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# PERSISTENT RESPIRATORY PROBLEMS, NEUROLOGIC, CARDIAC, MUSCLE AND JOINT SYMPTOMS OF OCCUPANTS IN WATER AND MICROBE DAMAGED BUILDINGS

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SAIJA HYVÖNEN: Persistent respiratory problems, neurologic, cardiac, muscle and joint symptoms of occupants in water and microbe damaged buildings
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## ABSTRACT

Objectives: The aim of this study was to estimate the risks of respiratory, neurologic, cardiac, musculo-skeletal, and infectious symptoms, multiple chemical sensitivity (MCS), and asthma severity and persistence in patients working in water damaged buildings with verified microbe growth.

Methods: This thesis includes a descriptive study of an asthma cluster during a renovation of a microbe damaged building. The severity of asthma was evaluated by 12 and 30 months after the exposure had ended. A microbiological work-up of the building was evaluated and toxicological analysis of the dust samples were made. Three cross-sectional retrospective studies compare morbidity risks in occupants of water- and microbe-damaged vs. non-water-damaged buildings. Statistical models were used to calculate risk ratios (RR) for the symptoms. Exposure to toxic microbiota was evaluated by microbiological and/or toxicological methods investigated in the microbiota-damaged premises.

Results: New asthma cases that manifested during the renovation of a microbe-damaged work-environment were still symptomatic after 12 and 30 months with 81% of the cases resistant to ordinary asthma treatment. All workspaces of the employees with diagnosed asthma had toxicity in the indoor dust specimens. Compared to the unexposed groups, all three study groups had elevated risks to neurologic symptoms, respiratory symptoms, and fatigue. Adult groups had elevated risk to cardiac symptoms and multiple chemical sensitivity (MCS). The children´s group showed elevated risks of otitis, gastrointestinal symptoms, and musculo-skeletal symptoms, which was also elevated in the other adult group.

Conclusions: The results of this study verify that asthma resulting from water damaged building toxic microbiota exposure may be severe and persistent. It also supports the hypothesis that toxic microbiota exposure in water damaged buildings may cause multiple organ symptoms.

KEYWORDS: Asthma, indoor air, microbe damaged building, mold, multi organ symptoms, multiple chemical sensitivity, neurology, water damage

UNIVERSITY OF TURKU
Lääketieteellinen tiedekunta
Kliininen laitos, Työterveyshuolto
SAIJA HYVÖNEN: Pitkäaikaiset hengitystieongelmat sekä neurologiset, sydän-lihas- ja niveloireet työntekijöillä ja koululaisilla, jotka työskentelivät kosteus- ja mikrobivaurioituneissa rakennuksissa.
Väitöskirja, 125 p.
Turun kliininen tohtoriohjelma
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## TIIVISTELMÄ

Tavoite: Tutkimuksen tavoitteena oli arvioida kosteus- ja mikrobivaurioituneissa kiinteistöissä työskentelevien henkilöiden hengitystieoireiden, neurologisten oireiden, sydänoireiden, nivel- ja lihasoireiden ja monikemikaaliherkkyyden esiintyvyyttä sekä puhjenneen astman vaikeusastetta ja astmaoireiden pysyvyyttä.

Menetelmät: Väitöstutkimuksessa kuvataan kosteusvaurioituneen kiinteistön remontin aikana sairastuneiden potilaiden taudinkuvaa ja vaikeusastetta. Astman vaikeusaste arvioitiin Global Initiative for Asthma -luokituksen perusteella vuoden ja 2,5 vuoden kuluttua altistumisen päättymisestä. Rakennuksesta tehdyt mikrobiologiset selvitykset arvioitiin ja remontin aikana astmaan sairastuneiden työhuoneista otettiin pölynäytteet toksisuuden määritystä varten. Lisäksi tutkimuksessa verrattiin kolmen kosteus- ja mikrobivaurioituneen kiinteistön työntekijöiden ja oppilaiden oirekuvia ei-kosteusvaurioituneissa kiinteistöissä työskentelevien ja opiskelevien oirekuviin. Altistumista toksisille kosteusvauriomikrobeille arvioitiin kiinteistöjen rakenne-, pöly- ja ilmanäytteistä.

Tulokset: Kiinteistön korjausten aikana astmaan sairastuneiden taudinkuvat olivat vaikeita ja 81 % sairastuneista ei saanut vastetta tavanomaiselle astman lääkitykselle. Kaikki remontin aikana sairastuneet jatkoivat astmalääkitystä 12 ja 30 kuukautta myöhemmin. Kaikkien astmaan remontin aikana sairastuneiden työtiloista löytyi testatuille soluille myrkyllistä pölyä. Kaikissa tutkimusryhmissä oli kohonnut riski neurologisiin oireisiin, hengitystieoireisiin ja uupumisasteiseen väsymykseen. Aikuisten ryhmässä oli myös kohonnut riski sydänoireisiin ja monikemikaaliherkkyyteen. Lasten ryhmässä oli kohonnut riski korvatulehduksiin, maha-suolikanavan oireisiin sekä lihas- ja niveloireisiin.

Johtopäätökset: Tutkimuksen tulokset vahvistavat johtopäätöstä siitä, että kiinteistöjen toksisille kosteusvauriomikrobeille altistumisen yhteydessä ilmenevä astma on vakava ja pysyvä sairaus. Se tukee myös havaintoja siitä, että kiinteistöjen myrkyllisille kosteusvauriomikrobeille altistuminen voi aiheuttaa usean eri elinryhmän oireita. Kosteusvauriomikrobit eivät ainoastaan aiheuta hengitysteiden oireilua ja astmaa, vaan myös muiden elinryhmien oireet ovat mahdollisia.

AVAINSANAT: Astma, home, kosteusvaurio, mikrobivauriot rakennuksissa., monielinoireet, monikemikaaliherkkyys, neurologia, sisäilma.

Te potilaat, joiden surulliset kohtalot ovat saaneet jatkamaan tätä matkaa työn ja kirjan parissa. Tämä on teille.

Kiitos Heli Heikkinen, kun olet sanoittanut niin monen tunteet.

Te omat, tiedätte kyllä, kiitos.

Oulussa 27.4.2022

Saija Hyvönen

*Oot töissä*
*yskit, kurkkua kuumottaa*
*ääni lähtee*
*päähän koskee, iho oireilee*
*tuntuu että leijut*
*Vähättelevät katseet*
*kohtaa*
*Siinä vaiheessa*
*haluaisit mennä maanrakoon*
*olla piilossa ja pyydellä*
*anteeksi olemassa oloasi*
*tunnet itsesi pieneksi*
*Mietin miksi minun*
*piti altistua huonolle*
*sisä ilmalle*
*Miksi minä en voisi*
*olla joku noista joka ei*
*oireile*
*Surullista*
*Onhan teidän vaikea*
*ymmärtää ja tukea*
*ennen kuin se kolahtaa*
*omalle kohdalle*
*niin kuin minulle*
*on käynyt*
*ja valitettavan*
*monelle muulle*
*On vain jaksettava*
*katsoa eteenpäin*
*Periksi en anna*
*Älä sinäkään* *by Heli*

# Table of Contents

# Abbreviations

| | |
|---|---|
| ABPA | allergic pulmonary aspergillosis |
| ACOS | asthma COPD overlap |
| BAL | broncho alveolar lavage |
| BRI | building related intolerance |
| cfu | colony forming unit |
| CInt | confidence interval |
| CI | chemical intolerance |
| CS | chemical sensitivity |
| CNS | central nervous system |
| CRS | chronic rhinosinusitis |
| COPD | chronic obstructive pulmonary disease |
| DG18 | dechlorane-glycerol-18 |
| DMHS | dampness and mold hypersensitivity syndrome |
| EC | effect concentration |
| FEV1 | forced expiratory volume in one second |
| FVC | forced vitality capacity |
| GINA | Global Initiative for Asthma |
| HITEA | Health Effects of Indoor Pollutants: integrating microbial, toxicological and epidemiological approaches. |
| ICS | inhaled corticosteroid |
| IEI | idiopathic environmental intolerance |
| Ig | immunoglobulin |
| IL | interleukin |
| IOM | Institute of Medicine |
| LABA | long-acting beeta-2 agonist |
| LMW | low molecular weight |
| M | mild toxicity |
| MCS | multiple chemical symptoms |
| NRU | neutral red uptake |
| NT | not toxic |
| OA | occupational asthma |

| | |
|---|---|
| OI | odor intolerance |
| O-RCSS | odor-related chronic somatic syndrome |
| PEF | peak expiratory flow |
| PGW | Persian Gulf War |
| RH | relative humidity |
| RR | risk ratio |
| S | strong toxicity |
| SBS | sick building syndrome |
| THG | trypsin glucose yeast |
| THL | Finnish Institute of Health and Welfare |
| THP-1 | human monocytic cell line |
| TLR | toll-like receptor |
| TNF | tumor necrosis factor |
| VOC | volatile organic compound |
| VS | very strong toxicity |
| WDB | water-damaged building |
| WHO | World Health Organization |
| WST-1 | [2- (4-iodophenyl) -3- (4-nitrophenyl) -5- (2,4-disulfophenyl) -2H-tetrazolium, monosodium salt |

# List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Hyvönen S, Syrjälä H. Asthma case cluster during renovation of a water-damaged and toxic building. Microorganisms 2019; 7: 642.

II Hyvönen S, Poussa T, Lohi J, Tuuminen T. High prevalence of neurological sequelae and multiple chemical sensitivity among occupants of a Finnish police station damaged by dampness microbiota. Archives of Environmental & Occupational Health 2020; 16: 1–7.

III Hyvönen S, Lohi J, Tuuminen T. Moist and mold exposure is associated with high prevalence of neurological symptoms and MCS in a Finnish hospital workers cohort. Safety and Health at Work 2020; 11: 173–177.

IV Hyvönen S, Lohi J, Räsänen L, Heinonen T, Mannerström M, Vaali K, Tuuminen T. Association of toxic indoor air with multi-organ symptoms in pupils attending a moisture-damaged school in Finland. American Journal of Clinical and Experimental Immunology 2020; 9(5):101–113.

# 1 Introduction

In 2016, a renovation process was started in a police department building in Oulu. During a six-month period working on the premises, almost 50% of the 290 occupants contacted health care for symptoms including cough, eye irritation, rash, loss of voice, bronchitis, and sinusitis.

Looking back, health complaints before the renovation had been typical of occupants of a damp building; fatigue, eye and nose irritation and absence from work due to upper airway infections. Repeated water damage and inadequate renovations with problematic constructions made the working environment unhealthy. Before the renovation, building investigations revealed microbe damage, but the hedging during the renovation was not sufficient. The first study group is from this workplace.

Occupants of a Finnish hospital in Helsinki contacted us in 2017, stating that employees had suffered from health problems due to dampness for several years. The second study group is from this hospital. Simultaneously with our studies of the adult groups, parents of students from one Finnish school contacted us with a request to investigate the morbidity of their children, who presented multiple non-specific symptoms.

Molds and bacteria act as saprotrophs in organic materials, and they are an essential part of nature´s cycle of life. Microbial growth starts in days when water reaches a building´s construction materials. Microbes are very modest in their nutritional needs, and they are able to restart growth even after years of hibernating. In large quantities in indoor environments, water-damaged-building (WDB) microbiota can create health problems. In 2012, remarkable water and microbe damage was estimated to appear in 6–26% of all Finnish buildings, 12–18% of schools, 20–26% of health and social care premises, and 2–5% of office buildings. In addition, 25–50% of hospitals were estimated to have an urgent need for remediation because of these damages. Hundreds of thousands (559 000–958 000) of individuals were exposed to WDB microbiota daily in Finland – [Rakennusten kosteus- ja homeongelmat, eduskunta 2012], and it has been estimated that WDB microbiota exposure in private homes causes 800 asthma cases yearly. Also, 50 000 occupants were estimated to have upper airway problems due to WDB exposure and

20 000 people suffered from lower airway problems because of WDB exposure [Asikainen et al. 2013]. The prevalence of dampness and mold in European housing was evaluated in 2012, revealing 10% with water damage, 10.3% with mold and 12.1% with dampness [Haverinen-Shaugnessy 2012].

According to scientific medical literature, respiratory consequences of remarkable indoor WDB microbiota exposure are undisputable. WDB microbiota in the indoor environment are known to cause airway problems, such as wheezing, new asthma, asthma exacerbation, allergic rhinitis and alveolitis, also known as hypersensitivity pneumonitis [Kuhn et al. 2003, Hardin et al. 2003, WHO 2009, Fisk et al. 2010, Mendell et al. 2011, Karvala et al. 2011, Quansah et al. 2012, Jaakkola et al. 2013, Miller et al. 2014, Hernberg et al. 2014, Karvonen et al. 2015, Cox-Cancer et al. 2015, Mendell et al. 2017, Mendell et al. 2018, Sinclair et al. 2018, Choi et al. 2019, Fisk et al. 2019, Holme et al. 2020]. The prevalence of asthma and allergic respiratory diseases is increasing among the Finnish population [Jousilahti et al. 2016]. It has also been claimed that nonrespiratory organs are influenced by microbes and their metabolic substances. Studies with animals [Fan et al. 2011] show that cognitive and neurologic problems are relevant; several other studies show that both children and adults exposed to moldy environments are at risk of affecting their neurocognitive capacity [Baldo et al. 2002, Crago et al. 2003, Gordon et al. 2004, Jedrychowski et al. 2011, Ratnaseelan et al. 2018] and immune system [Campbell et al. 2004, Shoemaker et al. 2013, Ratnaseelan et al. 2018, Harding et al. 2020]. Dermal effects [Dooley et al. 2020], adenotonsillar hypertrophy [Atan et al. 2016], fatigue, headache and ocular, throat, and dermal symptoms [Zhang et al. 2019] are associated with damp homes, but knowledge of permanent health effects outside of respiratory tract is still controversial. Association between indoor mold exposure and multiple chemical sensitivity (MCS) or Environmental/Chemical Intolerance (EI/CI) has been suggested [Valtonen 2017].

Finnish studies reveal that the risk of joint and muscle symptoms is elevated in moldy environments [Myllykangas-Luosujärvi et al. 2002, Luosujärvi et al. 2003]. Autoimmune system may also be affected by microbes and their metabolites [Ratnaseelan et al. 2018].

This study explores the multi organ health effects in three groups of occupants II-III) and schoolchildren (IV) working in water- and microbe- damaged environments, it looks at the prevalence of adverse health effects and long-lasting health damage and explains the possible mechanisms for the symptoms.

We have previously described the symptoms of four patients working in microbe-damaged offices, including two patients from the police department, which is part of this study. These patients had severe neurological or cardiac symptoms and joint pain, which were mostly relieved after exposure in the workplace ended. All

four patients suffered from severe respiratory problems in relation to water and microbe damage in their workplaces. The patient´s symptom history was explored from their medical records [Hyvönen et al. 2019].

# 2 Review of the Literature

## 2.1 Risk evaluation and methods of investigating indoor air affected by microbes

Water damage always poses a risk for microbial colonizing and adverse health effects. According to the National Supervisory Authority for Welfare and Health in Finland in 2016 (Asumisterveysasetuksen soveltamisohje), remediation of water damaged, decayed material, sensory detected or verified microbial growth inside a building, in reconstruction or insulating materials is mandatory if exposure inside the premises is possible. Instructions for risk evaluation were given by the Ministry of Social Affairs and Health in 2009 (**Table 1**).

Indicator microbes of construction water damage are presented in **Table 2**. Indoor air specimens most frequently show *Penicillium- Aspergillus-,* and *Cladosporium* -species, and a specimen collected from indoor air never excludes microbial growth in the constructions.

During the demolition of microbe-damaged constructions, the concentrations of mold and bacteria in the air are remarkably higher than in normal circumstances. These concentrations can cause negative health effects in humans [Rautiala 2004]. Therefore, the workers doing the demolition work need to be protected from exposure to WDB microbiota [Suomaa 2009].

**Table 1**. Ministry of Social Affairs and Health 2009:18. Probability of harmful exposure to microbes in construction materials.

| | |
|---|---|
| Harmful exposure unlikely | No water damage, no risk constructions, no intense negative indoor air pressure, and no leakage of air from unconventional microbial sources. |
| Harmful exposure possible | Marks of water or moisture in constructions, repaired water damage, occasional negative indoor air pressure and/or possible leakage of air from unconventional microbial sources. |
| Harmful exposure plausible | Visible (moisture) damage indoors, microbial growth in materials or constructions, abnormal microbial findings (air or dust indoors), negative air pressure and/or air taken from damaged construction or space. |

A Finnish study of hospital personnels´ indoor air -related symptoms shows that a good ventilation system diminishes symptoms and complaints of the indoor air, and therefore it is important that hospital ventilation systems are properly and regularly maintained and renovated at least when their technical lifespan expires. A poor ventilation system, performance, and modernity were found in 40% of the ten central hospitals in Finland [Hellgren et al. 2011]. Increased ventilation in classrooms lowered endotoxin, β (1,3)-glucan and particle levels significantly [Rosbach et al. 2016].

The Finnish recommendation in treatment of a patient with symptoms of moisture and mold recognizes and guides the treatment of airway symptoms and asthma in water and mold damage exposure [Kosteus ja homevauriosta oireileva potilas Käypä hoito 2017].

### 2.1.1 Microbial methods

The National Supervisory Authority for Welfare and Health in Finland (Asumisterveysasetuksen soveltamisohje 2016) gives accurate instructions for taking samples of microbial cultures and making analyses from construction material, indoor air, and indoor surfaces. The material specimens need to be taken widely enough from visibly damaged locations or nearby the assumed wet or moist structure, where the odds of damage are most likely. Several samples are always needed, and the sampling should be designed carefully based on the history of water damage and knowledge of the risky constructions, to define the health risks of microbial damage in a building.

**Table 2**. Indicator microbial species of microbial damage in construction materials [Sosiaali ja terveysministeriön selvityksiä 2009:18] with toxins that are known to be produced by these microbial species.

| Mold/ Bacteria species | Genera | Toxin | Toxin health effects |
|---|---|---|---|
| *Acremonium* | *Sarocladium, gliocladium* | Acrebols | Inhibits respiration chain and ATP production of the boar spermatozoids<br>In the mouse insulinoma cell caused necrosis-like cell death [Kruglov et al 2009] |
| *Actinomycetes* | *Streptomyces*<br>*Nocardia* | Valinomycin | Inhibits motility of boar spermatozoa (EC50 10–63 ng/mL) causes edema of mitochondria, disruption of cellular membranes inhibits ATP production. [Andersson et al 1998] |
| *Aspergillus* | *Versicolor* | Aflatoxins | Carcinogenic, genotoxic, enhance malignant cell proliferation and invasion.<br>Inhibits the function of dendritic cells and phagocytosis at concentrations of 10 ng/mL that are relevant in indoor air environment [Marchese et al 2018, Mehrzad et al 2018] |
| | *Ochraceus* | Ochratoxin | Renal failure, may cause urethra carcinoma<br>Genotoxic, causes epigenetic changes and oxidative stress. [Malir et al 2016] |
| | *Terreus* | Citrinin | Nephrotoxic to many animal species [Bennett et al 2003] |
| | | Patulin | Antiviral, antibacterial and antiparasitic properties; WHO: the maximum tolerable concentration is 0.4 mg/kg/day. [Bennett et al 2003] |
| | *Eurotium*<br>*Restricti*<br>*Sydowii*<br>*Fumigatus*<br>*Usti* | | |
| *Chaetomium* | *botrytrichum humicola* | Chaetomin | Toxic to pulmonary tissue [Ammann et al 2005] |
| | | Chaetoglobosin<br>Sterigmatocystein | Carcinogenic [Nieto et al 2018] |

| Mold/ Bacteria species | Genera | Toxin | Toxin health effects |
|---|---|---|---|
| *Engyodontium* | | | |
| *Exophiala* | *phaeococcomyces rhinocladiella ramichloridium* | | |
| *Fusarium* | | Fumonisines | Interfere with metabolism of sphingolipids.<br>Leucoencephalomalacia in horses and hares<br>Causes pulmonary edema and hydrothorax in pigs.<br>Hepatotoxic and carcinogenic to rats and may cause esophagus carcinoma in humans.<br>Strong inhibitors of protein synthesis by interfering with initiation, elongation, and termination steps. [Bennett et al 2003] |
| *Geomyces* | *Pseudogymnoascus oidiodendron Paecilomyces Purpureocillium* | | |
| *Paecilomyces* | | | |
| *Phialophora* | | | |
| *Scopulariopsis* | | | |
| *Sporobolomyces* | | | |
| *Sphaeropsidales* | | | |
| *Stachybotrys* | | Trichotecenes T-2 | |
| | | Satratoxin | Nose exposure caused olfactory sensor neuron apoptosis and acute inflammation of the nasal mucosa and brain.<br>Caused acute rhinitis, olfactory epithelium apoptosis in a dose-dependent manner, and mucosal damage when inoculated into rhesus macaques' nostrils [Carey et al 2012] |
| *Trichoderma* | | Peptaibols | |
| *Trichitrachium* | | | |
| *Ulocladium* | | | |
| *Wallemia* | | | |

### 2.1.2 General toxicity of dust/indoor air

Animal studies show adverse health effects associated with exposing to microbes producing toxic metabolites [Yike et al. 2002, Bennett et al. 2003, Rocha et al. 2005, Versilovskis et al. 2010].

Methods for indoor air and health effect investigations have been presented in recent years [Andersson et al. 2010, Salo 2014, Mannerström et al. 2019]. Toxicity tests from indoor dust, microbial cultures, and water vaporized in air are used in health risk evaluation in microbe-damaged buildings. Spores and microbes end on surfaces, where they can be taken for analysis. Scientific research of the association of dust toxicity and symptoms of occupants has been published [Salin et al. 2017, Salin et al. 2021]. Toxicity of house dust or vaporized water in air can be evaluated by using several probe cells like boar spermatozoa, *Eschericia coli*, and cultured human cells like fibroblasts and macrophages. Nutrition-contained mycotoxins in Finland are evaluated by the Finnish Food Authority (Evira) with similar tests.

Research by several universities in Finland showed no significant association between dust toxicity and water and microbe damaged buildings [Toksikologisen menetelmän kehittämissunnitelma 2010–2012]. Authorization from the National Supervisory Authority for Welfare and Health and the Ministry of Social Affairs and Health for health risk evaluation is missing and the authorities cannot accept toxicity tests as evidence for giving recommendations on the safety and health of water- and microbe -damaged buildings.

## 2.2 Clinical perspectives in WDB microbiota exposure

Multiple symptoms are possible in indoor WDB microbiota exposure (Figure 1). Some patients present with debilitating multiple symptoms: fatigue, neurocognitive symptoms, myalgia, arthralgia, headache, insomnia, dizziness, anxiety, depression, irritability, gastrointestinal problems, tremor, balance disturbance, palpitations, vasculitis, angioedema, and autonomic nervous system dysfunction, rhinosinusitis, allergic rhinitis including allergic fungal rhinitis, and sinusitis, asthma (new onset and exacerbations), conjunctivitis, invasive, and allergic pulmonary aspergillosis (ABPA), hypersensitivity pneumonitis, and sarcoidosis, pain syndromes, movement disorders, delirium, dementia, and disorders of balance and coordination [Hope 2013]. In addition, weakness, cramps, untypical pain, morning stiffness, skin sensitivity, light sensitivity, red eyes, blurred vision, tearing, sinus problems, cough, shortness of breath, numbness, tingling, metallic taste, disorientation, memory loss and cognitive problems were reported in a study of 26 patients exposed to WDB microbiota in a clinic specialized to these patients [Shoemaker et al. 2006]. Clinical outcomes of prolonged WDB microbiota exposure have also been described by

others [Valtonen 2017, Tuuminen et al. 2017, Tuuminen et al. 2018, Tuuminen et al. 2019].

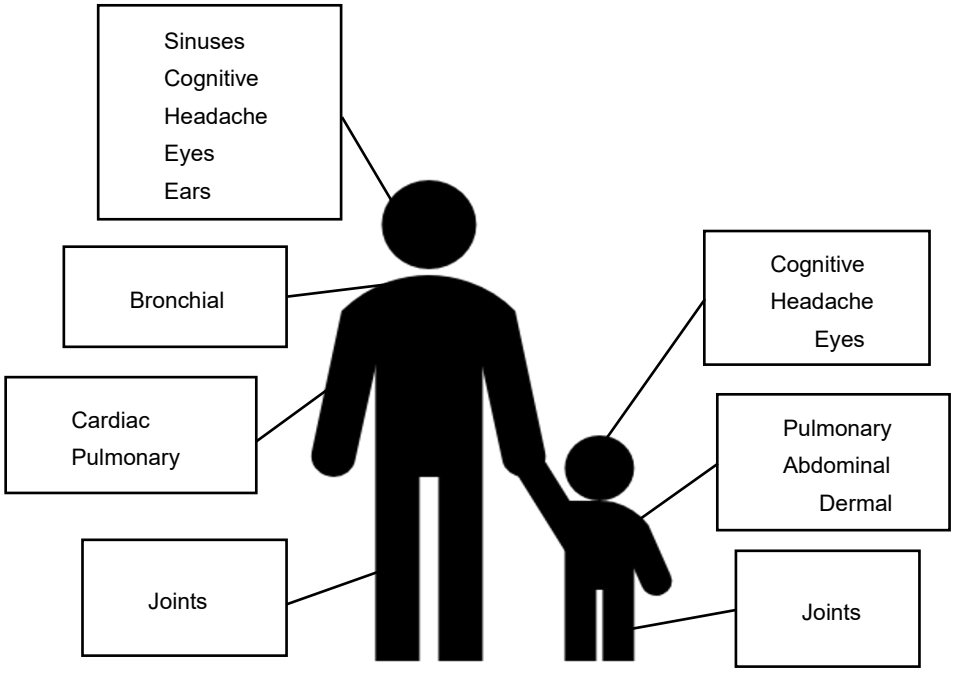


**Figure 1**. Multi organ symptoms in WDB microbiota exposed adults and children.

### 2.2.1 Respiratory symptoms in WDB microbiota exposure

Asthma is the best-known health effect of WDB microbiota exposure. Reports of asthma and allergic rhinitis association with mold exposure support a causal relationship [IOM 2004, WHO 2009, Caillaud et al. 2018]. From the point of immunopathology, asthma can be divided into eosinophilic, non-eosinophilic, and mixed granulocytic disease [Russell et al. 2017]. Patients with eosinophilic inflammation type asthma regulated by the T helper 2 (T2), or cytokines IL-4, IL-13 and IL-5 respond well to inhaled corticosteroids (ICS). In low -type 2 immunity asthma with increased concentrations of the T1 cytokines IL-17 and IL-23, neutrophils predominate in bronchial specimens [Papi et al. 2018]. This late-onset asthma is usually associated with a poor response to ICS treatment [Green et al. 2002]. About 5–20% of adult-onset asthma cases are work-related [Baur et al. 2012, Lau et al. 2019]. More than 300 causative agents in occupational asthma (OA) have been identified and they can be classified into high-molecular-weight proteins (≥ 5000 g/mol) of vegetal or animal origin, which produce eosinophilic inflammation

[Maestrelli et al. 2009, Vandenplas et al. 2019] and low-molecular-weight (LMW) proteins as causative asthma agents involve poor steroid response with neutrophilic inflammatory response [Choi et al. 2019].

In addition to asthma, chronic pulmonary obstructive disease (COPD) has also been associated with indoor mold exposure. In a recent population-based study of all new asthma diagnoses (521) in one hospital district during a 2.5-year study period, asthma-COPD overlap syndrome (ACOS) was significantly associated with the presence of mold odor in the workplace [Jaakkola et al. 2020]. An indoor *Stachybotrys* -exposed group of 25 individuals reported cough, shortness of breath and chest tightness that persisted for more than six weeks in 91% of the exposed individuals. Among this group 17 obtained a follow-up questionnaire 3 years later and persisting asthma-like symptoms, sick building symptoms and concentration problems were significantly more commonly reported compared to a control group [Al-Ahmad et al. 2010].

One investigation clinically assessed 694 mold exposed patients with a specific inhalation challenge test using mold extracts and serial peak expiratory flow recordings in Finland between 1995–2004. At the 6-month follow-up, examinations of 136 patients with probable occupational asthma showed that symptoms persisted, and no improvement in spirometry was noted despite adequate treatment. Only 58% of the patients had returned to work [Karvala et al 2010]. Patients (1267) with previously examined suspected occupational respiratory disease related to exposure to damp and moldy indoor environments were followed up with a questionnaire. Patients with workplace moisture and mold -induced asthma were associated with lower quality of life compared to the controls, which is related to being unemployed (due to disability, retirement, job loss, or another reason) and needing medication [Karvala et al. 2013].

Microbe, fungi, endotoxin and ergosterol exposure in a 20-story water-damaged building led to excessive prevalence of building-related rhinosinusitis, which also statistically significantly increased the risk for asthma [Park et al. 2012]. Hospitalization of the elderly was associated with indoor mold exposure in the residences among 14000 asthma patients in 40 states in the USA [Hsu et al. 2018], and asthma risk was at least 2-fold in poverty-stricken areas with elevated mold contamination compared to the wealthier areas in California Valley [Sinclair et al. 2018].

### 2.2.2 Infections in WDB microbiota exposure

Mucosal dysfunction increases susceptibility to infections [Kuhn et al. 2003, WHO 2009, Fisk et al. 2010, Lanthier-Veilleux et al. 2016, Wong et al. 2016, Polyzois et al. 2016, Valtonen 2017]. Mycotoxins are known to strengthen the infectious

capability of fungus. For example, *Aspergillus fumigatus* -produced gliotoxin helps it grow in the respiratory organs [Sugui et al. 2007]. Recurrent middle ear otitis in children is common [Pettigrew et al. 2004]. Schoolteachers suffering from recurrent sinusitis due to moisture damage had elevated *Chaetomium* IgG levels compared to control schoolteachers [Patovirta et al. 2004]. The mechanism of chronic sinusitis as the most frequently occurring chronic inflammatory disease by the activation of sensory C and A fibers was discussed in 2003. Activation of these fibers leads to release of multiple neuropeptides [Lacroix 2003].

Chronic rhinosinusitis (CRS) prevalence in Europe has been evaluated as 10.9%. Dampness at home was independently related to CRS (OR 1.71) and allergic rhinitis (OR 1.24) in a questionnaire study of 26577 adults [Pind et al. 2017].

Allergic fungal sinusitis is analogous to ABPA; both are chronic inflammatory respiratory tract disorders driven by hypersensitivity responses to the presence of small amounts of extramucosal fungi growing within airways, impacting allergic mucin [Schubert 2009].

Lung or airway infections by fungi or aspergillus can be diagnosed with a respiratory sample culture from sputum or bronchial specimens or from serum IgG testing. Antifungal treatment is indicated in patients with chronic invasive and granulomatous rhinosinusitis and aspergillus bronchitis. Pulmonary and sinus fungal disease prevalence in non-immunocompromised patients in several countries has been introduced [Denning et al. 2017]. Prevalence of chronic bronchitis among First Nations people in Canada was positively associated with moldy odor in the home (RR=2.33) [Pahwa et al. 2017].

Deep tissue infections are also possible and mostly seen in immuno-compromised patients, but they can also occur in previously healthy patients. The most severe forms of *Aspergillus* infections are ABPA and invasive aspergillosis, which have high mortality rates especially in patients with immune suppression or underlying lung diseases [Tomee et al. 2001]. Gliotoxin produced by *A. fumigatus* inhibits the mucociliary system and acts as an immunosuppressive agent in the host tissue [Tomee et al. 2000].

### 2.2.3 Musculo-skeletal, cardiovascular, neurologic, neuropsychological, gastrointestinal, and psychological symptoms in WDB microbiota exposure

Joint and muscle pain is a common symptom when exposed to WDB microbiota in Finnish studies. Arthritis rheumatoides has been associated with *Chaetomium* exposure in Finnish patients [Myllykangas et al. 2002, Myllykangas-Luosujärvi et al. 2002]. The role of neurogenic inflammation has been described in the pathogenesis of arthritis pain [Krustev et al. 2015, Seidel et al. 2013]. Musculo-

skeletal symptoms, such as myalgia and arthralgia are also recognized in reviews in association with mold exposure [Hope 2013, Dooley et al. 2020].

Cardiac symptoms because of indoor mold exposure are rarely reported in scientific literature. Most literature is focused on invasive mycoses [Yuan 2016], which are mostly seen in immunocompromised patients. Palpitations as a symptom of mold exposure have been acknowledged [Curtis et al. 2004, Rea et al. 2004, Hope 2013].

In several studies, exposure to WDB microbiota leads to symptoms as malaise, fatigue, and cognitive impairment, which appear to be related to the duration of the exposure [Baldo et al. 2002, Crago et al. 2003, Campbell et al. 2004]. In one study mold-exposed patients showed impaired cognitive measures, such as verbal learning, visuospatial learning and memory, psychomotor speed, and emotional functioning [Baldo et al. 2002]. In two more studies mold-exposed patients had similar symptoms of neurologic dysfunction compared to controls; motoric deficiencies, short-term memory loss, verbal recall impairments, and reaction time [Kilburn 2003, Rea et al. 2003]. Confirmed toxigenic mold-exposure history patients reported moderate to severe levels of cognitive, physical, and emotional symptoms, like depression. Simultaneously taken quantitative EEG results showed hypoactivation in the frontal cortex. Neuropsychological testing also indicated that impairment in cognitive functions was similar to what is seen in mild traumatic brain injury [Crago et al. 2003]. A study of 10 children chronically exposed to toxic mold showed abnormal findings in objective neurophysiological measurements indicating significant neurological deficits in all the patients [Anyanwu et al. 2003].

Gastrointestinal symptoms are more often linked to the alimentary mycotoxin exposure from food [Nesic et al. 2014, Alshannaq et al. 2017], but there are reports that indoor mold exposure may also cause abdominal problems [Johnson et al. 2009, Hope 2013]. Mycotoxins induce the mast-cell mediating mechanism, which increases the permeability of the gut-blood barrier through the abnormal secretion of neuropeptides [Lieberman et al. 1998].

It is clinically impossible to separate neurophysiological and psychological influences of mold exposure on health. Depression and other mental illnesses have already been widely accepted as an inflammatory disease [Liu et al. 2017, Lee et al. 2019]. Neurological and neurobehavioral symptoms are common in mold-exposed patients [Baldo et al. 2002, Kilburn 2003, Rea et al. 2003, Campbell et al. 2004, Empting 2009, Hope 2013, Shoemaker et al. 2016, Tuuminen et al. 2018]. Psychological consequences are inevitable, due to health and social outcomes, which restrict the lives of the patients through their loss of work ability and through social consequences [Karvala et al. 2013, Karvala et al. 2014].

## 2.3 Mechanisms of symptoms in WDB microbiota exposure

### 2.3.1 Allergic, inflammatory

The mold health effects of *Aspergillus* species are best known because these species are quite allergenic and pathogenic [Kyrup et al. 2000, Greenberger et al. 2002]. In moist environments people are exposed not only to microbes but also to their components and metabolic products as toxins. Bioactive components are recognized in the human immune system by receptors as toll-like receptors and lectin-binding receptors [Netea et al. 2004]. When these receptors are activated, an inflammation process gets started. During their lifetimes, 5% of individuals are predicted to have a mold allergy [Hardin et al. 2003].

Moldy environments consist of several species of microbes, bacteria, and fungi, even amoebae, which potentiate the cytotoxicity and proinflammatory properties of each other [Yli-Pirilä et al. 2006]. Cocultivation with amoebae increased the cytotoxicity of *Streptomyces californicus* and *Penicillium spinulosum*. Macrophages exposed to these cultures produced up to 10 times higher concentrations of NO after exposure to these cocultures. Production of cytokines (IL-6) was two times higher and TNF- α four times higher than after exposure to individually grown microbes [Yli-Pirilä et al. 2007]. A study of moisture-damaged and control schools showed significant elevations of IL-1, TNF-α and IL-6 in a nasal lavage and IL-6 in induced sputum of the subjects from the moisture-damaged school compared to the control school. Symptoms reported by the exposed workers were sore throat, phlegm, eye irritation, rhinitis, nasal obstruction, and cough [Roponen et al. 2001]. Toll-like receptors (TLR) TLR 2 and TLR 4 are implicated by *A. fumigatus* and are a sign of immune responses. Mammal immune systems identify pathogen-associated molecular patterns which are in the cell wall of fungi [Roeder et al. 2004]. This process leads to the secretion of proinflammatory mediates. In a study among 34 confirmedly indoor dampness microbiota -exposed patients in the Indoor Clinic, Helsinki University Hospital showed a significant peripheral blood CD19 leucocyte decrease and lymphocytosis in broncho-alveolar fluid (BAL) samples [Wolff et al. 2009]. The most recent study of 159 serum samples from microbe damaged and reference buildings showed higher complement activity as a sign of inflammation in users of the damaged building than in the controls [Atosuo et al. 2021].

### 2.3.2 Toxic

Several molds are known to produce mycotoxins, which are secondary metabolites produced by microfungi that can cause disease and death in humans and other

animals. Some toxins or their derivates are used as antibiotics, growth promotants and other kinds of drugs and chemical warfare agents [Bennett et al. 2003]. There are at least 21 different mycotoxin classes [Cole et al. 1981] with over 400 individual toxins produced by at least 350 fungi [Kuhn et al. 2003]. A constant need for new therapeutic compounds ensures studies among fungi, seen as an excellent and underexplored source for biologically active compounds with therapeutic potential. Secondary metabolites from more than 10 000 species of fungi were explored using zebrafish embryos, and scientists found 1526 fungal strains producing secondary metabolites with biological activity [Hoeksma et al. 2019]. A standardized method has been available for screening 474 fungal metabolites and mycotoxins in culture extracts since 2003 [Nielsen et al. 2003].

The most important mycotoxins associated with human and veterinary diseases are aflatoxin, citrinin, ergot alcaloids, fumonisins, ochratoxin A, patulin, trichothecenes and zearalenone. Mycoses are the best-known diseases of fungal etiology, but toxic secondary metabolites produced by saprophytic species are also an important health hazard. Mycotoxins are toxigenically active in their carcinogenity, inhibition of protein synthesis, immunosuppression, dermal irritation, and metabolic perturbations. The most usual way for toxins to enter the human body is by contaminated food, but inhalation of toxigenic spores and dermal contact are also considered important routes [Bennett et al. 2003].

*S. chartarum* can produce potent toxins, such as trichotechen satratoxin, which activates the intracellular inflammasome and causes a strong proinflammatory response in human macrophages [Kankkunen et al. 2009] and spirocyclic drimanes which are known to have immunosuppressive effects [Pestka et al. 2008].

Fumonisin from *Fusarium verticillioides* disturbs the metabolism of sphingolipids by inhibiting the enzyme ceramide synthesis. Fumonisin is neurotoxic, hepatotoxic, and nephrotoxic in animals and is classified as a possible carcinogen to humans [Stockmann-Juvala et al. 2008].

Ochratoxin A exposure reduces mitochondrial function and could lead to apoptosis in neurons [Aleo et al. 1991, Gautier et al. 2001, Zhang et al. 2009] and dysfunctional responses in cultured murine microglia and astrocytes [Hong et al. 2002, Zurich et al. 2005]. In studies with mice, satratoxin G produced apoptosis in sensory neurons in the olfactory bulb [Islam et al. 2006a] and encephalitis associated with persistently elevated levels of pro-inflammatory cytokines in the frontal brain region [Islam et al. 2006b].

Aflatoxin B1 produced by *Aspergillus Versicolor* is genotoxic, immunotoxic, hepatotoxic and mutagenic; it is considered one of the most toxic and potent naturally occurring carcinogenic substances and is the leading cause of liver cancer in many developing countries [Klich 2009].

Trichothecene mycotoxins cause emesis, diarrhea, weight loss, nervous disorders, cardiovascular alterations, immune depression, haemostatic dysregulation, decreased reproductive function and bone marrow damage [Rocha et al. 2005]. Some mycotoxins can up- or downregulate several genes, inhibit DNA, RNA, and protein synthesis, and cause oxidative stress [Doi et al. 2011]. Some mycotoxins are known to be carcinogenic [Clark et al. 2006]. Mycotoxins also cause neuroinflammation [Meggs 2017].

Cocultivation with amoebae increased the cytotoxicity of *Streptomyces californicus* and *Penicillium spinulosum* [Yli-Pirilä et al. 2007]. Co-exposure of toxins with lipopolysaccharide or β-glucan induced mitochondrial damage and autophagocytosis. [Korkalainen et al. 2017]. Amylosin caused dose-dependent potassium ion efflux from all tested mammalian cells (human monocytes and keratinocytes and porcine sperm cells). Exposure also inhibited the motility of porcine sperm cells and depolarized the mitochondria of human keratinocytes [Rasimus-Sahari et al. 2015].

Actinomycetes as *Streptomyces* are gram positive bacteria, which are commonly found in moist built environments. *Streptomyces* produces several toxic agents as antibiotics, immunosuppressive agents, and enzyme inhibitors [Andersson et al. 1997].

Toxigenic indoor microbes as *Stachybotrys* emit toxins as liquid vesicles (micro-vesicles, exomes). In these exomes, toxin concentrations are more than 1000-fold higher than in the same microbial particle (spores, hyphae fragments) emissions. From a water-damaged building, *Trichoderma sp* isolated on indoor surfaces has been shown to emit 40 biotoxins in guttation droplets [Castagnoli et al. 2018]. Inhaled vaporized water may include these toxin guttation droplets [Salo et al. 2019]. *Penicillium expansum* from fungal colonies of a damp building material was grown on gypsum in a laboratory, and it emitted mycotoxin-containing guttation liquids. These liquids were more than 100-fold more toxic to mammalian cells in bioassays than guttation droplets produced by the genera *Aspergillus, Chaetomium, Stachybotrys* and *Paecilomyces*. Toxic exudates containing communesins A, B, and D and chaetoglobosins were transferable by air [Salo et al. 2019]. A study investigating mycotoxin production by *Penicillium brevicompactum*, *A. versicolor*, and *S. chartarum* during their growth on wallpaper and the possible aerosolization showed that mycophenolic acid, sterigmatocystein and macrocyclic trichothecenes (sum of 4 major compounds) could be produced at levels 1.8, 112.1 and 27.8 mg/m$^2$. Part of the produced toxins were aerosolized from the substrate [Aleksis et al. 2017]. Microbe biomasses cultured from common indoor mold were able to emit liquid soluble bioreactive metabolites into the indoor air in a recent Finnish study [Andersson et al. 2020].

A comparative study with 200 respiratory cases and 152 asymptomatic employees in an office with a history of water damage analysed dust samples for culturable fungi, ergosterol, endotoxin and cat and dog allergens. Mold and ergosterol levels in dust were associated with new-onset asthma [Park et al. 2008].

A case study of serious building-related ill-health symptoms revealed toxic substances of *Bacillus simplex* and the actinobacterial genera *Streptomyces* and *Nocardiopsis*, which were mitochondriotoxic by dissipating mitochondrial membrane potential of boar spermatozoa. Substances from isolates of *Trichoderma harzianum* Rifai and *Bacillus pumilus* damaged the cell membrane barrier function of sperm cells [Peltola et al. 2001].

A report from Finland described an association between indoor dust toxicity and morbidity in school classrooms [Salin et al. 2017]. The method used was described in an article in 2010 [Andersson et al. 2010]. Indoor dust toxicity and microbial toxicity were analysed by a boar spermatozoa motion inhibition test, and high dust toxicity associates with building-related symptoms (BRS).

There are also controversial opinions of toxic molds and their health effects. A clinical review of 50 cases of purported mold-induced toxic effects did not recognize conditions that could explain presenting symptoms. The review acknowledges three mechanisms as well-accepted mold-induced diseases: allergy, infection, and oral toxicosis. Patients reported upper respiratory tract (80%), lower respiratory tract (94%), systemic (74%), and neurologic (84%) symptoms. Patients were carefully examined, and their medical records reviewed. Most patients showed several pre-existing diagnoses that could plausibly explain their presenting symptoms. [Khalili et al. 2005].

## 2.4 Multiple chemical sensitivity, chemical sensitivity, chemical intolerance

### 2.4.1 Definition

Multiple chemical sensitivity (MCS) was already described in 1952 [Randolph 1952]. MCS is described as a condition, where a person experiences a complex array of recurrent nonspecific symptoms, when exposed to low doses of chemicals that are well tolerated by most people. The person may react to perfumes, deodorants, detergents, tobacco or other smoke, fresh printed matters, paints, varnishes, glues, hairdressers´ products, dust, formaldehyde, or some other known chemicals, and spices. [Valtonen 2017].

A synthesis of the MCS literature in 1992 suggests that the phenomenology of MCS patients overlaps with that of affective spectrum disorders, and both involve dysfunction of the limbic pathways. Animal studies demonstrate that repeated low-

level environmental chemical exposures, like pesticides, cause limbic kindling. Kindling is one central nervous system (CNS) mechanism, that could amplify reactivity to low levels of inhaled and ingested chemicals. This mechanism may initiate persistent affective, cognitive, and somatic symptomatology [Bell et al. 1992].

CNS symptoms are central to the pathology of MCS [Lacour et al. 2005]. Although the disease has been known for decades [Randolph 1952, Cullen 1987, Bartha et al. 1999, Lacour et al. 2005], it´s mechanisms and etiology are still not unanimous and are considered missing [Rossi et al. 2018]. It is possible, however, that MCS will develop after prolonged or cumulative exposure to WDB microbiota [Valtonen 2017]. Epidemiological studies of MCS in association with WDB microbiota exposure are not found.

A 1999 review of MCS with a systematic literature search considered two questions: does MCS exist, and what causes MCS? The conclusion was that there is no unequivocal epidemiological evidence, since no quantitative exposure data exist; MCS seems to exist, but its prevalence seems to be exaggerated. Many causal mechanisms have been proposed, and the evidence seems to support a physical mechanism involving the sensitization of the limbic system most strongly with the recognition of psychological effects on physical illness [Graveling et al. 1999]. The US definition of MCS was published in 1999 [Bartha et al. 1999], and a systematic literature review of MCS was published in 2005 [Lacour et al. 2005], where the definition was extended. The conclusion was that the patients report nonspecific complaints of the CNS and other organ systems. Pathophysiological mechanisms of MCS have been studied [Reid et al. 2001, Pall et al. 2004, Pall 2009, Meggs 2017, Meggs et al. 1996b].

A 2017 review of MCS states that chemical sensitivity refers to the reactivity to environmental chemicals at levels below the toxic level and that are tolerated by most people [Meggs 2017]. Health outcomes are multi-organ: respiratory, gastrointestinal, dermatological, musculo-skeletal, and nervous systems. Chemicals bind receptors on nerve fibers, which release substances that produce sensory stimuli and neurogenic inflammation. Inflammation can lead to a remodeling of tissues in a way that the threshold for symptomatic reactivity is lowered. It is typical to experience sensitivity to low-concentration chemical agents, such as combustion products like tobacco smoke, diesel exhaust, wood smoke, and vehicle exhaust; dusts, such as grain dust, cotton dust, cement dust; fragrances and perfumes; pesticides; and noxious gases, such as sulfur dioxide, ozone, chlorine, oxides of nitrogen, chlorine dioxide, ammonia, chloramine, bleach (sodium hypochlorite), sulfuric acid, ethylene oxide, cleaning agents, and complex mixtures [Meggs 2017].

A systematic review of the MCS literature shares the same definition of MCS and states that etiology, diagnosis, and treatment are still debated among researchers. Even a diagnosis with absolute certainty is considered impossible because of the

non-specific symptoms common to various physical and psychic pathologies. Female gender, and association with medium-high social and cultural categories are considered as basically agreed-upon personal risk factors. Experimental studies have ended in uncertain and controversial conclusions, although involvement of the autonomic nervous and limbic system at the expense of cortical areas is broadly confirmed [Rossi et al. 2018].

### 2.4.2 Epidemiology

The overlap between asthma or allergies and hypersensitivity or intolerance to chemicals has been reported in several epidemiological studies. Epidemiology has been studied in several countries, as presented in **Table 3**.

**Table 3.** Prevalence of MCS, CI or CS in population studies.

| Country | Definition | Size of study | self-reported symptoms | Reference |
|---|---|---|---|---|
| USA 1996 | CI | 1027 | 33% | Meggs et al. 1996a |
| USA 1999 | CI | 4046 | 11.9% | Kreutzer et al. 1999 |
| USA PGW-veterans 2000 | MCS | 3695 | 3.4% | Black et al. 2000 |
| USA 2003 | CS | 1582 | 12.6% | Caress et al. 2003 |
| Japan 2003 | MCS | | 0.74% | Uchiuyama et al. 2003 |
| USA 2004 | MCS | 1057 | 31.1% | Caress et al. 2004/2005 |
| Sweden 2005 | OI | 1387 | 33% | Johansson et al. 2005 |
| Sweden 2008 | CS | 326 | 15.6% | Andersson et al. 2008 |
| South Korea 2014 | MCS | 379 | 19.7%/allergic* 11.3%/not allergic** | Jeong et al. 2014 |
| USA 2012 | CI | 400 | 20.3% | Katerndahl et al. 2012 |
| Japan 2015 | CI | 7245 | 7.5% | Azuma et al. 2015 |
| Norway 2015 | O-RCSS | 13 799 | 0.6/1.4% male/female | Gundersen et al. 2015 |
| USA 2018 | CS | 1137 | 25.9% | Steinemann et al. 2018a |
| Australia 2018 | CS | 1098 | 18.9% | Steinemann et al. 2018b |
| Finland 2018 | CI | 1535 | 15.2% | Karvala et al. 2018a |
| Sweden 2018 | CI | 3406 | 12.2% | Karvala et al. 2018a |

MCS=multiple chemical sensitivity
CI=chemical intolerance
CS= chemical sensitivity
OI= odor intolerance
O-RCSS= odor related chronic somatic syndrome
*Participants with allergies reporting MCS
** non-allergic participants reporting MCS

# 3 Aims

The aim of this study is to explore the multi-organ health effects in three groups of occupants and schoolchildren working in water-damaged and toxin -producing microbe growing environments compared to occupants in buildings with no known water damage history, to explore the prevalence of adverse health effects and long-lasting health damage such as asthma severity and MCS in WDB environments and to explain the possible mechanisms for symptoms.

# 4 Materials and Methods

Three independent groups from three public buildings showed similar symptoms and health complaints with their own characteristics. This study is made by descriptive, clinical methods and cross-sectional retrospective-based methods to estimate morbidity risks in the exposure to moldy and toxic indoor environments.

## 4.1 Patient data

### 4.1.1 Police department study

The first (I) part of the study is made among occupants in a Finnish police station. 138 workers experiencing health symptoms contacted the occupational health care unit over 6 months in 2016–2017 during the renovation of a water-damaged workplace. Asthma onset with workers in the workplace was examined in their occupational health unit or in the hospital pulmonary disease department. The asthma-diagnosed patients were interviewed by telephone 12 and 30 months later. Asthma medication and asthma -symptoms were inquired, and asthma severity was evaluated by the Global Initiative for Asthma (GINA) -classification [GINA 2018].

Data for the second (II) part of the study were collected with a questionnaire provided by Google Forms -software (Appendix 1). The questionnaire was sent by the employer to all occupants (290) in the police department in February 2018 and 116 answers (40%) were received. Data about symptoms and doctors´ diagnosed diseases were inquired. The participants were working in the police station, which was constructed in 1989 and renovated in 2016-2017 because of the problems with indoor air. Data about the building are given in **Table 8**.

The control group for both adult groups (studies II and III) was from a Finnish office building with no known exposure to bad indoor air or water and microbe damage in their workplace. The selected comparison workplace was suggested by an occupational health care colleague. Of approximately 150 employees, 45 (30%) volunteered to participate in the study. The questionnaire (Appendix 1) was sent to the participants in March 2018 via Google Forms. The researchers tried to find a control -police department but had no success.

The questionnaire used was designed basing on clinical experience and literature describing the health problems of indoor water and microbial damage exposure and is presented in Appendix 1. The questionnaire was based on a prior questionnaire [Sukeva-Hakanpää 2017] and modified with additional questions according to clinical experience to study any relevant symptoms in WDB exposure.

### 4.1.2 Hospital study

The third (III) part of the study collected data from employees of a Finnish obstetric hospital. The questionnaire (Appendix 1) of symptoms and doctors´ diagnosed diseases was sent to the participants in February 2018 using an e-mail list collected by a contact worker of the workplace. Data were collected via Google Forms. The employer stated that they did not have an opportunity to contact their workers after the hospital had been shut down and the employees transferred to other parts of their hospital. We were able to contact 90 of approximately 700 (13%) workers in January-March 2018.

The control group to both adult groups (II and III) is described in chapter 4.1.1. Researchers also tried to get a control group from another hospital building, but no volunteers were found among our contacts.

### 4.1.3 School study

The fourth (IV) study group consisted of 47 students aged 6–15 years. Most of the participants had multiple symptoms during the school day. The school administrators refused to distribute the questionnaire to the pupils and the personnel. Three parents affiliated with the school collected the e-mail addresses from the families that were willing to participate. We were able to enroll only 47/400 (12%) of the occupants of the school in the study, but none of the teachers. Participating children with help from their parents provided their health data using a questionnaire (Appendix 1) from Google Forms in January 2020.

The control group (n=56) was from a school in the same region with no history of water damage. The health data were collected via questionnaire (Appendix 1) provided by Google Forms, and it was distributed by the school administration to all the pupils´ parents and to personnel in February 2020. The age and gender of the respondents were matched to the study group.

## 4.2 Microbial and toxicity analyses

### 4.2.1 Police building

During several years of damage evaluations of the police department building, 40 material samples had previously been analyzed from floors 2–5 to identify water-damage indicator microbes. The samples had been cultured in an accredited environmental laboratory in October 2015. The culture method has been described by the Supervisory Authority for Welfare and Health in Finland. Cultures were made directly from the material samples on a 2% malt agar -extract plate, a dechlorane-glycerol-18-agar plate (DG18), and a trypsin-glucose yeast agar plate. Seven more samples had been taken from floors 1–2 in spring 2017 after the renovation had continued for six months and the samples had been cultured with the same methods.

Specimens for the toxicity analyses in the police building were collected in May 2017 and August 2017 from the rooms and spaces where the asthma patients from the study had been working. Dust samples were taken from floors 1–5. The dust was collected by wiping the horizontal surfaces >1m above the floor level with a sterile microfiber cloth. Dust samples were divided into three parts: The first part was extracted in ethanol, evaporated to dryness, then re-dissolved in ethanol to a concentration of 10mg/ml [Andersson et al. 1997, Andersson et al. 2010]. The other parts of the dust samples were cultured in malt extract (70,167, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany), agar (05039, Sigma-Aldrich Merck KGaA, Darmstadt Germany) and tryptic soy agar (22,091, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) plates for fungi and bacteria, for four weeks. The microbe biomass was then collected, extracted to ethanol, and evaporated to dryness, and then re-dissolved in ethanol to a concentration of 10mg/mL [Andersson et al. 2010].

Dust samples were analysed for three kinds of toxicity: straight dust toxicity, fungal toxicity from cultured dust, and bacterial toxicity from cultured dust. The toxicity analysis assay was based on the inhibition of boar spermatozoa motility. The exposed spermatozoa were assessed by microscopy for motility until identification of the lowest exposure concentration causing >50% of the spermatozoa to lose motility compared to the vehicle only (ethanol). The half maximal effect concentration or EC50 was expressed in ug/mL and a lower EC50 indicates higher toxicity [Andersson et al. 2010].

### 4.2.2 Hospital building

Conventional microbe cultures from the hospital building material samples were performed in April 2017. An accredited environmental microbiology laboratory had analyzed 46 samples taken from various floors and wings of the building. Cultures

were made directly from the material samples on a 2% malt agar extract plate, a dechlorane-glycerol-18-agar plate (DG18) and a trypsin-glucose yeast agar plate (THG). Public data from these reports were used in this study. The capability for toxin production of the cultured microbes was estimated based on species in scientific literature. No toxicity tests were performed from the hospital, while authorization to perform the tests was not approved by the building owner.

### 4.2.3 School building

The school´s microbe cultures and toxicity were investigated in spring 2020. One material sample from the wettest area in the basement floor staircase was performed. Precipitation plates were collected for one hour from the water-damaged classroom in the basement, the basement classroom next to the water-damaged classroom, and in the upper floor classrooms, two samples were taken from the second floor and one sample from the third.

The microbiological work-up was performed in an accredited microbiological laboratory (Bionautit, Helsinki, Finland). Two replicate dilutions from the material sample were made and cultured on three different media: i) malt extract agar (MEA), ii) dechlorane-glycerol-18 (DG18) favoring fungal species that thrive in dry environments, and iii) a bacterial-favored trypsin-glucose yeast medium (THG). Colonies were evaluated for total fungal and bacterial content after 7, 10, and 12 days of cultivation. Growth of actinobacteria on the THG plates was checked 2 weeks after the inoculation. Microbe colonies grown on precipitation plates were rejuvenated by inoculation on malt, DG18, and THG media. Microbial growth was quantified as a colony-forming unit per gram of the sample (cfu/g). The colonies were identified from the original and rejuvenated growth by microscopic examination, studying morphology under the microscope after 4–8 days of cultivation. The colonies were stained, examined, and photographed.

Condensed water samples for toxicity analysis were collected from the school basement floor (5) and from the first floor (2). Cottonwood swaps (2) were taken and packed in sterile plastic tubes to be tested for straight dust toxicity from the basement floor furniture.

Indoor air condensed water sampling is based on the following: A steel box, called “E-collector” is assembled on a stable stand in the room. A block of dry ice (appr. 1kg) is placed inside the box and covered with a lid to facilitate frosting. Water molecules from the indoor air condense on top of two cold surfaces on the metal plates. The frost melts at room temperature and is collected into an Eppendorf tube from a tray below the plates and sent to the laboratory for analysis.

Toxicological studies were performed at Finnish Center of Alternative Methods (FICAM), University of Tampere, Finland. Cottonwood samples were extracted,

sterilized by autoclaving and extracted for 72 hours in a cell culture incubator. Cytotoxicity analysis of the cottonwood swabs used human BJ fibroblasts and the neutral red uptake (NRU) assay, which shows lysosomal activity and cell membrane integrity. The extraction solution without the cottonwood swab was used as a negative control, extracts of polyurethane film containing 0.1% zinc diethyldithiocarbamate were used as a positive control and a clean cottonwood swab was a reference for cottonwood toxicity. Living cells incorporate neutral red. The light absorbance (560nm) is directly proportional to the number of living cells. Cells were incubated for 48 hours before the NRU assay.

The toxicity of indoor air condensed water samples was studied using human THP-1 macrophages/WST-assay, which is based on the mitochondrial activity of living, metabolically active cells that reduce WST-1 to a colored product. The optical density of the enzymatic product is measured at 450 nm. The higher the absorbance value, the higher the metabolic activity and the number of living cells. The viability of the cells is compared to control cells that are not treated with condensed water samples.

The water samples were sterile filtered and stored until use. THP-1 cells were seeded into 96-well plates at a density of 10 000 cells/well and differentiated to THP-1 macrophages for 48hours. The indoor air -condensed water samples and control samples (sterile distilled water) were pre-warmed to 37 °C for one hour before use. THP-1 macrophages were exposed to two sample concentrations: 10% and 25% at 6 replicates. Nickel II sulphate was used as a positive control of toxicity. Cells were incubated for 24 hours before the WST-1 assay.

## 4.3 Data analysis

All patients in study I with consistent lower respiratory symptoms were examined, and cases of asthma were diagnosed and treated according to Finnish recommendations of asthma [Astma käypä hoito 2012]. Data were collected from the patients` health records and from interviews. The patients were interviewed by telephone to explore their treatment and severity of asthma at 1 and 2,5 years after their diagnoses. The severity of asthma was evaluated by the GINA -classification [GINA 2018].

The primary variables utilized in the police study (II) were doctor-diagnosed asthma, self-reported MCS, and a predefined list of symptoms, dysfunction of the peripheral nervous system, fatigue, and muscle or joint pain. Log-binomial regression models were used to compare the study group with respect to the primary variables. There was a significant mismatch in the distributions of potential prognostic factors such as age and gender. Age was not included as a covariate due to a high number of missing values. The results were calculated as gender-adjusted

risk ratios (RR) with 95% confidence intervals (CInt). The chi-squared test was used to compare the groups with respect to other diagnoses and symptoms. All statistical tests used were two tailed, and p-values of <0.05 were considered statistically significant. The analyses were performed using IBM SPSS Statistics for Windows (version 26.0, Armonk, NY, USA, IBM Corp).

Asthma, self-reported respiratory, cardiac, neurologic, musculo-skeletal, and fatigue symptoms, and MCS were defined as primary variables in the hospital study (III).

The log-binomial regression analysis was a valid method to estimate the adjusted risk ratios (RR). However, the Mantel-Haenszel test was chosen because it is much easier to understand. The log-binomial regression method is still very rarely mentioned in the basic textbooks. In Mantel-Haenszel method the subjects were divided into two strata (0-1 diagnoses and 2 diagnoses).

The primary variables in the school study (IV) were headache, gastrointestinal symptoms, respiratory symptoms, fatigue, eye irritation, rash, ear infections, sub febrility, neurological symptoms, muscle, or joint pain, and MCS. Risk ratios were calculated to compare the exposed study group to the non-exposed control group with respect to the primary variables. The risk ratio is the proportion of subjects with symptoms in the exposed group divided by the proportion of subjects with symptoms in the non-exposed group. For symptoms with zero prevalence in the non-exposed group, a zero-count adjustment was done by adding 0.25 to all four cell counts. All statistical tests were two-tailed, and p-values <0.05 were statistically significant. The analyses were performed using NCSS 2019 Statistical Software. NCSS, LLC. Kaysville, Utah, USA.

In the cell culture studies of air condensates, the absorbance values were normalized, i.e., the viability of the untreated control (0% extract concentration) was set at 100%, and viability of the cells treated with different swab extract concentrations were calculated relative to this control value, either as a % decrease in cell viability (negative values) or as a % increase in mitochondrial dehydrogenase activity (positive values). In the swab studies, the absorbance values were normalized as the viability of untreated control (0% extract concentration) was set at 100% and viabilities of the cells treated with different swab extract concentrations were calculated in comparison to this control value. Samples were considered toxic at either concentration when statistically significant changes between the samples of indoor air condensate or cottonwood swab samples to the respective controls were compared in the Student`s t-test (Sigma Plot 14.0) and achieved $p<0.05$.

## 4.4 Ethical considerations

The first study among police occupants was approved by Police Administration (Poliisihallitus) 29.11.2017 (ID 17311158). At the first study initiation, the ethical committee secretary was consulted; she stated that no ethical approval was needed for a retrospective study, and the permissions from the participants were enough. All the participants and pupils´ guardians have given their permission to use the health record and questionnaire information. Data-protecting authorities of the data register were informed of the data collection in 2017 and a further privacy statement according to the legislation of the European Union´s General Data Protection Regulation was formulated in May 2018. The Ethical Committee from Northern Ostrobothnia has also approved a study plan searching for biomarkers on mold-related disease among school children of the study; EETTMK 10/2020.

# 5 Results

## 5.1 Patient data

There were 21 workers diagnosed with asthma according to the Finnish criteria in 2017 and the prevalence of asthma elevated from approximately 10% to 17% among the workers. At the 30-month follow-up, there were no cured individuals. Among the patients, 1 was having GINA 1 -level symptoms, 3 had GINA 2, 14 had GINA 3, and 3 patients had GINA 4 -level treatment 2,5 years later (**Table 4**).

The risk ratios of the multi-organ symptoms of the police study (II) are presented in **Table 5**. Risk ratios for central neurologic symptoms (CNS) (RR 2.85), respiratory (RR 2.66), and fatigue (RR 2.82) are similar to those in the other study groups. All the symptoms´ risks are within 95% confidence intervals (CI). In addition, risks for cardiac symptoms (RR 9.58) and MCS (RR 2.81), also within 95% CI are like those of the midwives´ group (III). The prevalence of airway infections during work history is also higher than in the control group: otitis 16% vs 4%, sinusitis 47% vs. 16%, bronchitis 34% vs 13%, and pneumonia 13% vs. 4%.

Results of the hospital study (III) are also presented in **Table 5**. Risk ratios for CNS (RR 4.94), respiratory (RR 2.56), and fatigue (RR 3.05) symptoms are elevated as in all other study groups compared to the controls. In addition, risk ratios for muscle and joint pain (RR 2.02), MCS (RR 3.44), and cardiac arrhythmia (RR 19.75) are also elevated. All the risk ratios are within 95% confidence intervals. When compared to the female controls only, the results are slightly different, but again with statistical significance (**Table 6** (unpublished data)).

An overlap in neurological symptoms was common; in the hospital study, 44% of the study group experienced four or more neurological symptoms, as did 15% in the police building group, whereas none of the nonexposed individuals had four or more symptoms of neurological origin. None of the children`s group had more than three neurological overlapping symptoms.

As in the adult groups (II, III), risk ratios in the school study (IV) for neurologic (RR 63.04) respiratory (RR 22,64), fatigue (RR 21.45), and muscle and joint pain (RR 58.28) symptoms are remarkably elevated when compared to a school without any known water damage and problems with indoor air. All these risk ratios are within 95% confidence intervals. Headache (RR 31.00), ear infections (RR 7.74),

rash (RR 5.96), eye irritation (RR 6.35), and sub febrile temperature show significantly elevated risks compared to the control school. Results show remarkable morbidity in the group of schoolchildren exposed to indoor water and microbe damage. MCS was rare in this group (**Table 7**). Sinusitis, bronchitis, and pneumonia in the children´s group were too rare to be reported.

**Table 4**. Clinical data for 21 patients who acquired occupational asthma during 8-month renovation of their building. Modified from original publication I.

| No | Sex | Smoking | Atopy | Allergic Rhinitis | FVC (l) | FEV1 (l) | Diagnostic Criteria | Asthma Medication | GINA 2019 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Male | no | no | no | 4.98 (92%) | 4.10 (89%) | 2 | ICS, LABA, salbutamol daily | G3 |
| 2 | Male | no | no | no | 5.53 (102%) | 4.32 (99%) | 2 | ICS, LABA, montelukast, salbutamol daily | G3 |
| 3 | Female | no | no | yes | 4.24 (94%) | 3.12 (84%) | 3 | ICS, LABA, salbutamol | G3 |
| 4 | Female | no | no | no | 4.17 (86%) | 3.54 (88%) | 2 | ICS and LABA re-started in spring 2018 | G3 |
| 5 | Female | no | no | no | 2.87 (87%) | 1.34 (51%) | 1 | ICS, LABA, montelukast, salbutamol weekly | G3 |
| 6 | Female | yes | N.A.* | no | 2.51 (75%) | 1.48 (54%) | 1 | ICS, LABA, tiotropium, salbutamol weekly | G4 |
| 7 | Female | no | no | no | 6.36 (147%) | 5.00 (135%) | 2 | ICS, LABA, salbutamol during exercise | G3 |
| 8 | Male | no | no | no | 4.34 (82%) | 3.78 (87%) | 2 | ICS, LABA, salbutamol during exercise | G3 |
| 9 | Male | no | no | no | 4.45 (93%) | 3.00 (75%) | 2 | salbutamol occasionally | G1 |
| 10 | Male | no | no | no | 3.79 (79%) | 2.96 (77%) | 1 | ICS, LABA, salbutamol weekly | G3 |
| 11 | Male | no | no | no | 5.73 (96%) | 4.40 (94%) | 2 | ICS, LABA, montelukast | G3 |
| 12 | Male | no | no | no | 4.02 (76%) | 2.93 (71%) | 1,2 | ICS, LABA, montelukast | G3 |

| No | Sex | Smoking | Atopy | Allergic Rhinitis | FVC (l) | FEV1 (l) | Diagnostic Criteria | Asthma Medication | GINA 2019 |
|---|---|---|---|---|---|---|---|---|---|
| 13 | Male | no | no | N.A. | 2.59 (51%) | 2.10 (51%) | 1 | ICS, LABA restarted 2019 | G3 |
| 14 | Male | no | no | no | 4.29 (84%) | 3.10 (73%) | 1 | ICS, LABA, montelukast, tiotropium, salbutamol weekly | G4 |
| 15 | Female | no | no | no | 3.52 (83%) | 2.92 (83%) | 4 | ICS, LABA, tiotropium | G4 |
| 16 | Female | no | no | no | 3.60 (96%) | 2.90 (96%) | 2 | ICS, LABA | G3 |
| 17 | Male | no | N.A.* | yes | 5.27 (91%) | 3.45 (74%) | 2 | ICS, salbutamol weekly | G2 |
| 18 | Male | yes | no | no | 5.60 (96%) | 4.50 (101%) | 4 | ICS, salbutamol during exercise | G2 |
| 19 | Female | no | no | no | 2.76 (89%) | 2.19 (88%) | 2 | ICS, LABA, salbutamol during exercise | G3 |
| 20 | Male | no | yes | yes | 5.44 (96%) | 4.23 (92%) | 4 | ICS, montelukast | G2 |
| 21 | Female | no | no | no | 3.05 (93%) | 2.13 (81%) | 4 | ICS, LABA | G3 |

Diagnostic criteria:
1: Forced expiratory volume in one second (FEV1)/Forced vital capacity (FVC) + 12% or more with salbutamol (increase in FEV1 > 12% with salbutamol).
2: Peak expiratory flow (PEF) + 15%/60 l or more; minimum 3 times with salbutamol.
3: PEF + 20%/60l or more; minimum 3 times day variation, excessive variability in twice-daily PEF over 2 weeks (>20%).
4: histamine provocation test positive.
ICS: Inhaled corticosteroid.
LABA: Long-acting $\beta$ 2-agonist.
*not available
GINA= global initiative for asthma -classification. G1: need for occasional medication with salbutamol, G2: need for regular ICS medication, G3: need for regular ICS and LABA medication, G4: need for regular ICS, LABA and tiotropium medication.

**Table 5**. Symptoms and RR`s reported by predominantly female and male group of occupants in workplaces colonized by WDB microbiota. Modified from original publications II and III.

| Symptom/disease | symptom prevalence, midwives/police officers n=90/119 | symptom prevalence, controls* n=45 | RR midwives/police officers | 95% CI midwives/police officers | *p*-value midwives/police officers |
|---|---|---|---|---|---|
| CNS symptoms** | 56/90 (62%)<br>35/115 (30%) | 5/44 (11%) | 4.94/<br>2.85 | 2.72–6.91/<br>1.19–6.85 | <0.001/<br><0.02 |
| Symptoms of autonomous nervous/peripheral nervous system*** | 45/90 (50%)<br>23/115 (20%) | 4/44 (9%) | 4.36/<br>2.57 | 1.90–7.41/<br>0.95–6.95 | 0.001/<br>0.06 |
| Asthma | 51/90 (57%)<br>35/116 (30%) | 9/45 (20%) | 1.86/<br>1.56 | 0.86–3.13/<br>0.81–3.00 | 0.11/<br>0.18 |
| Multiple chemical sensitivity | 36/90 (40%)<br>25/114 (22%) | 4/43 (9%) | 3.44/<br>2.81 | 1.39–6.44/<br>1.06–7.46 | 0.01/<br>0.04 |
| Fatigue | 69/90 (77%)<br>67/113 (59%) | 10/42 (24%) | 3.05/<br>2.82 | 2.19–3.64/<br>1.55–5.11 | <0.001/<br>0.001 |
| Muscle or joint pain | 46/90 (51%)<br>29/103 (28%) | 9/41 (22%) | 2.02/<br>1.50 | 1.11–3.02/<br>0.75–3.00 | 0.02/<br>0.25 |
| Respiratory symptoms**** | 72/90 (80%)<br>79/111 (71%) | 12/42 (29%) | 2.56/<br>2.66 | 1.84–3.04/<br>1.58–4.48 | <0.001/<br><0.001 |
| Cardiac arrhythmia | 51/90 (57%)<br>23/101 (23%) | 1/41 (2%) | 19.75/<br>9.58 | 4.47–36.30/<br>1.33–68.81 | <0.001/<br>0.02 |

*The comparison was done to the same control group. **problems with concentration, brain fog, memory difficulties, dizziness, balance problems. ***muscle traction, muscle weakness, hearing abnormalities, numbness of limbs, face, or tongue. **** cough, dyspnea, difficulty in breathing.

**Table 6.** Comparison of the risk ratios (RR) for different symptoms among the midwives (female gender) to female controls only (subgroup analysis)

| Primary variable | Midwives | Female controls | Midwives vs. female controls | | |
|---|---|---|---|---|---|
| | n=90 | n=24 | RR* | 95% CI | p-value |
| Asthma | 51/56.7% | 4/16.7% | 3.40 | 1.36–8.47 | <0.001 |
| Respiratory symptom | 72/80% | 7/30.4% | 2.63 | 1.40–4.92 | <0.001 |
| Cardiac symptom | 51/56.7% | 1/4.5% | 12.47 | 1.82–85.32 | <0.001 |
| MCS | 36/40% | 4/17.4% | 2.30 | 0.91–5.81 | 0.04 |
| CNS symptom | 73/81.1% | 3/12.5% | 6.49 | 2.24–18.79 | <0.001 |
| Peripheral nervous system symptom | 45/50.0% | 3/13% | 3.83 | 1.31–11.23 | 0.001 |
| Brain fog | 56/62.2% | 1/4.3% | 14.31 | 2.09–97.9 | <0.001 |
| Fatigue | 69/76.7% | 6/27.3% | 2.81 | 1.41–5.61 | <0.001 |
| Muscle or joint pain | 46/51.1% | 5/23.8% | 2.15 | 0.97–4.74 | 0.02 |

*RR was calculated using Mantel-Haenzel test because the sample size of the female control is small.

**Table 7**. School study symptom RR`s. From original publication IV.

| Disease or symptom | Study group (exposed) n=47 | Control group (non-exposed) n=56 | RR | 95%CI | *p*-value |
|---|---|---|---|---|---|
| GI symptoms* | 24 (51%) | 2 (4%) | 14.30 | 3.73–86.25 | <0.001 |
| Respiratory symptoms** | 19 (40%) | 1 (2%) | 22.64 | 3.60–453.3 | <0.001 |
| Neurologic symptoms*** | 7 (15%) | 0 (0%) | 63.04 | 3.03–850.2 | <0.001 |
| Headache | 26 (55%) | 1 (2%) | 31.00 | 5.16–612.4 | <0.001 |
| Fatigue | 18 (38%) | 1 (2%) | 21.45 | 3.38–430.5 | <0.001 |
| Eye irritation | 16 (34%) | 3 (5%) | 6.35 | 1.93–26.79 | <0.001 |
| Rash | 15 (32%) | 0 (0%) | 5.96 | 1.78–25.29 | <0.001 |
| Sub febrility | 13 (28%) | 1 (2%) | 15.49 | 2.32–316.8 | <0.001 |
| Ear infections | 13 (28%) | 2 (4%) | 7.74 | 1.82–49.11 | <0.001 |
| Muscular-skeletal pain | 12 (26%) | 0 (0%) | 58.28 | 2.77–787.1 | <0.001 |
| Asthma | 5 (11%) | 3 (5%) | 1.99 | 0.43–10.25 | 0.32 |
| Multiple chemical sensitivity (MCS) | 4 (9%) | 1 ((2%) | 1.59 | 0.53–4.91 | 0.11 |
| Cardiac symptoms**** | 2 (4%) | 0 (0%) | 10.71 | 0.31–155.4 | 0.15 |

*Pain in the abdominal area, diarrhea, nausea, vomiting, abdominal swelling, obstipation. **Cough, dyspnea, difficulty in breathing. ***Problems with concentration, brain fog, memory difficulties, muscle traction, dizziness, hearing abnormalities, balance problems. ****Tachycardia, palpitations.

## 5.2 Microbiological and toxicity data

Descriptions of the three buildings in the study are presented in **Table 8**. The control group´s buildings have not been evaluated.

In the police department building several episodes of water damage have appeared over the decades. Damage has been observed in the thin-cell concrete walls near the windows due to faults in the seams and significant leaks in the roof drainage pipes were reported over the years. In addition, air leaks were present near the windows and inner wall panels, opening the way to airborne microbial impurities from the wet and damaged structures. In some office rooms this manifested as an odor of mold. Reports of sewer leakages caused by the jail customers in the jail floors were also frequent. The air-pressure in the building was highly negative because of the ventilation system. Negative air pressure increases air leaks through the structures; the ventilation was also inadequate because of the increase in personnel working in the office building over the last decades, and the ventilation capacity had been greatly exceeded. No other respiratory disease-causing agents such as organic dust, animal allergens or industrial chemicals were reported as exposure.

A thorough renovation was started in 2016, producing a large amount of dust. Protection for the occupants was insufficient, and the dust was spread throughout the building via technical chases and inlets.

The hospital was constructed in 1960 and had suffered from significant water damage problems and bad indoor air. An evaluation of standardized constructional and microbiological methods of damage evaluation was carried out and published in April 2017. The hospital was shut down in 2018 over wide public concern of health effects.

The school was built in several phases from 1953 to 1969. The most recent damage was discovered in summer 2019, when a hot water pipe under the building was found to be leaking. The damage report was made in April 2020.

Of the 47 of the material samples from the police building study (I and II) examined, 14 revealed microbial growth. Species and genera and the number of cultures that revealed remarkable growth of moisture indicator microbes are presented in **Table 9**.

Of the 46 material samples taken from the hospital (study III), 45 showed microbial growth, indicating moisture damage according to the National Supervisory Authority for Welfare and Health in Finland 2016. In 12 of the 46, there was mold, actinomyces, or other water damage, indicating that microbial growth was considered remarkable, cfu >50/culture (**Table 9**). Species and genera recovered in microbe cultures in the hospital study (III) are presented in **Table 9**. Most of the species discovered in the cultures can produce toxic metabolites.

In the school study (IV) a total of six fungi of different genera of fungal groups were recovered from the sediment and cottonwood samples. Two of the fungi discovered are listed as moisture damage indicators by the National Supervisory Authority for Welfare and Health in Finland 2016. Only 9 samples were analyzed.

**Table 10** shows the results of boar-sperm toxicity analyses of dust and cultured dust in the police building study (I and II). Each of the 21 asthma patients had worked in an area where strong toxicity of at least one kind was demonstrated.

Strong dust toxicity (EC50 = 6.5–12.5 µg/mL) was observed in 14 cases (66.6%), four with very strong toxicity (EC50 < 6 µg/mL). In cultured dusts, strong mold toxicity (EC50 ≤16 µg/mL) was observed in five cases (23.8%), and strong bacterial toxicity (EC50 ≤9 µg/mL) in 20 cases (95.2%). The following strong toxicity combinations were observed: all three methods in five cases (23.8%); toxicity of dust together with cultured dust mold in five cases (23.8%), and together with cultured bacterial dust toxicity in 14 cases (66.7%); and in cultured dust, both mold and bacterial toxicity in seven cases (33.3%).

The indoor air-condensed water toxicity results in the school study (IV) are presented in **Table 11**. Indoor air samples from the two basement classrooms, the other one with the water leakage were mildly toxic, and the basement floor corridor sample showed moderate toxicity. All tested samples were toxic.

The results of the toxicity studies from the cottonwood swab in the school study (IV) are shown in **Table 12**. The swab sample taken from the basement corridor

furniture (piano) was most toxic, and the other basement furniture (bench) sample was moderately toxic. With the same concentrations (100% extracts), the toxicities were as follows: A piano swab specimen caused 77% cell toxicity (23% viability), a bench swab specimen caused 43% cellular death (57% viability), and the cottonwood swab alone caused 41% cell death (59% viability). The interpretation of the cellular toxicity is, that dust collected from the piano, increased cell death by 36% compared to the pure cottonwood control.

No samples were taken from the control buildings. The data of the buildings are not available, and no water damage or remarkable complaints of indoor air- or building -related symptoms were given by the occupational healthcare of the adults control group nor from the head principal of the school of the children´s control group.

**Table 8**. Description of the three buildings in studies I–IV.

| Building | Police (I-II) | Hospital (III) | School (IV) |
|---|---|---|---|
| Year | 1988 | 1960 | 1953-1969 |
| Renovation years | 2009, 2016 | Not known | 1984/1998-2002 |
| Area/$m^2$ | 9000 | Not known | 4400 |
| Floors | 0–5 | 0–11 | 0–2 |
| Constructure | Concrete, clinker tile surface | Tile, concrete | Tile/concrete |
| Rainwater sewerage | Inside steered | Not known | Outside steered |
| Insulation | Mineral wool | Siporex, toja, mineral wool, cork | Toja, mineral wool, cutter, turf |
| Ventilation | Forced | Forced | Forced in classrooms |
| Measured air pressure/ | Mainly negative | Both negative and positive | Both negative and positive |
| Water damage | Plumbing, rainwater | Several | Under the school for months |
| Sewer damage | Several | Several | Not reported |
| Air leaks from constructures | Verified | Verified | Verified |

**Table 9**. Microbial findings of the three buildings in studies I-IV.

| **Police department 2015** | | | |
|---|---|---|---|
| No of samples with growth | Microbe | Cultured colonies >10000cfu/g | Spot |
| 7/40 | *Penicillium* | | Window wall insulation |
| 6/40 | *Aspergillus versicolor* | 1/40 | Window wall insulation |
| 4/40 | *Cladosporium* | | Window wall insulation |
| 4/40 | *Aspergillus restricti* | 2/40 | Window wall insulation |
| 2/40 | *Mucor* | | Window wall insulation |
| 1/40 | *Rhizopus* | | Window wall insulation |
| 1/40 | *Eurotium* | | Window wall insulation |
| No of samples with no growth | | | |
| 31/40 | | | |
| **Police department 2017** | | | |
| No of samples with growth | Microbe | Cultured colonies >10000cfu/g | Spot |
| 3/7 | *Streptomyces* | | Paint inside, window wall insulation material, inside floor material |
| 1/7 | *Cladosporium* | | Table inside |
| 1/7 | *Engyodontium* | | Table inside |
| No of samples with no growth | | | |
| 2/7 | | | |
| **Hospital 2017** | | | |
| No of samples with growth | Microbe | Cultured colonies cfu >50/culture | Spot |
| 43/46 | *Penicillium spp* | 9/46 | Insulation materials |
| 40/46 | *Actinomyces* | 12/46 | Insulation materials |
| 14/46 | *Aspergillus sp* | | Insulation materials |
| 11/46 | *Paecilomyces variotii* | | Insulation materials |
| 5/46 | *Aspergillus niger* | | Insulation materials |
| 1/46 | *Chaetomium* | | Insulation materials |
| 1/46 | *Alternaria* | | Insulation materials |
| 1/46 | *Exophiala sp* | | Insulation materials |
| 1/46 | *Aspergillus sydowii* | | Insulation materials |
| 1/46 | *Paecilomyces sp* | | Insulation materials |
| 1/46 | *Cladosporium sp* | | Insulation materials |
| 1/46 | *Rhizopus sp* | | Insulation materials |
| No of samples with no growth | | | |
| 1/46 | | | |

| **School 2020** | | | |
|---|---|---|---|
| No of samples with growth | Microbe | Cultured colonies cfu>50/culture | Spot |
| 5/9 | *Aspergillus sp* | 2/9 | Concrete ground floor |
| 5/9 | *Penicillium spp* | | Insulation materials, concrete, passive air sample |
| 3/9 | *Scopulariopsis sp* | 1/9 | Concrete ground floor |
| 3/3 | *Actinomyces* | 3/3 | Insulation materials, concrete |
| 2/9 | *Cladosporium sp* | | Insulation materials, passive air sample |
| 2/9 | *Tritirachium* | | Concrete ground floor |
| 2/9 | *Eurotium sp* | | Inside wall paint |
| 1/9 | *Fusarium* | | Inside wall paint |
| 1/9 | *Aureobasidium* | | Passive air sample |
| 1/9 | *Rhodotorula* | | Passive air sample |
| No of samples with no growth | | | |
| 0/9 | | | |

**Table 10**. Toxicity data for dust and cultured dust samples from the offices of 21 patients who acquired occupational asthma during a renovation process (Police building study I). Modified from original publication I.

| Patient Number | Dust Toxicity | Fungal Toxicity of Cultured Dust | Bacterial Toxicity of Cultured Dust |
|---|---|---|---|
| 1 | S (10) | NT (61) | S (5) |
| 2 | VS (6) | S (1) | S (5) |
| 3 | S (10) | N.A. | S (1) |
| 4 | VS (6) | S (1) | S (5) |
| 5 | S (9) | M (19) | S (9) |
| 6 | VS (6) | S (1) | S (5) |
| 7 | VS (6) | S (1) | S (5) |
| 8 | S (9) | NT (42) | S (1) |
| 9 | S (9) | NT (42) | S (1) |
| 10 | NT (19) | M (36) | S (5) |
| 11 | M (13) | S (14) | S (5) |
| 12 | M (16) | S (12) | S (5) |
| 13 | N.A. | NT (38) | S (3) |
| 14 | S (9) | S (1) | S (5) |
| 15 | S (10) | N.A. | S (1) |
| 16 | N.A. | NT (42) | S (2) |
| 17 | S (9) | NT (42) | S (1) |
| 18 | S (9) | NT (42) | S (1) |
| 19 | S (10) | NT (54) | S (2) |
| 20 | M (17) | M (37) | S (7) |
| 21 | NT (73) | S (9) | M (17) |

The criteria for toxicity are based on previous research [Andersson 2010, Salin 2017]. The categories of degrees of toxicity (VS, S, M, NT) are based on the customer database by dividing the material into quartiles (Inspector Sec Ltd. Research Services). Dust toxicity: VS = very strong toxicity ($EC_{50}$ ≤ 6 µg/mL); S = strong toxicity ($EC_{50}$ = 6.5–12.5 µg/mL); M = mild toxicity ($EC_{50}$ =13–18 µg/mL); NT = not toxic ($EC_{50}$ > 18 µg/mL). Fungal toxicity of cultured dust: S = strong toxicity ($EC_{50}$ ≤ 16µg/mL); M = mild toxicity ($EC_{50}$ =17–37 µg/mL); NT = not toxic ($EC_{50}$ >37 µg/mL). Bacterial toxicity of cultured dust: S = strong toxicity ($EC_{50}$ ≤9 µg/mL), M = mild toxicity ($EC_{50}$ = 10–19 µg/mL), NT = not toxic ($EC_{50}$ >19 µg/mL). N.A. = not available.

**Table 11**. Toxicity of the water condensed from indoor air tested in THP-1 macrophage viability assay. From original publication IV.

| Sample | Conditions at sampling site | | Water inhaled ml/day* | The % change in THP-1 macrophage viability after exposure to indoor air samples | | Interpretation |
|---|---|---|---|---|---|---|
| | RH % | T °C | | 10% condensate | 25% condensate | |
| Basement classroom (damaged space, dryer on) | 45.5 | 17.5 | 109.00 | 1.80±5.00 | -15.90±5.90*** | Toxic |
| Basement Classroom (damaged space, dryer on) | 45.5 | 17.5 | 109.00 | 1.50±5.50 | -11.10±10.20* | Toxic |
| Basement Classroom (wooden work) | 32.5 | 18.4 | 43.30 | -3.00±5.30 | -12.70±3.30*** | Toxic |
| Basement Corridor (downstairs) | 31.2 | 22.0 | 127.10 | -6.10±1.9** | -5.60±3.30** | Toxic |
| Basement Piano room | 31.7 | 22.1 | 128.90 | -1.90±2.40 | -4.40±2.30** | Toxic |
| Upstairs (130) | 33.0 | 21.8 | 128.40 | -2.90±2.10 | -9.60±2.60*** | Toxic |
| Upstairs (130) | 34.6 | 21.3 | 126.50 | -2.00±3.70 | -7.00±2.20*** | Toxic |

RH% = relative humidity. Two volumes of the condensate were used: 10% and 25% of the total culture volume. The results are normalized against (untreated) control and expressed as % change in cell viability, mean±stdev, as compared to the control (0% change in cell viability). Negative values refer to decreased viability, positive values refer to increased mitochondrial activity, both are adverse effects. Each sample was tested at six replicates. The statistically significant changes in viability as compared to the respective control are indicated as *p<0.05; **p<0.01 and ***p<0.001.

**Table 12**. The toxicity of cottonwood swabs tested in BJ fibroblast viability assay. From original publication IV.

| Extract concentration % | Control sample (pure cottonwood) | The swab from the window bench | The swab from the piano |
|---|---|---|---|
| 0 | 100.00 ± 5.72 | 100.00 ± 4.65 | 100.00 ± 5.34 |
| 0.47 | 101.27 ± 4.17 | 107.58 ± 3.61 | 101.57 ± 6.69 |
| 1.01 | 96.11 ± 3.11 | 102.67 ± 5.37 | 99.20 ± 7.26 |
| 2.18 | 101.53 ± 1.76 | 101.54 ± 3.96 | 103.90 ± 6.61 |
| 4.68 | 101.56 ± 6.60 | 98.59 ± 3.03 | 94.19 ± 4.65* |
| 10.06 | 99.04 ± 4.11 | 96.96 ± 2.87 | 68.08 ± 15.44*** |
| 21.6 | 90.69 ± 3.32** | 87.47 ± 4.53*** | 67.44 ± 18.68*** |
| 46.5 | 79.66 ± 3.98*** | 80.07 ± 2.31*** | 42.16 ± 13.33*** |
| 100.0 | 59.45 ± 2.02*** | 56.79 ± 1.65*** | 22.93 ± 5.81*** |

Eight different cottonwood swab extract concentrations, i.e., 0.47-100%, were tested in six replicates each. The results are normalized against the control (0% extract concentration and 100% cell viability) and expressed as % cell viability, mean ± stdev. The statistically significant changes in viability as compared to the respective control are indicated as *p<0.05; **p<0.01 and ***p<0.001.

# 6 Discussion

## 6.1 Asthma, other respiratory and infectious outcomes of indoor WDB microbiota exposure

The first part of this thesis underlines, that WDB microbiota exposure creates a risk for severe asthma. Of the occupants, 21 (7.2%) got symptoms leading to an asthma diagnosis while working in a water-damaged building under renovation in 2016-2017. Asthma and allergic rhinitis caused by WDB exposure are considered verified [IOM 2004, WHO 2009, Caillaud et al. 2018, Fisk et al. 2019]. In non-sensitized populations, human challenge, and epidemiological studies of mold exposure consistently show the lowest observed effect levels of $10^5$ spores/$m^3$, but mycotoxin-producing and pathogenic species need to be detected specifically because of their higher toxicities [Eduard 2009]. During a demolition of microbe damaged constructions, the concentrations of mold and bacteria in the air are remarkably higher than in normal circumstances. These concentrations can cause negative health effects in humans [Rautiala 2004]. Therefore, it is recommended that workers doing the demolition work should be protected from exposure to the WDB microbiota [Suomaa 2009]. This was not done during the demolition work of the building in the first study (I), so it is possible that the workers were exposed to high levels of WDB microbiota. An association between microbial exposure, symptoms, and changes in pro-inflammatory mediators is suggested in a school study, in which the workers reported sore throat, phlegm, eye irritation, rhinitis, nasal obstruction, and cough [Roponen et al. 2001]. In the police building case presented in this thesis, 138 occupants contacted the health care unit during the renovation work because of these kinds of symptoms (I). Unfortunately, no measures of these responses have been made in this group.

In all the study groups (II-IV) respiratory symptoms´ RR´s are significantly high (2.56, 2.66, 22.64). A recent review hypothesizes that continuous exposure to mold particles may result in chronic low-grade pro-inflammatory responses contributing to respiratory diseases. Hyphal fragments in indoor air are more common than airborne spores, and fragments from both pathogenic and non-pathogenic mold species are more potent in pro-inflammatory responses in human airways than mold spores. Studies in the review reveal that exposure to spores and hyphal fragments

may act as allergens and pro-inflammatory mediators. The damage of the airways is possible by the production of toxins, enzymes, and volatile organic compounds (VOC) from the dampness microbiota [Holme et al. 2020]. Mold and ergosterol levels in dust were associated with new-onset asthma and the writers suggest hydrophilic fungi and ergosterol as measures of fungal biomass as promising markers of building-related respiratory risk in damp indoor environments [Park et al. 2008]. Water-damaged schools may contain larger numbers of microbial secondary metabolites at higher levels compared to non-damaged schools [Peitzsch et al. 2012]. Inflammational phenomena with elevated complement activation when exposed in microbe-damaged buildings has also been verified in a recent article from Finland [Atosuo et al. 2021]. The study among confirmedly mold-exposed patients in Finland showing peripheral blood B lymphocytes ($CD19^+$ leucocytes) decrease and lymphocytosis in BAL samples probably indicates an active immune response in the lungs [Wolff et al. 2009].

Airway infections are also a possible consequence of indoor mold exposure [Kuhn et al. 2003, WHO 2009, Fisk et al. 2010, Lanthier-Veilleux et al. 2016, Wong et al. 2016, Valtonen 2017]. Infections are present in the adult study groups in this thesis: sinusitis 47% and 39% vs. 16% in controls, bronchitis 34% and 29% vs. 13%, otitis 16% and 22% vs. 4%, pneumonia 13%, and 10% vs. 4%. In unpublished data from the writer, which collected symptoms from 297 self-reported WDB-exposed participants, 2 participants reported having 100 or more antibiotic courses during their history of exposure, and 15% of the participants had been treated with more than 20 antibiotic courses. Whereas WDB microbiota exposure leads to excessive prevalence of building-related (BR) rhinosinusitis, a statistically relevant risk for asthma also occurs. BR upper respiratory illness due to WDB exposure may predict future endemic asthma [Park et al. 2012], which is seen in the first article (I) in this thesis. As indoor mold elevates the risk for asthma exacerbation, mold remediation was encouraged in 2018 in an asthma seminar summary in Lancet [Papi et al 2018]. Remediation of the buildings, however, is not simple, and many of them fail to ease the occupants´ symptoms [Sauni et al. 2015, Park et al. 2018].

Asthma, bronchitis, and allergic rhinitis attributable to WDB exposure have enormous annual costs according to an American study: $3.7 billion of the annual $24.8 billion costs of allergic rhinitis, $1.9/$13.5 billion of acute bronchitis, $15.1/$94.5 billion of asthma morbidity and $1.7/$10.8 billion of asthma mortality, and $22.4 billion for the respiratory health effects yearly [Mudarri 2016]. In Finland, the costs of WDB microbiota exposure were estimated to be approximately €0.5 billion in 2012. It has been estimated that it would be cost-effective to invest €1.5 billion over three years perspective to remediate water-damaged constructions to benefit the national economy to procure fewer healthcare and social insurance expenses [Rakennusten kosteus- ja homeongelmat, eduskunta 2012].

A summary of a multi-national workshop, “Reactive indoor air chemistry and health,” reminds us that there is still a need for improved understanding of the possible link between indoor air chemistry and observed acute and chronic health effects and long-term effects such as work-related asthma. Potential exposure investigation targets are indoor oxidants, the use of derivatization techniques, atmospheric pressure detection and improved complex modelling techniques [Wells et al. 2017]. Study I showed that at least toxin-producing microbes and toxicity were present in the building.

The prevention of health problems is always the priority, and if there are symptoms in occupant´s airways related to their occupancy, clinicians´ first principle “primum non nocere”, should be obeyed, and the exposure should be terminated. Asthma is still a severe disease, and according to the literature, these WDB-related non-IgE -mediated asthmas with probable LMW protein exposure as a causative agent seem difficult to treat because of a reluctancy to inhaled corticosteroids [Choi et al. 2019] and a possible asthma-COPD (ACOS) overlap [Jaakkola et al. 2020]. A review of asthma triggers finds molds as an allergen to be an important reason for poor asthma control and suggests it for a global checklist among other triggers for use in research and clinical practice [Vernon et al. 2012]. Finnish studies with probable WDB-microbiota -related occupational asthma show that patients have persisting symptoms [Karvala et al. 2010] and lower quality of life [Karvala et al. 2013]. This phenomenon was also seen in the first article of this thesis; the asthma cluster of 21 employees of one workplace that were diagnosed in 2017 showed the severity of asthmas according to their reluctancy to ordinary asthma treatment. Of the asthma patients, 81% needed medication of GINA 3 or GINA 4 classification with LABA and/or tiotropium with ICS. At the 30-month follow-up, most asthma cases that became symptomatic during the remediation of the workplace were still in need of this medication.

## 6.2 Multi-organ outcomes of WDB exposure

The symptoms of WDB-exposed groups in this thesis are multi-organ with respiratory RR´s of 2.66 (II), 2.56 (III), and 22.64 (IV); neurologic RR´s of 2.85 (II), 4.94 (III), and 63.04 (IV), cardiac RR´s of 9.58 (II) and 19.75 (III), musculo-skeletal RR´s of 2.02 (III) and 58.28 (IV), and dermal RR 5.96(IV). Scientific literature is in line with these findings originating from WDB microbe exposure.

The mechanism of water damage microbiota´s disadvantage to mammal organisms have been widely studied [Roponen et al. 2001, Yike et al. 2002, Leino et al. 2003, Roeder et al. 2004, Pall 2007, Theoharides et al. 2007, Wolff et al. 2009]. Exposure initiates nitrosative stress reactions [Pall 2007] and chronic inflammation [Hope 2013]. Toll-like receptors are implicated by *A. fumigatus* as a sign of immune

response. The mammal immune system identifies the pathogen-associated molecular patterns found in the cell wall of fungi. This process leads to the secretion of proinflammatory mediates [Roeder et al. 2004]. The autoimmune system is affected by microbes and their metabolites [Ratnaseelan et al. 2018]. The mechanisms may involve antibody development. Indoor mold -exposure increases IgG, IgM, and IgA anti-mold antibody levels, and can also lead to high mycotoxin antibody-levels. The writers suggest that these antibodies could be used in epidemiologic investigations of fungal exposure [Vojdani et al. 2003a]. Salival IgA -antibodies are also suggested to indicate the late phase of type-1 hypersensitivity reaction or type-2 and type-3 delayed sensitivities [Vojdani et al. 2003b]. Elevated neural autoantibodies have been found in indoor water and microbial damage exposed patients. Measuring these autoantibodies is considered relevant in symptomatic patients exposed to toxic mold or other environmental toxicants [Abou-Donia et al. 2018]. Exposure to water damage microbiota may increase the risk of developing neural autoantibodies, peripheral neuropathy, and neurophysiological abnormalities [Campbell et al. 2004]. In our recent study of 25 WDB-exposed sick building syndrome (SBS) patients with neurological and/or cardiac symptoms, there is a significant difference between 25 non-symptomatic controls in two types of IgG and IgM autoantibodies of 13 examined autoantibodies [Tuuminen et al 2021].

Toxicity mediated by inhaled indoor air is under suspicion of the multiple symptoms in water-damaged buildings. The health effects of toxins are difficult to estimate. Possible colonization of building materials by toxigenic fungi is considered as subsequent exposure risk to aerosolized mycotoxins for occupants [Aleksis et al. 2017]. *Fusarium* mycotoxin has been considered to affect humans in moisture-damaged buildings [Stockmann-Juvala et al. 2008]. Toxic exudates containing communesins A, B and D and chaetoglobosins are transferable by air, which is considered a novel mechanism of mycotoxin dispersal in indoor environments [Salo et al. 2019, Andersson et al. 2020]. *Streptomyces* produces several toxic agents as antibiotics, immunosuppressive agents, and enzyme inhibitors and is often considered a possible cause of the symptoms in moldy environments [Andersson et al. 1997].

Neurologic and especially neuropsychologic symptoms during mold exposure have been widely acknowledged [Baldo et al. 2002, Kilburn 2003, Rea et al. 2003, Crago et al. 2003, Empting 2009, Dooley et al. 2020], and neurological disturbances from neurogenic inflammation have been discussed [Pall 2007]. Overlapping neurological symptoms were prevalent in the adult groups in this thesis: 44% in the midwives´ group (III) suffered from four or more neurological symptoms as well as 15% in the police building group (II), while only 2% of the control group had four neurologic symptoms. A common symptom in indoor mold exposure, “brain fog”, was experienced by 30% and 62% of the participants in the adult study -groups,

compared to 16% in the control group (unpublished data), has been implicated in mast cell-microglia interactions [Skaper et al. 2014, Theoharides et al. 2015, Theoharides et al. 2016, Girolamo et al. 2017].

Neuropathophysiological processes behind clinical features of mold exposure mimicking classical neurological disorders need further scientific exploration [Empting 2009]. There seem to be multiple reasons for fatigue as a symptom, with RR´s of 2.85 (II), 3.05 (III), and 21.45 (IV) in this thesis. Possible explanations for fatigue are recurrent infections [Valtonen 2017], dysregulation of immune and neuroendocrine systems, neuroinflammation, dysregulation of the autonomous nervous system with decreased activation of the sympathetic nervous system or mitochondrial damage by inhaled toxins [Tuuminen et al. 2019, Tuuminen et al. 2018], thyroid-hypophysis-hypothalamus and hypophysis-pituitary-adrenal axis, and untreated nonthyroidal illness [Somppi 2017]. Fatigue was also a common symptom in a recent study in Finland that showed an association between indoor dust and microbial toxicity and BRS [Salin 2021].

It has been claimed that the maximum calculated dose of mycotoxins (aflatoxins B1 and B2, satratoxins G and H, fumitremorgens B and C, verruculogen and trichoverrols A and B), which could be inhaled in 24 hours in indoor environments is not high enough to cause any adverse health effects [Kelman et al. 2004], and it is claimed that exposure below 30 ng/$m^3$ is tolerated without health hazards even when exposed continuously throughout a 70-yr lifetime [Hardin et al. 2009]. However, the toxins cause inflammation [Yarom et al. 1987], and as relatively nonpolar and hydrophobic, they can penetrate through the blood-brain -barrier, thereby perturbing the function of synapses [Campbell et al. 2004, Bauer et al. 2018], which makes neurological outcomes possible. It has been shown that the toxins are transferred in droplets, and therefore, methods of dust testing or measuring gases might underestimate the amounts of microbial toxins indoors [Salo et al. 2019]. Moreover, whereas a single mycotoxin may not produce any effect, a combination of mycotoxins could induce synergistic toxicity even at low levels [Alassane-Kpembi et al. 2013]. Exposure to satratoxin H has been shown to increase susceptibility to other neurotoxic mycotoxins [Thrasher et al. 2009, Karunasena et al. 2010]. Hippocampal and neuroendocrine systems are affected by endotoxins [Sava et al. 2007].

There was an elevated risk of musculo-skeletal problems in moldy environments in earlier Finnish studies [Myllykangas-Luosujärvi et al. 2002, Luosujärvi et al. 2003]. Two of the study groups in this thesis (III, IV) revealed an elevated risk (RR`s 2.02 and 58.28) for musculo-skeletal symptoms. Immunological responses by the innate immune system may be involved in developing these symptoms [Ratnaseelan 2018]. Incremental selection and prevalence of autoimmune diseases can also have partial environmental causes [Germolec et al. 2012, Tuuminen et al. 2017].

Controversial interpretation of toxic mold exposure and patients´ permanent symptoms has been aero irritation, mimicking other pseudo diagnostic categories, such as sick building syndrome, and idiopathic chemical intolerance. This phenomenon is commonly seen in patients with a psychogenic predisposition [Khalili et al. 2005]. Symptoms of mold exposure have also been interpreted as a mass hysteria or somatoform [Stone et al. 2006, Borchers et al. 2017]. Functional disorders and psychological origin descriptions have been discussed [Nordin 2020, Tuuminen et al. 2020]. It is noteworthy that these observations can only be observative and theoretical, no causality can be proven and therefore these interpretations should be precautious before the actual health hazards in the environment have been examined.

In this study, the toxicity of indoor settled dust or indoor air -vaporized water was shown in three of the studies (I, II, IV). Toxin production -capable microbes were found in all three buildings in this thesis (I-IV); therefore, exposure to dampness microbiota and to toxicity in the study groups is possible. The tests that were used are accredited for commercial use but are not accepted by the government authorities for health risk evaluation in WDB exposure. These toxicity tests were selected because of their availability during our studies, and they do not prove the toxicity as an outcome of WDB microbiota. No tests or microbial evaluations were made in the buildings of the comparison groups.

Exposure to WDB microbiota indoors is probably related to chemical exposure. The atmosphere indoors is an entity. Even in normal conditions indoor air contains several chemicals (VOCs, formaldehyde, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc.) [THL, ympäristöterveys]. WDB microbiota decomposes materials in the buildings, which leads to chemical release proliferation. It is impossible to separate these exposures from each other. The role of molds is difficult to disentangle from other water damage related exposure as non-biological particles and chemical pollutants according to the recent review [Holme et al. 2020].

Cardiac and neurological symptoms in WDB microbiota exposure may be attributed to neurogenic inflammation because of exposure to both; toxic mold, and chemicals. Cytokine production and histamine release are contributions of this exposure [Yike et al. 2002, Leino et al. 2003, Islam et al. 2007, Korkalainen et al. 2017]. Histamine release can induce spasms in coronary arteries [Kounis et al. 1991]. Chemically intolerant individuals report cardiopulmonary problems [Baldwin et al. 1998]. Atrial fibrillation, supraventricular tachycardia, and premature ventricular contractions can be triggered when exposed to chemical irritants [Rea 1978]. Even cardiac arrests have been found to correlate with air pollution in a dose-response manner [Kang et al. 2016]. Both adult groups (II, III) also showed elevated risk for cardiac symptoms (RR´s 9.58 and 19.75). Cardiac and neurological symptoms may be attributed to neurogenic inflammation, or they may be a result of exposure to both,

toxic mold, and chemicals [Islam et al. 2007, Korkalainen et al. 2017, Yike et al. 2002, Leino et al. 2003].

Gastro-intestinal symptoms in the children´s group, RR 14.30 (IV) were also reported in connection to mold exposure. Mast cells may play a key role in this outcome of dampness microbiota exposure, while mycotoxins can increase the permeability of the gut-blood barrier [Liebermann 1998]. The permeability increase of this barrier may also be the underlying mechanism of irritable bowel syndrome [Hunt et al. 2002, Stasi et al. 2012]. WDB exposure and its connection to increasing rates of inflammatory bowel diseases, which have more than tripled in Finnish adolescents during 1987-2014, with suspected environmental triggers [Virta et al. 2017], should be investigated in future studies.

The multinational research, Health Effects of Indoor Pollutants: Integrating microbial, toxicological, and epidemiological approaches (HITEA) concludes that dampness in buildings is likely linked to elevated exposure to some microbes and their partly toxic metabolites. School dampness was associated with respiratory tract symptoms in students and teachers, and the researchers suggest that these findings should alert national and European authorities to take action to improve safety and health and controlling indoor biological and chemical pollutants should be prioritized to avoid health problems [HITEA website]. The Finnish recommendation of treating mold and water damage -exposed patients does not recognize multi-organ symptoms and health problems outside of respiratory problems. The recommendation also guides doctors to not evaluate the cause of the symptoms before the buildings are thoroughly investigated [Kosteus ja homevauriosta oireileva potilas Käypä hoito 2017], which hampers early prevention of possible health damage in WDB`s. There is a need for biomarkers of both; the damaged building and the symptomatic individual, to identify the harmful environment and the occupant at risk of permanent health effects of WDB exposure.

In a human body it is impossible to separate mold and its metabolite effects to different organs. Effects are multi-organ, and they vary between individuals. It is a considerable risk to interpret symptoms in relation to any environmental factors as somatoform or idiopathic instead of resolving the possible environmental exposure, which prolongs symptoms and individuals deteriorating condition [Rossi et al. 2018]. A recent review of dampness and mold literature encourages the interpretation of the potential role of mold exposure by evaluating information from experimental studies to elucidate possible mechanistic links [Holme et al. 2020]. An elevated risk of multi-organ symptoms is found in all three groups of this thesis.

## 6.3 Approaches to MCS and DMHS etiology and pathology

One of the aims in our studies was to find the prevalence of MCS among the study participants. Two studies of this thesis showed significantly elevated risk ratios of MCS in WDB exposed occupants compared to controls: RR 2.82 (II) and 3.44 (III), (UNPUBLISHED female subgroup 2.30, **Table 5**).

Linking to MCS, dampness and mold hypersensitivity syndrome (DMHS) has been described as a systemic condition as a response to long term exposure to dampness and mold. Criteria for DMHS were introduced by professor Valtonen in 2017; he described the course of the disease as a) history of exposure to water-damaged buildings, b) a re-occurrence of infections, c) Sick Building Syndrome, d) MCS, and e) increased scent sensitivity such as the ability to smell a moldy odor [Valtonen 2017]. DMHS criteria were fulfilled by 27% and 15% of the adult groups vs. 0% of the controls in this thesis (unpublished data). Developing DMHS may be a consequence of prolonged exposure to indoor dampness microbiota, which was on average 11.3 years among the police building group (II) and 7.8 years among the hospital group (III) with MCS, (RR´s 2.82 and 3.44/2.30). The length of the true exposure is impossible to evaluate, but as the buildings had constructional problems, it is speculated that indoor mold exposure in these buildings may have been possible for decades. DMHS was rare in the children´s group (1/47) and was not found among their controls. Exposure time in children´s group has naturally been shorter, and their reports of neurological symptoms were also more modest: None of the study group children had more than three neurologic symptoms during exposure to WDB microbiota, and only one had MCS symptoms.

In the 1980´s MCS was suspected to be an outcome of exposure to organic compounds [Brooks et al. 1985]. Neurogenic inflammation is suggested as a possible origin of symptoms in multiple chemical exposure [Meggs 2017]. MCS patients´ nasal biopsies show a proliferation of sensory nerve fibers and a breakdown of the epithelial barrier [Meggs et al. 1996b]. Hyperactivation of the sensory receptors as the C-fibers are considered to be one of the MCS mechanisms [Pall et al. 2004, Pall 2009].

The still unclear pathophysiological mechanisms and processes in MCS are based on immunological findings and activation of peripheral and central inflammatory and oxidative stress pathways [Meggs 2017]. There are some reports on measuring or evaluating neurogenic inflammation and reactions, which are considered important mechanisms in MCS. Nerve growth factor is a mediator that causes an increase in nerve fibers with chemoreceptors in them. When exposed to chemical irritants, these nerve fibers release substance P, which is a major mediator of neurogenic inflammation. Chemicals bind receptors on nerve fibers, which release substances that produce sensory stimuli and neurogenic inflammation. Inflammation

can lead to the remodeling of tissues in a way that the threshold for symptomatic reactivity is lowered [Meggs 2017]. Altered cytokine profiles, decreased function of natural killer cells, the presence of autoantibodies, reduced response of T-cells to mitogen and other specific antigens, functional or genetic defects on endogenous enzymes detoxifying peroxides, or stable toxic products of lipid oxidation may cause chronic oxidative stress and metabolic alterations. Elevated peroxynite levels may cause mitochondrial dysfunction and elevated pro-inflammatory cytokine levels, inducing nitric oxide and superoxide production with additional peroxynite formation. Chronically persistent toxic compounds may chemically modify proteins to form autoantigens, which is suspected based on symptoms that overlap with classical autoimmune diseases. Further research on these mechanisms is suggested to validate possible related biomarkers in MCS [de Luca et al. 2011].

The prevalence of MCS or CI has been estimated to be equivalent to allergies [Meggs et al 1996a]. In South Korea, the estimated prevalence of MCS was higher in allergic patients (20%) compared to non-allergic patients (11%) [Jeong et al. 2014]; in Japan CI yielded a prevalence of 7.5% [Azuma et al. 2015]; in East-Carolina USA, clinically diagnosed MCS prevalence was 13% [Steinemann 2018a]. Intolerance to chemicals was reported by 12% of the Swedish and 15% of the Finnish study participants [Karvala et al. 2018b]. In Australia, the estimate is that MCS affects one million Australians and CS affects another two million. Reducing chemical exposure is strongly suggested [Steinemann et al. 2018b].

A remarkable part of the researchers shares the opinion, that MCS, CI, BRI, or IEI seem to share features with functional somatic syndromes and relate to psychiatric disorders rather than exposure to indoor mold or chemicals [Altenkirch 2000, Staudenmayer 2001, Bornschein et al. 2002, Staudenmayer et al. 2003a, Staudenmayer et al. 2003b, Bailer et al. 2005, Das-Munshi et al. 2006, Hausteiner et al. 2006, Papo et al. 2006, Baliatsas et al. 2015, Karvala et al. 2018a, Nordin 2020]. There is also a suspected reporting bias when assessing non-specific symptoms, which may explain many of the associations found in cross-sectional studies on SBS symptoms and indoor environment factors [Brauer et al. 2006]. In an American study, just 1.4% of the MCS symptomatic patients had a history of prior emotional problems but almost 38% developed these problems after the physical symptoms emerged, which according to the writers suggests that MCS has a physiologic etiology, not a psychologic one [Caress et al. 2003].

A review of CI in 2015 discussed theories of explaining the condition, which includes changes to the immune system, central nervous system, olfactory, and respiratory system, as well as altered metabolic capacity, behavioural conditioning, and emotional regulation. The writers remind, that there are still uncertainties regarding the underlying mechanisms behind symptom elicitation [Dantoft et al. 2015]. A review of MCS literature in 2018 concludes that broadly confirmed

evidence exists on the activity of the limbic system and autonomic nervous system at the expense of cortical areas. Writers find important that analysis of the patient at anamnestic and etiological levels is of significant importance. Differences in timing and the mode of symptom manifestation are crucial to separate the symptoms caused by chemicals from psychiatric syndromes [Rossi et al. 2018]. The more recent review of CI in 2019 concluded that there is consistent evidence for the altered neurological processing of sensory information in individuals with CI, and the writers suggest further neuropsychological research exploring the processing of extrinsic stimuli and cognition of sensation through the limbic system and related cortices in CI patients [Azuma et al. 2019]. A review of idiopathic approaches to MCS in 2020 concluded that only a subset of individuals exposed to certain building environment or everyday indoor products develop CI, which refers to learning-based protective functions that all humans and many other species possess: If an individual associates a certain type of exposure to negative health effects, the exposure is interpreted as threatening and results in a physiological stress response. Initially, this response will probably manifest itself as unpleasant emotions and cognitions. However, the writer also reminds that additional mechanisms may also be involved in CI - as neurogenic inflammation, or neural sensitization due to allergy, other types of functional somatic disorders, or psychological stress which may cause immunologic or neurogenic responses [Nordin 2020].

Avoidance of chemical substances is considered the best way to prevent the symptoms of MCS [de Luca et al. 2011]. Dalton and Jaen take a further step in their review, in which they summarize the mounting evidence of airborne chemical odor and irritation; they suggest, that managing odors and irritants in the workplace is critical maintaining the health and wellbeing of workers, even among individuals without chemical sensitivity [Dalton et al. 2010].

If we separate human physiological and psychological health into their own compartments and ignore the patients´ experiences with WDB exposure, treating it as mass-hysteria or a somatoform symptom [Stone et al. 2006, Borchers et al. 2017], the result is that patients are treated irrationally, and it is to their detriment. There have been several examples of this throughout history. The unknown continues being interpreted as psychological, as has been the case in the history of epilepsy, lead poisoning, asthma, and as recent research has shown, with mental disorders, in which the inflammation mechanism has been recognized only in recent years [Liu et al. 2017, Lee et al. 2019]. Studies with a presumed idiopathic explanation commonly report a comorbidity, which might be a consequence of a physical disease. Physical illnesses are confirmed to have mental consequences and social effects. Psychiatric patients often have somatic disorders, which are commonly neglected. Even Finnish health authorities claim that the relationship between microbes and nonspecific symptoms should be interpreted with considerable caution to avoid strengthening the

assumed nocebo-related mechanisms of symptoms. A response to our article of the Hospital study reminds us of neglecting the current knowledge of psychological origin of environmental intolerance, which was not our subject when evaluating exposure to indoor molds, a known cause to several negative health effects. They also remind us of low response rate, selection bias and reporting bias with statistical analysis problems, which result in unsupported conclusions of the article [Pekkanen et al. 2020]. In our response to the letter, we have once more explained the circumstances, which were present in this study, and we have also corrected the name of the statistical method falsely mentioned in the results section in the article [Hyvönen et al. 2020]. The mentioned confounding effect of gender distribution is included in this thesis (**Table 6**) and it has been shown that female gender has only minor effect to the results.

Evidence to justify preventive actions to WDB indoor environments [HITEA conclusion] is solid. Preventing health effects from indoor water and microbe damage is crucial. Inflammation processes [Hope 2013, Atosuo et al. 2021] and stressors that initiate nitrosative stress reactions [Pall 2007] induced by indoor water damage microbiota exposure are considered to lead to hypersensitivity of sensory and immunological systems and should be avoided.

Acknowledging the pathologic processes in WDB-exposed or MCS-patients and developing alternative treatment [Hope 2013] is necessary, instead of offering them scientifically unsupported psychological and educational treatment [Skovbjerg et al. 2012, Hauge et al. 2015, Vuokko 2019, Selinheimo et al. 2020].

If a patient suffers from unexplained multi-organ, respiratory or neurologic symptoms, joint and muscle pain, fatigue, or constant sub febrile temperature increase, the first question should be whether they are being exposed to indoor water and microbe damage and whether it is possible to avoid it. The odds for this are plausible, as 10-18% of occupants in Finland were estimated to have been exposed daily to indoor mold damage in 2012 and the problem was evaluated to be growing without remarkable investments in remediating buildings [Rakennusten kosteus- ja homeongelmat, eduskunta 2012].

## 6.4 Weaknesses and strengths of this thesis

The groups in the studies are small, and the fraction of participating occupancies stay small, which is typical for questionnaire studies. The participation is low, with only 12-40% of the occupants responding. The unexpected challenge was the reluctancy of the workplace and school authorities to take part in the study, and therefore the conclusions of the study are limited.

Self-reported symptoms include possibilities for misunderstandings, which is always the case in real life. Memory bias can also affect the results. The effect of

having only one control group in the adult studies limits the conclusions, but similar symptoms in all three groups strengthens the likelihood that the symptoms were caused by WDB microbiota, which is also described in scientific literature. Because there was no authorization for the toxicity tests, there is a restriction in conclusions regarding whether indoor toxicity existed in the hospital study. However, several of the microbe species found were toxin producers.

The toxicity tests in this study are accredited to commercial use but are not accepted by the government authorities for use in health evaluation of WDBs. These toxicity tests were selected because of their availability during our studies, not by any scientific basis. The toxicity in our samples has not been verified originating from the microbes discovered. Nevertheless, the microbes recovered are capable to produce toxins and other sources of toxicity are unlikely.

No microbial evaluation or toxicity tests were made in the buildings of the comparison groups, and the determination that there was no water damage in the buildings was based only on the evaluation of the school´s head principle and the occupational doctor of the workplace in the office building.

Due to lack of funding, we have not been able to have serological testing of inflammatory responses from the study or control groups. However, these kinds of tests are easy to execute in the future to verify inflammation processes during WDB exposure.

## 6.5 Conclusions

Moisture in the building, even in the past, has most likely made it possible for the water-damage microbiota to flourish. In all three buildings in this thesis, there was a widespread and prolonged water and/or sewer damage and exposure, and the occupants showed a wide variety of symptoms.

Asthma and respiratory symptoms are understood to be a result of WDB exposure. In this thesis, the asthma cases (I) were severe, mostly responding insufficiently to ordinary asthma treatment, which raises suspicions about whether there was an undiagnosed co-morbidity, which needs further studies.

In this thesis, it is claimed, that neurological as well as cardiac, respiratory, musculoskeletal, and infectious symptoms in WDB-exposed individuals are possible because of toxic WDB microbiota exposure. Significantly elevated risk ratios for neurological and respiratory symptoms in three separate groups, as well as musculo-skeletal and cardiac symptoms and MCS in two groups support the likelihood of causality. Risk ratios for neurological symptoms are particularly concerning in the children´s group, while their neurological system is just developing and is more vulnerable to disturbances.

According to the literature and this thesis, developing long-term and permanent health damage is possible when exposure to WDB microbiota is continued or reoccurring. Available data underline the fact that health outcomes in water damage microbiota exposure are obvious, and exposure to indoor water and microbe damage should be prevented. To prevent permanent health damage, clinicians should recognize the possibility of exposure to indoor toxic microbiota behind multiple symptoms of unidentified cause. If the patient has a chronic cough, recurrent rhinosinusitis, neurological symptoms, or joint and muscle pain without an obvious reason, possible indoor WDB microbiota exposure should be acknowledged.

The next step in our studies is to explore the neural autoantibodies association to SBS, which we have already done in a small group of SBS patients and their controls.

It has been stated that it takes an average of 17 years for research to translate into clinical practice. This thesis proposes immediate action to change clinical practice according to the severity of WDB microbiota exposure. Proven causality is too late to prevent health damage. Health risks should be acknowledged when exposing inhabitants and occupants to indoor WDB microbiota, and we need further studies to recognize buildings where health may be endangered.

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# Appendices

**Appendix 1.** Questionnaire on the symptoms related to workplace indoor air.

1. Cover letter to the control group

We study symptoms related to moisture and mold problems in workplaces. The problem is significant and was acknowledged in a report by the Finnish Parliament in 2012. It is estimated that the expenses of water damages are approximately €953 million yearly.

This questionnaire intends to collect data on health effects and symptoms from workers at office buildings without severe moisture damage. The data will be run in parallel with another study of workers exposed to moisture damage. This research is independent of any organization and has no funding. We do not collect any identity data or email addresses. We use the data for statistics only, and no single respondent can be identified.

2. Cover letter to the study group

We study symptoms related to moisture and mold problems at workplaces. The problem is significant and was acknowledged in a report by the Finnish Parliament in 2012. It is estimated that the expenses of water damages are approximately €953 million yearly.

We study the health effects of bad indoor air at workplaces. This questionnaire intends to collect data on the health effects and symptoms from occupants of buildings with severe moisture damage. The data will be run in parallel with another study of workers without exposure to water damage. Your participation is confidential. If you want to inform us of your name in the questionnaire, it will help in our future studies. Later, we hope to have a method to measure the toxicity of indoor air to estimate a possible association between air toxicity and health effects.

The results will be published in peer-reviewed scientific journals. This study has no funding, and the scientists have no conflicts of interest.

Questions to both groups

1. Personal data
   1.1 Age
   1.2 Sex
   1.3 Smoking yes/no
   1.4 Allergies yes/no
   1.5 Moist or mold problem causing symptoms at home yes/no
   1.6 Pets yes/no
   1.7 The name of the building you are working in
   1.8 Since when you have been working in this building

2. Doctor ´ s diagnosed diseases and work ability yes/no
   2.1 Asthma
   2.2 Allergic rhinitis
   2.3 Atopy
   2.4 Urticaria
   2.5 Rheumatoid disease
   2.6 Fibromyalgia
   2.7 Hypothyroidism
   2.8 Intestinal disease (Mb Crohn or Colitis ulcerosa)
   2.9 Cardiac problems, arrhythmia
   2.10 Migraine
   2.11 Diabetes
   2.12 Multiple sclerosis
   2.13 Epilepsy
   2.14 Cancer
   2.15 Anxiety disorder
   2.16 Depression
   2.17 Insomnia, sleep disorder
   2.18 Chronic fatigue syndrome
   2.19 Hematologic disease
   2.20 High blood pressure
   2.21 Infertility
   2.22 Pension because of no work ability
   2.23 Part time pension because of work ability deterioration
   2.24 Other illnesses (describe below)
   2.25 No diagnoses

3. Medication used

4. Common symptoms you experience during working in this building
    4.1 Headache
    4.2 Itching, pain, or other symptoms in your eyes
    4.3 Skin rash
    4.4 Nose bleeding
    4.5 No symptoms described above
    4.6 Lower respiratory tract symptoms
        4.6.1 Cough
        4.6.2 Dyspnea
        4.6.3 Difficulty in breathing
        4.6.4 No lower respiratory tract symptoms
    4.7 Abdomen area symptoms
        4.7.1 Swelling
        4.7.2 Pain
        4.7.3 Diarrhea
        4.7.4 Constipation
        4.7.5 No abdomen area symptoms
    4.8 Pain in the muscle or joints
        4.8.1 Joint
        4.8.2 Muscle
        4.8.3 No pain in muscle or joint region
    4.9 Cardiac symptoms
        4.9.1 Arrhythmia
        4.9.2 Elevated heart rate while sitting
        4.9.3 Elevated heart rate while standing up
        4.9.4 Pressure or pain in the thoracic region
        4.9.5 No cardiac symptoms
    4.10 Neurologic symptoms
        4.10.1 Dizziness, disorientation
        4.10.2 Memory difficulties
        4.10.5 Numbness of limbs
        4.10.6 Numbness of face or tongue area
        4.10.7 Speech disorders
        4.10.8 Unintentional muscle contraction
        4.10.9 Muscle weakness
        4.10.10 Vertigo
        4.10.11 Hearing sensitivity
        4.10.12 Problems with body balance

4.10.13 Problems with concentration
4.10.14 No neurologic symptoms
4.11 Fluctuation of body temperature
4.11.1 Too high body temperature above 37 degrees Celsius
4.11.2 Too low body temperature
4.11.3 Perspiration
4.11.4 No symptoms
4.12 Need of medication during working hours
4.12.1. Inhaled salbutamol or terbutaline
4.12.2 Allergy medication
4.12.3 Inhaled corticosteroid
4.12.4 Tablet corticosteroid
4.12.5 No need for medication
4.13 Infections you have had during your work history in this building
4.13.1 Recurrent otitis
4.13.2 Maxillary sinus infections
4.13.3 Bronchitis
4.13.4 Pneumonia
4.13.5 How many courses of antibiotics during work history/year
4.14 Unusual exhaustion
4.15 Feeling of sickness
4.16 Decreased work ability

Could you please answer a few more questions:

5. If you are aware of previous exposures to moist or mold e.g., at home, day care, school, or other workplaces, please make an estimation of the duration.

6. Do you feel being sensitive to chemicals
If you answer "yes", please answer the questions blow:
Sensitive to:
Perfumes, perfumed detergents
Deodorants, after shave lotions
Tobacco smoke, other smokes
Fresh prints as newspapers
Paints, glue, varnish
Grit, other dusts
Traffic dust, exhaust, gasoline, oil
Windshield detergents
Formaldehyde or other chemicals

Spices, other supplements of food

7. Do you smell the moldy scent from peoples nearby you yes/no

8. Do you have following symptoms in other buildings with probable moist or mold damage. Please indicate your symptoms yes/no.
Asthma exacerbation
Neurological symptoms
Skin problems
Symptoms in the eyes
No symptoms
Other symptoms, describe here

Thank you for your participation. If you have any questions, don ´ t hesitate to ask me.

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