.84 (1.01, 3.37)*	2.72 (1.70, 4.35)***	1.42 (0.87, 2.33)
N2	N2 variables	2.65 (1.94, 3.60)***	2.13 (1.20, 3.81)(**)	3.20 (1.67, 6.15)***	1.30 (0.60, 2.83)	2.32 (1.32, 4.07)**	2.23 (1.32, 3.77)**
Indoor air pollution							
New furniture	Yes vs. no	1.40 (1.14, 1.72)**	0.82 (0.55, 1.21)	1.30 (0.85, 1.99)	0.77 (0.48, 1.24)	1.08 (0.76, 1.54)	1.32 (0.91, 1.91)
Redecoration	Yes vs. no	1.45 (1.13, 1.86)**	1.02 (0.63, 1.64)	1.01 (0.59, 1.75)	0.85 (0.47, 1.53)	0.95 (0.61, 1.48)	1.32 (0.86, 2.03)
ETS at home	Yes vs. no	0.93 (0.75, 1.16)	1.34 (0.89, 2.02)	1.10 (0.70, 1.71)	1.97 (1.19, 3.26)**	1.75 (1.16, 2.62)**	1.19 (0.80, 1.77)
Ventilation							
Window opening habits	F vs. N/S	0.80 (0.66, 0.98)*	0.98 (0.68, 1.42)	0.65 (0.44, 0.98)*	0.55 (0.36, 0.83)**	0.85 (0.61, 1.19)	1.04 (0.72, 1.48)
Mechanical ventilation in kitchen	Yes vs. no	1.20 (0.97, 1.49)	1.00 (0.69, 1.46)	0.87 (0.57, 1.34)	1.24 (0.79, 1.95)	1.10 (0.78, 1.58)	0.94 (0.65, 1.36)
Exhaust fan in bathroom	Yes vs. no	0.87 (0.69, 1.11)	0.77 (0.51, 1.18)	0.78 (0.48, 1.27)	0.94 (0.55, 1.62)	1.00 (0.66, 1.52)	0.65 (0.43, 0.97)*

*p b 0.05, ** p b 0.01, (**) p = 0.01, *** p b 0.001, (***) p = 0.001.

[a] Dermal symptoms were defined by any weekly dermal symptom among dry/flushed facial skin, scaling/itching scalp or ears, and hands dry/itching/red skin.

[b] Adjusted for gender, current smoking, history of atopy, outdoor T, WS, and SO_2 exposure during last three months by multiple logistic regression models.

et al., 2013a; Wang et al., 2013b). Some Swedish studies suggested that emissions from newly painted indoor surfaces or renovations in dwellings were associated with SBS symptoms (Norbäck et al., 1996; Sahlberg et al., 2009; Sahlberg et al., 2012; Sahlberg et al., 2013). Previous experimental studies have found that complex mixtures of volatile organic compounds (VOCs) contributed to general symptoms including headaches, fatigue, and dizziness (Molhave, 1992; Molhave et al., 1993). A number of studies about SBS in the home environment from Japan found that recent indoor painting (Takaoka and Norback, 2011), chemicals emitted from renovations in newly built house (such as formaldehyde) (Takigawa et al., 2010), coating materials and volatile organic compounds (VOC) as well as semi-volatile organic compounds (SVOC) (Kanazawa et al., 2010; Nakayama and Morimoto, 2009) were all associated with the risk of SBS symptoms. Moreover, another Japanese study found significant associations between home-related mucous symptoms and some specific microbial volatile organic compounds MVOCs including 1-octen-3-ol and 2-pentanol (Araki et al., 2010). A study in Taiwan found that VOC was associated with urinary 8-OHdG which was significantly higher in participants with SBS symptoms than in those without such complaints (Lu et al., 2007).

Our finding that window opening habits and the use of exhaust fan in bathroom were associated with less nose and dermal symptoms is agreement with some previous studies regarding building ventilation and SBS symptoms. Building ventilation can be measured either as CO_2 levels, personal outdoor air flow or air exchange rate (Norback, 2009). In the present study, frequent opening windows and use of exhaust fan in bathroom could increase outdoor air flow and air exchange rate (Fabi et al., 2012), and thus dilute or remove CO_2 and other indoor air pollutants. A Swedish study has found that buildings with mechanical supply/exhaust ventilation, and consequently the highest airflow rate, had least dampness, which indicates that the good ventilation in dwellings could reduce the risk of SBS symptoms. A review study found that higher ventilation rates in offices, up to about 25 l/s per person (Sundell et al., 2011). This review emphasized the need for more

Table 8
Associations of exposure to outdoor air pollution and meteorological parameters during last three months with weekly SBS symptoms using stepwise multiple logistic regression models (n = 3.485).

	OR (95% CI)	Wald	p
Fatigue			
Window pane condensation in winter	1.73 (1.30, 2.31)	14.09	<0.001
Mold/dampness on floor/ceiling	1.60 (1.11, 2.30)	6.28	0.012
Redecoration	1.49 (1.07, 2.06)	5.68	0.017
Moldy odor	1.59 (1.07, 2.37)	5.33	0.021
WS (per 0.83 m/s)	1.07 (1.00, 1.14)	3.34	0.068
Dampness on bed/clothing	1.30 (0.98, 1.73)	3.24	0.072
History of atopy	1.56 (0.96, 2.54)	3.24	0.072
Gender	0.88 (0.60, 1.31)	0.39	0.533
Current smoking	0.96 (0.58, 1.59)	0.03	0.858
Headache			
History of atopy	2.43 (1.30, 4.53)	7.73	0.005
Mold/dampness on floor/ceiling	1.80 (1.07, 3.04)	4.84	0.028
Gender	2.01 (0.93, 4.38)	3.11	0.078
Window pane condensation in winter	1.47 (0.95, 2.28)	2.93	0.087
Current smoking	1.33 (0.52, 3.42)	0.36	0.551
Itching, burning or irritation of eyes			
History of atopy	5.50 (3.43, 8.84)	49.77	<0.001
Window opening habits	0.69 (0.46, 1.03)	3.31	0.069
Gender	1.75 (0.85, 3.59)	2.30	0.130
Current smoking	0.69 (0.25, 1.91)	0.51	0.477
Irritating, stuffy or runny nose			
History of atopy	16.56 (10.86, 25.26)	170.10	<0.001
Window opening habits	0.54 (0.36, 0.82)	8.26	0.004
Gender	1.71 (0.83, 3.52)	2.09	0.148
Current smoking	1.03 (0.40, 2.61)	0.00	0.957
Hoarse, dry throat			
Dampness on bed/clothing	1.62 (1.09, 2.40)	5.63	0.018
History of atopy	2.01 (1.09, 3.73)	4.95	0.026
Window pane condensation in winter	1.53 (1.01, 2.31)	4.00	0.045
Gender	1.68 (0.85, 3.32)	2.26	0.133
Current smoking	1.68 (0.75, 3.74)	1.59	0.208
PM_{10} (per 12 $\mu g/m^3$)	0.91 (0.75, 1.09)	1.07	0.301
Dermal symptoms[a]			
History of atopy	2.58 (1.51, 4.42)	11.87	0.001
Mold odor	1.91 (1.21, 3.02)	7.59	0.006
Exhaust fan in bathroom	0.66 (0.44, 0.99)	4.01	0.045
Gender	1.76 (0.93, 3.35)	2.99	0.084
Current smoking	1.58 (0.74, 3.37)	1.41	0.235

The models include variables retained in a stepwise logistic regression model (forward regression Wald statistics, $p < 0.10$). These variables were entered in a multiple logistic regression model (enter) adjusting for gender, current smoking, and history of atopy.

[a] Dermal symptoms is defined as any weekly dermal symptom among dry/flushed facial skin, scaling/itching scalp or ears, and hands dry/itching/red skin.

studies of the relationship between ventilation rates and health, especially in diverse climates, in locations with polluted outdoor air and in buildings other than office. It has been suggested that window opening behavior has the largest effect on air change rates, causing increases ranging from a few tenths of an air change per hour to approximately two air changes per hour (Wallace et al., 2002). One study stated that opening of a single window increased the air change rate by an amount roughly proportional to the width of the opening, reaching increments as high as 1.3 h^{-1}, and multiple window openings increased the air change rate by amounts ranging from 0.10 to 2.8 h^{-1} (Howard-Reed et al., 2002). Our finding highlights that frequent window opening and use of exhaust fan in bathroom could reduce SBS by increasing the ventilation flow.

There are several limitations of this study that should be acknowledged. Firstly, epidemiological studies can be affected by selection bias. In this study, we included parents (one per child) from a cross-sectional study, with no prior information on parents' health status. The sample size was reasonably large, and the response rate was good (78%) and the 36 kindergarten were from all five major districts of Changsha. Thus, selection bias is fairly unlikely but the proportion of males in the study population was only 23% since the mothers answered the questionnaire more often the fathers. However, since the study population consisted of young parents, expected to be mostly between 25 and 35 years old, results may not be representative for the entire adult population in Changsha. Moreover, information on socioeconomic status (SES) was limited and not used in this study since we had only self-reported data on the mother's occupation during pregnancy. Secondly, recall bias is another potential problem; subjects may overestimate or underestimate their personal symptoms and/or signs of indoor environment risk factors. Recall bias for moldy odor perceptions, and SBS symptoms should not be a big issue in this study, since the recall period was short (past three months). Information bias, in which subjects are aware that certain factors have previously been identified as risks, is another potential problem. However, the indoor factors studied in this paper (e.g. moldy odor), are not well known as creating indoor health problems among the Chinese population. The prevalence of weekly and sometimes perceptions of moldy odor perceptions were not high in our study (0.5% and 11.3%). Thirdly, we only had information on three air pollutants, PM_{10}, SO_2 and NO_2, to represent the ambient air pollution. In China, these pollutants have been paid great attention and have been routinely monitored and the levels have been published to the public (Kan et al., 2012). However, it is important to further investigate the roles of other pollutants, particularly $PM_{2.5}$, which can be a better proxy for traffic air pollution (Gehring et al., 2002; Hertz-Picciotto et al., 2007). Fourthly, exposure to air pollution was modeled by using an inverse distance weighted (IDW) method based on the data from ambient air quality monitoring stations. This modeling method may lead to bias of exposure because the number of monitoring stations was few and the sources of the air pollution and the land use conditions were ignored. Finally, as the first step of the nationwide project (CCHH), our study only included one city. Additional research in other cities is needed to confirm the persistence and generalizability of the results, given that the concentration levels of the air pollutants in our study were comparable to those recorded in a number of cities in China (Chan and Yao, 2008; Kan et al., 2012). Finally, the cross-sectional study design limits the possibility to draw conclusions on causality.

5. Conclusions

In conclusion, adults' SBS symptoms can be associated with a history of asthma and allergic rhinitis (atopy). Indoor factors, especially mold/dampness, could play an important role for SBS symptoms in Chinese adult residents in this area of China. Furthermore, indoor redecoration could increase fatigue, and high ventilation flow rate could reduce the risk of nose and dermal symptoms. Our results suggest that it is beneficial with respect to SBS symptoms to reduce household mold/dampness, control air pollution emissions from home renovations, and encourage improving building ventilation through frequent opening windows and use of an exhaust fan in bathroom.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.04.033.

Acknowledgements

The research was supported by the National Science Foundation of China (No. 51178466 and No. 51576214) and the Doctoral Program of Higher Education of China (No. 20120162110011).

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Indoor Air 2012; 22: 514–522
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INDOOR AIR
doi:10.1111/j.1600-0668.2012.00781.x

Microbiological evaluation of ten French archives and link to occupational symptoms

Abstract Fungi that damage documents in archives may harm workers' health, depending on which mold species are inhaled, the concentrations of fungal species inhaled, and individual factors. Our aim was to identify and quantify fungi in archives and to investigate possible links with the symptoms experienced by workers. Ten French archives were sampled using an air impactor and electrostatic dust collectors. Allergies and general symptoms felt by 144 workers were reported using a self-report questionnaire. Utilizing culture-based analysis methods along with qPCR, *Penicillium chrysogenum*, *Cladosporium sphaerospermum,* and *Aspergillus versicolor* were the three main fungi in air and dust in terms of quantity and frequency. Median fungal concentrations in storage areas, ranged from 30 to 465 CFU/m^3. People working in the most contaminated archives did not report more symptoms of allergy than others. However, workers in contact with moldy documents reported more headaches (odds ratio, 2.4; 95% confidence interval, 1.1–5.3), fatigue (OR, 2.9; 95% CI, 1.2–6.7), eye irritation (OR, 5.4; 95% CI, 1.9–14.9), throat irritation (OR, 2.4; 95% CI, 1.0–5.7), coughing (OR, 3.2; 95% CI, 1.2–8.4), and rhinorrhea (OR, 2.6; 95% CI, 1.0–6.4) than others. Other parameters such as dust levels and concentrations of metabolites and chemical substances should be considered as confounding factors in further investigations to isolate the role of molds.

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Key words: Archives; Airborne; Fungi; Respiratory symptoms; Allergy; Dust.

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Received for review 15 September 2011. Accepted for publication 9 March 2012.

Practical Implications

Most studies about fungi and archives deal with the conservation of manuscripts and documents, and few discuss workers' health problems. Our study shows that archives do not represent a highly contaminated environment. Symptoms felt by workers were more often linked to direct contact with moldy documents than to high concentrations of mold in the air of archive storage areas. This study provides data on concentration levels in archives that could be used to interpret microbiological investigations in this type of environment in the future.

Introduction

The desire to preserve historical records and to have access to knowledge has made the conservation and management of archive collections a major point of interest in all countries. Books and documents are subject to deterioration because of chemical (airborne pollutants), physical (light, temperature, and dampness), and biological (insects, rodents, fungi, etc.) agents. The presence of microfungi, i.e. molds, is a recurrent and major problem, and workers worry about the effects that mold exposure may have on their health.

Fungi are prevalent outdoors and enter archive buildings through doorways, windows, ventilation, air-conditioning and heating systems and can also be transported indoors by workers or by the collections themselves. If environmental conditions are favorable, fungi grow and proliferate. Molds essentially require moisture, nutrients (a source of carbon), and a suitable place to grow. The critical factor in mold development is water activity (A_W). The water activity in a substrate necessary for fungal growth ranges from 0.61 to 0.96 (Pitt and Hocking, 2009; Samson et al., 1995, 2011). Although they can survive at low temperatures, the optimal growth temperature for fungi is often between 18 and 25°C (De Hoog et al., 2001). To prevent mold growth in archives, the recommended temperature and relative humidity for archive storage areas are, respectively, 18 ± 2°C and 55 ± 5%. Below this humidity level, paper and some organic material-based artifacts can become desiccated and brittle.

Molds also have harmful effects on human health. In a review of the literature, Zyska (1997) estimated the number of librarians worldwide at one million and underlined the need for further investigations into the health effects of fungal exposure in libraries. Spores of molds are present everywhere, including on books, and it is not possible to eliminate them from the environment. Curators, conservators, and all workers in archives are therefore exposed to molds on a daily basis. High concentrations can lead to illness in sensitive individuals. For these people, molds can cause symptoms such as a runny nose, eye irritation, wheezing, coughing, nasal congestion, or skin irritation. In some people, fungi can cause respiratory and skin diseases, especially allergic skin diseases (Bush et al., 2006; Kuhn and Ghannoum, 2003; Gebbers and Glück, 2003). Most mold species associated with contaminated books and documents are potential allergens, and inhalation of their spores and mycelium fragments may be responsible for immune responses resulting in diseases such as allergic bronchopulmonary aspergillosis, hypersensitivity pneumonitis, asthma, rhinitis, allergic bronchitis, and perhaps even Sick Building Syndrome (Crook and Burton, 2010). Mycotoxins and microbial volatile organic compounds (MVOCs) produced by fungi and components of the cell wall such as b(1,3) glucans can also modulate allergic reactions (Straus, 2009; Schram et al., 2005). Inhalation of molds can occur if mold concentration in the air is increased or if people directly handle moldy materials (Singh et al., 1995). Mold concentrations in air increase when contaminated materials dry out, causing spores and other mold cells to be released into the air from where they may be subsequently inhaled.

The objective of this study was to evaluate fungi quantitatively and qualitatively in 10 French archives and investigate a possible link with the symptoms experienced by workers.

Material and methods

Archives

A call for volunteers was published on the French National Archives website. To participate, each archive center had to have suspected problems with molds in storage areas and agree to microbial investigations in archives and to a medical questionnaire for employees. The first ten centers to volunteer were included in the study. Nine of the archives were located in metropolitan France and one in the French Antilles. Seven archives were visited between October 2008 and May 2009 and three in January and February 2010.

Environmental samples

Each archive was visited by a researcher from the Mycology Department of Besançon University Hospital to take air and dust samples. Air samples were taken in all storage areas of each archive (148 air samples in all) with the MAS 100™ Impactor (Merck®, Darmstadt, Germany) for 1 min at 100 l per min. The sampler was placed in the center of each room, 1 m above floor level. Dust samples were taken using electrostatic dust collectors (EDC) deposited for 4 weeks in archives and retuned by mail to the laboratory. Five EDCs were utilized in each archive, with each EDC placed on a shelf near the center of a different storage area, except for archive number 8 where only two EDCs were used (47 EDCs in total). Electrostatic dust collectors were mainly used in storage areas suspected to be contaminated by molds. If the archive was not known to be contaminated by molds, EDCs were randomly placed. The EDCs were similar to those used in other environmental studies on allergy (Noss et al., 2008). Electrostatic wipes, 22 · 30 cm and composed of 90% polyester and 10% polypropylene fibers (Super U®, Rungis, France), were first sterilized (124°C, 30 min) and placed in a plastic folder cleaned with disinfectant (Aniosurf®, France).

Microbiological analyses by culture

Concerning air samples, microorganisms from the air were impacted on culture media (100 l per plate). Electrostatic dust collectors were analyzed as follows: 20 ml of 0.1% Tween 80 solution (Merck®, Darmstadt, Germany) were added to wipes removed from EDCs and blended for 10 min using a Stomacher™ (AES®, Combourg, France). Liquid was cultured on culture media (100 ll per plate). For each air and dust sample, four culture media were used in Petri dishes: (i) DG18 (dichloran glycerol 18% agar) incubated at 30°C (Oxoid®, Basingstoke, Hampshire, UK), (ii) 3% malt extract agar (Difco®, Detroit, MI, USA) with chloramphenicol at 20°C, (iii) 3% malt extract agar with 10% NaCl (Sigma-Aldrich®, NY, USA) and chloramphenicol at 15°C, and (iv) 0.5% cellulose agar [0.5% cellulose microcrystalline powder (Sigma-Aldrich®), 0.11% KH_2PO4, 0.01% $MgSO_4$, and 0.01% NH_4Cl] at 20°C.

Mold species were identified according to macroscopic and microscopic criteria as described in reference mycology manuals. *Penicillium* species were identified by morphological study and by sequencing the gene coding for b-tubulin (Samson and Frisvad, 2004). For each archive, two to six morphologically different *Penicillium* colonies were isolated, and extracted DNA was sequenced. Other *Penicillium* were macroscopically and microscopically described and identified in reference to the sequenced ones. Results were expressed in colony-forming units per cubic meter of air (CFU/m^3) after correction with the 'positive hole conversion table MAS 100' or in CFU/cm^2.

Quantitative real-time PCR (qPCR)

Washing liquids from the EDC were analyzed by real-time quantitative PCR. DNA extraction was performed as follows: 200 ll of washing liquid was placed into 2-ml lysing matrix tube containing glass beads (Lysing Matrix E™; MP Biomedical®, OH, USA) and 200 ll of brain heart infusion (BBL™; Becton Dickinson®, Sparks, MD, USA). The tubes were shaken in a MagNA Lyser Instrument™ (Roche Applied Science®, Mannheim, Germany), heated in a boiling water bath, and then placed on ice. The tube was centrifuged, and the supernatant was used for subsequent analysis.

DNA from *Cladosporium sphaerospermum*, *Alternaria alternata, Stachybotrys chartarum*, and *Aspergillus fumigatus* was detected by qPCR using primers and TaqMan probes previously described by Haugland et al. (2004) (http://www.epa.gov/microbes/moldtech.htm). These species were responsible for allergic (*Alternaria alternata*), toxic (*Stachybotrys chartarum*), or infectious (*Aspergillus fumigatus*) diseases in humans (Horner et al., 1995; Kuhn and Ghannoum, 2003; Knutsen and Slavin, 2011). The PCRs contained 10 ll of Gene Expression Master Mix (Applied Biosystems, Foster City, CA, USA), 1 lM of fungal primers, 80 nM of probes, and 5 ll of template DNA in a total volume of 20 ll. PCR was run on a 7500 Fast Real-Time PCR System (Applied Biosystems). A sterile distilled water sample was used as a negative control in each extraction series. Positive controls and calibrated spore suspensions for quantification were performed as previously described with reference strains of fungi and were used in each PCR assay to calculate spores concentration in each EDC (Bellanger et al., 2009). Quantification cycle (Cq) values above 42 were considered to be negative.

Medical questionnaire

A questionnaire was used to assess allergic and general symptoms, as well as time, duration of work in the archive and individual prevention systems used. Questionnaires were distributed during visits to archives, were anonymously completed, and were returned by mail. Questions about symptoms were expressed as follows: 'Have you had _*x symptom*_ at any time in the last 12 months?' The question used to determine whether employees were in contact with contaminated documents was as follows: 'Do you work directly with moldy documents?' Contamination by molds is a common problem in archives that preserve documents, and people working in these places can detect the presence of molds on documents. It appears as a cotton or powder-like layer together with a change in the color of the paper and sometimes a mold-like odor. Questions about potential confounders such as age, gender, smoking habits, and known exposure to allergens (other than occupational exposure) were included.

Statistics

Concentrations of airborne microorganisms were first tested for normality (skewness tests) and were found to be non-normally distributed. We therefore performed a nonparametric Kruskal–Wallis test to compare fungal concentrations among archives.

Results of fungal concentrations in the air of storage areas were classified into four concentration levels according to a previous study in houses with the same sampling method (Reboux et al., 2009). Three thresholds were specified to define these four concentration levels (low <170 CFU/m^3; 170< moderate <560 CFU/m^3; 560< high <1000 CFU/m^3; and very high >1000 CFU/m^3).

Answers to medical questionnaires (that were identified by archive name) were coded and entered into a database. Two people checked the database independently. Archives where the median concentration of total fungi in the air was below 170 CFU/m^3 were classified as 'less-contaminated archives' and those where median concentration was >170 CFU/m^3 were classified as 'contaminated archives'. In the same way, archives where the median concentration of total fungi in EDC was below 2 CFU/cm^2 were distinguished from those where the median concentration was ‡2 CFU/cm^2. The risk for those working in the more contaminated archives of suffering more symptoms compared to others was evaluated using the odds ratio (OR) with a 95% confidence interval. The same method was used to calculate the risk for those working directly in contact with moldy documents of suffering more symptoms compared to others. Symptoms were tested one by one and all together in logistic regressions adjusted for age, gender, and smoking habits (smoker/

non-smoker). STATA 9.0 SE (Stata Corporation, College Station, TX, USA) was used for statistical analyses. The statistical significance was set at 5%.

Results

Archives

All the archive centers had storage areas, work rooms, and a reading room open to the public. Our investigations focused on storage areas. During fieldworker visits, the temperature in the storage areas ranged from 16 to 24 C and relative humidity ranged from 35.5% to 44%. Five types of problems were identified in the archives. For archives N 1 and N 7, the buildings were old and did not allow for sufficient air circulation. Archives N 2, N 4, and N 8 suffered sudden water damage caused by a breakage in the plumbing system. For archives N 3, N 6, and N 10, the air-conditioning system was dysfunctional, leading to temperature and humidity variations (humidity levels rose to over 70% in one of these archives). For archive N 5, molds were already present on the documents when they were given to the archive. For archive N 9, there were insulation problems in the building leading to dampness and molds on the walls.

Fungal microflora in the air of archives

Air samples were taken from 148 storage areas. The main fungi isolated are presented in Table 1. Only species isolated in more than 1% of samples (air and EDC) are presented; 31 other fungi (not shown) were also isolated in the air. Four molds were specifically isolated in storage areas of archives located in the Antilles (*Curvularia* sp., *Exophiala* sp., *Drechslera* sp., and *Penicillium decaturense*). The median total mold concentration in the air of storage areas was 70 CFU/m^3 (interquartile range IQR = 30–170). The differences among archives were significant (P = 0.0001, Kruskal–Wallis test). Distributions of concentrations for each archive are shown in Figure 1. Three thresholds defining four concentration levels are indicated on the graph to compare results with concentrations in reference houses previously described (Reboux et al., 2009). Seven storage areas presented high concentrations of molds and four presented very high concentrations. Other air samples showed low or moderate concentrations.

Specific cases of highly contaminated storage areas

Although archives were not highly contaminated environments on average, there were variations within centers, and four samples were highly contaminated (more than 1000 CFU/m^3). In archive number 5, a very high concentration of *Stachybotrys chartarum* (19500 CFU/m^3) was found in one air sample and was associated with a moldy odor. In this storage area, moldy documents were stored that had originally come from a building suffering water damage. These documents were, therefore, moldy before arrival in this archive. Documents were highly contaminated by *Stachybotrys chartarum* (40 CFU/cm^2). In archive number 7, a high concentration (2860 CFU/m^3) of molds was identified, and *Penicillium chrysogenum* was the main isolated fungus. There were no moldy odors and no visible molds on archive boxes, but several documents in these boxes were moldy. In archive number 9, two air samples showed high concentrations of molds (2010 and 3000 CFU/m^3). *Penicillium chrysogenum* was the main fungus, and there was nothing obvious to explain these concentrations.

Table 1 Isolation frequencies of molds in the air and dust samples

Species	Air samples (N = 148)	EDC (N = 47)	Total (N = 195)
Fungi isolated in more than 5% of samples			
Penicillium chrysogenum	88	38	126
Cladosporium sphaerospermum	91	12	103
Aspergillus versicolor	38	11	49
Rhodotorula sp.	33	2	35
Yeasts	27	6	33
Eurotium spp.	22	9	31
Alternaria spp.	25	3	28
Basidiomycetes	24	4	28
Aspergillus niger	8	14	22
Wallemia sebi	17	1	18
Aureobasidium pullulans	11	4	15
Mycelia sterila	13	0	13
Acremonium spp.	11	2	13
Penicillium corylophilum	11	1	12
Penicillium crustosum	2	9	11
Dematiaceae	9	1	10
Fungi isolated in 1–5% of samples			
Aspergillus fumigatus	8	1	9
Cladosporium herbarum	8	1	9
Penicillium spp.	6	3	9
Aspergillus ochraceus	7	1	8
Rhizopus spp.	6	0	6
Trichoderma spp.	2	4	6
Ulocladium spp.	5	0	5
Cladosporium cladosporioides	5	0	5
Phoma sp.	5	0	5
Aspergillus chevalieri	4	0	4
Penicillium citreonigrum	1	3	4
Penicillium olsonii	4	0	4
Scopulariopsis spp.	4	0	4
Verticillium spp.	4	0	4
Penicillium citrinum	2	1	3
Aspergillus sydowii	2	1	3
Botrytis cinerea	2	1	3
Tritirachium oryzae	3	0	3

EDC, electrostatic dust collectors.

Self-reported symptoms among workers

Of the 268 self-report questionnaires distributed, 144 (54%) were returned. Employees had a mean age of 45

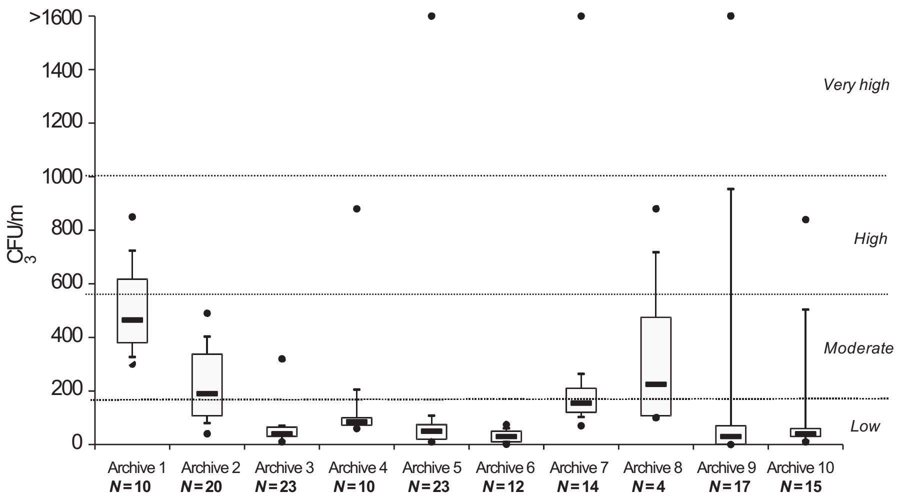

Fig. 1 Graph box presenting distribution of mold concentration in storage areas for each archive (maximum, 90th centile, third quartile, median, first quartile, tenth centile, minimum). According to a previous study with the same sampling method, three thresholds were given defining four concentration levels (Low <170 CFU/m3; 170< moderate <560 CFU/m3; 560< high <1000 CFU/m3; very high >1000 CFU/m3). *N*, number of air samples

[standard deviation (s.d.) = 10], and 73% of them were women. No employees could identify exposure to highly allergenic substances outside the work context that should have caused their symptoms. The amount of time spent with the archives was at mean 8 h per working day (s.d. = 0.8). The odds ratio (OR) was calculated for each reported symptom, comparing the 110 employees working in archives with a median contamination level <170 CFU/m^3 (archive numbers 3, 4, 5, 6, 7, 9, and 10) to the 34 working in archives with a median contamination level over 170 CFU/m^3 (archive numbers 1, 2, and 8). No OR was statistically significant (Table 2). The same ORs were calculated to compare employees working in contact with moldy documents to the others. Work with moldy documents was associated with significantly more headache, fatigue, eye irritation, throat irritation, coughing, and rhinorrhea (Table 2). In a multivariate regression analysis, including significant symptoms and adjusted for age, gender, and smoking status, only eye irritation was also significant (OR, 5.7; 95% CI, 1.8–18.5).

Fungal microflora in archive dust

Dust samples were taken by EDC from five storage areas per center (except for archive 8 where two dust samples were taken), including those suspected of being contaminated by molds, and were analyzed by culture and qPCR. The main fungi isolated by culture are presented in Table 1. Medians for concentration of total fungi were 1 CFU/cm^2 (IQR = 0–1), 0 CFU/cm^2 (IQR = 0–1), 0 CFU/cm^2 (IQR = 0–2), 1 CFU/cm^2 (IQR = 1–8), 0 CFU/cm^2 (IQR = 0–2), 5 CFU/cm^2 (IQR=0–6), 3 CFU/cm^2 (IQR = 3–3), 8 CFU/cm^2 (IQR = 0–16), 1 CFU/cm^2 (IQR = 0–1), and 4 CFU/cm^2 (IQR = 4–4) for archives 1–10, respectively. The OR was calculated for each symptom, comparing the 91 employees working in archives with a median concentration of molds in dust under 2 CFU/cm^2 (archive numbers 1, 2, 3, 4, 5, and 9) to the 53 working in archives with a median contamination level over 1 CFU/cm^2 (archive numbers 6, 7, 8, and 10). No OR was statistically significant (Table 2).

Table 2 Association between concentrations of fungi in archives, handling moldy documents, and symptoms reported by workers

	Comparison in relation to air results[a]	Comparison in relation to electrostatic dust collectors results[b]	Contact with moldy documents[c]
Headache	1.4 (0.7–3.1)	0.8 (0.4–1.6)	2.4 (1.1–5.3)*
Fatigue	0.8 (0.4–1.8)	1.6 (0.7–3.2)	2.9 (1.2–6.7)*
Eye irritation	0.8 (0.3–1.7)	0.8 (0.4–1.6)	5.4 (1.9–14.9)*
Sneezing	1.1 (0.5–2.5)	1.8 (0.9–3.8)	1.5 (0.7–3.1)
Throat irritation	1.4 (0.7–3.2)	1.0 (0.5–2.2)	2.4 (1.0–5.7)*
Coughing	1.5 (0.6–3.3)	1.5 (0.7–3.4)	3.2 (1.2–8.4)*
Rhinorrhea	1.5 (0.7–3.4)	1.3 (0.6–2.8)	2.6 (1.0–6.4)*
Skin irritation	0.8 (0.3–2.0)	1.2 (0.6–2.8)	2.6 (0.9–6.8)
Sinusitis	1.3 (0.5–3.1)	0.7 (0.3–1.5)	2.6 (0.9–7.4)
Nose irritation	1.4 (0.6–3.3)	0.8 (0.3–1.8)	1.6 (0.6–4.0)
Feeling dizzy	1.4 (0.5–3.8)	1.0 (0.4–2.6)	2.7 (0.8–9.9)
Nausea	1.1 (0.3–3.6)	0.8 (0.3–2.3)	1.7 (0.5–6.4)
Eczema	1 (0.3–3.3)	0.5 (0.2–1.4)	0.7 (0.2–2.1)
Shortness of breath	2.9 (1–8.5)	0.8 (0.3–2.3)	0.6 (0.2–1.9)
Wheezing	2.8 (0.7–11.1)	1.7 (0.3–8.8)	3.4 (0.4–28.4)
Asthma[d]	1.5 (0.5–4.8)	0.8 (0.3–2.4)	1.8 (0.5–6.8)

*Significant symptoms.

[a] Odds ratio comparing employees working in archives with a median contamination level in air over 170 CFU/m^3 to other workers.

[b] Odds ratio comparing employees working in archives with a median contamination level in dust over 1 CFU/cm^2 to other workers.

[c] Odds ratio comparing employees working in contact with moldy documents to others.

[d] Diagnosed by a doctor.

The results obtained from EDC with culture and qPCR methods were compared. *Aspergillus fumigatus* was isolated by culture in one sample (0.5 CFU/cm^2)

and no EDC showed a positive result by qPCR. In the same way, *Alternaria sp* was isolated in three samples by culture (5, 10, and 15 CFU/cm^2), and DNA for *Alternaria alternata* was detected in one sample (equivalent to 110 spores/cm^2). *Stachybotrys chartarum* was not isolated by culture and was detected in seven EDC samples by qPCR in low quantities (mean = 1.37 CFU/cm^2, s.d. = 0.86). *Cladosporium sphaerospermum* was detected by culture in 12 EDCs and by qPCR in 22 EDCs. There was no linear correlation between CFU/cm^2 obtained by culture and spores/cm^2 obtained by qPCR. The qPCR method was more sensitive in detecting the *Stachybotrys chartarum* and *Cladosporium sphaerospermum* species than culture, but no correlation between quantities was obtained by either method.

Discussion

Ten archives were investigated by culture and molecular methods using two sampling systems. Our investigations led to almost 50 genera of molds being identified. *Penicillium chrysogenum*, *Cladosporium sphaerospermum*, and *Aspergillus versicolor* were the three main fungi isolated in air and dust samples. Qualitatively, these molds were similar to those previously described in archives and documents (Zyska, 1997; Montemartini-Corte et al., 2003; Maggi et al., 2000; Zottia et al., 2008). The optimal temperature for the growth of fungal species isolated in this study is between 18 and 30°C, except for *Aspergillus fumigatus* (Samson et al., 1995; De Hoog et al., 2001). Compared to airborne microflora in houses (Reboux et al., 2009; Fairs et al., 2010; Vesper et al., 2007b; Lignell et al., 2008), large quantities of white yeasts, *Rhodotorula* spp., and *Aureobasidium pullulans* were found in the air. These microorganisms are characterized by a requirement of high water activity ($A_w > 0.90$), and their presence can be explained by the high humidity rate contained in books and maintained in the air.

Very few recent publications report on air contamination in archives. Borrego et al. (2010) found concentrations of 493 and 230 CFU/m^3 in two archives sampled by sedimentation during 5 min on Petri dishes. This sampling method is questionable because the sedimentation speed of fungal spores is not the same depending on the species. The same authors suggest that environments with microbial concentrations above 1000 CFU/m^3 should be considered as 'contaminated'. Based on critical reviews of epidemiological studies mainly in farming environments, Eduard (2009) suggests that the lowest observed effect level is approximately 10^5 spores/m^3 for diverse fungal species in non-sensitized populations. In Brazil, a government guideline established the alert limit at 750 CFU/m^3 for fungi (Nunes et al., 2005). In domestic environments, Reboux et al. (2009) suggested three thresholds (170, 560 and 1000 CFU/m^3) to define four concentration levels (low, moderate, high, and very high), established by examining fungal contamination in unhealthy houses, houses occupied by allergic patients, and matched control houses. The highest of these concentration levels would be considered to present a potential health hazard. This scale was used as a reference for the present study because activities in archives are similar to domestic activities, unlike labor activities such as in agriculture or industry.

Of 148 storage areas, 93% showed low or moderate concentrations. Although archives were not highly contaminated environments on average, there were variations within airborne samples, and 3% showed very high concentrations of fungi. These fungal concentrations can be a health risk, but other factors must also be taken into account. Indeed, simply walking through a room with highly contaminated air does not cause significant exposure. Duration of exposure per day, number of years of exposure, individual protection systems used, and individual sensitivity to microorganisms are also the important factors. Identification at the genus or species level of the main airborne fungi is important because not all species have the same effects on health. *Stachybotrys chartarum* was highly isolated in one storehouse and was detected in 15% of EDC by qPCR. Interest in this fungus is because of its ability to produce toxic substances. More than 30 mycotoxins belonging to six chemical classes (trichothecenes, phenylspirodrimanes, stachybocins, cyclosporins, atranones, and stachylysin) were produced by *Stachybotrys chartarum* (Systems, 2004). *Stachybotrys chartarum* was reported to have inflammatory and toxic effects on the lungs in mice and rats (Lichtenstein et al., 2010; Pestka et al., 2008). Toxic strains of *Stachybotrys chartarum* were suspected to be involved in pulmonary hemorrhage among children in Cleveland, Ohio (Dearborn et al., 2002), and its involvement in Sick Building Syndrome is often discussed (Straus, 2009). *Penicillium chrysogenum* was isolated in three highly contaminated storage areas. This fungus is known as being allergenic for sensitive subjects (Chou et al., 2003) and has caused cases of hypersensitivity pneumonitis (Fergusson et al., 1984). Identifying species of fungi in highly contaminated samples may help to specify the risk to health and give clear indications on how to prevent contamination.

Health problems in employees were assessed by self-reported questionnaires. This method allows health problems to be observed in a large number of subjects. Although self-reported questionnaires have been widely used in cohort studies (von Mutius and Schmid, 2006; Bjerg et al., 2011; Weinmayr et al., 2010), it has certain limitations. Indeed, the answers given by those questioned may have been influenced by negative feelings about the working environment. Exposure is a multifactorial measurement and includes dose, biologically relevant time period, and substance types that can be numerous in archives (dust, microorganisms, mites, glues, and chemical substances). In our study, people working in the most contaminated archives did not report more symptoms of allergy than others, but the medical questionnaires were anonymous and did not reveal individual history of exposure. We also showed that contact with moldy documents was significantly linked to a high prevalence of headache, fatigue, eye irritation, throat irritation, coughing, and rhinorrhea. These symptoms are usually reported in cases of Sick Building Syndrome (Chang et al., 1993). In the multivariate model, only eye irritation was significant. However, these symptoms may be caused by molds or else by other domestic or occupational factors (mites, dust, air-conditioning system, low temperature, dust level, lack of light, glue exposure, etc.). Further investigation including medical visits, respiratory investigations, and immunological tests as well as measurements of additional parameters (dust data, MVOC, mycotoxins, chemical substances, etc.) is necessary to isolate the role of molds.

Quantitative PCR assays were performed on dust to detect *Alternaria alternata*, *Aspergillus fumigatus*, and *Cladosporium sphaerospermum*. These three fungi are commonly found indoors and are related to allergic disorders in humans (Cruz et al., 1997; Horner et al., 1995). Using qPCR to analyze EDC allowed us to detect 15% and 21% more positive samples than when using culture. For detection of *Alternaria alternata*, culture methods detected fungus more efficiently than qPCR, but colonies of *Alternaria* obtained by culture were not identified at species level. Only one part of the spores is able to grow on culture medium, and the interactions between spores in culture medium lead to growth inhibition in some species (Haugland et al., 1999). Furthermore, dead spores, mycelium fragments, chlamydospores, or other fungal components that cannot or do not easily germinate also contain DNA. These fungal components, which are highly present in the air, may also be antigenic and may cause health problems (Gorny et al., 2002). One disadvantage of qPCR results is the lack of reference data. This method is becoming widely used in environmental studies (e.g., Haugland et al., 2004; Vesper et al., 2007a), but databases are lacking for comparing quantities of DNA in several different environments and situations. The second disadvantage is the high specificity of analysis: only the target fungi were detected, and other potentially pathogenic fungi were not detected.

Our assessment was performed by collecting air by impaction and settled dust using EDC. Air samples provided the advantage of focusing on microorganisms that are contaminating the air and that are inhaled by workers. However, bioaerosol emissions are known to be highly variable from one measurement to another, even for samples taken over a short period of time (Douwes et al., 2003). Electrostatic dust collectors, which consists of an electrostatic wipe to collect deposited dust, can make a long-term assessment of airborne microorganisms. As for qPCR dosage, databases for comparing quantities obtained by EDCs in several different environments and situations are lacking. In our study, we sampled between October and May, when fungal concentration was at its lowest and when outdoor influence was minimal. Indeed, mold concentration in the air changes with the seasons, tends to be higher in the warmest season (between June and October), and lower in the cold seasons (Ruga et al., 2008; Maggi et al., 2000). Taking samples during the cold season allows us to obtain more accurate measurements of fungi that are attributable to indoor sources.

Conclusion

The evaluation of fungal contamination in archives reporting problems with mold shows that concentrations in the air were moderate and that microflora have specific characteristics linked to high humidity level and presence of cellulose. To evaluate fungi in archives, we recommend air sampling using an impactor and EDC for long-term sampling, and analyzing samples by culture and by qPCR to investigate species of fungi that grow slowly or with difficulty in culture medium (such as *Stachybotrys chartarum*). Employees in contact with moldy documents reported several symptoms. Of these, eye irritation was the most frequent.

Acknowledgement

This study was supported by the National research program for the knowledge and conservation of cultural heritage (Programme National de Recherche sur la Connaissance et la Conservation des Matériaux du Patrimoine Culturel PNRCC) 2008, from the French Ministry for Culture and Communication. The authors are grateful to Frances Sheppard of the Clinical Investigation Center (Inserm) of Besançon for her editorial assistance.

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Received: 31 May 2018 | Revised: 23 October 2018 | Accepted: 24 October 2018

DOI: 10.1111/ina.12517

ORIGINAL ARTICLE

WILEY

Dampness and mold in homes across China: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue among adults

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Funding information
the National Key Research and Development Program of China, Grant/Award Number: 2017YFC0702700; the State Key Basic Research Program (973) project, Grant/Award Number: 2015CB553404; Natural Science Foundation of Shanxi Province of China, Grant/Award Number: 2016011080; Shanxi One Hundred Excellent Experts Project, Grant/Award Number: nr 9; the Swedish Research Council Formas, Grant/Award Number: 348-2011-7402

Abstract

We studied dampness and mold in China in relation to rhinitis, ocular, throat and dermal symptoms, headache and fatigue. A questionnaire study was performed in six cities including 36 541 randomized parents of young children. Seven self-reported signs of dampness were evaluated. Multilevel logistic regression models were used to calculate odds ratios (ORs). Totally, 3.1% had weekly rhinitis, 2.8% eye, 4.1% throat and 4.8% skin symptoms, 3.0% headache and 13.9% fatigue. Overall, 6.3% of the homes had mold, 11.1% damp stains, 35.3% damp bed clothing, 12.8% water damage, 45.4% window pane condensation, 11.1% mold odor, and 37.5% humid air. All dampness signs were associated with symptoms (ORs from 1.2 to 4.6; $P < 0.001$), including rhinitis (ORs from 1.4 to 3.2; $P < 0.001$), and ORs increased by number of dampness signs. The strongest associations were for mold odor (ORs from 2.3 to 4.6) and humid air (ORs from 2.8 to 4.8). Associations were stronger among men and stronger in Beijing as compared to south China. In conclusion, dampness and mold are common in Chinese homes and associated with rhinitis and ocular, throat and dermal symptoms, headache and fatigue. Men can be more sensitive to dampness and health effects of dampness can be stronger in northern China.

Xin Zhang and Dan Norbäck contributed equally to the work.

KEYWORDS
dampness, home environment, mold odor, rhinitis, sick building syndrome, water damage

1 | INTRODUCTION

The home is the indoor environment where we spend most (65%) of our time.[1] One of the most well-documented indoor risk factors for impaired respiratory health is dampness, which includes water damage, damp spots, visible mold, and mold odor. Many epidemiological studies have been published, linking dampness to an increased risk of asthma,[2] asthmatic symptoms,[3,4] respiratory infections,[3,5] and rhinitis.[3,6] Mold odor has been pointed out as the dampness indicator with the strongest association with asthma[2] and rhinitis.[6] Associations between dampness in the home environment and mucosal symptoms (eg, eye, nose, and throat symptoms), general symptoms (eg, headache and fatigue), and dermal symptoms have been demonstrated in studies from northern Europe[7-14] and Japan.[15-20] A few studies have investigated these symptoms among adults in relation to dampness in workplace buildings.[21-24]

China is the most populated country in the world, a middle-income country with rapid changes in indoor environmental exposures linked to modernization and urbanization. There are only a few population studies available reporting data on self-reported dampness and mold in Chinese homes. Parents of preschool children in the city of Taiyuan in northern China reported that 19.5% of the homes had water leakage or floor dampness and 6.9% had visible indoor mold growth.[25] The large SNEC study from seven north east cities in China reported that 10.5% of the homes had indoor mold growth.[26] One investigation of dorm rooms of university students in Tianjin reported that 12.2% of the rooms had visible indoor mold and 31.4% had a history of water damage.[27] However, few previous studies exist from China on the consequences of dampness and mold in homes for adult health.[27]

China is divided into northern and southern China with the Yangze River as the border. Buildings in northern China (including Beijing) have a central heating system. Residential buildings in southern China have no central heating system installed but the residents may use their air conditioner or electric radiators to heat the home in winter. Most residential buildings in Chinese cities are high-rise apartment buildings constructed after 1990. The wall construction in modern buildings in southern China consists of a frame of reinforced concrete with bricks as the wall material. Recently, mineral insulation on the outside of the walls has started to be used. The façade material is usually ceramic tiles. The wall has an inside layer and an outside layer of waterproof cement mortar as dampness barriers.[28] Residential buildings in Beijing have a similar construction but with thicker walls and mineral insulation and with a central heating system.

The CCHH study (China Children Home Health) is a multicenter observational study across China on associations between children's health and the home environment.[29-32] The parentally administered questionnaire in CCHH asked aboutthe children's health butcontained some information on parent's health (one parent per child answered the questionnaire). Two cities within the CCHH study (Chongqing and Changsha) have reported associations between dampness and rhinitis, eye, nose, throat and dermal symptoms, headache and fatigue among parents of young children.[33,34] The aim was to study associations between different signs of indoor dampness and mold in homes across China and medical symptoms (including rhinitis, ocular, throat and dermal symptoms, headache and fatigue) among parents of young children in Beijing and five cities in south China (Shanghai, Nanjing, Wuhan, Chongqing, Changsha). Our hypothesis is that there are positive associations between the seven signs of dampness and the medical symptoms (including three merged symptom categories).

Practical Implications

- There is an obvious need for improvements of dampness conditions in Chinese homes.
- Since there are mostly weak correlations between different signs of dampness, it is important to assess different types of dampness indicators in indoor epidemiology to cover all aspects of dampness.
- More detailed technical investigations are needed in China to identify underlying causes of dampness in homes in China to be able to improve the home environment and to build homes with less dampness in the future.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

Consents were obtained from parents or guardians of participating children before the study. The study and the consent procedure were approved by the Ethic Committee of School of Public Health, Fudan University, China.

2.2 | Study subjects

The present article is based on merged data from six cities from the CCHH study performed in 10 Chinese cities from December 2010 to January 2012. The participation rate in the CCHH study was 77%. Eight cities contributed to the merged database and six of the cities had the version of 12 medical symptoms in the last 3 months used in this article. The study population consisted of the parents of children attending randomly selected day care centers in the six cities (Beijing, Shanghai, Nanjing, Wuhan, Chongqing, and Changsha). One parent per child (1-8 years) answered the questionnaire. The questionnaires were mostly completed by the child's parents, but in some cases by grandparents or other persons. Totally, 36 541 were answered. We excluded questionnaires answered by grandparents (n = 1945) or other persons (n = 378) and those without information on who had answered it (n = 1659). The current study was restricted to the parents in order to get a more homogenous population with respect to age range and home environment exposure. Grandparents and other persons who answered the questionnaire may not live permanently in the dwelling. Finally, 32 559 questionnaires were included.

2.3 | Questionnaire

The questionnaire included questions on the current home environment, health information on the child and demographics and health among the adults answering the questionnaire.[29] There were questions on gender, current smoking (Yes/No), ever had allergic asthma (Yes/No), and ever had allergic rhinitis (Yes/No). The questions on asthma and allergies were combined to one (Yes/No) variable in the data analysis. The questions on current medical symptoms were from the standardized Swedish Örebro questionnaire.[35]

The questions were as follows: In the last 3 months, have you had any of the following symptoms: (a) fatigue; (b) feeling heavy headed; (c) headache; (d) nausea/dizziness; (e) difficulties concentrating; (f) eye symptoms (itching, burning, or irritation of the eyes); (g) rhinitis (irritating, stuffy, or runny nose); (h) throat symptoms (hoarse throat or dry throat); (i) cough; (j) dry or flushed facial skin; (k) scaling/itching skin in scalp or ears; and (l) dermal symptoms from the hands (dry skin, itching, red skin)? There were three possible responses: weekly; sometimes; and never. For some statistical analysis, we combined the symptoms into three groups as in previous publications.[10,11] General symptoms included fatigue, feeling heavy headed, headache, nausea/dizziness, and difficulties concentrating. Mucosal symptoms included itching, burning, or irritation of the eyes; irritating stuffy or runny nose; hoarse throat, dry throat, and cough. Skin symptoms included dry or flushed facial skin, scaling/itching scalp or ears, and dry itching or red skin on the hands. Rhinitis, eye symptoms, throat symptoms, headache, and fatigue were considered as core symptoms and were analyzed as single items as well.

Questions on demographics included gender, current smoking (yes/no), and a history of asthma or allergic rhinitis (atopy) (yes/no). Information on the current home used in this article included the following:

(1) House size (<40 m^2/41-60 m^2/61-75 m^2/76-100 m^2/101-150 m^2/150 m^2).
(2) Construction year of the building (before 1980/1980-1990/1991-2000/2001-2005/after 2005).
(3) Ownership of the building (Yes/No).
(4) Mold spots on floor, walls, or ceiling (Yes/No/Not known).
(5) Damp stains on floor, walls, or ceiling (Yes/No/Not known).
(6) Damp bed clothing in the last year (Sometimes/Frequently/Never).
(7) Water damage (Yes, in the past years/Yes, in the last 12 months/No/Not known).
(8) Window pane condensation in winter (Yes/No).
(9) Moldy odor indoors in the last 3 months (Weekly/Sometimes/No).
(10) Perception of humid indoor air in the last 3 months (Weekly/Sometimes/No).

Size of the home has been demonstrated to be a good socioeconomic indicator related to family income[36] and has been used previously in China as an indicator of socioeconomic status (SES).[31]

2.4 | Dampness/mold index

A dampness/mold index was created from four variables (mold spots, damp stains, water damage, and mold odor) by adding the coded numbers of the four variables (range 0-6). Mold spots and damp stains were coded as "1" and "0" (yes and no), water damage as (0-No/not known, 1-Yes, in the past years, 2-Yes, in the last 12 months), and moldy odor (0-Never, 1-Yes, sometimes, 2-Yes, weekly). The dampness/mold index ranged from 0 to 6.

2.5 | Statistical analysis

In all statistical analysis, sometimes symptoms or never symptoms were coded 0 and weekly symptoms were coded 1. In the logistic regression models, "not known" (an option used for the question on water damage only) was coded as 0. Chi-square test was applied to compare the prevalence of symptoms between groups. Factor analysis was applied to the seven signs of indoor dampness and mold, size of the home, construction year of the building, and ownership of the home, using principal component analysis and varimax rotation. Kendal Tau beta rank correlation test was used to measure correlations. Mann-Whitney *U* test was used to study differences between two groups. Moreover, we used multilevel logistic regression (two levels: individual level and city level) to evaluate the associations between dichotomous dampness indicators (dependent variable) and city level exposure indicators (outdoor temperature and GDP per capita). Multilevel ordinal regression (two levels: individual level and city level) was used to evaluate the associations between three-level dampness indicators (dependent variable) and city level exposure indicators (outdoor temperature and GDP per capita). Finally, we used multilevel logistic regression (two levels: individual level and city level) to evaluate associations between symptoms (dependent variable) and indoor dampness and mold. Models were adjusted for gender, asthma or allergy, smoking and house size. Sensitivity analysis was performed, stratifying for gender, asthma or allergy and city (Beijing vs southern cities). Data analysis was conducted with SPSS 20.0 (IBM, Armonk, NY, US) and STATA 12.0 (STATA Corp, College Station, TX, US; multilevel logistic regression). The associations were expressed as odds ratios (ORs) with a 95% confidence interval (CI) for logistic regression. In all statistical analyses, a two-tailed test and 5% level of significance were applied.

TABLE 1 Prevalence of weekly symptoms in the last three months stratified by gender, atopy, and smoking (N = 32 559)

Types of symptoms	Tota	Gender			Atopy (asthma or allergy)			Current smoking		
	(N = 32 559) (%)	Male (N = 8467) (%)	Female (N = 24 092) (%)	*P*-value	No (N = 28 984) (%)	Yes (N = 2539) (%)	*P*-value	No (N = 27 792) (%)	Yes (N = 3935) (%)	*P*-value
Any general symptom[a]	4951 (17.1)	1117 (15.0)	3834 (17.8)	<0.001	4205 (16.1)	607 (27.0)	<0.001	4295 (17.2)	580 (16.7)	0.515
Fatigue	4308 (13.9)	1015 (12.6)	3293 (14.3)	<0.001	3654 (13.1)	538 (22.1)	<0.001	3725 (14.0)	516 (13.7)	0.650
Heavy headedness	992 (3.4)	202 (2.7)	790 (3.6)	<0.001	831 (3.2)	127 (5.6)	<0.001	865 (3.4)	110 (3.2)	0.424
Headache	890 (3.0)	151 (2.0)	739 (3.3)	<0.001	759 (2.8)	106 (4.6)	<0.001	787 (3.0)	83 (2.3)	0.022
Nausea/dizziness	314 (1.1)	64 (0.9)	250 (1.2)	0.039	262 (1.0)	42 (1.9)	<0.001	276 (1.1)	31 (0.9)	0.332
Difficulties concentrating	810 (2.7)	197 (2.6)	613 (2.8)	0.391	657 (2.5)	125 (5.5)	<0.001	692 (2.7)	103 (2.9)	0.476
Any mucosal symptom[b]	2579 (8.9)	572 (7.7)	2007 (9.3)	<0.001	1884 (7.2)	612 (26.9)	<0.001	2248 (8.9)	285 (8.2)	0.191
Eye symptoms	838 (2.8)	169 (2.2)	669 (3.0)	<0.001	617 (2.3)	194 (8.4)	<0.001	752 (2.9)	68 (1.9)	0.001
Rhinitis	921 (3.1)	214 (2.8)	707 (3.2)	0.093	491 (1.8)	407 (17.3)	<0.001	802 (3.1)	98 (2.8)	0.276
Throat symptoms	1211 (4.1)	238 (3.1)	973 (4.4)	<0.001	967 (3.6)	201 (8.6)	<0.001	1058 (4.1)	131 (3.7)	0.239
Cough	538 (1.8)	191 (2.5)	347 (1.6)	<0.001	395 (1.5)	120 (5.2)	<0.001	421 (1.6)	107 (3.0)	<0.001
Any skin symptom[c]	1412 (4.8)	303 (4.1)	1109 (5.1)	<0.001	1123 (4.3)	245 (10.9)	<0.001	1238 (4.9)	146 (4.2)	0.078
Dry facial skin	712 (2.4)	106 (1.4)	606 (2.8)	<0.001	559 (2.1)	140 (6.1)	<0.001	639 (2.5)	52 (1.5)	<0.001
Scaling scalp or ears	776 (2.6)	210 (2.8)	566 (2.6)	0.360	624 (2.4)	119 (5.3)	<0.001	661 (2.6)	100 (2.9)	0.369
Hand eczema	258 (0.9)	65 (0.9)	193 (0.9)	0.938	187 (0.7)	66 (2.9)	<0.001	226 (0.9)	27 (0.8)	0.556

[a] At least one weekly general symptom (fatigue, feeling heavy headed, headache, nausea/dizziness, and difficulties concentrating).

[b] At least one weekly mucosal symptom (itching, burning, or irritation of the eyes; irritating, stuffy, or runny nose; hoarse, dry throat, or cough).

[c] At least one weekly skin symptom (dry or flushed facial skin, scaling/itching scalp or ears and hands dry, itching, red skin).

TABLE 2 Signs of dampness and mold, home characteristics and ETS at home (for Beijing vs the five southern cities) (N = 32 559)

Home environment variables	Beijing, N = 5267 (%)	Southern cities, N = 27 292 (%)	*P*-value	All six cities, N = 32 559 (%)
Mold spots	202 (3.8)	1793 (6.9)	<0.001	1995 (6.3)
Damp stains	299 (5.7)	3201 (12.2)	<0.001	3500 (11.1)
Damp bed clothing	744 (14.5)	10 413 (39.3)	<0.001	11 157 (35.3)
Water damage				
Yes, in the past years	234 (4.4)	1843 (7.2)	<0.001	2077 (6.8)
Yes, in the last 12 mo	201 (3.8)	1636 (6.4)		1837 (6.0)
Window pane condensation in winter	2057 (40.2)	10 754 (46.5)	<0.001	12 811 (45.4)
Mold odor				
Yes, sometimes	327 (6.4)	2765 (11.2)	<0.001	3092 (10.4)
Yes, weekly	39 (0.8)	165 (0.7)		204 (0.7)
Perception of humid air				
Yes, sometimes	655 (12.9)	10 128 (40.6)	<0.001	10 783 (35.9)
Yes, weekly	53 (1.0)	434 (1.7)		487 (1.6)
Construction year				
Before 1980	356 (7.1)	1803 (6.9)	<0.001	2159 (6.9)
1980-1990	709 (14.1)	3466 (13.2)		4175 (13.4)
1901-2000	1157 (23.0)	7602 (29.0)		8759 (28.1)
2001-2005	1888 (37.5)	8150 (31.1)		10 038 (32.2)
2006-	931 (18.5)	5150 (19.7)		6081 (19.5)
Size of the home (m^2)				
≤40	416 (8.2)	3972 (14.9)	<0.001	4388 (13.8)
41-60	791 (15.6)	3573 (13.4)		4364 (13.8)
61-75	652 (12.8)	3405 (12.8)		4057 (12.8)
76-100	1182 (23.3)	7365 (27.7)		8547 (27.0)
101-150	1435 (28.2)	6686 (25.1)		8121 (25.6)
>150	606 (11.9)	1619 (6.1)		2225 (7.0)
Ownership of the home				
Rented	1822 (36.2)	9856 (37.1)	0.220	11 678 (37.0)
Self-owned	3213 (63.8)	16 708 (62.9)		19 921 (63.0)
ETS at home	2609 (49.5)	16 106 (59.0)	<0.001	18 715 (57.5)

N and % (within the parentheses) were calculated when the missing data were excluded.

3 | RESULTS

Of the 32 559 participants included in the study, 74.0% were women. Data on the prevalence of weekly symptoms in the last 3 months (12 symptoms and 3 combined variables) are given in Table 1, stratified by gender, asthma/allergy, and smoking. All symptoms, except cough, difficulties concentrating, skin symptoms from the scalp/ears, and skin symptoms from the hands were more common among women. All symptoms were more common among subjects with allergic asthma or rhinitis. Smokers had a lower prevalence of headache, eye symptoms, and facial skin symptoms, but a higher prevalence of cough (Table 1).

As a next step, we compared building-related data between Beijing and the southern cities (Table 2). All signs of dampness and mold were more common in the southern cities. In the total material from the six cities, window pane condensation in winter (45.4%), perception of humid air (37.5%), and damp bed clothing (35.3%) were the most common signs of dampness. A total of 6.3% reported mold spots, 11.1% damp stains, 12.8% water damage, and 11.1% mold odor. Most (67.4%) lived in smaller homes (≤100 m^2), and more than half (63.0%) were the owners of the home. More than half of buildings (51.7%) were constructed after 2000.

Factor analysis was performed to understand to what degree different dampness signs were related to each other and to other building characteristics (construction year, size of the home, type of ownership). The factor analysis of building-related data identified 3 factors. Factor 1 consisted of construction year, size of the home, and ownership of the home. Homes in newer buildings ($P < 0.001$) and homes owned by the inhabitants ($P < 0.001$) had larger living area and homes owned by the inhabitants were newer buildings ($P < 0.001$) (calculated by Mann-Whitney *U* test). Factor 2 consisted of mold spots and damp stains. Mold spots and damp stains were positively associated (Kendal Tau beta 0.55; $P < 0.001$). Factor 3 consisted of damp bed clothing and perception of humid air. Damp bed clothing and perception of humid air were positively associated (Kendal Tau beta 0.32; $P < 0.001$). Water damage, window pane condensation in winter, and mold odor did not belong to any factor. There were low rank correlations between other dampness factors (Kendal Tau beta < 0.3).

Data on annual mean outdoor temperature and annual mean GDP per capita in each city from 2010 to 2012 were collected, and mean values for the three year period were calculated. The mean outdoor temperature was 11.8°C for Beijing, 15.9°C for Nanjing, 17.2°C for Shanghai, 17.7°C for Changsha, 16.8°C for Wuhan, and 18.2°C for Chongqing. The mean GDP income per capita (ReMinBe) was 79161 RMB for Beijing, 76059 RMB for Nanjing, 81619 RMB for Shanghai, 78194 RMB for Changsha, 81890 RMB for Wuhan, and 51732 RMB for Chongqing. A higher outdoor temperature was associated with a higher prevalence of mold spots (OR 1.10; 95% CI 1.19-1.18; $P = 0.02$), damp stains (OR 1.12; 95% CI 1.00-1.20; $P = 0.046$), mold odor (1.10; 95% CI 1.02-1.19; $P = 0.02$), damp bed clothing (OR = 1.27; 95% CI 1.25-1.28; $P < 0.001$), and perception of humid air (OR = 1.25; 1.10-1.42; $P < 0.001$). ORs for outdoor temperature were expressed per 1°C change of temperature. A higher GDP per capita was associated with window pane condensation in winter, only (OR 1.29; 95% CI 1.05-1.18); $P = 0.01$). ORs for GDP per capita were expressed per 10 000 RMB/y.

TABLE 3 Associations [OR (95% CI)] between dampness and mold, ETS at home and three symptom categories by two-level logistic regression models (N = 32 559)

Dampness indicators	Any general symptom[a] OR (95% CI)	Any mucosal symptom[b] OR (95% CI)	Any skin symptom[c] OR (95% CI)
Mold spots	1.66 (1.47-1.86)***	1.78 (1.54-2.07)***	1.87 (1.55-2.25)***
Damp stains	1.62 (1.47-1.77)***	1.73 (1.54-1.95)***	2.03 (1.75-2.35)***
Damp bed clothing	1.42 (1.33-1.52)***	1.52 (1.39-1.67)***	1.79 (1.59-2.01)***
Water damage			
No (reference)	1.00	1.00	1.00
Yes, in the past years	1.54 (1.37-1.74)***	1.43 (1.22-1.67)***	1.70 (1.40-2.06)***
Yes, in the last 12 mo	1.72 (1.53-1.95)***	1.48 (1.25-1.74)***	1.63 (1.33-2.01)***
Window pane condensation in winter	1.40 (1.31-1.50)***	1.56 (1.42-1.71)***	1.41 (1.25-1.60)***
Mold odor			
No (reference)	1.00	1.00	1.00
Yes, sometimes	1.67 (1.51-1.84)***	1.64 (1.45-1.87)***	1.89 (1.61-2.21)***
Yes, weekly	2.60 (1.88-3.59)***	3.49 (2.41-5.07)***	4.60 (3.06-6.91)***
Perception of humid air			
No (reference)	1.00	1.00	1.00
Yes, sometimes	1.36 (1.27-1.46)***	1.28 (1.17-1.41)***	1.68 (1.49-1.90)***
Yes, weekly	3.22 (2.61-3.97)***	3.87 (3.03-4.94)***	4.78 (3.58-6.37)***
ETS at home	0.93 (0.87-1.01)	1.04 (0.94-1.15)	1.08 (0.95-1.23)

All models were adjusted for gender, atopy, current smoking, and house size (one model per exposure).

[a] At least one weekly general symptom (fatigue, feeling heavy headed, headache, nausea/dizziness, and difficulties concentrating).

[b] At least one weekly mucosal symptom (itching, burning, or irritation of the eyes; irritating, stuffy, or runny nose; hoarse, dry throat, or cough).

[c] At least one weekly skin symptom (dry or flushed facial skin, scaling/itching scalp or ears and hands dry, itching, red skin).

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

Associations between the dampness variables and the three merged health variables (any weekly general, any weekly mucosal, and any weekly skin symptoms) were analyzed by multilevel logistic regression (Table 3). All seven dampness variables were associated with the merged health variables ($P < 0.001$). The highest ORs were found for mold spots, weekly mold odor, and weekly perception of humid indoor air. Table 4 shows associations for five core symptoms (weekly eye symptoms, weekly rhinitis symptoms, weekly throat symptoms, weekly headache, and weekly fatigue). All seven dampness variables were associated with all five core symptoms (most P-values < 0.001). The ORs for rhinitis ranged from 1.23 to 3.19. The highest ORs were for weekly mold odor (OR = 2.81) and weekly humid air (OR = 3.19). Similar ORs were found for the other symptoms.

Then, we analyzed health associations for the dampness/mold score (0-6). Due to low prevalence of higher scores, scores higher than 2 were combined. The score was significantly associated with all types of symptoms, with increasing ORs by increasing score numbers (Table 5).

We performed sensitivity analysis, stratifying by gender. Associations between mold odor and health were stronger among men. For general symptoms and weekly weekly mold odor, OR was 3.82 (95% CI 1.95-7.48) among men and 2.34 among women (95% CI 1.61-3.40). For mucosal symptoms and weekly mold odor, OR was 12.69 (95% CI 6.19-26.01) among men and 2.23 among women (95% CI 1.40-3.57). For mucosal symptoms and weekly mold odor, OR was 11.99 (95% CI 5.79-24.85) among men and 3.27 (95% CI 1.97-5.44) among women (Figure 1 and Table S1). Moreover, associations for water damage in the last 12 months were stronger among men, especially for skin symptoms (OR = 2.92; 95% CI 1.94-4.38 for men and OR = 1.38; 95% CI 1.08-1.76 for women) (Figure 1 and Table S1). Most other ORs were similar among men and women.

Then, we made a sensitivity analysis with respect to location (Beijing vs the southern cities) (Figure 2 and Table S2). ORs for mold odor were higher in Beijing. For mucosal symptoms and weekly mold odor, OR was 4.63 (95% CI 2.22-9.64) in Beijing and 3.14 (95% CI 2.03-4.84) in the southern cities. For skin symptoms and weekly mold odor, OR was 10.13 (95% CI 4.93-20.80) in Beijing and 3.39 (95% CI 2.03-2.65) in the southern cities. For general symptoms and weekly mold odor, associations were similar in Beijing and in the southern cities (Figure 2 and Table S2).

Finally, we performed sensitivity analysis, stratified by atopy (defined as allergic asthma or allergic rhinitis). Most associations were similar, but associations between mold odor and general and dermal

TABLE 4 Associations [OR (95% CI)] between dampness and mold and weekly symptoms (for five core symptoms) by two-level logistic regression models (N = 32 559)

Dampness indicators	Fatigue	Headache	Eye symptoms	Rhinitis	Throat symptoms
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Mold spots	1.63 (1.44-1.84)***	1.90 (1.51-2.38)***	2.00 (1.59-2.51)***	1.73 (1.37-2.19)***	1.75 (1.43-2.15)***
Damp stains	1.55 (1.40-1.70)***	1.71 (1.42-2.07)***	1.93 (1.60-2.33)***	1.63 (1.35-1.97)***	1.67 (1.42-1.98)***
Damp bed clothing	1.41 (1.31-1.51)***	1.40 (1.21-1.62)***	1.73 (1.49-2.01)***	1.41 (1.22-1.63)***	1.50 (1.33-1.71)***
Water damage					
No (reference)	1.00	1.00	1.00	1.00	1.00
Yes, in the past years	1.55 (1.37-1.75)***	1.34 (1.03-1.75)*	1.47 (1.14-1.91)**	1.44 (1.12-1.84)**	1.19 (0.95-1.50)
Yes, in the last 12 mo	1.63 (1.44-1.85)***	1.98 (1.57-2.51)***	1.76 (1.37-2.27)***	1.51 (1.17-1.95)***	1.25 (0.98-1.59)
Window pane condensation in winter	1.45 (1.35-1.56)***	1.17 (1.01-1.36)*	1.66 (1.42-1.94)***	1.51 (1.29-1.76)***	1.59 (1.39-1.81)***
Mold odor					
No (reference)	1.00	1.00	1.00	1.00	1.00
Yes, sometimes	1.56 (1.41-1.73)***	1.63 (1.33-2.00)***	1.96 (1.61-2.39)***	1.59 (1.30-1.96)***	1.58 (1.32-1.90)***
Yes, weekly	2.27 (1.63-3.17)***	4.33 (2.66-7.04)***	4.59 (2.80-7.52)***	2.81 (1.58-5.02)***	3.76 (2.40-5.90)***
Perception of humid air					
No (reference)	1.00	1.00	1.00	1.00	1.00
Yes, sometimes	1.32 (1.23-1.43)***	1.17 (1.00-1.37)*	1.25 (1.06-1.47)**	1.23 (1.05-1.44)**	1.37 (1.19-1.56)***
Yes, weekly	3.14 (2.54-3.88)***	2.82 (1.90-4.17)***	4.40 (3.11-6.22)***	3.19 (2.18-4.66)***	3.89 (2.84-5.33)***

All symptoms were weekly symptom in the last 3 mo. All models were adjusted for gender, atopy, current smoking, and house size (one model per exposure).

$^{*}P < 0.05$.

$^{**}P < 0.01$.

$^{***}P < 0.001$.

TABLE 5 Associations [OR (95% CI)] between dampness/mold index and weekly symptoms by two-level logistic regression models (N = 32 559)

	Dampness/mold index[a]			
Types of symptoms	**0, OR (95% CI)**	**1, OR (95% CI)**	**2, OR (95% CI)**	**>2, OR (95% CI)**
Any general symptom[b]	1.00	1.44 (1.31-1.58)***	1.77 (1.57-1.99)***	2.35 (2.05-2.71)***
Fatigue	1.00	1.41 (1.27-1.56)***	1.69 (1.49-1.91)***	2.15 (1.86-2.49)***
Headache	1.00	1.18 (0.94-1.47)	1.68 (1.32-2.15)***	2.94 (2.29-3.79)***
Any mucosal symptom[c]	1.00	1.45 (1.28-1.65)***	1.72 (1.47-2.01)***	2.19 (1.82-2.62)***
Eye symptoms	1.00	1.42 (1.14-1.76)**	1.78 (1.39-2.29)***	2.86 (2.19-3.74)***
Rhinitis	1.00	1.33 (1.08-1.64)**	1.57 (1.22-2.01)***	2.22 (1.68-2.93)***
Throat symptoms	1.00	1.48 (1.24-1.77)***	1.72 (1.39-2.13)***	1.87 (1.44-2.44)***
Any skin symptom[d]	1.00	1.56 (1.33-1.84)***	1.68 (1.37-2.06)***	2.88 (2.32-3.56)***

[a] Dampness/mold index: Damp stains and mold spots on floor/ceiling were coded as “1” and “0” (yes and no), water damage and moldy odor were coded as “0”, “1”, and “2”, by adding numbers of home indoor dampness problems as the dampness/mold index (ranging between 0 and 6).

[b] At least one weekly general symptom (fatigue, feeling heavy headed, headache, nausea/dizziness, and difficulties concentrating).

[c] At least one weekly mucosal symptom (itching, burning, or irritation of the eyes; irritating, stuffy, or runny nose; hoarse, dry throat, or cough).

[d] At least one weekly skin symptom (dry or flushed facial skin, scaling/itching scalp or ears and hands dry, itching, red skin).

Models were adjusted for gender, atopy, current smoking, and house size.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

symptoms were stronger in the atopy group. For general symptoms and weekly mold odor, OR was 3.63 (95% CI 1.70-7.74) in the atopy group and 2.40 (95% CI 1.67-3.46) in the nonatopy group. For skin symptoms and weekly mold odor, OR was 6.38 (95% CI 2.83-14.39) in the atopy group and 4.14 (95% CI 2.56-6.79 in the nonatopy group. In contrast, associations between mold odor and mucosal symptoms were stronger in the nonatopy group. For mucosal symptoms and weekly mold odor, OR was 2.31 (95% CI 1.03-5.13) in the atopy group and 3.90 (95% CI 2.59-5.88) in the nonatopy group. Finally, window pane condensation was a significant risk factor for general and dermal symptoms mainly in the nonatopic group (Table S3).

4 | DISCUSSION

Signs of dampness and indoor mold were common in homes in China, especially in southern China. We found associations between seven different signs of dampness or mold at home and rhinitis, throat symptoms, cough, and nonrespiratory symptoms (eye and skin symptoms, headache, and fatigue). The strongest health associations were found for mold spots, mold odor, and perception of humid air. Health associations for mold odor and water damage were stronger among males. Health associations for water damage and moldy odor were stronger among subject with atopy while health associations for window pane condensation in winter were stronger in the nonatopic group. Association between dampness indicators and health were stronger in Beijing as compared to the southern cities, especially for mold odor. To our knowledge, this is one of the largest studies on rhinitis and nonrespiratory symptoms among adults in relation to dampness and mold in the home environment.

Selection bias can be a problem in epidemiological studies. Our study is based on parents (one per child) of preschool children recruited from randomly selected day care centers in six cities across China. All parents in the selected day care centers were invited. The sample size was quite large, and the overall participation rare in the CCHH study was good (77%).[29] In China, about 80% of all children aged 3-5 years attended a day care center, and in urban areas, the day care attendance is even higher.[30] One limitation is that our study covers mainly urban areas and only six cities. The one child policy was still valid when the study was performed, and thus, most of the children had no siblings. The study subjects were young parents and more mothers than fathers answered the questionnaire. This could have caused a selection bias related to gender, with symptomatic fathers tending to participate in the study. On the contrary, we found that most symptoms were more common among females, and none of the symptoms were more common among men. The higher prevalence of symptoms among women agrees with previous studies.[37,38] Bearing these limitations of our study in mind, we can conclude that it should be reasonably representative for parents of young children in urban areas of China.

Another problem in epidemiological studies can be reporting bias. The “auto-suggestion” effect can influence subjective report on exposure as well as symptoms. In China, the main concern is health risks of outdoor air pollution. Health risks associated with indoor dampness or mold have not been a public issue in China. Moreover, we found different ORs for different types of symptoms and different types of dampness indicators. In conclusion, it is less likely that our results are severely influenced by recall bias but the lack of objective assessment of dampness and mold and the cross-sectional study design limits the possibility to draw conclusions on causality.

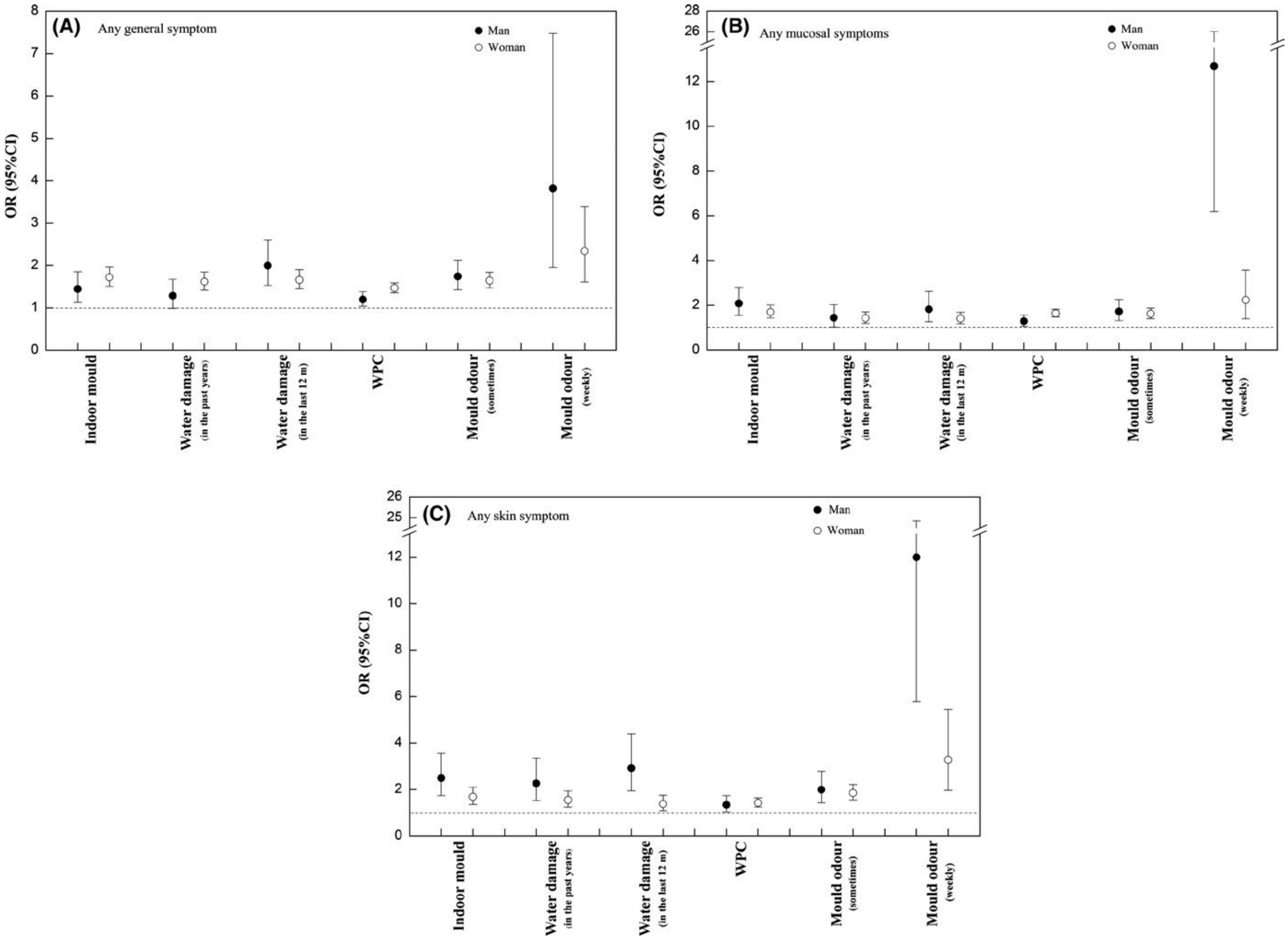

FIGURE 1 Associations between different dampness indicators and any general symptom (A), any mucosal symptom (B), and any skin symptom (C) by two-level logistic regression models, stratified by gender. Models were adjusted for atopy, current smoking, and house size

Finally, confounding is an important issue in epidemiology. A confounder is a variable related to the health variables as well as the exposure variables. The socioeconomic status (SES) of the family has been suggested to be a potential confounder (linked to health as well as dampness or mold at home). We do not have data on family income in our study but we adjusted for size of the home in our models. Size of the home has been demonstrated to be an indicator (proxy variable) of family income and socioeconomic status in USA[36] as well as in China.[31] Associations between socioeconomic status and dampness or mold at home have been investigated in a study multicenter study across Europe. Manual workers reported less water damage but more mold at home as compared to managerial/professional workers.[39]

We found that many subjects reported symptoms in the last 3 months but few reported weekly symptoms. The most common weekly symptom was fatigue, and the prevalence of other individual weekly symptoms was rare (1%-4%). Our results concerning symptom prevalence in China are similar as in two previously published articles from the Changsha[31] and Chongqing[30] centers of the CCHH study. The prevalence of weekly fatigue in our study was 13.9%, which is 1.6 times higher than that in the Chongqing study but similar as in the Changsha study.

We found that most symptoms were more common among women. This result agrees with many previous studies on symptoms included in the so-called sick building syndrome (SBS).[40-42] One explanation could be differences in the indoor or occupational exposure. Women with children can spend more time at home and the job exposure in nonindustrial workplaces (eg, at office work) can be higher among women.[37] Other researchers have suggested that sex differences in personality traits could explain the differences in symptom prevalence.[38] Moreover, we found that subjects with a history of atopy (allergic asthma or allergic rhinitis) had a higher prevalence of all type of symptoms. This is in agreement with previous studies on this topic.[40-42] Tobacco smoking was not a major determinant of symptoms, except for cough, which is in agreement with conclusions from previous review articles.[40-42]

Our main results were consistent associations between reported dampness and mold at home and rhinitis as well as nonrespiratory symptoms (eg, eye symptoms, skin symptoms, headache, and fatigue). Our results concerning an association between dampness and rhinitis are consistent with the conclusions from a recent review article.[6] However, it should be noted that this conclusion is mainly based on studies on childhood rhinitis. Only two studies on adult

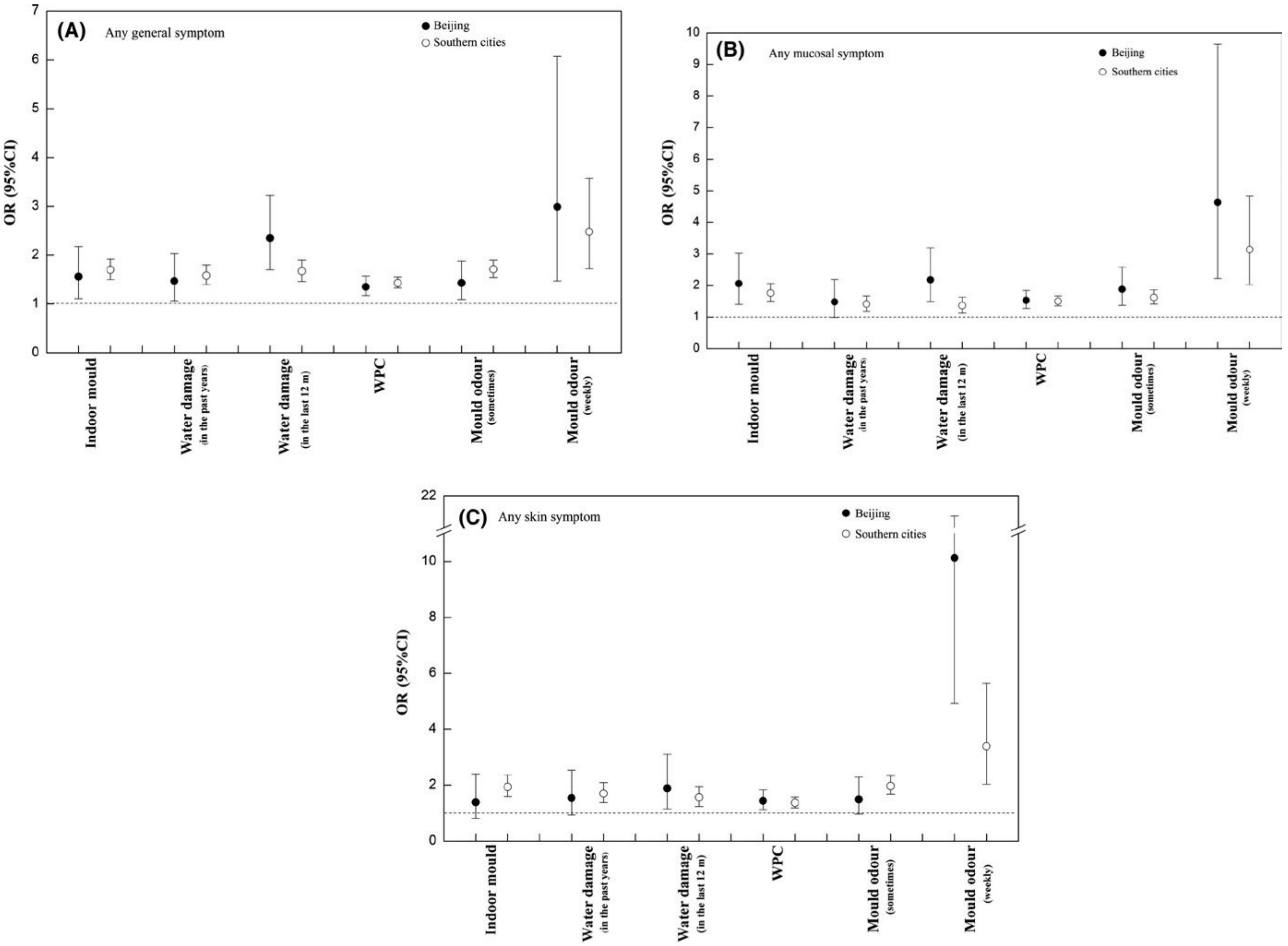

FIGURE 2 Associations between different dampness indicators and any general symptom (A), any mucosal symptom (B), and any skin symptom (C) by logistic regression models, stratified by Beijing vs southern cities. Models were adjusted for gender, atopy, current smoking, and house size

rhinitis were identified and included in this review. Our findings concerning associations between dampness or mold and nonrespiratory symptoms are in agreement with previous studies from northern Europe[7-14] and Japan.[15-20] However, our study is one of the largest studies on this topic and one of few studies from China.

We found the most pronounced health associations for mold odor, for rhinitis as well as for nonrespiratory symptoms. The results concerning mold odor and rhinitis are in agreement with the review article on rhinitis. They found that mold odor had the strongest association with rhinitis.[6] Moreover, we found that the health associations for mold odor were stronger for men as compared to women. To our knowledge, this is a new finding. On the contrary, one article from Canada reported that women were more sensitive to dampness at home.[43] Moreover, we found stronger health associations for mold odor and water damage in Beijing, as compared to the southern cities. One explanation could be that due to the cold climate in northern China, homes have less ventilation, with tighter building constructions and less window opening, especially in winter. A lower ventilation flow can increase the effects of air pollutants linked to water damage and mold odor.

Window pane condensation in winter was associated with rhinitis and nonrespiratory symptoms. Window pane condensation in wintertime is an indicator of high air humidity and occurs at low ventilation flow and low surface temperature on the windowpane (linked to the dew point). Window pane condensation in winter at homes in Sweden and United Kingdom has been reported to be associated with higher relative air humidity and increased levels of house dust mite allergens in dust.[44,45] Previous studies have found an association between window pane condensation at home in winter and rhinitis in Japan[18] and China.[46] Moreover, window pane condensation at home in winter has been reported to be associated with mucosal, general, and dermal symptoms.[19] Moreover, one Swedish study reported positive associations between window pane condensation in winter and fractional exhaled nitric oxide (FeNO), a biomarker of airway inflammation.[47]

In this study, there were health associations for all seven dampness indicators, and the ORs increased by number of dampness signs in the home, indicating a dose-response relationship. We created a dampness/mold index by adding the number of signs of dampness or mold at home. Similar dampness indexes have been used in previous studies.[46,48] Since the correlations between different dampness indicators were relatively weak, our conclusion is that it is important to use different types of dampness indicators in indoor epidemiology to cover all aspects of dampness. However, three factors were identified in the factors analysis. One factor related to variables linked to the type of building (construction year, home size, and ownership of the home) but not linked to any dampness indicators. It means that we cannot predict dampness or indoor mold from building age or socioeconomic indicators. The second factor was linked to mold spots and damp stains. It is reasonable that these two variables are connected since dampness on indoor surfaces can created mold growth as well as damp stains. The third factor included indicators related to high relative air humidity (damp bed clothing and perception of humid air). Water damage, window pane condensation in winter, or mold odor did not belong to any factor. Our study indicated that indoor dampness and mold in homes are more common in warmer climate zones in China. This is in agreement with one European multicenter study[39] and one literature review on dampness studies from Europe.[49] Moreover, we found that home dampness and indoor mold were more common at higher development level of the city (measured as annual GDP/capita). It is important to avoid further increase of dampness and indoor mold in Chinese homes in the future linked to the increased economic development and the increased ambient temperature due to the global warming.

Dampness and indoor mold can have different causes. China is a large country with different climate zones and causes of dampness and indoor mold could be different in different climate zones. Living in an area with a higher annual precipitation may increase the risk of dampness and indoor mold in residential buildings.[39] Accidental events such as flooding or water leakage from the roof or from water or sewage pipes can cause dampness in building materials. Living on the ground floor may increase the risk of water damage from the ground and from heavy rains or flooding. Living on the top floor may increase the risk of water damage from leaking roofs. Overcrowding or certain personal habits may increase the indoor dampness load (eg, drying of clothes indoors, keeping aquarium, frequent showering, or cooking.). The indoor dampness load is increased in homes with poor ventilation. Poor constructions and poor maintenance are other risk factors for dampness and mold in residential buildings.

More detailed technical building investigations are needed to identify the main causes of dampness and indoor mold in Chinese homes. Due to the rapid urbanization, modern Chinese buildings are often low quality buildings not expected to last for very long time. This may increase the risk of dampness and indoor mold due to poor constructions and poor maintenance. One study from Shanghai reported that living on the ground floor or the top floor and having an aquarium were risk factors for home dampness-related exposures.[50] Another study measured indoor carbon dioxide (CO_2) and other indoor air pollutants in homes in Shanghai.[51] Indoor CO_2 is an indicator of ventilation flow in relation to personal load and should be below 1000 ppm. Most living rooms had CO_2 levels below 1000 ppm, whereas over half of the child's bedrooms had CO_2 levels slightly over 1000 ppm during night. They concluded that air quality among most residences in Shanghai could meet the national standard for indoor air quality in warm seasons; but ventilation status in winter should be improved.[51] Another publication from the same home inspection study in Shanghai reported indoor concentrations of airborne fungi.[52] The mold levels were higher in spring and winter than in other seasons. Higher levels of airborne culturable fungi in the child's bedroom were positively associated with location in a rural area, living in buildings constructed before 2000, and ground floor or top floor location in the building. They concluded that indoor airborne culturable fungi during spring and winter require more attention than in other seasons.[52]

5 | CONCLUSIONS

Dampness and mold in Chinese homes can increase the risk of rhinitis, ocular, throat and dermal symptoms, headache and fatigue among younger adults. It is important to use different types of dampness indicators in indoor epidemiology to cover all aspects of dampness. The prevalence of dampness and mold can be higher in southern China, linked to a warmer and more humid climate. However, the health risk linked to water damage and mold odor can be higher in northern China, possible due to less ventilation in the homes because of the cold climate. Our study supports the view that dampness and mold are the risk factor for adult rhinitis, throat symptoms, and nonrespiratory medical symptoms. It is important to reduce dampness and mold in dwellings to improve indoor environment and health.

ACKNOWLEDGEMENTS

This study was supported by the National Key Research and Development Program of China (2017YFC0702700), the State Key Basic Research Program (973) project (2015CB553404), Natural Science Foundation of Shanxi Province of China (2016011080), Shanxi One Hundred Excellent Experts Project (nr 9) and the Swedish Research Council Formas (348-2011-7402).

CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Zhang X, Norbäck D, Fan Q, et al. Dampness and mold in homes across China: Associations with rhinitis, ocular, throat and dermal symptoms, headache and fatigue among adults. *Indoor Air*. 2018;00:1–13. https://doi.org/10.1111/ina.12517

AMERICAN JOURNAL OF INDUSTRIAL MEDICINE 55:844–854 (2012)

Comparison of Work-Related Symptoms and Visual Contrast Sensitivity Between Employees at a Severely Water-damaged School and a School Without Significant Water Damage

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Background *The National Institute for Occupational Safety and Health (NIOSH) conducted a health hazard evaluation (HHE) of a water-damaged school in New Orleans (NO), Louisiana. Our aim in this evaluation was to document employee health effects related to exposure to the water-damaged school, and to determine if VCS testing could serve as a biomarker of effect for occupants who experienced adverse health effects in a water-damaged building.*

Methods *NIOSH physicians and staff administered a work history and medical questionnaire, conducted visual contrast sensitivity (VCS) testing, and collected sticky-tape, air, and dust samples at the school. Counting, culturing, and/or a DNA-based technology, called mold-specific quantitative PCR (MSQPCR), were also used to quantify the molds. A similar health and environmental evaluation was performed at a comparable school in Cincinnati, Ohio which was not water-damaged.*

Results *Extensive mold contamination was documented in the water-damaged school and employees (n ¼ 95) had higher prevalences of work-related rashes and nasal, lower respiratory, and constitutional symptoms than those at the comparison school (n ¼ 110). VCS values across all spatial frequencies were lower among employees at the water-damaged school.*

Conclusions *Employees exposed to an extensively water-damaged environment reported adverse health effects, including rashes and nasal, lower respiratory, and constitutional symptoms. VCS values were lower in the employees at the water-damaged school, but we do not recommend using it in evaluation of people exposed to mold.* Am. J. Ind. Med. 55:844–854, 2012. *Published 2012. This article is a U.S. Government work and is in the public domain in the USA.*

KEY WORDS: mold; school; visual contrast sensitivity; respiratory; asthma; health hazard evaluation

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Disclosure Statement: The authors report no conflicts of interests.

[y] The MSQPCR technology was patented (#6,387,652) by the U.S. Environmental Protection Agency (EPA) and commercial applications can result in royalties paid to the EPA. The EPA, through its Office of Research and Development, partially funded and collaborated in the research described here. It has been subjected to the Agency's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

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Accepted 6 April 2012

DOI 10.1002/ajim.22059.

INTRODUCTION

The National Institute for Occupational Safety and Health (NIOSH) conducted a health hazard evaluation (HHE) at a 1930s public high school in New Orleans (NO), LA. Employees requested the HHE because they were concerned about visible mold and flaking paint. Employees reported respiratory symptoms and difficulty concentrating, irritability, and trouble remembering things.

In 2009, the World Health Organization (WHO) published guidelines for protection of public health from mold and other exposures in damp buildings [WHO, 2009]. Based on its review of the scientific literature, the WHO concluded that there was sufficient epidemiologic evidence that occupants of damp buildings are at risk of developing upper and lower respiratory tract symptoms (e.g., cough, wheeze, and dyspnea), as well as respiratory infections, asthma, and asthma exacerbation. The WHO also concluded that limited evidence suggested associations between bronchitis and allergic rhinitis and damp buildings.

Visual contrast sensitivity testing (VCS) measures deficits in visual perception as a result of effects on the central nervous system even though visual acuity, which is a function of the eye itself, is normal [Regan, 1989]. VCS testing has been reported as useful in ''diagnosing'' and monitoring treatment of ''biotoxin-related illness'' among individuals working or living in water-damaged buildings [Shoemaker and House, 2005, 2006]. ''Biotoxin-related illness'' is not a generally accepted medical condition, and reportedly consists of multiorgan system symptoms, among which neurobehavioral symptoms are prominent. Interpretation of these studies is hampered by methodological limitations, including a nonrepresentative sample, medical conditions that often present with multiple system symptoms (fibromyalgia and chronic fatigue syndrome), the lack of a comparison group, and poor exposure characterization. These limitations could account for the lower VCS values in the participants of these studies, rather than illness from working or living in water-damaged buildings.

VCS is adversely affected by exposure to toxic substances that affect the central nervous system such as solvents [Frenette et al., 1991; Broadwell et al., 1995; Schreiber et al., 2002; Boeckelmann and Pfister, 2003; Gong et al., 2003; Hitchcock et al., 2003]. In addition to toxic chemical exposures, VCS test results can be affected by a number of factors/conditions, for example, hypertension, diabetes, head injury, alcohol consumption, attention deficit hyperactivity disorder, depression, and various eye conditions such as cataract, glaucoma, LASIK, and other eye surgery [Atkin et al., 1979; Sokol et al., 1985; Roquelaure et al., 1995; Pearson and Timney, 1998, 1999; Trick et al., 1988; Nomura et al., 2003; Hammond et al., 2004; Shamshinova et al., 2007; Bartgis et al., 2009; Bubl et al., 2009]. Therefore, any assessment of possible chemical or toxin exposures by VCS testing must take into consideration these other factors/conditions.

Our aim in this evaluation was to document employee health effects related to exposure to the water-damaged school, and to determine if VCS testing could serve as a biomarker of effect for occupants who experienced adverse health effects in a water-damaged building.

MATERIALS AND METHODS

This evaluation was conducted under a blanket institutional review board approval for the HHE program because HHEs are generally not considered research but workplace evaluations. In April 2005, NIOSH physicians and staff conducted a walk-through assessment of the water-damaged school and held confidential, open-ended interviews with school employees. In addition, preliminary environmental sampling (collection of tape samples for microscopic analysis for fungal spores, collection of bulk paint samples for lead analysis, and use of a moisture meter to qualitatively assess wall moisture levels) was performed. In May 2005, a work and health history questionnaire and VCS testing were performed at the water-damaged school. We also collected environmental samples for mold. We went to a comparison school (without water-damage) in Cincinnati, OH in February 2006 to do a similar environmental evaluation, a questionnaire survey, and VCS testing.

Medical Questionnaire

All employees at both schools were invited to participate in the evaluation. Each participant gave full informed consent and filled out a questionnaire about his or her age, medical history, work history, exposure to mold or moisture in the home, and symptoms experienced during the last month while working in the school. These included upper respiratory and mucus membrane, lower respiratory, constitutional, neurobehavioral, and dermal symptoms. If the participant reported having the symptom during the previous month while in the school and that the symptom improved or went away on days not at school, the symptom was considered ''work-related.'' The presence of work-related wheezing or whistling in the chest, or two of the following three symptoms: cough, chest tightness, or unusual shortness of breath defines symptoms consistent with work-related asthma.

Visual Contrast Sensitivity

VCS testing [Ginsburg, 1993] was conducted with the Functional Acuity Contrast Test (F.A.C.T.^{TM}) handheld chart by NIOSH physicians. The F.A.C.T. sine-wave grating chart tests five spatial frequencies (1.5, 3, 6, 12, 18 cycles per degree) and nine levels of contrast. Cycles per degree refers to the number of alternating bands within one degree of visual angle and contrast refers to the difference in intensity between the light and dark bands. Because high levels of visual sensitivity for spatial form are associated with low contrast thresholds, a reciprocal measure (1/threshold), termed the contrast sensitivity score, is computed. Measures of VCS, rather than measures of refractory visual acuity, have been presented as better appraisals of visual dysfunction resulting from chemical exposures. However, if visual acuity is poor, then performance on the F.A.C.T. will also be poor. Therefore, we also measured visual acuity with a handheld Snellen chart. Results from persons with visual acuity of 20/50 or less were removed from further analysis.

The tests were conducted monocularly and binocularly under standard daylight illumination in the library of each school (68 and 239 candelas per square meter, as specified by the Ginsburg method). Participants who used corrective lenses were asked to wear them during testing. The test results place the VCS ability of the employee on the scale of average performance for 90% of the general population [Ginsburg, 1993] (to learn about the VCS test and to see how it is performed, go to: http://www.youtube.com/watch?v¼lpvCoPqWb0Q).

Environmental Sampling

Environmental analyses during the first site visit at the water-damaged school included moisture measurements using a TRAMEX Moisture Encounter meter (Tramex Ltd., Littleton, CO). Also, 14 sticky tape samples (SKC Inc., Eighty-Four, PA) were collected in multiple rooms for microscopic analysis of mold spores and other indications of mold growth. In addition, five vacuum dust samples were collected after class, two from fourth floor and three from third floor classrooms, using a filter sock with an average pore size of 6.7 mm (Midwest Filtration Company, Fairfield, OH) attached to a high-efficiency particulate air vacuum, 497A JIC (3M, St. Paul, MN) for approximately 5 min each (time depends on filling of the sock). These five dust samples were analyzed via a DNA-based method called mold-specific quantitative polymerase chain reaction (MSQPCR) [Haugland et al., 2002, 2004; Brinkman et al., 2003].

During the second site visit to the NO school, additional environmental samples were collected. In four classrooms and one outdoor location, triplicate air samples were collected at a flow rate of 28.3 liters per minute (lpm) using Andersen N-6 samplers (Thermo Electron Corporation, Waltham, MA) in conjunction with malt extract agar (MEA) plates and triplicate spore trap samples were also collected with Air-o-Cell^1 samplers (Zefon International, Inc., Ocala, FL) at a flow rate of 15 lpm. All samples were collected for 5 min each. The vacuum sampling pumps were pre- and post-calibrated with a DryCal (Bios International Corporation, Butler, NJ).

At the comparison school in Cincinnati, one outside and four inside air samples were collected (during school) at a flow rate of 3.5 lpm for approximately 5 hr with SKC Inhalable Button samplers (SKC Inc.) in conjunction with a 2.0-mm pore size polycarbonate filters. These samples were analyzed using MSQPCR. Eight sticky tape samples were collected for microscopic analysis. In addition, triplicate samples were collected by both Anderson samplers and Air-o-Cell^1 samplers (as described above for the NO school) at one outside location (just outside the school entrance) and in four rooms, for 5 min each.

Lead concentrations in the water-damaged school paint were assessed by collecting flaking paint samples in seven rooms and analyzing them for lead content with NIOSH Method 7300, that is, inductively coupled plasma atomic emission spectrometry [NIOSH, 2012]. At the comparison school, no peeling paint was found and, therefore, no samples were collected for lead analysis.

Statistical Analysis

Statistical analysis was performed with SAS Version 9.1.3 software (SAS Institute, Cary, NC) and StatXact Version 6 software (Cytel Software Corporation, Cambridge, MA). Results with *P*-values ::: 0.05 were considered statistically significant. Chi square or Fisher's exact tests were used to compare the prevalence of symptoms, certain demographic characteristics, and percent of abnormal VCS scores between schools. To examine the relationship between work-related symptoms and VCS test results, the symptoms were combined into the following symptom complexes: upper respiratory (sinus problems, dry or irritated eyes, nosebleeds, sore or dry throat, frequent sneezing, stuffy nose, or runny nose); and lower respiratory (cough, wheezing or whistling in the chest, chest tightness, or unusual shortness of breath).

The Wilcoxon two-sample test was used to compare alcohol consumption and VCS values between employees at each school and to examine the relationship between symptom complexes (i.e., upper or lower respiratory symptoms) and VCS values. MSQPCR mold concentration data having a minimum detection limit of 1 cell/mg dust were treated as left-censored data with appropriate statistical methods applied [Helsel, 2005]. Procedurally, nondetects were set at half the minimum detection limit, and given equal and lowest rank for nonparametric rank-based analyses [Helsel, 2005].

RESULTS

In the water-damaged school, condensation was observed in classrooms near the top of walls and in the adjacent hallways. Rusty spots on the light fixtures and stained or missing ceiling tiles were observed in fourth floor classrooms and hallways of the water-damaged school (Figs. 1 and 2). The moisture readings of the plaster walls were 70–90% in the third floor stairwell, 80% on the fourth floor exterior wall, and 10–30% on the lower floors. These observations and moisture readings indicated past and current water problems. In the comparison school, there was no visual evidence of on-going water problems with the exception of the band rooms located underneath a patio area.

Demographic and background health data are presented in Table I. Of 119 employees at the water-damaged school, 95 (80%) participated in the evaluation and of 165 employees at the comparison school, 110 (67%) participated. Participants at both schools were similar in sex, age, history of psychiatric disorder, atopy (the predisposition to allergic disease), smoking history, and reporting mold or moisture problems in their home. Some of the relevant differences included a significantly higher percentage of employees at the water-damaged school had hypertension but a significantly higher percentage of comparison school employees reported head injuries. The median number of alcoholic drinks among comparison employees was higher than those at the water-damaged school.

Employees from the water-damaged school had higher prevalences of work-related rash and lower respiratory, upper respiratory, constitutional, and neurobehavioral symptoms in the previous month than employees from the comparison school reported (Table II). Thirteen employees at each school reported currently having asthma, but 69% of the asthmatics at the water-damaged school reported their asthma was worse at work, compared to 23% at the comparison school (P ¼ 0.02). We removed employees with current asthma from our analyses for lower respiratory symptoms. Again, the water-damaged school employees had a significantly higher prevalence of work-related cough ($P < 0.01$), wheezing or whistling in the chest, ($P < 0.01$), chest tightness ($P < 0.01$), and unusual shortness of breath ($P < 0.01$). After excluding those employees who reported current, physician-diagnosed asthma, 20 employees at the water-damaged school met the definition of symptoms consistent with work-related asthma.

FIGURE 1. Picture of mold growing along hallway ceiling at the water-damaged school.

FIGURE 2. Picture of classroom wall and ceiling showing active mold growth at the water-damaged school.

Fifteen individuals were excluded from analyses of VCS because of conditions that could affect their VCS such as glaucoma, cataract, LASIK surgery, or retinal surgery (4 from the water-damaged school and 11 from the comparison school). If near visual acuity was 2:20/50 for a particular eye, these specific-eye results were excluded (16 eyes from the water-damaged school and 21 eyes from the comparison school). Three individuals (two from the water-damaged and one from the comparison school) were excluded from analyses because their binocular near visual acuity was 2:20/50. Near monocular and binocular visual acuity did not differ significantly between the remaining employees (80 from the water-damaged school and 81 from the comparison school).

Monocular and binocular VCS values were significantly lower at all spatial frequencies among employees at the water-damaged school ($P < 0.01$). We repeated the analyses excluding diabetics, and results were similar. We compared VCS values between schools among only hypertensives, and again among only nonhypertensives. We found lower values at all frequencies among employees at the water-damaged school in both groups, although the

TABLE I. Selected Characteristics of Participants, by School

Variable	Water-damaged school (n ¼ 91^95[a])	Comparison school (n ¼ 98^109[a])
Age (mean)	46 years	48 years
Tenure (median)	4 years	8 years
Number of alcoholic beverages in the past 30 days (median)	0	3
	Water-damaged school, N (%)	Comparison school, N (%)
Female	49 (52)	59 (55)
Mold or moisture problem at home	6 (6)	9 (8)
Ever had asthma	20 (21)	13 (12)
Physician diagnosed asthma	19 (20)	12 (11)
Currently have asthma	13 (14)	13 (12)
Atopy[b]	69 (73)	75 (69)
Diabetes	8 (8)	4 (4)
Hypertension	37 (39)	28 (26)
Physician-diagnosed anxiety	15 (16)	15 (14)
Physician-diagnosed depression	15 (16)	17 (16)
Physician-diagnosed obsessive compulsive disorder	1 (1)	2 (2)
Physician-diagnosed bipolar disorder	3 (3)	1 (1)
History of eye surgery	3 (3)	10 (9)
History of head injury	10 (11)	23 (21)
Ever smoked cigars, cigarettes, or pipes	32 (34)	35 (33)

[a] Denominators vary due to missing information.

[b] Atopy is a self-reported history of asthma, allergic rhinitis or hay fever, or eczema. Atopy signifies a predisposition to allergic disease.

differences were not statistically significant among the hypertensives at spatial frequencies 1.5 cpd (*P* ¼ 0.12) or 18 cpd (*P* ¼ 0.07) in the left eye or spatial frequencies 12 cpd (*P* ¼ 0.12) and 18 cpd (*P* ¼ 0.16) binocularly among nonhypertensives. A significantly higher percentage of employees at the water-damaged school had scores at all spatial frequencies in the both the right eye and left eye that fell below the average performance for 90% of the general population (i.e., in the lower 10th percentile) than employees at the comparison school (Table III).

We also assessed whether VCS deficits were associated with upper or lower respiratory symptom complexes (Table IV). Persons meeting the designation of lower respiratory symptom complex had significantly lower mean VCS values at all spatial frequencies than those reporting no lower respiratory symptoms. In addition, persons reporting one or more lower respiratory symptom had significantly higher prevalences of VCS scores below 90% of the population (i.e., in the lower 10th percentile) at all spatial frequencies in the left eye, and the majority of frequencies in the right eye and binocularly (Table V).

The environmental analyses and observations (Figs. 1 and 2) revealed widespread evidence of water-damage and mold contamination in the water-damaged school. MSQPCR analysis of the dust showed that nearly every one of the 36 molds tested for was detected in this school (Table VI). In addition, the Environmental Relative Moldiness Index (ERMI) values were high, ranging from 13.8 to 19.1 (The ERMI scale ranges from about - 10 to 20, lowest to highest; Table VI). Fifteen of the 16 sticky-tape samples contained abundant mold spores, primarily *Cladosporium*. More culturable mold and spores were seen in the air samples from the water-damaged school (Table VII) than the comparison school (Table VIII).

At the comparison school, no water-damage or mold growth was observed except in the band rooms. Six of the eight sticky tape samples showed no mold spores and the other two samples, collected in the band rooms had only a few mold spores and hyphae. Air samples from the comparison school for spores, culturable molds, and those analyzed by MSQPCR showed low levels of airborne molds (Table VIII). The outdoor mold concentration was much lower in Cincinnati than NO (Tables VII and VIII).

Lead concentrations in the peeling paint chips collected in seven rooms in the water-damaged school averaged 937 mg lead per gram dust (range: 11–1,730 mg). Since there was no peeling paint in the comparison school, no comparison samples were obtained for lead analysis.

DISCUSSION

Visual observation of flaking paint and extensive mold growth, high moisture readings, and the results from analytical tests for molds documented the environmental

TABLE II. Prevalence of Work-Related Symptoms in the Last Month, by School

Symptom	Water-damaged school (n ¼ 81^88[a]), N (%)	Comparison school (n ¼ 102^107[a]), N (%)	Prevalence ratio (95% confidence interval)	*P*-value
Lower respiratory				
Cough	35 (43)	11 (10)	4.16 (2.26, 7.68)	<0.01
Wheezing or whistling in chest	19 (23)	2 (2)	12.13 (2.91, 50.62)	<0.01
Chest tightness	22 (27)	0	þinf (7.69, þinf)[b]	<0.01
Unusual shortness of breath	19 (24)	4 (4)	6.22 (2.20, 17.56)	<0.01
Upper respiratory				
Sinus problems	27 (33)	14 (13)	2.44 (1.37, 4.35)	<0.01
Dry or irritated eyes	16 (20)	12 (11)	1.72 (0.86, 3.44)	0.12
Nosebleeds	3 (4)	1 (1)	3.70 (0.53, 47.02)	0.33
Sore or dry throat	21 (24)	13 (13)	1.95 (1.04, 3.67)	0.03
Frequent sneezing	17 (20)	4 (4)	5.23 (1.83, 14.96)	<0.01
Stuffy nose	25 (29)	10 (10)	3.09 (1.57, 6.07)	<0.01
Runny nose	22 (25)	7 (7)	3.87 (1.73, 8.62)	<0.01
Constitutional				
Fever or sweats	14 (16)	4 (4)	4.10 (1.40, 12.01)	<0.01
Aching all over	12 (14)	4 (4)	3.71 (1.24, 11.08)	0.01
Unusual tiredness or fatigue	25 (31)	18 (17)	1.78 (1.04, 3.03)	0.03
Headache	30 (35)	21 (20)	1.74 (1.08, 2.81)	0.02
Neurobehavioral				
Difficulty concentrating	15 (18)	4 (4)	4.63 (1.60, 13.44)	<0.01
Confusion or disorientation	8 (10)	2 (2)	5.05 (1.25, 29.56)	0.02
Trouble remembering things	15 (17)	5 (5)	3.59 (1.36, 9.47)	<0.01
Irritability	19 (22)	15 (14)	1.51 (0.82, 2.80)	0.18
Depression	6 (7)	2 (2)	3.74 (0.87, 20.82)	0.14
Change in sleep patterns	16 (19)	4 (4)	4.99 (1.73, 14.37)	<0.01
Rash, dermatitis, or eczema (on face, neck, arms, or hands)	12 (14)	4 (4)	3.70 (1.24, 11.06)	0.01

[a] Denominators vary because of missing information.
[b] Positive infinity or undefined.

problems in the water-damaged school. The ERMI scale was based on the MSQPCR analysis of 36 molds in dust samples from a random national sampling of homes in the US [Vesper et al., 2007a]. Of these 36 molds, there are 26 Group 1 molds associated with mold growth in water-damaged buildings and 10 Group 2 molds that are found commonly in buildings, even without water damage [Vesper et al., 2011; Vesper, 2011]. High ERMI values (e.g., ERMI > 5) in homes have been associated with childhood development of wheeze, rhinitis, asthma, and asthma exacerbation [Vesper et al., 2006, 2007b; Reponen et al., 2011]. Although the ERMI scale was developed for homes in the United States, finding very high ERMI values in other buildings would indicate that these buildings were water-damaged [Meklin et al., 2004; Yap et al., 2009].

Some employees of the water-damaged school reported work-related symptoms shown to be associated with occupancy in damp and/or moldy buildings, including upper and lower respiratory symptoms and possibly development of asthma. Twenty of these employees without current, physician-diagnosed asthma met our case definition of work-related asthma, and employees at the water-damaged school who reported current asthma were significantly more likely to report that their asthma was worse at work than were employees with asthma at the comparison school. Employees at the water-damaged school had significantly elevated prevalences of constitutional symptoms such as fever, body aches, and unusual tiredness, which, along with cough and shortness of breath, could indicate a history of hypersensitivity pneumonitis.

We attempted to address methodological limitations of prior studies of VCS and water-damaged buildings. We compared VCS scores and symptoms of employees in the water-damaged school to those of employees in a school without water-damage. All employees were asked to participate. Participation was relatively high at both schools,

TABLE III. Prevalence of Visual Contrast Sensitivity Scores Below 90% of the General Population

	Water-damaged school (n ¼ 80), N(%)	Comparison school (n ¼ 81), N(%)	*P*-value
Left eye (cycles per degree)			
1.5	10(13)	1(1)	<0.01
3	11(14)	1(1)	<0.01
6	23(29)	4(5)	<0.01
12	23(29)	3(4)	<0.01
18	22(28)	2(3)	<0.01
Right eye (cycles per degree)			
1.5	7(9)	0(0)	<0.01
3	10(13)	1(1)	<0.01
6	21(26)	2(3)	<0.01
12	22(28)	4(5)	<0.01
18	23(29)	6(7)	<0.01
	Water-damaged school (n ¼ 86), N(%)	Comparison school (n ¼ 95), N(%)	*P*-value
Both eyes (cycles per degree)			
1.5	0(0)	0(0)	
3	2(2)	0(0)	0.22
6	11(13)	1(1)	<0.01
12	10(12)	2(2)	0.01
18	11(13)	1(1)	<0.01

so that not only persons who had significant health issues made up our study population. Although the significant decrements in VCS scores in the water-damaged school may be due to mold contamination, these effects also may be the result of other factors. We did not examine all possible exposures that may be present in damp buildings, and it is still unclear exactly what exposures in damp buildings are responsible for health effects [WHO, 2009]. Dust mites, bacteria, and chemical emissions can be present in damp buildings.

TABLE IV. Comparison of Binocular VCS Values Between Participants From Both Schools Who Reported One or More Symptoms of the Upper Respiratory or Lower Respiratory Symptom Complex and Those Who Reported No Symptoms

Work-related symptom complex	Spatial frequency (cycles per degree)	Both schools[a] (n ¼ 159^164), *P*-value
Upper respiratory	1.5	<0.01
	3	<0.01
	6	0.07
	12	0.15
	18	0.09
Lower respiratory	1.5	<0.01
	3	<0.01
	6	<0.01
	12	<0.01
	18	<0.01

[a] All findings in anticipated direction (i.e., mean VCS scores were lower for those with symptom complex).

We postulated that upper and lower respiratory symptoms and their potential treatment may have led to constitutional and neurobehavioral symptoms and the lower VCS scores among these employees. Studies have clearly demonstrated that some persons with allergic rhinitis or asthma complain of fatigue, sleepiness, poor concentration, poor work or school performance, poor sleep, and irritability [Bender, 2005; Leander et al., 2009; Williams et al., 2009]. In addition, objective evidence of cognitive impairment (impaired mood, decreased reaction time, attention, and memory) has been demonstrated in persons with allergic rhinitis and asthma [Fitzpatrick et al., 1991; Weersink et al., 1997; Bender, 2005].

We do not recommend using VCS testing in a clinical setting to diagnose illness in occupants of water-damaged buildings because of its nonspecificity. VCS is adversely affected by a multitude of conditions that are common in general population. We were able to detect significant differences in VCS between these two groups of employees, but most employees at the water-damaged school had normal contrast sensitivity, that is, that which would be seen in 90% of the population. VCS testing is part of a panel of neurobehavioral tests recommended by the Agency for Toxic Substances and Disease Registry (ATSDR) for use in community studies of residents exposed to neurotoxins, as a nonspecific screening tool [Amler et al., 1996; Sizemore and Amler, 1996]. ATSDR also stated there is no evidence that the tests will identify past exposures to neurotoxins, but they ''will detect, without specificity, subtle neurobehavioral changes that may be consequent to many insults'' [Amler et al., 1996]. VCS testing has not been validated as a standalone test for diagnosing neurobehavioral deficits in individuals. In this study, lower VCS or abnormal VCS was mostly related to lower respiratory symptoms. Because asthma and other respiratory symptoms are known to be associated with occupancy in water-damaged buildings, it is more important to remove affected individuals from the building until remediation is complete, and to diagnose and treat their respiratory symptoms or asthma using standard methods such as spirometry and peak flows.

There were several limitations to this evaluation. It is possible that the use of a comparison school from a different region of the country biased our study. This makes it difficult to use outdoor samples for comparisons. The higher outdoor air mold concentrations from NO compared to Cincinnati probably reflects the sub-tropical compared to temperate climates. We had planned a third visit

TABLE V. Prevalence of Visual Contrast Sensitivity (VCS) Scores Below 90% of the General Population Between Those Reporting One or More Work-Related Lower Respiratory Symptom and Those Reporting No Lower Respiratory Symptoms

Spatial frequency in cycles per degree	2:1 work-related lower respiratory symptom	VCS below 90% of population in left eye	VCS below 90% of population in right eye	VCS below 90% of population in both eyes
1.5	Yes	8/52 (15)[a]	4/53 (8)	0/58 (0)
	No	3/94 (3)	1/94 (1)	0/105 (0)
3	Yes	7/52 (14)[a]	6/53 (11)[a]	2/58 (3)
	No	3/94 (3)	2/94 (2)	0/105 (0)
6	Yes	14/52 (27)[a]	12/53 (23)[a]	7/58 (12)[a]
	No	8/94 (9)	6/94 (6)	2/105 (2)
12	Yes	15/52 (29)[a]	13/53 (25)[a]	8/58 (14)[a]
	No	7/94 (7)	7/94 (7)	2/105 (2)
18	Yes	14/52 (27)[a]	14/53 (26)[a]	8/58 (14)[a]
	No	6/94 (6)	10/94 (11)	2/105 (2)

[a] Statistically significantly higher prevalence.

TABLE VI. Fungal Spore Equivalents in Dust Samples (Cells per Milligram Dust) From the Water-Damaged School and Their Calculated Environmental Relative Moldiness Index (ERMI)[a] Value

Fungal species	Room 321	Room 317	Room B	Room 322	Room 400
Group 1[b]					
Aspergillus flavus	6	5	31	7	ND
Aspergillus fumigatus	14	3	94	13	7
Aspergillus niger	9	2	16	7	22
Aspergillus ochraceus	ND	ND	ND	ND	ND
Aspergillus penicillioides	743	211	351	47	55
Aspergillus restrictus	ND	ND	63	ND	ND
Aspergillus sclerotiorum	76	38	196	104	ND
Aspergillus sydowii	1,174	214	67	ND	113
Aspergillus unguis	4,504	237	375	52	2,631
Aspergillus versicolor	ND	ND	ND	ND	ND
Aureobasidium pullulans	754	1,273	5,036	431	2,288
Chaetomium globosum	49	2	21	14	51
Cladosporium sphaerospermum	2,464	1,758	2,647	578	1,119
Eurotium group	512	409	762	173	224
Paecilomyces variotii	31	11	174	82	148
Penicillium brevicompactum	39	14	48	ND	ND
Penicillium corylophilum	ND	ND	ND	ND	36
Penicillium group 2	ND	ND	ND	ND	ND
Penicillium purpurogenum	ND	ND	ND	ND	3
Penicillium spinulosum	ND	ND	ND	ND	ND
Penicillium variabile	12	4	37	16	95
Scopulariopsis brevicaulis	4	18	2	ND	10
Scopulariopsis chartarum	ND	ND	ND	ND	ND
Stachybotrys chartarum	ND	ND	11	ND	ND
Trichoderma viride	10	5	16	135	64
Wallemia sebi	75	43	250	42	57

(*Continued*)

TABLE VI. (*Continued*)

Fungal species	Room 321	Room 317	Room B	Room 322	Room 400
Group 2[c]					
Acremonium strictum	ND	ND	4	ND	ND
Alternaria alternate	ND	22	841	72	159
Aspergillus ustus	252	62	88	138	392
Cladosporium cladosporioides-1	611	502	1,820	556	453
Cladosporium cladosporioides-2	19	3	12	6	17
Cladosporium herbarum	36	12	619	42	86
Epicoccum nigrum	20	16	499	19	95
Mucor group	25	6	8	3	7
Penicillium chrysogenum type 2	112	114	37	43	400
Rhizopus stolonifer	7	21	45	ND1	21
Sum of the Logs Group 1	32.66	29.32	39.05	27.88	33.39
Sum of the Logs Group 2	13.62	14.24	19.93	14.05	18.1
ERMI values	19.0	15.1	19.1	13.8	15.3

ND, not detected.

[a] ERMI values are calculated by subtracting the sum logs of Group 2 molds from the sum logs of Group 1 molds.

[b] Molds associated with mold growth in water-damaged buildings.

[c] Molds that are found commonly in buildings, even without water damage.

to NO to perform similar testing at a local school that did not have water intrusion or mold growth. However, Hurricane Katrina and the subsequent flooding prevented access to an appropriate comparison school in NO. Therefore, an alternative school and location were needed.

Although the schools were of a similar age (both built around 1930) and made of similar materials (stone, brick, and concrete), the comparison was not optimal. The ventilation systems in were different. The NO school was originally designed with a natural ventilation system using large exterior windows and windows at the top of the interior walls adjacent to the hallways to provide cross-ventilation. In 2002, individual ventilation units (Scholar QV^{TM}, Marvair Cordele, GA) were installed in each classroom and hallway. Each unit was placed on an exterior wall; the ventilation unit's louvered outside air intake replaced an existing window. In the comparison school, the ventilation system consisted of steam heat in the winter and air conditioning in the summer.

Another difference in the schools was the occurrence of flaking paint in the NO but not in the Cincinnati school. It may have been present in both schools based on the age of the two buildings, but no peeling paint was observed at the comparison school. It would have been ideal to have been able to separate water-damage and flaking paint exposures but this was not possible. However, lead paint in buildings generally poses a risk to children because of ingestion, and not adults (except from improper removal exposures). We do not think that lead exposures were the source of the VCS deficits, although it cannot be ruled-out. We did not observe any other obvious contaminants that might explain the VCS results.

Another limitation of our study was the difference in racial and socioeconomic status of the two employee populations. Unfortunately, we did not collect information on our specific participant employees because we had planned to use another school in NO as our comparison. However, some city-wide comparisons were available

TABLE VII. Results of Air Sampling for Mold at the Water-Damaged School

	Room 205	Room 316	Room 420	Room 427	Outside
Culturable analysis		Average number of colony forming units per cubic meter of air			
Molds present in order of prevalence (*Cladosporium*, *Penicillium*, *Alternaria*, *Aureobasidium*, *Aspergillus*)	888	321	246	1,000	1,179
Spore trap analysis		Average number of mold spore counts per cubic meter of air			
Molds present in order of prevalence (*Cladosporium*, *Alternaria*, *Epicoccum* *Penicillium/Aspergillus*)	1,311	388	98	1,911	4,000

TABLE VIII. Results of Air Sampling for Mold at the Comparison School

	Room 102	Room 333	Library	Room 260	Outside
Culturable analysis	Average number of colony forming units per cubic meter of air				
Molds present in order of prevalence (*Cladosporium, Penicillium, Epicoccu, Aureobasidium, Aspergillus*)	8	82	159	12	140
Spore trap analysis	Average number of mold spore counts per cubic meter of air				
Molds present in order of prevalence (*Cladosporium*, *Penicillium/Aspergillus*)	27	187	253	93	520
Mold-specific quantitative polymerase chain reaction analysis	Spores per cubic meter of air				
Group 1 Molds					
Aspergillus fumigates	ND	ND	34	ND	35
Aspergillus niger	ND	ND	7	ND	ND
Eurotium group	ND	61	20	ND	210
Penicillium brevicompactum	ND	ND	ND	23	13
Group 2 Molds					
Cladosporium cladosporioides type 1	1	4	ND	ND	ND
Penicillium chrysogenum type 2	ND	ND	5	ND	ND

ND, none detected.

from databases. Cincinnati was 84% white and 13% black and New Orleans was 55% white and 37% black [CensusScope, 2010]. The salaries for teachers in the NO School District ranged from $25,439 to $41,478 and those of Cincinnati Public School teachers ranged from $34,888 to $69,609 [US Cities, 2009]. This implies a lower socioeconomic status for the NO school employees, which may be associated with poorer health overall and perhaps substandard housing. However, a similar proportion of employees at both schools reported mold or moisture problems at home (6–8%). Another limitation is the possibility of reporting bias, meaning that those participants in the water-damaged school may be more likely to report symptoms than those in the comparison school. Finally, the cross-sectional nature of our evaluation does not allow for determination of cause–effect relationships.

ACKNOWLEDGMENTS

Industrial hygiene field assistance was provided by Srinivas Durgam, Andrea Markey, Rob McCleery, and Perianan Periakaruppan. Medical field assistance was provided by Judith Eisenberg and Marilyn Radke. Analytical support was provided by Ronnee N. Andrews, Chemical Exposure Monitoring Branch, Division of Applied Research and Technology, and EMLab P&K Laboratories, Cherry Hill, New Jersey. Health communication assistance was provided by Stefanie Evans. Editorial assistance was provided by Ellen Galloway.

Notice: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health. The U.S. Environmental Protection Agency (EPA) through its Office of Research and Development partially funded and collaborated in the research described here. It has been subject to the Environmental Protection Agency's peer review and has been approved as an EPA publication. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA or NIOSH for use. EPA can receive royalties based on the commercial use of it patented mold-specific quantitative PCR.

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