Extract from Europe WHO Guidelines for Indoor Air Quality: Dampness and Mould, 2009

Mycotoxins, or fungal toxins, are low-relative-molecular-mass biomolecules produced by fungi, some of which are toxic to animals and human beings. **Mycotoxins are known to interfere with RNA synthesis and may cause DNA damage**. Some fungal species may produce various mycotoxins, depending on the substrate. In the case of *Penicillium*, one such compound is penicillin, a strong antibiotic.

Several mycotoxins, e.g. aflatoxin from *Aspergillus flavus* and *Aspergillus parasiticus*, are potent carcinogens. Many mycotoxins are immunotoxic, but the trichothecene mycotoxins are immunostimulating at low doses (Eduard, 2006). Numerous mycotoxins have been classified by their distinct chemical structures and reactive functional groups, including primary and secondary amines, hydroxyl or phenolic groups, lactams, carboxylic acids, and amides.

The mycotoxins that have perhaps received most attention are the trichothecenes, produced by *Stachybotrys chartarum.* Bloom et al. (2007) showed that several mycotoxins produced by *S. chartarum* and *Aspergillus versicolor* (i.e. macrocyclic trichothecenes, trichodermin, sterigmatocystin and satratoxin G) could be present in most samples of materials and settled dust from buildings with current or past damage from damp or water. Charpin-Kadouch et al. (2006) compared the levels of macrocyclic trichothecenes in samples from 15 flooded dwellings known to be contaminated with *S. chartarum* or *Chaetomium,* and a group of nine dwellings without visible mould. The level of macrocyclic trichothecenes was significantly higher in floor dust from the mouldy houses than from the reference dwellings; the levels in wall samples from mouldy houses were also higher (of borderline statistical significance), but no statistically significant difference in air concentrations was observed. In a study by Brasel et al. (2005a) in seven buildings known to be contaminated with *S. chartarum*, the airborne level of macrocyclic trichothecenes was significantly higher than that in four control buildings (i.e. with no detectable *S. chartarum* or history of water damage).

The same authors also showed that *S. chartarum* trichothecene mycotoxins can become airborne in association with both intact conidia and smaller fungal fragments (Brasel et al., 2005a,b). Sterigmatocystin was shown to aerosolize from a finishing material (Moularat, Robine, 2008), at an airflow rate of 100 cm/s and a relative humidity of 30%. These studies demonstrate that mycotoxins are present in the indoor environment and that the levels may be higher in buildings affected by mould or damp

Some exposures with adverse health effects associated with damp indoor environments include emissions of volatile organic compounds from damp and mouldy building materials (Claeson, Sandstrom, Sunesson, 2007). Emissions are a consequence of competition between moisture and some chemicals for adsorption sites. Volatile organic compounds can be similar to microbial ones, as both often occur in the same environment. The main difference is the source of emission, i.e. mould or building materials. Damp concrete floors have been shown to increase chemical degradation of the plasticizer in polyvinyl chloride floor coatings and glues, resulting in emissions of volatile organic compounds such as 2-ethyl-1-hexanol (Norback et al., 2000; Tuomainen, Seuri, Sieppi, 2004). Similarly, damp concrete floors may emit ammonia from the self-levelling flooring compound used in the late 1970s and early 1980s in Europe. Furthermore, the **offgassing of formaldehyde from composite wood products and the rate of formation of ozone increase with relative air humidity** (Arundel et al., 1986; Godish, ish.

Rouch, 1986). Formaldehyde concentrations may also be elevated in damp indoor environments because moist air holds more formaldehyde. The levels of semi-volatile compounds, such as

pentachlorophenol (a wood preservative) and other pesticides, may also be elevated in damp indoor environments.

Several other effects of exposure to mould have been discussed in the context of indoor air, including toxic, immunological, reproductive and neuropsychiatric symptoms and syndromes. A Finnish group studied the occurrence of rheumatic diseases associated with dampness. In two studies, the authors found clustering of cases of rheumatic disease in water-damaged buildings (Myllykangas-Luosujarvi et al., 2002; Luosujarvi et al., 2003) and suggested that the symptoms could be attributed to exposure to mould spores. In a later publication (Lange, 2004), the author proposed that endotoxins and other triggers of the innate immune response might play a role, although the exposure levels are much lower than those in situations where joint pain is more prevalent, as on farms and among bird fanciers. Rheumatic diseases among people exposed in damp buildings and the possible role of endotoxins was also reported by Lorenz et al. (2006).

This brief review of toxicological studies on microbial exposure in damp buildings is based mainly on studies published between 2000 and mid-2007; when appropriate, references are also be made to earlier publications, including those in the review of the Institute of Medicine (2004). The data reviewed originate from studies in vitro (i.e. experimental studies in a controlled environment outside a living organism, such as a test tube), summarized in Table A2.1, and studies in experimental animals in vivo (Table A2.2). Although direct extrapolation from experimental data to human risk is not possible, the studies that are described provide important information about the possible toxicological mechanisms behind the observed health effects in damp buildings. This review focuses on the **ability of microbial exposures associated with damp buildings to activate the following potential toxicological mechanisms: immunostimulation and allergies, cytotoxicity and immunosuppression, autoimmunity, irritation, neurotoxicity, genotoxity and reproductive toxicity**. Novel toxicological data on the role of microbial interactions are also included.

The variety of respiratory symptoms and diseases observed in damp and mouldy indoor environments suggests that the airways are the primary route of entry for agents. Therefore, studies in experimental animals were limited to those in which the airways were used as the pathway of exposure, thus excluding the extensive literature on the induced toxic effects of bacterial toxins and mycotoxins, associated, for example, with ingestion of mould-contaminated food. Most of the in vitro and in vivo studies included in this review addressed the effects of microbial components found in damp buildings, such as fungal spores, bacterial spores and cells, and the toxic components or products of microbes (e.g. fungal mycotoxins and endotoxins) (see Tables A2.1 and A2.2). Possible toxic effects due to released non-microbial chemicals are not addressed, because experimental data on exposures to chemicals in damp buildings were missing or limited.

In damp buildings, people are exposed to constantly changing concentrations of different microbial species, their spores, metabolites and components, and other compounds in indoor air, including chemical emissions from building materials. This complex mixture of exposures inevitably leads to interactions, which may change the toxic characteristics of the inhaled particles, causing different outcomes in different situations. Furthermore, the effects of microorganisms, microbial substances or dampness-related chemical compounds seen in experimental animals or cells often result from exposures that are orders of magnitude higher than the average doses that reach the human lungs under normal conditions in indoor air. Nevertheless, the surface doses within the lungs of patients with respiratory conditions can vary a thousandfold, due to uneven particle deposition (Phalen et al., 2006), resulting in even larger maximal surface doses in human lungs than in those used in experimental toxicological studies. Moreover, many other factors, such as exercise, can result in larger-than-average doses in the human lung. Thus,

experimental toxicological studies are essential for clarifying cellular mechanisms and identifying causative compounds, but the dosage must be considered in interpreting the findings and attempting extrapolation to the range of human exposures indoors.

There is evidence that dampness in buildings increases the risks of asthma, sensitization and respiratory symptoms, as extensively reviewed by Bornehag et al. (2001, 2004). Many of the health effects may result from recurrent activation of immune defence, leading to exaggerated immune responses and prolonged production of inflammatory mediators. Overproduction of these compounds damages the surrounding tissues and may manifest itself as chronic inflammation and inflammation-related diseases, such as asthma (Martin, Frevert, 2005). The central role of inflammatory responses is corroborated by studies reporting increased levels of inflammatory mediators in nasal lavage fluid and induced sputum from the occupants of damp buildings (Hirvonen et al., 1999; Purokivi et al., 2001; Walinder et al., 2001).

The immunostimulatory activity of Gram-negative bacterial lipopolysaccharide is well established, but several other bacteria, fungi and isolated mycotoxins associated with damp buildings have been shown to induce inflammatory responses in vitro (Huttunen et al., 2001; Nielsen et al., 2002; Huttunen et al., 2003; Pylkkanen et al., 2004; Johannessen, Nilsen, Lovik, 2005; see also Table A2.1). In line with the findings in vitro, the same microbial species activate acute (Nikulin et al., 1996; Rao, Brain, Burge, 2000; Jussila et al., 2003; Leino et al., 2003; Rand et al., 2006) and sustained inflammation in the lungs of experimental animals (Jussila et al., 2002a) (Table A2.2). Furthermore, it has been shown in an animal model that immunological status plays an important role in airway inflammation induced by *S. chartarum*, enhancing the effects of the mould (Leino et al., 2006). The results imply that sensitized people are more susceptible to exposure to mould than nonatopic people. Different microbial species differ significantly in their immunostimulatory potency in both mouse and human cells in vitro (e.g. Huttunen et al., 2003). Furthermore, it has been clearly demonstrated that different growth conditions and competition between microorganisms for the same habitat in vitro change their inflammatory potency, protein expression and toxin production (Ehrlich, 1987; Meyer, Stahl, 2003; Murtoniemi et al., 2003).

One of the mechanisms underlying the health effects of exposure to microbial agents may be IgE-mediated allergic responses. Although many of the reported symptoms are similar to those of allergy, only a small percentage of exposed people actually develop allergies to mould (Taskinen et al., 1997; Immonen et al., 2001). The most prevalent fungal genera associated with mould allergy are *Aspergillus*, *Cladosporium* and *Penicillium* (Ledford, 1994). Sensitization to *A. alternate* has been linked to the development, persistence and severity of asthma (Zureik et al., 2002; Bush, Prochnau, 2004; Salo et al., 2006). **Some fungal species can induce histamine release by other mechanisms (Larsen et al., 1996); thus, allergy-like symptoms can also occur in non-sensitized people**. Some of the chemical compounds associated with damp and degrading materials, such as phthalates and their metabolites, can stimulate the immune system by acting as allergens or adjuvants (Hansen et al., 2007).

Increased frequencies of common respiratory infections have been observed in people living or working in damp buildings (Aberg et al., 1996; Pirhonen et al., 1996; Kilpelainen et al., 2001), suggesting that agents present in the indoor air of these buildings can suppress immune responses, leading to increased susceptibility to infections. Several microbes originating from damp buildings or their toxins have been shown to have immunosuppressive effects in vitro mediated – for example, by impaired particle clearance (Pieckova, Jesenska, 1996, 1998) or by cytotoxicity (Huttunen et al., 2004; Penttinen et al., 2005a,b). **The immunosuppressive effects of mycotoxins have been confirmed in experimental animals.** Trichothecenes T-2 and deoxynivalenol (vomitoxin) impair

immune responses to respiratory virus infection, increasing the severity of infection (Li et al., 2006; Li M et al., 2007). Some airborne fungi and bacteria may act as opportunistic human pathogens, causing upper or lower airway, pulmonary and in some cases systemic infectious diseases in immunocompromised people (Bush et al., 2006). The acute cytotoxicity of fungal strains in damp buildings has been found to be due to the metabolite profile produced in vitro, although their biological activity may not depend solely on toxin production (Nielsen et al., 2002; Huttunen et al., 2003). Fungal spores appear to have toxic effects other than those that cause the inflammatory reaction. Studies of Gram-positive and -negative bacteria (e.g. *Streptomyces californicus*, *Pseudomonas fluorescens, Mycobacterium terrae, Bacillus cereus*) have shown that the significant difference in cytoxicity among strains (Huttunen et al., 2003) is due at least partly to differences in inflammatory activity. Spores and toxins of the fungus *S. chartarum* have been shown to activate the apoptotic pathway (programmed cell death) (Islam et al., 2006; Wang, Yadav, 2006; Penttinen et al. 2007), whereas the spores of *S. californicus* induce cell cycle arrest (Penttinen et al., 2005b). Studies in experimental animals with the same fungal or bacterial species confirm the in vitro findings for cytotoxic effects, showing increases in total protein and lactate dehydrogenase in bronchoalveolar lavage fluid from exposed animals, as well as lung tissue damage (Nikulin et al., 1996; Rao, Brain, Burge, 2000; Rao, Burge, Brain, 2000; Jussila et al., 2001, 2002a, 2002c; Yike et al., 2002; Jussila et al., 2003; Rand et al., 2006).

Cases of autoimmune diseases and related symptoms have been reported among the occupants of damp buildings (Myllykangas-Luosujarvi et al., 2002; Luosujarvi et al., 2003), but there are no toxicological data on autoimmune responses caused by microorganisms or microbial substances found in damp buildings. Microbial fragments can, however, cause autoimmune reactions by molecular mimicry, acting as microbial superantigens or by enhancing the presentation of autoantigens (Wucherpfennig, 2001).

Neurotoxicity - Such health effects as fatigue, headache and difficulties in concentration (Johanning et al., 1996; Koskinen et al., 1999b) indicate that microbes or other agents present in damp buildings have neurological effects. Many pure microbial toxins, such as the products of *Fusarium* (fumonisin B1, deoxynivalenol), *Stachybotrys* (satratoxin G), *Aspergillus* (ochratoxin A) and *Penicillium* (ochratoxin A, verrucosidin), have been shown to be neurotoxic in vitro and in vivo (Rotter, Prelusky, Pestka, 1996; Belmadani et al., 1999; Kwon, Slikker, Davies, 2000; Islam, Harkema, Pestka, 2006; Stockmann-Juvala et al., 2006).

Heavy occupational exposure by inhalation to mycotoxins in mouldy grain may be linked to an increased risk of cancer (Olsen, Dragsted, Autrup, 1988; Kristensen, Andersen, Irgens, 2000), but there is no epidemiological evidence for an association between exposure in damp buildings and cancer. Some of the microbial toxins produced by bacteria and fungi are known to be genotoxic and carcinogenic (IARC, 1993), but the relevance of these findings to exposure by inhalation in damp buildings is not known. It has been shown, however, that microbial isolates from damp buildings have genotoxic activity in vitro. Wang and Yadav (2006) showed that toxins of *S. chartarum* extracted from spores cause DNA damage, *p53* accumulation and apoptosis in murine alveolar macrophages. Another report suggested that the spores of *S. californicus* produce genotoxically active compound(s) that induce DNA damage, and that the production of this compound is potentiated when the microbe grows together with *S. chartarum* (Penttinen et al., 2007). Microbial compounds may not only produce genotoxic compounds but may also increase the risk of cancer

through secondary mechanisms (e.g. by inducing oxidative stress in chronic inflammation) (Fitzpatrick, 2001).

The immunostimulatory properties of the fungal and bacterial strains typically found in moisture-damaged buildings are synergistically potentiated by microbial interactions during concomitant exposure in vitro (Huttunen et al., 2004). These interactions are not limited to microbes grown separately (Penttinen et al., 2005a) but also occur also when microbes are cultivated together (Penttinen et al., 2005b). Interactions between *S. californicus* and *S. chartarum* during co-cultivation stimulated the production of currently unidentified cytostatic compound(s) (Penttinen et al., 2006), which significantly potentiate the abilities of the spores to cause apoptotic cell death (Penttinen et al., 2005b). Interactions during co-cultivation stimulate these microbes to produce highly toxic compounds, which can damage DNA and provoke genotoxicity (Penttinen et al., 2007). In addition, concomitant exposure in vitro with amoebae potentiates the cytotoxic and inflammatory properties of the microbial spores of *S. californicus* or *Penicillium spinolosum* isolated from damp buildings (Yli-Pirila et al., 2007). These findings point to the importance of considering microbial interactions when investigating the causative agents and mechanisms of the adverse health effects observed in damp buildings.

References attached.