

Environmental Mycology in Public Health

Fungi and Mycotoxins
Risk Assessment and Management



Edited by
Carla Viegas, Ana Catarina Pinheiro, Raquel Sabino,
Susana Viegas, João Brandão, and Cristina Veríssimo



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Dedication

**The editors wish to acknowledge the vision of
Dr. Laura Rosado, who recognized environmental exposure
to fungi as part of a wider medical mycology approach.
Her enthusiasm, passed on at the National Institute of Health
Dr. Ricardo Jorge in Portugal, was an inspiration to
the editors of this book.**

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Chapter 18

Occurrence of Mycotoxins in Indoor Environments

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INTRODUCTION AND SCOPE

Moisture damage, dampness, and visible mold in buildings are consistently associated with adverse health outcomes. A 2011 review of the epidemiological evidence has concluded that conditions of dampness and mold are associated with multiple allergic and respiratory effects, including asthma development and exacerbation, current asthma, upper and lower respiratory tract symptoms, respiratory infections, allergic rhinitis, and eczema.¹ Although the link between building dampness and adverse health is well established the causal agents and mechanisms underlying the observed health effects are not well understood. Microbial proliferation on indoor surfaces and in dust following the increased availability of water in damp buildings is one of the main mechanisms in generating dampness-related indoor pollutants and links to observations of visible mold in such buildings. The World Health Organization considers, in their *WHO guidelines for indoor air quality: dampness and mould*, the following dampness-related indoor pollutants as most relevant: allergens (from house dust mites and fungi), bacteria, bacterial and fungal cell wall components (such as endotoxin and fungal β -D-glucans), mycotoxins, and microbial and other volatile organic compounds.² This WHO document concludes that even though causative agents of adverse health effects in damp buildings have not been clearly identified, excess levels of various microbial agents, including mycotoxins, in the indoor environment need to be considered as a potential health hazard; microbial growth in response to moisture problems in buildings needs to be avoided or removed.

The WHO report and other reviews¹⁻⁴ provide the background in front of which essentially all of the research on mycotoxins in indoor environments is placed: dampness and visible mold are consistently associated with ill health, but our knowledge as concerns the causal agents and disease mechanisms is poor (Figure 1). Mycotoxins (and also the bacterial equivalents, i.e., toxic bacterial secondary metabolites) are among the candidates that have been suggested to be involved in health problems observed in damp buildings. In this chapter,

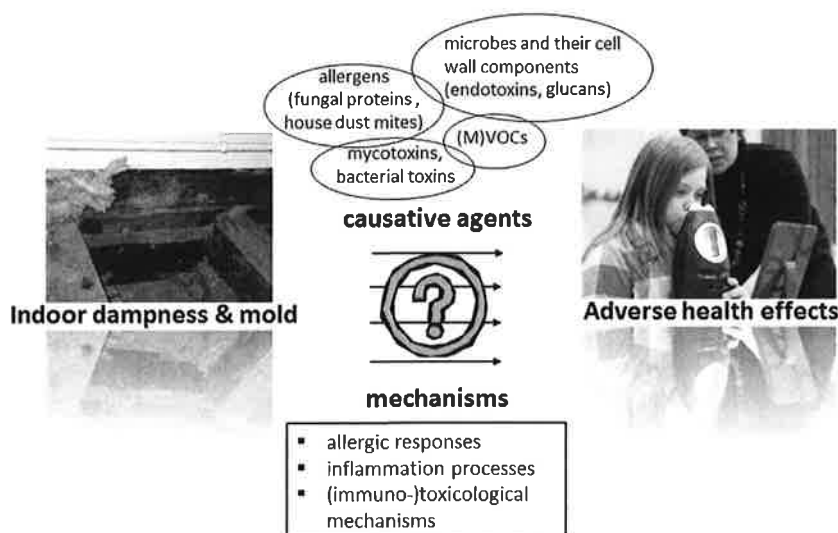


FIGURE 1 Moisture damage, dampness, and visible mold in indoor spaces and the related exposures and adverse health.

we attempt to summarize what is known today from scientific studies that have reported mycotoxins specifically in indoor environmental samples.

The potential involvement of mycotoxins in building dampness-related illness was probably first clearly postulated in 1986, when Croft et al. reported an outbreak of trichothecene mycotoxicoses in a family living in a Chicago home.⁵ The family members suffered from symptoms resembling those of stachybotryotoxicosis in livestock, with central nervous symptoms that included neuropsychiatric manifestations. Exposure to *Stachybotrys* spores and to macrocyclic trichothecene mycotoxins produced by these molds was linked to the health effects. In the late 1990s, the “toxic black mold” *Stachybotrys chartarum* and its partly highly toxic secondary metabolites, that is, mycotoxins such as satratoxins, roridins, verrucarins, and spirocyclic drimanes, hit the newspaper headlines. This came after reports of a cluster of cases of pulmonary hemorrhage in infants in Cleveland, Ohio, and several other cases that linked severe health outcomes to exposure to *S. chartarum* and its mycotoxins.^{6–10} Subsequently, mycotoxins produced by *Stachybotrys* strains became the major target of research on indoor mycotoxins for almost two decades. Only more recently, analytical methods targeting mycotoxins in indoor environmental samples have been developed with the aim of detecting multiple different mycotoxins rather than only a few specific target compounds, for example, Refs 11–13.

In the context of this book, more detailed discussion on what mycotoxins are and why these compounds are produced by fungi is provided elsewhere (Chapter 1.1). Here, mycotoxins are defined as fungal secondary metabolites that pose a potential health risk to humans and/or animals when introduced

by a natural route.¹⁴ Generally, secondary metabolites either have an intrinsic function within the producing species, as for example initiation of growth and differentiation, or act on targets external to the producing species. These compounds are proposed to improve survival fitness of the producing species.¹⁵ In this concept, mycotoxins may in many cases be relevant in the mediation of competitive interactions between microorganisms, for example, in competition for nutrients on a building material surface.

To date, several hundreds of different mycotoxins have been identified and characterized, the majority of which in regard to food and feed contaminants in agricultural settings.¹⁶ Estimates, however, reach from 20,000 up to 300,000 unique mycotoxins being present in the environment.¹⁷ Characteristic for mycotoxins is that they are nonvolatile, low-molecular-weight natural products that are typically very stable. In indoor environmental settings—even though not volatile—these compounds do get airborne attached to spores, fragments, and particulate matter. Mycotoxins comprise a wide variety of chemical structures and subsequently different biological activities and act on various organ systems in the human or animal body. Most of our knowledge on the modes of action of mycotoxins relates to ingestion exposure; for example, inflammatory, immune-suppressive, cytotoxic, and carcinogenic effects in various organs have been described (detailed in Chapter 3.6).

Oral ingestion of contaminated food stuffs is the most studied, most recognized, and main route for human exposure to mycotoxins. Whereas the health threat posed by food-borne mycotoxins is a worldwide phenomenon, it is a severe problem in countries with lower agricultural standards, high pressure in terms of nutritional needs, and environmental conditions favoring fungal growth. The topic of food-borne exposure to mycotoxins is addressed in Chapters 3.2 and 3.6 of this book. Dermal contact to mycotoxins as an exposure route is primarily a problem in occupational settings, for example, through handling of mycotoxin-contaminated grains in farms, dealing with mycotoxin-containing materials in laboratories, or in the process of remediating fungal- and mycotoxin-contaminated building structures. Symptoms of irritation upon dermal exposure have been reported for several mycotoxins, but the health relevance of such exposure is little explored. Interestingly, skin irritation is also very commonly reported in residents of “moldy buildings.” It is, however, unclear whether such symptoms are actually caused by the mycotoxins present in these buildings or by other exposures. Workers in farms, sawmills, or the feed processing industry can also be exposed to high levels of very potent mycotoxins, such as aflatoxins and ochratoxin A, via inhalation of heavily contaminated dusts.^{18–20} Such exposure situations are considered occupational health risks, and research on the potential health implications is ongoing (Chapter 3.4).

In this chapter, we consider non-occupation-related exposure to mycotoxins in indoor environments—such as residential homes, schools, and offices—through inhalation. The focus is on mycotoxins but also other fungal secondary metabolites, for which human health risks are not yet well established, that is, compounds

for which the definition “mycotoxin” is not applicable following strict definitions. Whereas most of the general discussion with respect to human exposure is restricted to fungal toxins—and so is the content of this chapter—it should be mentioned that also (toxic) bacterial secondary metabolites may be relevant in the context of indoor exposure. Bacteria produce an enormous variety of bioactive secondary metabolites; many thousands of such compounds have been characterized so far.²¹ Such metabolites are widely used in pharmacological products, exploiting their antibiotic, immune-suppressive, enzyme-inhibiting, antitumor, or antiparasitic potential, to give a few examples. Some of the most potent bacterial producers of pharmacologically active compounds are commonly encountered indoors and are particularly linked to conditions of indoor dampness, for example, species of the bacterial genus *Streptomyces*. The finding that toxic bacterial secondary metabolites co-occur alongside mycotoxins in indoor sample materials in damp buildings²² implies that these compounds should also be considered as part of a complex and diverse microbial exposure situation in mold-contaminated buildings.

MYCOTOXINS IN BUILDING MATERIALS, DUST, AND AIR FROM INDOOR ENVIRONMENTS

This chapter attempts to provide an overview of studies that report on the *actual indoor occurrence* of mycotoxins and fungal secondary metabolites, that is, studies that are supported by the detection of these compounds in naturally infested indoor sample materials. Mycotoxin production of a given fungal strain under laboratory conditions does not imply that any of the same mycotoxins are necessarily produced by this fungus under “real-life conditions”, for example in a damp building. The availability of nutrients and growth substrate on the indoor material and water, temperature, light, and other environmental factors,^{23–25} as well as species succession and interaction with other microbes present in this particular ecological niche, all are factors that might have an impact on the production of secondary metabolites. It is a well-established fact that toxigenic fungi, that is, fungi that have the potential to produce toxic secondary metabolites, occur in indoor environments and can proliferate on various building materials.^{26,27} However, the occurrence of a toxigenic fungus, for example, in an indoor air sample or on a building material does not necessarily mean that mycotoxins of this particular mold are present in the indoor environment as well. In fact, the opposite is also true, that is, detecting mycotoxins in an indoor sample does not necessarily predict the presence of the mycotoxin-producing molds.

There is a good body of literature that has documented mycotoxin production under laboratory conditions by fungal strains that have been isolated from moisture-damaged indoor environments or from species that are known to occur indoors for example, Refs 9,24,28–37. Some authors have taken another step forward to imitate real-life situations and investigated the secondary metabolite

production on artificially infested building materials, such as gypsum board or wood for example, Refs 24,25,37–41. In particular the work conducted by K.F. Nielsen and colleagues should be highlighted here. They have laid the foundations for indoor mycotoxin research in describing the vast variety of mycotoxins and other fungal secondary metabolites produced by indoor fungi on building materials under experimental conditions. These studies have not only shown that mycotoxin production can occur on building materials, but have also established that it is species or even strain specific, dependent on growth conditions and substrate (building material) and the competition/coculture with other microbes. It has also been established that one given mycotoxin may be produced by different fungal strains, and one fungal strain may produce different mycotoxins.

Table 1 provides an overview of studies that have reported mycotoxin occurrence in indoor sample materials. We have sorted the studies in chronological order and provided a summary description of the aspects of the study design or locations, sample materials, mycotoxins targeted, and analytical methodology used and the main findings as concerns the occurrence of mycotoxins in indoor environments. We list a total of 30 studies published between 1986 and 2013; although we have done our best to be complete, we cannot exclude the possibility that one or another study published may be missing from this table.

There are a few general observations that can be made from almost 30 years of studies on the indoor occurrence of mycotoxins. To start with it is obvious that mycotoxins do occur indoors, as they have been readily detected in various indoor sample materials in multiple studies, using different analytical methodologies. Most commonly, building materials (in 15 studies) and dust samples (in 17 samples) have been collected for analyses of mycotoxins. Where building materials are concerned, typically mold-infested or water-damaged materials have been sampled. House dust—be it from swabs of ventilation ducts, bulk, or vacuumed samples of floor dust, vacuum cleaner dust bag dust, or settled airborne dust collected from elevated surfaces—has been utilized extensively, in particular also in more recent studies. The rationale behind using house dust as a sample material is that indoor dust links to airborne exposure through mechanisms of deposition and resuspension. Such samples may be more representative in terms of human exposure indoors as they act as a sink for airborne particles from multiple sources (e.g., mold-affected areas in a home), whereas a material sample typically reflects the situation from a single spot in the building. House dust is moreover less affected by the known large temporal variation of microbial concentrations in indoor air.⁴² In particular vacuumed floor dust is commonly used in population health studies for determination of contaminants people might be exposed to in indoor environments. Concentrations reported for various mycotoxins in building material samples (or fungal matter scraped off mold-infested materials) are typically in the order of nanograms to micrograms per gram of building material (range pg–mg/g) or nanograms to micrograms per square centimeter of sampled surface. For ventilation duct dust, concentrations of picograms to nanograms per gram of dust (up to $\mu\text{g/g}$) and picograms to

TABLE 1 Overview of Studies that Report on the Occurrence of Mycotoxins (and Other Fungal and Bacterial Secondary Metabolites) in Indoor Sample Materials

No.	Study Sites and Design	Sample Materials (Number of Samples)	Target Compounds	Analytical Methodology	Main Findings Concerning Indoor Mycotoxin Occurrence	References
1	Residential house with moisture problems in Chicago, IL, USA	Ceiling fiberboard infested with <i>Stachybotrys chartarum</i> (N=1)	Trichothecenes	TLC, HPLC, GC-MS	Verrucarol, verrucarins B + J, satratoxin H, trichoverrins A + B detected	Croft et al. ⁵
2	3 office spaces with "sick building syndrome"; Montreal, QC, Canada	Dust from ventilation system, floor, surfaces (N=3)	Trichothecenes	TLC, HPLC	T-2 toxin, diacetoxyscirpenol, roridine A, T-2 tetraol detected in the dust samples	Smoragiewicz et al. ⁵⁷
3	Office building with water and mold damage in New York, NY, USA	Bulk samples of water-damaged, <i>Stachybotrys</i> -contaminated paper material (N=2)	Trichothecenes	HPLC	Satratoxin H (c. 1 µg in 60 mg of scrape-off sample), stachybotrylactone, stachybotrylactone acetate	Johannig et al. ¹⁰
4	Child day care center with moisture damage, Finland	Gypsum board liner from a site with and without water damage (N=2)	Satratoxins G and H, verrucarol	HPLC	Satratoxins G and H (17 µg/g) and verrucarol were found in water-damaged but not in undamaged gypsum board	Andersson et al. ⁷⁵

5	Courthouse and office buildings with moisture problems; Florida, USA	Moldy ceiling tiles and vinyl wall covering (N=3)	Satratoxins G+H, sterigmatocystin, deoxynivalenol	HPLC	Satratoxins G and H at 2 and 5 ppm in ceiling tiles; deoxynivalenol in vinyl wall covering	Hodgson et al. ⁵⁸
6	One school, one domestic residence with water/mold damage; Denmark	Moldy wall scrapings, insulation material, outer cardboard layer (N=3)	Verrucarol and trichodermol	GC-MS	Verrucarol (indicative of macrocyclic trichothecenes) in 3/3, trichodermol in 1/3 samples	Nielsen et al. ⁷⁶
7	Residential, moisture-damaged home of an infant with pulmonary hemorrhage; USA	10 cm ² of a <i>Stachybotrys</i> -contaminated closet ceiling (N=1)	Trichothecenes	HPLC	Roridin L-2 (0.5 ng/cm ²), roridin E (0.7 ng/cm ²), satratoxin H (3.2 ng/cm ²) on building material surface	Flappan et al. ⁸
8	23 water-damaged buildings; Denmark	<i>Stachybotrys</i> -infested building materials (N=4)	Trichothecenes, sterigmatocystins	GC-MS, HPLC, TLC	Macrocyclic trichothecenes (verrucarol-type) in 4/4 materials (2–15 ng/cm ²); trichodermol in 2/4 materials	Gravesen et al. ⁷⁷
9	Mold-infested domestic residence, several rooms/locations	Vacuumed samples or scrapings from wall paper (N=22)	Multiple mycotoxins	HPLC-DAD (diode array detection), TLC	Meleagrins, sterigmatocystin, 5-methoxysterigmatocystin, chaetoglobosins A + C detected in multiple samples of the home	Nielsen et al. ⁴⁰

Continued

TABLE 1 Overview of Studies that Report on the Occurrence of Mycotoxins (and Other Fungal and Bacterial Secondary Metabolites) in Indoor Sample Materials — cont'd

No.	Study Sites and Design	Sample Materials (Number of Samples)	Target Compounds	Analytical Methodology	Main Findings Concerning Indoor Mycotoxin Occurrence	References
10	Residential building with symptomatic residents and pets; USA	Composite and individual samples of dust (wipes) in the heating system (N = 7)	Ochratoxin A	HPLC, LC-MS, TLC	All samples from the heating ducts contained at least traces of ochratoxin A, up to a maximum conc. of >1500 ppb	Richard et al. ⁵⁹
11	Finnish buildings with moisture problems and symptomatic individuals	Bulk samples of interior finishing (N = 79; e.g., wallpaper, cardboard, wood, plasterboard)	17 mycotoxins	HPLC-MS/MS	43% of samples with at least one of the target compounds; most common sterigmatocystin (24%; 0.2 ng/g–31 µg/g), satratoxins G + H, verrucarol, diacetoxyscirpenol, deoxynivalenol (DON), 3-acetyl-DON, T2-tetraol, citrinin	Tuomi et al. ⁴⁹
12	Residential homes with visible mold/dampness problems; Germany	Carpet dust samples (N = 11)	Sterigmatocystin	HPLC-MS/MS	Sterigmatocystin detected in 2/11 carpet dust samples from mold-infested homes (2–4 ng/g)	Engelhart et al. ⁷⁸

13	Schools, residential homes, commercial building	Dust samples	Stachylysin (N=8)	ELISA	Stachylysin concentrations in dust samples ranging from 2.2 to 162 ng/mg	Van Emon et al. ⁶⁰
14	8 buildings with water damage and <i>Stachybotrys</i> contamination, 4 control buildings, outdoor air (USA)	High-volume liquid impaction bioaerosols samples; case buildings N=40; control buildings N=30; outdoor air N=4	Macrocytic trichothecenes	ELISA	Trichothecenes in contaminated buildings <10 to >1300 pg/m ³ of sampled air, significantly lower levels in control homes (<10–120 pg/m ³); not detected in outdoor air	Brasel et al. ⁴³
15	15 buildings with and 9 buildings without mold/moisture damage	Surface swab, floor dust, and air samples	Macrocytic trichothecenes	ELISA	Higher concentration of macrocytic trichothecenes in floor dust, surface swabs, and air from moldy dwellings vs controls, but significant only for floor dust	Charpin-Kadouch et al. ⁴⁴
16	Mold-contaminated indoor environments	Moldy material samples (N=15)	Satratoxins G+H	LC-MS/MS	Satratoxins G and H in 4 samples of wallpaper (max. 9.7 and 12 µg/cm ²); rosidin E and L-2, satratoxin F, verrucarol J	Gottschalk et al. ⁷⁹
17	Buildings with history of water damage	62 building materials (N=62), settled dust samples (N=8)	Verrucarol, trichodermol, sterigmatocystin, satratoxins G+H	GC-MS/MS, LC-MS/MS	Verrucarol (19–43 pg/mg), trichodermol (2.4–3.4 pg/mg), sterigmatocystin (17 pg/mg) in 3/8 settled dust samples; toxins in 45 of 62 building material samples	Bloom et al. ⁸⁰

Continued

TABLE 1 Overview of Studies that Report on the Occurrence of Mycotoxins (and Other Fungal and Bacterial Secondary Metabolites) in Indoor Sample Materials — cont'd

No.	Study Sites and Design	Sample Materials (Number of Samples)	Target Compounds	Analytical Methodology	Main Findings Concerning Indoor Mycotoxin Occurrence	References
18	Dwelling with water damage and <i>Stachybotrys</i> contamination	Air sample (5 m ³ /h) for 15 h onto 0.8 μm polycarbonate filter	Satratoxins G and H	LC-MS/MS	Satratoxins G (0.25 ng/m ³) and H (0.43 ng/m ³) detected in air sample from a water-damaged building	Gottschalk et al. ⁴⁵
19	5 severely mold-contaminated buildings in New Orleans, LA, USA	Bulk samples from floors from selected rooms (N = 7)	Verrucarol, trichodermol, satratoxins G+H, sterigmatocystin	GC-MS/MS, LC-MS/MS	Verrucarol in 3 samples (0.6–18 pg/mg), sterigmatocystin in 2 homes (18–28 pg/mg)	Bloom et al. ⁸¹
20	57 water-damaged buildings, Sweden	Building materials (N = 100), settled dust samples (N = 18)	Verrucarol, trichodermol, sterigmatocystin, gliotoxin, aflatoxin B1, satratoxins G+H	GC-MS/MS, LC-MS/MS	66% of material and 11% of settled dust positive for at least one mycotoxin; building materials: gliotoxin (0.43–1.12 pg/mg), sterigmatocystin (4.9–150,000 pg/mg), trichodermol (0.9–8700 pg/mg), verrucarol (8.8–17,000 pg/mg), satratoxins G+H; settled dust: verrucarol (0.6–1.7 pg/mg)	Bloom et al. ⁸²

21	3 schools with nonspecific indoor air problems, 1 control school; Finland	Dust swabs and filter materials from ventilation systems (N=26)	40 mycotoxins	LC-MS/MS	Mycotoxins detected in all intake and exhaust systems (pg-ng/g or pg-ng/cm ²), in total 10 compounds: beauvericin, enniatins, verrucarol most common; penicillic acid, sterigmatocystin, satratoxins, trichodermol, chaetoglobosin A, gliotoxin, aflatoxin B ₁	Hintikka et al. ¹¹
22	7 water damaged buildings in Belgium	Samples of air, dust, wall paper, mycelium, or silicone (N=99)	20 mycotoxins	LC-MS/MS, LC-Q-TOF (Time-Of-Flight)-MS	62/99 samples positive for at least one of the mycotoxins, mainly roquefortine C, chaetoglobosin A, and sterigmatocystin; also roridine E, ochratoxin A, aflatoxin B ₁ and B ₂ ; concentrations in air: 0.3 pg-3.4 ng/m ³ ; material samples: 8 pg-13 µg/cm ²	Polizzi et al. ¹²
23	Damp buildings in Slovakia and Austria	Mold-infested material samples (N=14; gypsum board, wall scrapings; paper; soil/wood, etc.)	186 fungal and bacterial secondary metabolites	HPLC-MS/MS	20 different compounds detected, all samples positive for min. 3 metabolites, concentration range ng-mg/kg: meleagrins, roquefortine C, sterigmatocystin, enniatins most common; alamethicin, alternariol, alternariol methyl ether, beauvericin, chaetoglobosin A, chaetomin, citrinin, cytochalasins (B, D), emodin, equisetin, meleagrins, stachybotrylactam, viridicatin	Vishwanath et al. ¹³

Continued

TABLE 1 Overview of Studies that Report on the Occurrence of Mycotoxins (and Other Fungal and Bacterial Secondary Metabolites) in Indoor Sample Materials — cont'd

No.	Study Sites and Design	Sample Materials (Number of Samples)	Target Compounds	Analytical Methodology	Main Findings Concerning Indoor Mycotoxin Occurrence	References
24	8 secondary schools in Malaysia	Cotton swabs of airborne settled dust (N = 32)	Aflatoxin B ₁ , satratoxins G + H, sterigmatocystin, verrucarol, trichodermol	LS-MS/MS, GC-MS/MS	Aflatoxin B ₁ in 1/32 classrooms (6.7 pg/m ²), sterigmatocystin in 2/32 samples (max. 50.5 ng/m ²), verrucarol in 4/32 samples (max. 467 pg/m ²)	Cai et al. ⁶³
25	9 residential homes and 2 public buildings with moisture problems; Finland, Sweden	Building materials (N = 42), dust bag dust (7), floor dust (13), and settled airborne dust (7)	186 fungal and bacterial secondary metabolites	LC-MS/MS	All samples positive for min. 1 of the target compounds; 33 different fungal and bacterial secondary metabolites; concentrations ranging from pg/g to µg/g; emodin, enniatin B, beauvericin, equisetin, physcion, sterigmatocystin, and meleagrins most common	Täubel et al. ²²
26	5 residential homes with moisture damage (Finland), 2 control house dusts (USA, India)	Vacuumed floor dust (N = 7)	186 fungal and bacterial secondary metabolites	LC-MS/MS	15 different metabolites in moisture-damaged homes, 10 in control dust; sterigmatocystin, equisetin, enniatins, cytochalasin D detected in floor dust of Finnish homes with water damage only	Vishwanath et al. ⁵⁰

27	97 school buildings with and without moisture damage in Spain, the Netherlands, Finland	Settled dust and surface swab samples (N=741)	186 fungal and bacterial secondary metabolites; verrucarol, trichodermol	LC-MS/MS, GC-MS/MS	42–58% of dust samples positive for min. 1 of the fungal and bacterial metabolites detected; emodin, emmiatins, physcion most common (up to 37% prevalence); higher number of mycotoxins at elevated concentration in moisture-damaged school buildings	Peitzsch et al. ⁵¹
28	A residential home with moisture/mold problems	Bulk samples from bathroom, bedroom, crawl space (N=6)	Trichothecenes, aflatoxins, ochratoxin A	ELISA	Trichothecenes (0.47–11.7 ppb), aflatoxins (3.5 ppb), and ochratoxin A (2.1–7.7 ppb)	Trasher et al. ⁶¹
29	5 residential homes and one office building	Dust specimens	Trichothecenes, aflatoxins, ochratoxin A	ELISA	Macroyclic trichothecenes in all dust samples, small amounts of ochratoxin A detected in 4/6 samples	Brewer et al. ⁶²
30	95 residential homes in eastern Finland, 14 with moisture damage	Living room floor dust samples (N=95)	330 fungal and bacterial secondary metabolites	LC-MS/MS	42 different mycotoxins and bacterial metabolites (in the order of magnitude of ng/g dust), up to 29 in a single home; only suggestive associations with moisture damage for individual compounds	Pekkanen et al. ⁵² Kirjavainen et al. ⁵³

ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MS, mass spectrometry; TLC, thin-layer chromatography; DAD, diode array detection; TOF, Time-Of-Flight.

nanograms per square centimeter of sampling area have been reported. Typical concentrations in floor dust or settled dust are in the range of nanograms per gram (can reach up to $\mu\text{g/g}$) or picograms per square centimeter of sampled surface, for settled dust.

There are only few reports of mycotoxin findings from actively sampled indoor air^{12,43–45} (see Table 1). Reasons for this are (1) active air sampling is far more cumbersome compared to, for example, obtaining a house dust or building material sample and (2) air concentrations of mycotoxins in indoor environments such as residential homes, schools, or offices are usually very low—disregarding here occupational environments with expected high exposure levels, such as grain-handling facilities. The expected low concentrations set the requirements for using highly sensitive analytical methodology and/or on obtaining high-volume air samples, which implies either extended sampling periods or more expensive equipment capable of high-volume sample collection.

All four studies that report mycotoxins from indoor air are rather consistent in that the concentrations reported are in the range of subnanograms to low nanograms per cubic meter of actively collected air. Based on these studies and also on some more experimental work⁴⁶ it can be concluded that even though mycotoxins are not volatile as such, they do get airborne on fungal spores, fragments, and other particles of inhalable size, so that exposure of occupants of mold-infested buildings takes place. Brasel et al.⁴³ and Charpin-Kadouch et al.⁴⁴ used a macrocyclic trichothecene-specific enzyme-linked immunosorbent assay (ELISA) as analytical methodology for determination of mycotoxins from indoor air samples. Whereas the authors of the first study reported significantly higher levels of airborne trichothecenes in water-damaged buildings with known *Stachybotrys* contamination compared to control buildings and outdoor air, the French study failed to show such significant difference in air samples, but reported the presence of macrocyclic trichothecenes (MCTs) also in buildings without mold problems. Gottschalk et al.⁴⁵ provided the first report of specific mycotoxins in indoor air determined with specific methodology, using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). These authors used a method fulfilling the prerequisites for being accepted as a “confirmatory method for organic residues and contaminants” according to guidelines provided by the European Commission.⁴⁷ The use of ELISA or other bio/immunoassays, on the other hand, is somewhat problematic in terms of specificity and assay interferences. Air sampling was performed in a moisture-damaged building with known *Stachybotrys* contamination, in which earlier satratoxins G and H had been found in a moldy wall paper. The same mycotoxins were detected also in indoor air, at concentrations of 0.25 and 0.43 ng/m^3 of air, respectively. Polizzi et al.¹² sampled air in seven moisture-damaged homes in Belgium and detected mycotoxins in 6 out of total 20 air samples, using LC-MS methodology. These authors report the detection of roquefortine C, roridin E, sterigmatocystin, chaetoglobosin A as well as ochratoxin A, and aflatoxins B₁ and B₂ from indoor air. These mycotoxins are likely to be produced by certain *Penicillium* spp., *Stachybotrys*

spp., *Chaetomium* spp., *Aspergillus versicolor*, and other *Aspergillus* spp. In particular the detection of aflatoxins (max. 0.15 ng/m³) in indoor air in the study of Polizzi et al.¹² is surprising as these mycotoxins are generally very rarely detected as indoor environmental contaminants.

When looking at the sampling sites listed for the individual studies in Table 1 it becomes obvious that the mycotoxin occurrence indoors is almost exclusively considered in the context of dampness and mold contamination in indoor spaces. The focus of the sample collection is on buildings (and materials) with severe moisture damage and/or known mold contamination. It is comprehensible to assume that moisture damage and mold contamination indoors relate to excess microbial proliferation, which ultimately increases the occurrence of mycotoxins, potentially to a level that might affect human health. It is, however, surprising that testing such a hypothesis has not been recognized in the design of almost any of the studies that have been conducted. Only a handful of studies have considered also sampling undamaged control environments in addition to damaged premises, and even fewer have done so with sufficient sample numbers. This implies that in fact very little is known about what is “normal” background with respect to the indoor occurrence of mycotoxins and which compounds are strongly related to conditions of dampness and mold. This issue is further discussed below.

Development of analytical techniques and instrumentation during the past decades with respect to their specificity and sensitivity has naturally reflected on the methods used in studies on indoor mycotoxins (Table 1). A good number of studies have attempted to follow recommendations to use chromatographic separation combined with mass spectrometry to imply specificity in the detection of the target compounds.^{47,48} For a more detailed discussion on analytical methodology in mycotoxin research and monitoring we refer here to Chapter 3.6 in this book. Mycotoxin determination from indoor samples is a complex and challenging task, given the multitude of potentially relevant secondary metabolites being produced by indoor molds and considering that house dust or building materials are very complex and sample matrices are difficult to deal with.⁴⁸ Some of the mycotoxin findings listed in Table 1 have been doubted as being false positive reports. For this more analytical discussion, however, we refer to the papers in which this criticism has been formulated.^{26,48}

Initial studies on indoor mycotoxin occurrence focused on a few, specific target analytes that were mostly selected based on their toxicological relevance and suggested adverse effects on human health. Until the late 1990s, MCTs were almost exclusively the mycotoxins of interest, owing to being partly highly toxic and being produced by *S. chartarum*, which had been linked to severe disease outcomes.^{6,7,9,10} Only since Nielsen and colleagues reported on the immense potential of indoor molds to produce numerous fungal secondary metabolites on indoor surfaces have multimetabolite methods been developed and applied more frequently in indoor studies.^{11–13,22,43,49–52} Nevertheless, the number of studies that have considered a large variety of mycotoxins and

sufficient sample numbers is still small. Target compounds and sample matrices only partly overlap between studies, and differences in detection limits and recovery rate for the various mycotoxins complicate an objective assessment of prevalence based on different studies. Therefore it is not possible yet at this point to present a final conclusion on the most important mycotoxins or fungal secondary metabolites regarding their indoor occurrence. Considering those studies that have used multimetabolite methods to analyze larger numbers of samples from several indoor locations so far, the following compounds have been listed repeatedly as being more prevalent in indoor samples: enniatins and beauvericin (depsipeptides produced by *Fusarium* spp.), meleagrin and roquefortine C (produced by *Penicillium* spp.), emodin and physcion (anthraquinone derivatives produced by *Eurotium* and *Aspergillus* spp.), sterigmatocystin and its precursors, as well as 3-nitropropionic acid (produced mainly by *A. versicolor* and *Aspergillus* spp., respectively), and chaetoglobosins (mostly produced by *Chaetomium* spp.). The detection of MCTs, most prominently satratoxins G and H, either directly or through their hydrolysis product verrucarol, is reported also in some of these later studies. Given the earlier, multiple literature reports on findings of MCTs in severely mold-affected indoor environments, this group of mycotoxins should be added to the list of mycotoxins most commonly reported indoors.

Concerning the compounds listed here, there are two points to be made: one, not all of them can be considered real mycotoxins in the strict sense, as their effects on human or animal health are little explored or not well established. Two, a source attribution for the occurrence of these metabolites indoors is not always straightforward. Although the production of most of these compounds by indoor molds on building materials or on cultivation medium has been shown, the high prevalence of compounds such as enniatins, emodin, or physcion in indoor dust samples suggests that also an influx from outdoor sources is relevant.^{22,51,53} Some plants produce secondary metabolites identical to fungal compounds, for example, the anthraquinone derivatives emodin and physcion. Indoor contamination by outdoor dust and soil particles and also by plant material may be sources of low levels of indoor mycotoxins.

MYCOTOXINS IN THE CONTEXT OF MOISTURE DAMAGE

As pointed out earlier in this chapter, the interest in mycotoxin occurrence indoors is closely linked to moisture damage and dampness in buildings. Obviously, indoor environments with moisture problems generally provide good growth conditions for microbes because of the higher availability of water, which explains general observations of higher microbial levels. However, molds are present and sustain metabolic activity also in “normal” buildings without moisture problems and may find microenvironments in which to proliferate. Thus, the occurrence of mycotoxins in buildings per se cannot be assumed to be a phenomenon of damp indoor environments only. Among the building-associated fungi with

mycotoxin-producing potential are various *Aspergillus* and *Penicillium* species, *Chaetomium globosum*, *Wallemia sebi*, *Eurotium* spp., *Trichoderma* spp., and *S. chartarum*, to give a few examples. Certainly, not all of those fungi are “moisture-damage molds”; several are recognized as primary colonizers in buildings based on their lower water activity requirements²⁶ and may be present also in buildings without moisture problems.

Against this background it is striking to notice how few of the studies conducted so far have used an index/reference design, that is, have collected sample materials from buildings with and without dampness and mold problems. Only studies of such design will ultimately allow the pinning down of mycotoxins that are actually associated with dampness observations in buildings. The vast majority of papers deal with the detection of a few selected mycotoxins in heavily moisture-damaged and/or mold-infested indoor spaces. As of this writing, we have very limited knowledge on which fungal secondary metabolites occur in *undamaged* indoor environments and at what levels; we have insufficient understanding of what can be considered a normal baseline in terms of the presence of mycotoxins indoors.

The studies that used active air sampling in buildings with severe moisture damage and mold contamination and in control buildings without such observations were briefly presented earlier.^{43,44} We wish to add here the work published by Yike et al.,⁵⁴ who collected a total of 15 air samples from seven mold-contaminated residential homes and six air samples from uncontaminated control rooms. In their paper they describe the development of a sensitive protein translation assay for the detection of trichothecene mycotoxin activity in airborne particles. The assay used measures one of the biological effects of trichothecenes, which is the inhibition of protein translation in target cells, but does not specifically measure the exact amount of a given mycotoxin in a sample. This is the reason we did not include this work in the list of studies in Table 1, as we restricted that review to reports on actual mycotoxin detection in indoor matrices. The authors found a strong inhibition in the assay by air particulate extracts from mold-contaminated homes, but not from control homes, and allocated this inhibition to the presence of trichothecene mycotoxins. *Stachybotrys* spp. were cultivated from the air in almost all of the case buildings, which makes the presence of (macrocytic) trichothecenes in indoor air in these homes plausible.

Brasel et al.⁴³ applied a macrocytic trichothecene-specific ELISA on 40 air samples collected in eight mold-contaminated buildings (from 16 rooms), 30 air samples from four buildings with no visible contamination and history of water damage (14 rooms), and four outdoor air samples; all samples were collected in the state of Texas in the United States. Whereas MCTs were also detected in the air of control environments (<10–120 pg of trichothecene equivalents per cubic meter of air), the authors reported significantly higher levels in air samples from *S. chartarum*-infested rooms (<10 to >1300 pg/m³). MCTs were not detected in samples of outdoor air.

Charpin-Kadouch et al.⁴⁴ also used the ELISA for determination of MCTs in samples of surface swabs from walls, floor dust, and indoor air from 15 French

buildings with and nine buildings without moisture and mold problems. MCTs were detected in all samples, both from index and from reference houses. Mean values for MCTs were higher in moldy buildings for all three sample types; however, a significant difference between index and reference homes was observed only for floor dust samples, not in air or surface swab samples.

The study in schools presented by Hintikka et al.¹¹ is not a study with a strict index and reference design, but is nevertheless briefly mentioned here. The “index” schools were schools that reported some rather nonspecific indoor air problems and were referred to as typical schools in Finland, whereas the “reference” school was chosen based on not having a history of moisture damage. Dust samples were collected in the ventilation ducts of these schools. The authors reported a variety of mycotoxins at concentrations of picograms to nanograms per square centimeter of swabbed surface in ventilation ducts from all schools, with the control school building actually showing the highest number of different mycotoxins in both the supply and the exhaust air. Compounds detected in the ventilation system of this school included MCTs (only in exhaust); chaetoglobosins, beauvericin, and enniatins in both exhaust and intake ducts; and penicillic acid, sterigmatocystin, gliotoxin, and aflatoxin B₁ in supply air only. The authors concluded—based on the presence of some of the mycotoxins in intake and exhaust air ducts—that a good part of the mycotoxins in school buildings might not originate from sources within the building, but either are introduced through outdoor air or may be produced in the ventilation systems, which would indicate insufficient maintenance.

A more recent study in schools compared the mycotoxin occurrence in moisture-damaged versus non-moisture-damaged school buildings following a robust, epidemiological design.⁵¹ The authors targeted more than 180 fungal and bacterial secondary metabolites and presented data from 675 settled dust samples collected in 66 index and reference schools. Index and reference status of the schools located in the Netherlands, Spain, and Finland were based on standardized school building inspections recording observations of moisture damage and dampness. The authors did not find clear statistically significant differences in the occurrence of individual mycotoxins in moisture-damaged versus non-moisture-damaged school buildings. However, they showed a tendency for the occurrence of a larger number of mycotoxins at elevated levels in moisture-damaged schools, reaching significance when considering all samples from three countries. The study concluded that microbial toxins are also present in undamaged buildings as part of the “normal” microbial flora indoors. The authors suggested that not only does moisture damage—by triggering microbial proliferation and metabolite production—act as a source of mycotoxins in indoor settled dust, but also outdoor air and particulate matter seem to have an impact on the indoor occurrence of microbial secondary metabolites. Indeed, the presence of mycotoxins in outdoor air particulate matter has been shown in a follow-up study conducted by some of the same authors.⁵⁵

Vishwanath and colleagues⁵⁰ report mycotoxin findings from floor dust from residential homes with moisture damage in Finland as well as from floor dust

from two reference homes in the United States and in India. The geographical difference in the origins of the samples forbids a direct comparison of mycotoxin occurrence in index and reference house dusts, as differences may be related to geographical/climatic differences. However, it can be mentioned here that in the reference house dust fungal secondary metabolites were also detected.

A Finnish birth cohort study presented multimetabolite analysis data from floor dust collected from 95 residential homes.^{52,53} Fourteen of these homes had inspection-assessed major moisture damage in the living rooms and kitchen. The authors found a nonsignificant tendency toward elevated total number and load of fungal and bacterial secondary metabolites in moisture-damaged homes compared to undamaged homes. Differences in the number of metabolites in index versus reference homes reached statistical significance when compounds occurring at elevated concentrations were considered (similar to the finding reported by Peitzsch et al. in schools⁵¹). There were suggestive associations of a number of mycotoxins with moisture damage, but these associations were weak and did not survive correction for multiple testing.

SUMMARY, CONCLUDING REMARKS, FUTURE CHALLENGES

This chapter identifies 30 reports in the literature that have attempted to determine mycotoxins from indoor sample materials, including building material, surface swab, dust, and active air samples. The majority of reports refer to residential homes; schools, offices, and other public buildings are also considered in a few studies. The research on the indoor occurrence of mycotoxins is essentially exclusively conducted in the context of dampness and mold contamination in buildings. Typically, samples are collected from severely moisture-damaged and/or mold-infested premises; where building materials are concerned, these are usually from mold-affected areas. The results of an analysis of samples collected in undamaged, control environments are rarely presented.

In particular earlier studies, but also some of the more recent reports, used analytical methodology that targeted only a few specific mycotoxins. Those target compounds have been selected primarily based on the toxicological properties of some of the fungi known to occur in damp indoor environments. Examples here are the MCTs produced by *S. chartarum* or also sterigmatocystin of *A. versicolor*—compounds that are partly highly toxic and are known to pose a threat to human health upon exposure. Reports of MCTs or also sterigmatocystin indoors are therefore frequently found in the literature. However, there is an enormous variety of mycotoxins produced by fungi in our environment, with estimates starting at 20,000 unique mycotoxins. Considering this and realizing the limited availability of occurrence data for a wider range of mycotoxins, it is unclear whether compounds that have been the focus of early indoor mycotoxin studies are also the most relevant targets with respect to their prevalence indoors. Not all mycotoxins that are detected in indoor dust or indoor air originate from indoor sources. The high prevalence of some fungal secondary

metabolites—such as enniatins, beauvericin, emodin, and phycion—observed in indoor samples in a number of studies suggests influx from outdoor sources.

Conclusions about which would be the most relevant mycotoxins in indoor spaces that are *associated with observations of indoor dampness and mold* are impossible at this stage. There is a striking lack of studies that would have considered a large variety of microbial secondary metabolites from a sufficient number of samples collected in a standardized manner from indoor environments with and without moisture damage. The few studies that provide such index/reference data have not shown very clear differences in the prevalence or levels of individual mycotoxins. Such studies rather indicate more subtle differences, for example, a higher number of mycotoxins occurring at elevated concentrations in damp compared to reference buildings. The respective studies were, however, limited to some extent as concerns the severity of the moisture damage conditions⁵¹ and/or the sample numbers.^{52,53} There is a need for future studies recruiting severely damaged indoor environments into a sound, epidemiological index/reference study design.

Ultimately, the aim of our research must be to answer the question “... if mycotoxins at concentrations found in mould damaged indoor environments make us sick,” as was formulated by Bloom in her Ph.D. thesis.⁵⁶ Despite some 30 years of research dealing with the indoor occurrence of mycotoxins, sound information on actual health effects is limited. Several of the studies on the occurrence of mycotoxins indoors listed in Table 1 present some information on health complaints or in a few cases clinical data for the occupants of these buildings.^{5,8,10,57–62} However, no formal statistical analyses relating to the measured mycotoxin exposure of the individuals to their health outcomes were performed in any of these studies, mostly for reasons of too few samples or insufficient patient or mycotoxin exposure data. Support from epidemiological studies in clarifying potential health effects upon indoor mycotoxin exposure is almost completely absent. The fact that there are no commonly accepted biomarkers of airborne exposure to multiple mycotoxins in non-occupational settings is certainly contributing to this situation. Cai et al.⁶³ analyzed associations of verrucarol (the hydrolysis product of MCTs) in surface dust collected in 32 classrooms on self-reported respiratory symptoms of 462 pupils in eight schools in Malaysia. The authors reported an inverse association of verrucarol with daytime breathlessness. The study was, however, limited by the fact that verrucarol was detected in only 4 of 32 classrooms. Two studies that used multimetabolite analysis methods in an epidemiological study setting are being prepared for publication as of this writing, with part of these data being published in conference proceedings. Kirjavainen et al.⁵³ (manuscript under review; see also Ref. 52) explored associations of mycotoxins in floor dust at early life with development of asthma in 95 pupils in a Finnish birth cohort study. The authors reported that neither sum load nor number of individual toxins were associated with a risk of doctor-diagnosed asthma; positive and negative indicative associations were observed for individual mycotoxins with the development of asthma. Zock et al.⁶⁴ (full manuscript under preparation see also Ref. 55) studied respiratory

symptoms in 645 teachers in relation to dampness and levels of microbial secondary metabolites in schools in Finland, the Netherlands, and Spain. Preliminary analyses indicated a significant dose–response association of mycotoxin load in dust with the asthma symptom score and nasal symptoms in Finnish teachers.

There is good knowledge of the toxicological mechanisms that mycotoxins may exert—those are discussed in another section of this book (Chapter 3.6). However, most of what is known refers to food-borne mycotoxins and ingestion as an exposure route. Apart from a few exceptions, much less information is available from the more indoor-relevant metabolites and their toxicology upon inhalation exposure. It has been suggested in the literature that inhalation of mycotoxins may be many times more toxic than ingestion,^{65,66} but this is in fact very little understood. Mycotoxins are present in indoor environments and inhalation exposure takes place. Mechanistic work^{46,67,68} has shown that not only fungal spores carrying mycotoxins but also fragments may be crucial in terms of exposure. Based on the few reports of mycotoxins in the air of mold-infested indoor spaces, “usual” exposure levels are very low, in the concentration range of picograms to nanograms per cubic meter of air. Thus—unless high-exposure occupational settings are concerned, or exceptionally severe cases of indoor contamination—acute toxic effects of mycotoxin exposure may be rare. Nevertheless, chronic low-level exposure to mycotoxins through inhalation could also represent a health hazard. For example, Miller et al.^{69,70} have shown alterations in the expression of inflammation-related genes in mouse lungs upon exposure to “real-world” levels of various indoor mycotoxins. There are *in vitro* studies that indicate that synergistic effects in evoking cellular responses may be highly relevant when considering multiple mycotoxins and mycotoxins and other microbial compounds.^{71–74} These findings also link to the initial scheme presented (Figure 1), illustrating the multitude of biological and chemical exposures present in damp buildings. Measurement of single agents is probably not sufficient if we want to attempt to elucidate the adverse health effects observed in exposed occupants, but rather we will have to take into consideration the complexity of the exposure situation in damp buildings in future assessments.

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