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Review

# ADVERSE HEALTH EFFECTS OF INDOOR MOULDS*

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Building associated illnesses – sick building syndrome (SBS) as a common example – are associated with staying in buildings with poor indoor air quality. The importance of indoor fungal growth in this phenomenon continues to be evident, even though no causative relation has been established so far. Indoor humidity is strongly associated with the symptoms of SBS. Fungal metabolites that may induce ill health in susceptible occupants comprise beta-D-glucan, mycotoxins, and volatile organic compounds as known irritants and/or immunomodulators. Indoor toxic fungal metabolites might be located in micromycetal propagules (endometabolites), in (bio-)aerosol, detritus, and house dust (exometabolites) as their particular carriers. It is highly probable that hyphal fragments, dust, and particles able to reach the alveoli have the strongest depository and toxic potential. Most fungal spores are entrapped by the upper respiratory tract and do not reach further than the bronchi because of their size, morphology, and the mode of propagation (such as slime heads and aggreggation). This is why studies of the toxic effects of fungal spores prefer directly applying metabolite mixtures over mimicking real exposure. Chronic low-level exposure to a mixture of fungal toxicants and other indoor stressors may have synergistic effects and lead to severe neuroendocrine-immune changes.

**KEY WORDS:** *inflammation, mycotoxins, respiratory tract, sick building syndrome, volatile organic compounds*

Different indoor factors (such as chemicals, microorganisms, allergens, and tobacco smoke) can contribute to many building-related illnesses (BRI) in exposed occupants. These include infections related to building ventilation (legionellosis), common cold, flu or, reactions to indoor chemicals, fungi, bacteria, and/or their toxins. A type of BRI known as sick building syndrome (SBS) includes a complex of health complaints and general discomfort (1, 2). Symptoms of SBS are non-specific and usually depend on indoor microclimatic parameters such as temperature, relative humidity, dust, cigarette smoke, ventilation, building materials and furnishing, and sensibility of affected persons. Inflammatory reactions to indoor toxicants are either local – in the airways, mediated by IgE (allergic mechanism) or by non-specific inflammation (e. g. cytotoxic effects) – or systemic through the release of lung cytokines (3). The role of fungi in the aetiology of health problems associated with staying in certain buildings has not been fully clarified. Indoor fungal exposure may lead to allergies such as fungal rhinitis, hypersensitivity pneumonia, and/or asthma (4, 5). Monitoring exposure to indoor fungi is rather complicated due to a lack of standard methods to evaluate how indoor microclimate, outdoor environment, and microscopic fungi affect each other. The ability of *Penicillium* sp. and *Aspergillus* sp., the so-called first colonisers, to grow on common house

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dust at relative humidity of 76 % to 80 % can probably explain why they prevail even in healthy buildings. Secondary colonisers (*Cladosporium* sp., *Alternaria* sp., *Chaetomium* sp.) that grow at 85 % relative humidity and tertiary colonisers (*Fusarium* sp., *Acremonium* sp., yeasts) that grow at >90 % humidity are able to biodeteriorate any building material under optimal growth conditions (6-8).

## MYCOTOXINS, VOLATILES, AND HAEMOLYSINS

Currently, a general approach to the study of the mechanism of fungal effects on human beings is becoming more urgent. Such an approach includes the immunosuppressive influence and (bioaerosoloic) inflammatory reactions to beta-glucans from fungal cell wall as well as toxic and irritative effects of their toxic exo- and endometabolites – mycotoxins and/or volatile organic compounds, and pathological conditions associated with fungal haemolysins (9). Regarding mycotoxins with rather well characterised toxicity (incl. carcino-, muta-, teratogenicity, cytotoxicity and immunosuppression) after ingestion or dermal exposure, an adverse biological effect can be caused by the inhalation of a dose at the minimum level of one tenth of the alimentary one. Indoor mycotoxins and their health effects have joined the most studied public health issues (10).

Microbial volatile organic compounds (MVOCs) such as alcohols, aldehydes, ketones, aromatic compounds, amines, terpenes, chlorinated hydrocarbons, and sulphuric compounds, (at indoor concentrations as high as 250 mg $m^{-3}$) cause typical mouldy odour (usually 2-octen-1-ol or geosmine), but also an irritation, swelling/inflammation of the airways in sensitive people, and/or cytotoxic effects (DNA damage). These effects are associated with invisible moulds usually growing under wallpaper, carpets, or mattresses. The most common MVOCs are xylene, toluene, 2-propanol, limonen, heptane, formaldehyde, and acetaldehyde. There is a positive correlation between MVOC production and the ability of fungi to synthesise mycotoxins. According to Larsen et al. (11) and Corpi et al. (12), *Aspergillus* spp., *A. versicolor, Cladosporium* spp. and *Penicillium* spp. are the strongest producers of such compounds, e. g. 2-ethylhexanol, cyclohexane (skin and/or mucose irritation), and carcinogenic benzene.

Although, many occupational pulmonary mycotoxicoses have been reported as adverse effects of inhaled organic dust contaminated by microbial toxins (13), there is no objective evidence of clinical diseases caused by indoor mycotoxins alone, produced by *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Trichoderma* sp. and *Stachybotrys chartarum*, as no serious epidemiological study has addressed the issue (14). During the fatal infant idiopathic pulmonary haemorrhage outbreaks in the USA, isolated fungi were analysed for toxicity *in vitro*. *S. chartarum* produced cytotoxic and immunosuppressive macrocyclic trichothecenes (stachybotryotoxins) and spirocylic drimanes that caused inflammation and haemorrhages in the respiratory tract and intestines of laboratory animals. *M. echinata* produced griseofulvins and *A. versicolor* carcinogenic sterigmatocystin (15). Toxins produced by stachybotrys (satratoxins, roridins, verrucarins) affect the cell by binding to 60S ribozomes, by inhibiting proteosynthesis, elongation, termination, and by inducing apoptosis, as they induce mitogen-activating proteinkinases (MAPK). Hintikka (16) also found that spirocyclic drimans of *S. chartarum* inhibited proteosynthesis and were immunosuppressive (inhibited the complement system and TNF-alfa release and stimulated plasminogen, fibrinolysis, and thrombolysis). Tobacco smoke was stressed by some epidemiologists as increasing the health risk of fungal toxins (14). *S. chartarum* isolated cardboard and vinyl ceilings of damp schools and dwellings in Denmark produced trichodermol trichothecenes (17). Acute intratracheal exposure of rats to atranone A, produced by *S. chartarum* isolated from mouldy walls in a Bratislava house, resulted in haematological changes, cell damage, and inflammatory pulmonary injury in those animals (18, 19). However, complex toxic mechanisms underlying the *in vivo* activity of atranones remain unclear. Simple trichothecens (such as trichodermol and trichodermin) that usually accompany atranones are neurotoxic (eye dysfunction, dyspnoea, tachycardia, nausea, tremors, dizziness, lowered concentration, confusion, loss of balance, hypotension, myelosupression, lower CNS activity bordering neurocognitive/behavioural deficits, and somnolence) and act as skin irritants (20).

In mice, Ren et al. (21) reported the adverse effects of mutagenic and foetotoxic mycotoxin alternariol and its monomethylether produced by *Alternaria alternata* isolated from ceiling tiles. This isolate was also able to grow on cardboard. An experimental culture of *Penicillium expansum* grown on wallpaper glue produced nephrotoxic citrinin and fagocytosis-

inhibiting patulin (22). Metabolite synthesis in fungi depends on the quality of construction materials (23, 24). In our earlier study (25) on tracheal cultures of 1-day-old chicks, varying ciliostatic activity was found in biomass extracts from building materials (mineral wool, plasterboard, cardboard) inoculated with pure isolates of indoor moulds *Penicillium chrysogenum, P. palitans, Trichoderma viride, Stachybotrys* sp., and *A. versicolor*. Generally, moulds growing on materials composed of finely divided cellulose were more active than those growing on mineral wool. The only available data on the ciliostatic activity of indoor mould metabolites refer to sterigmatocystin from *A. versicolor* and *Chaetomium* spp. even though 78 % of all indoor fungi (*Aspergillus clavatus, A. flavus, A. fumigatus, A. nidulans, A. niger, A. ochraceus, A. restrictus, A. ustus, A. versicolor, Alternaria* sp., *Chaetomium* sp., *Cladosporium cladosporioides, C. sphaerospermum, Phoma* sp., and *Stachybotrys chartarum*) isolated in Slovakia over the last 15 years produce complex metabolites capable of stopping tracheal ciliary beating in 24 hours (26-28). Viable spores of *A. versicolor* and *Penicillium* sp. were isolated from different plasters after 40 days to 3 months (7, 8), which means that they are able to produce toxins for a relatively long time.

Only a few animal studies addressed histological pulmonary changes after intranasal exposure to fungal spores and the time course of inflammatory and cytotoxic processes in the airways after intratracheal instillation of sporal suspensions (20, 29, 30). All toxic fungal metabolites are located in micromycetal propagules (endometabolites), in aerosol, detritus, and house dust (exometabolites) (6). It is highly probable that hyphal fragments, dust, and material particles able to reach the alveoli have the highest depository and toxic potential. Most of the fungal spores are entrapped by the upper respiratory tract and do not reach further than the bronchi, because of their size, morphology, and the mode of propagation (slime heads, aggregation, etc.). This is why studies of the toxic effects of fungal spores prefer direct use of metabolite mixtures over recreating real exposure to microorganisms.

Health damaging effects of fungal haemolysins (indoor e. g. stachylysin produced by *S. chartarum*,or chrysolysin by *Penicillium chrysogenum*) result from the activation of histamine- and cytokin-producing cells (inflammatory, cold-like SBS symptoms) and from vascular tissue lysis (headaches, bleeding, vertigo) (31).

## CONCLUSIONS

In the occupants of damp mouldy dwellings, complex toxic fungal metabolites can disrupt the self-cleaning airway system, induce inflammation, and damage cells and blood. These effects are enhanced by indoor contaminants such as cigarette smoke and can finally result in ill health including respiratory disorders and general intoxication, especially in children with quick metabolism. To determine the relationship between mycotoxins, their real mixtures, and other bio-, and non-biological factors in indoor environment and specific human health disorders after their inhalation, it is necessary to establish the minimal effective concentration of mycotoxin able to cause clinical symptoms, as opposed to the *in vitro* approach, to choose the best animal or other biological model for studying mycotoxin pathogenicity and pathophysiology (pulmonary deposition), and to characterise short- and long-term health damages (biomarkers) in the exposed people. Future studies should not neglect the potential of fungal toxins to cause multifactorial human diseases (immune, degenerative, and tumorous), whose incidence and prevalence are on the rise in modern societies.

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***Sažetak***

ŠTETNI ZDRAVSTVENI UČINCI PLIJESNI UNUTARNJIH PROSTORA

Bolesti povezane sa stanovanjem te sindrom bolesne zgrade (engl. *sick building syndrome, SBS*), kao jedan od njihovih tipičnih primjera, povezani su s boravkom u zgradama s lošom kvalitetom zraka. Premda još nije uspostavljena jasna uzročno-posljedična povezanost, važnu ulogu u spomenutom fenomenu ima rast plijesni u unutarnjim prostorima. Pojavnost simptoma sindroma bolesne zgrade snažno ovisi o vlažnosti prostora. U metabolite plijesni koji mogu izazvati poremećaje zdravlja u osjetljivijih ispitanika ubrajamo beta-D-glukan, mikotoksine te hlapljive organske spojeve koji su poznati kao iritansi i/ili imunomodulatori. Toksični metaboliti gljiva unutarnjih prostora mogu biti sadržani u propagulama mikromiceta (endometaboliti), (bio)aerosolu, detritusu te kućnoj prašini (egzometaboliti). Najjači potencijal za depoziciju te toksični potencijal imaju fragmenti hifa, prašina i čestice koje mogu doprijeti do alveola. Većinu gljivičnih spora zaustave gornji dišni putovi te one zbog svoje veličine, morfologije i načina razmnožavanja (npr. ljepljive glavice i agregacija) ne dopiru dalje od bronha. To je razlog zašto se u istraživanjima toksičnih učinaka gljivičnih spora češće primjenjuju smjese metabolita negoli oponaša stvarna izloženost. Kronična izloženost niskim razinama smjesa gljivičnih toksikanata i drugih stresora unutarnjih prostora može izazvati sinergijske učinke i dovesti do teških neuroendokrino-imunosnih promjena.

**KLJUČNE RIJEČI:** *dišni putovi, hlapljivi organski spojevi, mikotoksini, sindrom bolesne zgrade, upala*

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