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SANNA LAPPALAINEN

Occupational exposure to fungi and methods to assess the exposure

Doctoral dissertation

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ABSTRACT

Workers may be exposed to fungi in several occupational settings, for example, in agricultural environments, the wood industry, compost plants, and often even in indoor environments without productive fungal sources (such as in offices and schools). As a result of fungal exposure, occupational diseases develop annually in hundreds of workers in Finland, and the number of symptomatic workers is even many magnitudes higher. In this thesis, different fungal agents were determined first under laboratory conditions and then in agriculture and water-damaged schools. The production of microbial volatile organic compounds (MVOCs) and its possible connection with mycotoxin production were investigated in the laboratory. Culturable and non-culturable fungi, MVOCs, and mycotoxins were determined in the air and materials of eight farms and a horse stable. In addition, 83 farmers' exposure to *Fusaria* and *Wallemia sebi* was studied with two immunochemical techniques. Culturable fungi in the air and water-damaged building materials, as well as MVOCs, were analysed in nine school buildings. In the laboratory experiments, the most frequent MVOCs were 3-octanone, 2-hexanone, 2-heptanone, and 1-octen-3-ol. Most of these and some other possible MVOCs were observed also in the air of the horse stable and schools at low concentrations ($<4.5 \mu\text{g}/\text{m}^3$). However, reliable detection of fungal contamination on the basis of the occurrence of a few MVOCs is unlikely under field conditions because of other possible sources in the environment and the fact that proportion of MVOCs of the total load of volatile organic compounds is low. In agricultural environments, the concentrations of airborne culturable and total fungi were highest ($10^6 \text{ cfu}/\text{m}^3$ or $10^7 \text{ spores}/\text{m}^3$) during the feeding of animals and the milling of grain. Most of the grain samples collected from the farms contained trichothecenes, especially deoxynivalenol (DON) up to 11 mg/kg. Low DON concentrations (nanograms/ m^3) were also detected with chemical analyses in the air during the milling of grain. It is recommended to measure airborne mycotoxins if materials contain mycotoxins or potential toxin-producing species, and workers have symptoms referring to mycotoxicosis or suspected occupational diseases. The serological studies showed that the farmers' were widely exposed to *W. sebi* and *Fusaria*. *W. sebi* and *Fusarium* species should be included in serological test panels when fungal exposure is evaluated among farmers. In the schools, culturable fungal concentrations were $\approx 780 \text{ cfu}/\text{m}^3$ (geometric mean $\approx 132 \text{ cfu}/\text{m}^3$), even in extensively mould-damaged rooms. Both fungal flora and counts were valuable in the assessment of workers' exposure to fungi, although information of fungal flora was more advisable in the identification of abnormal indoor fungal sources. In conclusion, none of the methods and fungal agents measured are adequate by themselves to assess fungal exposure thoroughly. In addition, these methods are difficult to apply to indoor environments and may lead to underestimation of fungal exposure. In the development of new methods for indoor environments, independence from culturability, long sampling times with good recovery of fungi, and sufficiently low detection limits are some essential properties for appropriate methods.

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ABBREVIATIONS

| | |
|--------|--|
| AcDON | acetyldeoxynivalenol |
| AIHA | American Industrial Hygiene Association |
| BRI | building-related illness |
| BRS | building related symptoms |
| CAMNEA | collection of airborne microorganisms on Nucleopore filters, estimation and analysis |
| CEN | European Committee for Standardization |
| cfu | colony-forming units |
| DG18 | dichloran glycerol-18 |
| DNA | deoxyribonucleic acid |
| DON | deoxynivalenol |
| ECD | electron-capture detection |
| EIA | enzyme immunoassay |
| ELIFA | enzyme-linked immunofiltration assay |
| ELISA | enzyme-linked immunosorbent assay |
| EPS | extracellular polysaccharides |
| FBS | foetal bovine serum |
| FID | flame ionization detector |
| FL | feline foetal lung |
| GC | high resolution gas chromatography |
| GC-MS | gas chromatography with a mass spectrometry detector |
| HPLC | high-performance liquid chromatograph |
| IARC | International Agency for Research on Cancer |
| Ig | immunoglobulin |
| ISO | International Organization for Standardization |
| LAL | Limulus ameocyte lysate |
| MEA | 2% malt extract agar |
| MS | mass spectrometer detector |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test |
| MVOC | microbial volatile organic compound |
| NIV | nivalenol |
| ODTS | organic dust toxic syndrome |
| PBS | phosphate-buffered saline |
| PCR | polymerase chain reaction |
| RAST | radioallergosorbent test |
| RCS | Reuter centrifugal air sampler |
| RH | relative humidity of the air |
| RIA | radioimmunoassay |
| SBS | sick building syndrome |
| SCAN | scanning mode in GC-MS |
| SEM | scanning electron microscopy |
| SFC | supercritical fluid chromatography |
| SIM | selected ion monitoring mode in GC-MS |
| TCT | thermal desorption cold trap |
| TLC | thin-layer chromatography |
| TGY | tryptone-glucose-yeast |
| TVOC | total volatile organic compounds |
| VOC | volatile organic compounds |
| WHO | World Health Organization |

LIST OF ORIGINAL PUBLICATIONS

This dissertation is mainly based on data presented in following five articles. In the text, these articles are referred to by their Roman numerals:

I

Pasanen A.-L., Lappalainen S., Pasanen P. 1996. Volatile organic metabolites associated with some toxic fungi and their mycotoxins. *Analyst*. Vol 121, p. 1949-53.

II

Lappalainen S., Pasanen A.-L., Pasanen P., Kalliokoski P. 1997. Production of fungal volatile organic compounds in bedding material. *Agricultural and Food Science in Finland*. Vol 6, p. 219-27.

III

Lappalainen S., Nikulin M., Berg S., Hintikka E.-L., Pasanen A.-L. 1996. *Fusarium* toxins and fungi associated with handling of grain on eight Finnish farms. *Atmospheric Environment*, Vol. 30, p. 3059-65.

IV

Lappalainen S., Pasanen A.-L., Reiman M., Kalliokoski P. 1998. Serum IgG antibodies against *Wallemia sebi* and *Fusarium* species in Finnish farmers. *Allergy, Asthma & Immunology*. Vol. 81, p. 585-92.

V

Lappalainen S., Kähkönen E., Loikkanen P., Palomäki E., Lindroos O., Reijula K. 2001. Evaluation of priorities for repairing in moisture-damaged school buildings in Finland. *Building and Environment*, Vol. 36, p. 981-6.

Additional unpublished data are also included in the thesis.

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I INTRODUCTION

Agriculture, forestry, the wood industry, and many other types of industries have productive sources of fungi. According to the statistics (Tilastokeskus, 2000), agricultural and forest work employed 144 000 workers in Finland in 1999. About 130 000 dairy farms or farms with grain production existed in Finland in that year (Maa- ja metsätalousministeriön tietopalvelukeskus, 1999). A smaller number of employees works in other environments with productive fungal sources (e.g., at compost plants, landfills and waste treatment plants). In such environments, workers' exposure to fungi may be high, depending on their worktasks, the quality of material handled, and the length of exposure (Hurst et al., 1997). Biotechnological processes and laboratories have also created new work environments with potential fungal exposure (Lacey & Crook, 1988; Crook, 1996).

The assessment of fungal exposure has primarily been based on cultivation methods for culturable fungi or on the microscopic counting of total (viable and non-viable) fungal spores (Blomquist et al., 1984b; Kotimaa et al., 1984; Dutkiewicz et al., 1994; Crook & Sherwood-Higham, 1997; Eduard & Heederik, 1998; Kiviranta et al., 1999). However, these methods are not necessarily adequate in evaluations of health effects caused by fungal exposure. The viability of fungi, for example, is not essential for non-infectious health effects (e.g., mucous membrane irritation, chronic bronchitis, asthma, and hay fever) (Eduard, 1996). Recently, more attention has been paid to determining specific fungal agents, like allergens, mycotoxins, glucans, and metabolic products, that may provide more information on the causality between fungal exposure and health effects (Crook & Sherwood-Higham, 1997; Flannigan, 1997; Rylander, 1999a; Pasanen, 2001). However, more data on the occurrence of these agents in the environment is needed before conclusions can be drawn about the dose-response relationships.

Research in indoor air environments without productive fungal sources has recently focused on water-damaged buildings, where fungal growth may appear. According to some Finnish studies, moisture problems occur in 82% of terrace houses (Partanen et al., 1995; Nevalainen et al., 1998), 60% of apartments in blocks of flats (Koivisto et al., 1996), 70% of day-care centres (Ruotsalainen et al., 1995) and over 50% of schools (Teijonsalo et al., 1991). It has been estimated that repair measures are urgently needed in 55% of water-damaged terrace houses (Nevalainen et al., 1998) and in 40% of blocks of flats (Koivisto et al., 1996). It is also obvious that moisture problems are common in offices even though estimates of such occurrences are not available.

In Finland, 399 000 workers were employed in the office and administration sector, and 125 000 workers functioned in the education sector in 1999 (Tilastokeskus, 2000). Haahtela and Reijula (1997) estimated that 20 000–30 000 persons have symptoms associated with microorganisms in water-damaged dwellings and workplaces in Finland (Haahtela & Reijula, 1997). According to another estimate (Tuomisto, 2000), the corresponding number is as high as 50 000 persons. As a result, mould has been one of the commonest agents causing occupational diseases among teachers in the past six years (Karjalainen et al., 1996;

Karjalainen et al., 1997, 1998; Karjalainen et al., 1999; Karjalainen et al., 2000; Karjalainen et al., 2001).

In the indoor environment, airborne fungal spore concentrations often remain rather low, even though serious and extensive mould damage has been detected in building constructions (Haverinen et al., 1999a; Haverinen et al., 1999b). The release of spores into the air is affected by the humidity of growth media and the air, as well as by air velocity (Pasanen, 1992b; Eduard, 1996; Flannigan, 1997), and therefore airborne fungal levels vary greatly indoors. This variation is difficult to monitor with cultivation and direct counting methods. In addition, traditional methods are time consuming, expensive, and laborious. Thus new methods to detect fungal contamination and assess fungal exposure in indoor environments are required (Flannigan, 1997; Pasanen, 2001).

In this doctoral thesis, workers' exposure to several fungal agents (fungal spores, antigens, mycotoxins, and fungal volatile metabolites) is evaluated for both work environments with productive fungal sources (agriculture) and indoor work environments (schools). Bacteria and bacterial products have been excluded for the most part. The thesis includes laboratory experiments on the production of fungal volatile organic compounds; otherwise it is based on field investigations. In addition, the sampling and analytical methods used for various fungal agents are discussed, and methods used to assess fungal exposure are evaluated.

II REVIEW OF LITERATURE

2.1 Description of fungal agents

Fungal spores. The growth of fungi is controlled by the nutrients, humidity, temperature, and pH of growth media (Gravesen, 1979). The most restrictive factor for fungal growth is the water activity or the equilibrium-relative humidity of the substrate (Grant et al., 1989). During the growth phase, fungi produce spores (typically size of 2–10 μm) or reproductive cells (yeasts) for survival and dispersal via sexual reproduction or asexual proliferation. Fungal spores are released into the air either passively by mechanical disturbance or actively by special spore discharge mechanisms. Active spore dispersal often occurs during periods of high relative humidity of the air (RH). So-called dry spores, produced during the asexual stage, are primarily released passively by air movements or by gravity. Airborne spores can be dispersed over great distances and can settle slowly (Gravesen, 1979; Burge, 1985; Pasanen et al., 1991).

Microbial volatile organic compounds. Most fungi and bacteria obtain energy through aerobic respiration, producing carbon dioxide and water. Microorganisms excrete enzymes that split compounds in substrates to more appropriate forms to be used as nutritional sources for fungi. During the metabolism, volatile residues, microbial volatile organic compounds (MVOCs), are produced. These metabolites include alcohols, ketones, aldehydes, esters, carboxylic acids, lactones, terpenes, sulphur, and nitrogen compounds, as well as aliphatic carbons and hydrocarbons (Batterman, 1995; Sunesson, 1995; Korpi et al., 1998; Pasanen et al., 1998). The most frequent MVOCs reported in the literature include 2-methylpropan-1-ol, 2-methylbutan-1-ol, 3-methylbutan-1-ol, 3-octanol, 1-octen-3-ol, 2-butanone, 2-hexanone, 2-heptanone, 3-octanone, 2-methylisoborneol, geosmin, limonene, sesquiterpenes, 2,5-dimethylfuran, 3-methylfuran, and dimethyl disulphide (Kaminski et al., 1974; Börjesson & Schnürer, 1990; Börjesson et al., 1993; Sunesson, 1995; Sunesson et al., 1996; Bjurman et al., 1997; Korpi et al., 1998).

Mycotoxins. In addition to the primary metabolites, fungi can also produce complex secondary metabolites, mycotoxins, which are toxic to mammalian and other organisms (Betina, 1989). More than 400 mycotoxins have been estimated to exist. Some of them, such as ergotamin, are derivatives of amino acids. Mycotoxins derived from other precursors are divided into aromatic- and phenolic-related toxins (e.g., aflatoxins, zearalenone) and terpenoid toxins (e.g., trichothecenes and fusidanes) (Hurst et al., 1997). Many fungal genera are capable of producing mycotoxins under certain conditions. For example, trichothecenes are formed by *Fusarium* and *Stachybotrys*, aflatoxins are produced by some *Aspergillus* species, and *Penicillium* and *Aspergillus* species can produce ochratoxins (Betina, 1989; WHO, 1990).

Components of fungal cell walls. Antigens are molecules that react with antibodies. **Allergens** are antigens that elicit hypersensitivity or allergic reactions (Janeway et al., 1997). Fungal spores and mycelia contain allergens of a varying potency (Flannigan, 1987). Many of them are enzymes (Horner et al., 1995). Some of them have commercial uses. For example,

alpha-amylase, which basically originates from *Aspergillus oryzae*, is used as an additive of flour. Phytase is a phosphatase derived from *Aspergillus niger* and is used as an animal feed additive. Lactase is a derivative of *A. oryzae* and is used for pharmaceutical purposes, whereas cellulase (mainly originating from *Trichoderma viridea* and *A. niger*) is used for digestive aids (among others) in the textile industry. Several other fungal enzymes are utilized in the production of beer, wine, food processing, and food fermentation (Gravesen et al., 1994). **1,3-β-D-Glucans** are polyglucose polymers found in fungal cell walls (Rylander, 1998). **Ergosterol** is a primary metabolite in the cell membranes of filamentous fungi. It is also present in the cell walls of yeast and mitochondria (Matile et al., 1969; Axelsson et al., 1995).

Extracellular polysaccharides (EPS) are stable nonbranched carbohydrate polymers that fungi release when they degrade substrates (Eduard & Heederik, 1998).

2.2 Exposure to fungi in work environments with productive fungal sources

2.2.1 Airborne fungal spores

Agriculture is one of the most studied work environments regarding microbial exposure. Farmers are exposed to fungi during tilling, harvesting, animal feeding, and the handling of bedding materials (Malmberg, 1990). Fungal spores primarily originate from grain, hay, and straw (Kotimaa et al., 1984; Clarke & Madelin, 1987; Eduard et al., 1990; Kotimaa, 1990b; Kotimaa, 1990a). The quality of feed and fodder and the phases of work (feeding of cattle, grain milling, etc.) have a significant impact on the exposure to bioaerosols (Mustonen et al., 1983; Kotimaa, 1990a; Hanhela et al., 1995). Handling hay, grain, and straw raises the airborne level of biocontaminants for several hours (Pasanen et al., 1989). The culturable spore **concentrations** generally vary from 10^1 to 10^{11} colony-forming units (cfu)/m³ in the air of farm buildings, as shown in Table 1.

Table 1. Levels of airborne mesophilic fungi measured in different work environments with productive fungal sources.

| Work environment | Airborne spore concentrations of culturable mesophilic fungi (cfu/m ³) | Reference |
|---|--|---|
| Dairy farms | 10 ¹ –10 ¹⁰ | (Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Dutkiewicz et al., 1994; Hanhela et al., 1995) |
| Modern cubicle cow houses | 10 ¹ –10 ³ | (Louhelainen et al., 1997) |
| Horse stables | 10 ² –10 ⁶ | (Heikkilä et al., 1990), (Dutkiewicz et al., 1994) |
| Harvesting of grain and work with grain elevators | 10 ⁴ –10 ⁵ | (Beard et al., 1996) |
| Piggeries | 10 ⁵ –10 ⁶ | (Dutkiewicz et al., 1994) |
| Sawmill/handling of wood chips | 10 ³ –10 ⁷ | (Blomquist et al., 1984b; Eduard et al., 1993; Eduard et al., 1994) (Kotimaa, 1990a; Duchaine et al., 1999) |
| Waste sorting facilities | 10 ⁴ –10 ⁶ | (Bünger et al., 1999; Reinthaler et al., 1999) |
| Sorting of recyclable paper | 10 ¹ –10 ⁵ | (Hyvönen & Linnainmaa, 1999; Würtz et al., 1999a) |
| Landfills | 10 ¹ –10 ⁵ | (Bünger et al., 1999; Kiviranta et al., 1999) |
| Potato storages | 10 ⁴ –10 ⁵ | (Blomquist et al., 1984b) |
| Fuel-chip storages | 10 ⁴ –10 ⁸ | (Blomquist et al., 1984b) |
| Compost facilities | 10 ⁶ –10 ⁸ | (Fischer et al., 1998; Bünger et al., 1999; Fischer et al., 1999a) |
| Bakeries | 10 ¹ –10 ³ | (Levy et al., 1999) |
| Tobacco factories | 10 ¹ –10 ³ | (Reiman & Uitti, 2000) |

The concentrations of thermotolerant fungi have ranged from 10¹ to 10⁵ cfu/m³ on dairy farms (Kotimaa et al., 1984; Kotimaa, 1990b; Hanhela et al., 1995; Louhelainen et al., 1997) and from 10¹ to 10⁶ cfu/m³ in the handling of grains (Mustonen et al., 1983; Beard et al., 1996). The concentrations of total spores have been determined mainly on dairy farms, in modern cubicle cow houses, and in horse stables, where they have ranged from 10³ to 10¹¹, 10¹ to 10⁵, and 10¹ to 10⁷ spores/m³, respectively (Lacey & Lacey, 1964; Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Heikkilä et al., 1990; Malmberg et al., 1993; Dutkiewicz et al., 1994; Hanhela et al., 1995; Louhelainen et al., 1997; Eduard et al., 2001; Melbostad & Eduard, 2001). Eduard et al. (1990) reported the mean concentrations of total spores (spores/m³) to be 10⁷ in sawmills, 10⁸ during wood chip/straw handling, and 10⁹ in grain elevators.

Significantly higher concentrations of culturable fungi have been found on the farms of asthmatics or farmer's lung patients than on farms with healthy farmers. The levels have ranged from 10^5 to 10^6 cfu/m³ on the farms with farmer's lung or asthmatic patients and from 10^2 to 10^5 cfu/m³ on the reference farms. The corresponding levels for thermotolerant fungi were 10^0 – 5×10^5 and 10^0 – $<2 \times 10^5$, respectively (Kotimaa et al., 1984; Hanhela, 1999). On the farms of patients with organic dust toxic syndrome (ODTS), the exposure level of airborne culturable fungal spores has typically been very high: from 10^6 to 10^8 cfu/m³ for mesophilic fungi or total spore concentrations of $>10^9$ spores/m³ (Kotimaa et al., 1984; Lacey & Crook, 1988; Malmberg & Rask-Andersen, 1993; Malmberg et al., 1993; Weber et al., 1993; Rask-Andersen, 1995; Vogelzang et al., 1999).

Insufficient ventilation can increase the RH in the production buildings of farms and lead to the condensation of water, which, in turn, can result in fungal growth in constructions and materials. Severe defects in the planning and construction of ventilation systems have been observed in Finnish farm buildings (Louhelainen, 1997; Hanhela, 1999).

Species of *Aspergillus* (e.g., *A. glaucus* and *A. fumigatus*), *Penicillium*, *Cladosporium*, *Rhizopus*, *Mucor*, *Paecilomyces*, *Alternaria*, *Absidia*, *Botrytis*, *Scopulariopsis*, and yeasts are the commonest airborne fungi found in farm buildings, grain elevators, and animal sheds (cowsheds, horse stables and piggeries), according to the studies listed in Table 1. On the other hand, *Fusaria* are, for example, frequent in grain and straw but rare in air (Hanhela et al., 1995). Grains are first contaminated with field fungi, like *Cladosporium*, *Alternaria*, *Fusarium*, and *Botrytis*, to which farmers are exposed during harvesting. Gradually field fungi are replaced with storage fungi, like *Aspergillus* and *Penicillium*, which can easily become airborne. If the water content in the grain exceeds 15% during storage, the prevalence of thermotolerant fungi (e.g., *Aspergillus*, *Absidia*, and *Rhizopus*) increases as the temperature rises. Species of *Alternaria*, *Cladosporium*, and *Helminthosporium* are the most abundant in grain of good quality (water content <15%). *Wallemia sebi* is the most abundant xerophilic fungi in hay and in the air of farm buildings (Hocking & Pitt, 1980; Lappalainen, 1993; Hanhela et al., 1995). The weather and storage conditions affect the quality of fodder and thus, consequently, both the quantity and quality of farmers' exposure to fungi (Mustonen et al., 1983; Lacey, 1987).

High levels of airborne mesophilic fungi (up to 10^8 cfu/m³) are also found in **compost plants, fuel chip plants, sawmills, waste sorting facilities, landfills, the tobacco industry, the food industry, and the handling of paper**, as cited in Table 1. Concentrations as high as 10^6 cfu/m³ have been detected for thermotolerant species (e.g., *A. fumigatus*, *Emericella nidulans*, and *Paecilomyces variotii*) in compost plants, in waste treatment facilities, and during the handling of wood chips (Kotimaa, 1990a; Fischer et al., 1998; Reinthaler et al., 1999), while the levels have been much lower (10^1 – 10^3 cfu/m³) in tobacco factories (Reiman & Uitti, 2000). The most dominant fungi are *A. fumigatus* in compost plants (concentrations up to 10^8 cfu/m³), *Aspergillus* species in tobacco factories, *Aspergillus*, *Penicillium* and *Cladosporium* species in bakeries, *Aspergillus* (*A. fumigatus*, *A. niger*), *Penicillium*, *Cladosporium*, *Acremonium* and *Fusarium* in landfills, and *Rhizopus microsporus* and *Aspergillus* species in sawmills. (See the references given in Table 1.) The quality and age of the material (e.g., paper, wood) handled influence the airborne spore levels and thus workers' exposure (Kotimaa, 1990a; Würtz & Breum, 1997). In these settings, background levels are usually

low, but they can increase several orders of magnitude during certain tasks (Bünger et al., 1999; Duchaine et al., 1999; Kiviranta et al., 1999).

2.2.2 Mycotoxins

Humans can be exposed to mycotoxins by foodstuffs contaminated heavily with toxigenic fungi (Wyllie & Morehouse, 1978; WHO, 1990). Ingestion is the commonest route of mycotoxin exposure, but, because many trichothecenes and aflatoxins are fat soluble, they can also penetrate the skin (Kemppainen et al., 1988; Schiefer, 1990). Mycotoxins are not volatile compounds under normal conditions, but, as mycotoxins, they are concentrated on spores (Wicklów & Shotwell, 1982; Miller, 1990; Pasanen et al., 1993a; Pasanen et al., 1993b). Workers can also be exposed to toxins through inhalation.

Fungi may produce several mycotoxins; on the other hand, the same mycotoxin can be produced by different fungal species. Furthermore, not all strains of the toxigenic fungal species necessarily produce toxins. The growth conditions, like nutrient, humidity and pH, affect mycotoxin production (Betina, 1989). Sometimes, a rapid change in growth conditions (e.g., a drop in temperature) can stimulate toxin production (Austwick, 1984; Hintikka, 1984). Fungi produce mycotoxins mostly in their spores, although some mycotoxins have also been detected in hyphae filaments (Betina, 1989; Jarvis, 1990).

Mycotoxins have frequently been found in **cereals**, **animal feed**, and **human foodstuffs** throughout the world. Levels of mycotoxins are generally lower in human foodstuffs than in raw agricultural fodder (WHO, 1990; Rizzo, 1993). The commonest mycotoxins detected in grains are listed in Table 2. Trichothecenes, especially deoxynivalenol (DON), are the most frequent toxins found in Finnish grain (Rizzo, 1993). The concentrations of trichothecenes are generally below 1 mg/kg in grain samples and human foodstuffs. However, some very high levels of mycotoxins, up to dozens of milligrammes per kilogramme, have sometimes been detected in mouldy animal feeds and foodstuffs (e.g., peanuts) (WHO, 1990).

Table 2. Type and concentration of the mycotoxins most frequently found in grain.

| Material | Mycotoxin | Concentration, µg/kg | Reference |
|---------------------------------------|--|--|---|
| Grain (mostly oats) | DON, 3-AcDON, T-2-toxin, NIV | 1–6 300 2–210 5–240 1–1000 | (Rizzo, 1993) |
| Oats | T-2-toxin, DON | 10–50 3–20 000 | (WHO, 1990) |
| Maize | Fumonisin B ₁ , Aflatoxin B ₁ , Aflatoxin B ₁ or B ₂ , DON, NIV, Zearalenone, T-2-toxin, Ochratoxin A | 110–12 000 190–4900 13–310 10–20 000 4–2700 10–170 10–5000 5–5100 | (Burg et al., 1981, 1982; Chu & LI, 1994) (Luo et al., 1990; WHO, 1990; Chu & LI, 1994; Kedera et al., 1999) |
| Barley | DON, 3-AcDON, NIV, Zearalenone, Ochratoxin A | 4–40 000 10–170 10–38 000 40–1400 9–3800 | (WHO, 1990; Kim et al., 1993) |
| Wheat | Ochratoxin A, DON, NIV, Zearalenone, T-2-toxin | 5–140 0.00003–8500 0.00004–7800 <0.025 2 000–4000 | (Scott et al., 1984; WHO, 1990; Sugiura et al., 1993) |
| Animal fodder (contained 8% maize) | Sterigmatocystin | 4000 | (Pohjanvirta et al., 1984) |

DON = deoxynivalenol, 3-AcDON = 3-acetyl-deoxynivalenol and NIV = nivalenol

Mycotoxins detected in the **air** or settled **dust** of agricultural environments are presented in Table 3, which indicates that aflatoxins are generally the most often analysed. The airborne concentrations have usually been on the levels of nanogrammes per cubic metre, although somewhat higher levels have been measured in the air of animal buildings. Ghosh et al. (1997) observed that the levels of airborne aflatoxin were higher in particle fractions of < 7 µm when compared with those in total dust samples.

Table 3. Mycotoxins detected in the air or settled dust of agricultural environments.

| Sample | Worktask | Mycotoxin | Concentration | Reference |
|--------------|---|--------------------------|-----------------------------|---------------------------|
| Air | Harvest and grain unloading | Aflatoxin B ₁ | 0.04–90 ng/m ³ | (Selim et al., 1998) |
| Air | Feeding animals in farm buildings | Aflatoxin B ₁ | 5–420 ng/m ³ | (Selim et al., 1998) |
| Air | Bin cleaning in farm buildings | Aflatoxin B ₁ | 120–4 800 ng/m ³ | (Selim et al., 1998) |
| Air | Different tasks in a rice processing plant | Total aflatoxins | 0–0.025 ng/m ³ | (Ghosh et al., 1997) |
| Air | Different tasks in a maize processing plant | Total aflatoxins | 0.008–2.4 ng/m ³ | (Ghosh et al., 1997) |
| Air | Transport of mouldy maize into and out of a storage bin | Total aflatoxins | 5–107 ng/m ³ | (Burg et al., 1981, 1982) |
| Air | Handling of copra, cottonseed and maize gluten in feed production factories | Total aflatoxins | 0–1.1 ng/m ³ | (Kussak, 1995) |
| Settled dust | Handling of mouldy maize | Total aflatoxin | Max 540 ppb | (Burg et al., 1981, 1982) |
| Settled dust | Milling of maize | Aflatoxin B ₁ | 5–6 ppb | (Silas et al., 1987) |
| Settled dust | Different tasks in swine buildings | Aflatoxin B ₁ | 23–5100 ng/g | (Selim et al., 1998) |
| Settled dust | Work with elevators | Zearalenone | 25-100 ng/g | (Palmgren et al., 1983) |

Few concentrations of mycotoxins have been published for measurements directly from the air of any other work environments with productive fungal sources than agriculture. Some data have, however, been reported for **sawmills** and **compost plants**. Land et al. (1987) found strains of *A. fumigatus* isolated from sawmills to produce verruculogen and fumitremorgen C. The toxins caused tremorgenic reactions in animal experiments. Tryptoquivaline, another compound with tremorgenic properties, and trypacidin, whose toxic properties have not yet been described, both produced by *A. fumigatus*, have recently been found in extractions of dust samples collected from a compost facility (Fischer et al., 1999a).

2.2.3 Microbial volatile organic compounds

Several research groups have concluded that some alcohols (e.g., 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-methyl-1-butanol, 3-methyl-2-butanol, 2-ethyl-1-hexanol, 3-octanol, 1-octen-3-ol), ketones (e.g., 2-butanone, 2-hexanone, 2-heptanone, 3-octanone), and terpenes (e.g., 2-methylisoborneol, geosmin, pinenes, limonene, sesquiterpenes) can be regarded as MVOCs (Kaminski et al., 1974; Börjesson & Schnürer, 1990; Börjesson et al., 1993; Sunesson, 1995; Sunesson et al., 1996; Bjurman et al., 1997; Korpi et al., 1998). The presence of MVOCs in work environments with productive fungal sources has been published only in a few studies. Fischer et al. (1998, 1999a) found MVOCs, like 3-methyl-1-butanol, 2-methyl-1-butanol, 3-octane and many terpenes (e.g., limonene and camphene), in the air of compost facilities, although they did not report the concentrations of these compounds. In addition, fungal species isolated from the air of compost plants were detected to produce 3-methyl-1-butanol, 2-methyl-1-butanol, 3-octene, 1-octen-3-ol, limonene, styrene, and dimethyldisulphide in the laboratory (Fischer et al., 1998; Fischer et al., 1999b).

2.2.4 Fungal allergens, 1,3-β-D-glucan, ergosterol and extracellular polysaccharides

Only a few studies on airborne fungal **allergens** have been conducted in work environments. In one study, half of the workers in a biotechnology plant had occupational bronchospasm and reacted positively to *A. niger* in the skin prick test. Airborne *A. niger* antigen levels, measured by inhibition in the radioallergosorbent test (RAST), ranged from 90 000 to 710 000 ng/m³ in some areas of the plant (Topping et al., 1985). In another study, airborne allergen levels of *A. fumigatus* (determined by RAST inhibition) varied between 1000 and 72 000 ng/m³ before, during, and after bedding chopper operations in dairy farming (Pratt et al., 1990).

Exposure to fungal enzymes that are also allergenic compounds can occur in a variety of industries, for example, in bakeries, pharmaceutical and textile facilities (Houba et al., 1997; Bernstein et al., 1999; Kim et al., 1999). Alpha-amylase levels of < 620 ng/m³ (mostly < 40 ng/m³) have been determined in different types of bakeries during various work phases (Houba et al., 1997; Lillienberg et al., 2000; Elms et al., 2001). Another enzyme, also a derivative of *A. oryzae*, is a lactase used as a dietary aid for patients with lactose intolerance. The airborne levels of lactase have ranged from 30 000 to 300 000 ng/m³ in the processing rooms of a pharmaceutical facility. After the facility was redesigned, the airborne lactase levels decreased to 100 ng/m³ in areas where protective garments or respirators were not used and to 10 000 ng/m³ in areas where protective garments were required. (Bernstein et al., 1999)

The levels of airborne **1,3-β-D-glucan** in cotton cardrooms, paper mills, sawmills, and compost plants and on farms have ranged from 0.2 to 76 000 ng/m³ (Rylander et al., 1989; Rylander et al., 1999; Douwes et al., 2000a; Mandryk et al., 2000; Eduard et al., 2001). In addition, the geometric mean level of 1,3-β-D-glucan has been reported to be 2.3 ng/m³ in a woodchipping mill, 1.4 ng/m³ in sawmills, 2.0 ng/m³ on logging sites, and 0.4 ng/m³ in joineries (Alwis et al., 1999). Thorn et al. (1998, 2001) measured 1,3-D-glucan concentrations

up to 45 ng/m³ in the ambient air of waste collector workers, the highest concentrations being recorded during the warm season.

The levels of other components of fungal cell walls have been rarely measured. **Ergosterol** concentrations of 200-300 ng/m³ were reported in the air of a pig barn (Axelsson et al., 1995). Eduard et al. (2001) measured levels of <1–1000 µg units/m³ for **EPS** of *Aspergillus* and *Penicillium* in farm environments.

2.3 Exposure to airborne fungi in water-damaged buildings

2.3.1 Airborne fungal spores

Levels of airborne culturable fungal spores found in some recent studies on indoor environments without natural fungal sources are summarized in Table 4. In general, fungal spore concentrations often remain low (10²–10³ cfu/m³) in spite of serious and extensive fungal damage to building constructions. Species of *Penicillium*, *Cladosporium*, *Aspergillus*, and yeasts are the most frequent fungi found indoors in both mouldy and reference buildings (Table 4). Certain fungi, such as species of *Stachybotrys*, *Fusarium*, and *Acremonium*, that require high moisture conditions for growth (Samson & van Reenen-Hoekstra, 1998) are often detected only in mouldy buildings.

Table 4. Levels of airborne mesophilic fungi in some indoor work environments.

| Country | Indoor environment | Airborne culturable fungal concentration (cfu/m ³) | | Season | References |
|-------------------|------------------------|--|---------------------------------|---|---|
| | | Mouldy buildings | Reference buildings | | |
| United States | Office (n=91) | a) 48–116 b) 7–3490 c) 26–8000 | Not studied | a) Midsummer b) Not mentioned c) Winter | a. (Johanning et al., 1996), b. (Sudakin, 1998; Womble et al., 1999; Fung et al., 2000) c. (Hodgson et al., 1998) |
| Finland | School (n=22) | 5–780 | Below the detection limit – 204 | Winter | (Meklin et al., 1996) (Taskinen et al., 1997; Lappalainen et al., 1999; Taskinen et al., 1999; Haverinen et al., 1999b) |
| Norway/ Sweden | School (n=46) | a. Not studied b. 70–4500** | a. < 40 * | a. Winter b. Spring and summer | a. (Dotterud et al., 1996), b. (Smedje et al., 1996; Norbäck & Smedje, 1997) |
| United States | School (n=53) | Not studied | 16–6500 * | Winter | (Levetin et al., 1995; Bartlett et al., 1999; Scheff et al., 2000) |
| Finland | Office (n=159) | a. 2–360 | b. Mean 170 * | a. Winter b. Different seasons | a. (Lappalainen et al., 1999) b. (Kähkönen & Sunden, 1995) |
| Finland | Hospital (n=1) | 7–68 | 4–71 | Autumn and fall | (Haverinen et al., 1999a) |
| Finland | Day care centre (n=10) | 120–430 | 29–64 | Winter | (Koskinen et al., 1995) |
| | | 160–510 | 180–570 | Fall | |

* = absence of fungal damage not verified, ** = study included both mouldy and reference buildings

During the demolition and repair of moisture and fungal damage in buildings, the concentrations of airborne fungi can temporally increase even 100-fold and result in a risk of heavy fungal exposure for occupants and repair workers. Culturable fungal concentrations of 10^3 – 10^5 cfu/m³ and total spores concentrations of 10^5 – 10^6 spores/m³ have been measured during demolition work in Finnish studies (Rautiala et al., 1996; Rautiala et al., 1998).

Even though the ventilation system removes microorganisms (including fungi) from the indoor air (Parat et al., 1997; Burge et al., 2000), it can also act as a fungal source. Fungi are gradually accumulated onto the dust in supply air ducts: spore concentrations up to 10^7 cfu/g of dust have been reported (Pasanen et al., 1997). Water condensation can maintain fungal

growth in air ducts if the ducts are placed in unheated spaces of buildings (Pasanen et al., 1993c). Fungal growth has been detected also in supply air filters (Parat et al., 1996; Simmons et al., 1997). Fung et al. (2000) reported that remediation of an air conditioning unit contaminated by moulds decreased airborne fungal concentrations from 250 to 140 cfu/m³. Special attention should be paid to the control of fungal exposure in hospitals. Infections caused by fungi are dangerous, especially to immunosuppressed patients. Nosocomial aspergillosis (caused by *Aspergillus*) is usually of external origin and often related to fungal contamination in building construction, air conditioning systems, or humidifier water (Anderson et al., 1996; Fridkin et al., 1996; Simmons et al., 1997; Willinger & Aspöck, 1998). Poor planning of waste handling has also caused contamination of *A. fumigatus* in hospitals (Anderson et al., 1996).

In agricultural environments, exposure to higher fungal levels in homes, compared with levels found in urban buildings, is obvious because fungi are carried from farm buildings to dwellings (Pasanen et al., 1989). Concentrations of 30–1900 cfu/m³ of airborne mesophilic fungi have been found in new rural houses, and levels of 100–5700 cfu/m³ have been measured in old rural houses (Pasanen, 1992a). In another study, the concentrations of mesophilic fungi ranged from 11 to 3100 cfu/m³ in rural houses occupied by farmers with asthma and from 18 to 6300 cfu/m³ in houses of healthy farmers (Hanhela, 1999). Airborne thermotolerant fungi were also detected in low concentrations in the both types of houses in the study.

2.3.2 Mycotoxins

In recent years, increasing interest has been directed towards the occurrence of mycotoxins in water-damaged buildings. In several studies, mycotoxins have been found in water-damaged building materials and settled dust, as presented in Table 5. In these cases, moisture damage or water leaks had typically existed in the buildings for several years. The contamination was primarily due to the growth of *Stachybotrys chartarum* in wet cellulose-based building materials. Laboratory studies have shown that *S. chartarum* grows well and preferably produces toxins in materials with a high cellulose content (Pasanen et al., 1994; Nikulin, 1999). Other fungi, especially *Aspergillus* species, *Fusarium* species, *Penicillium* species, and *Trichoderma* species have also been suggested to produce mycotoxins in building materials (Tuomi et al., 2000). Despite mycotoxins having been determined in contaminated materials and settled dust, it has not been possible so far to measure airborne mycotoxins in indoor environments.

Table 5. Occurrence of mycotoxins in the indoor environment.

| Indoor environment | Contaminated material | Mycotoxin | Concentration | References |
|---------------------------|--|--|------------------------------|---------------------------------------|
| Dwelling | Extract from the filter used in an electrostatic precipitator | Trichothecenes | Not determined | (Croft et al., 1986) |
| Dwelling | Ceiling fibre board | Verrucarol, satratoxin H, trichoverrins A and B, verrucarins B and J | Not reported | (Croft et al., 1986) |
| Dwelling | Fibreglass, wall paper, gypsum board | Roridin A | 0–2500 ng/g | (Dietrich, 1999; Gareis et al., 1999) |
| Dwelling | Dust from heating ducts | Ochratoxin A | 58–1500 ppb | (Richard et al., 1999) |
| Office | Air handling insulation and paper products | Satratoxin H | Not reported | (Johanning et al., 1993) |
| Office | Ceiling tiles, vinyl wall | Satratoxins G and H, sterigmatocystins, DON | Satratoxins G and H: 2-5 ppm | (Hodgson et al., 1998) |
| Office | Dust from air ducts, drapes and ceiling fibre boards | T-2-toxin, DAS, roridine A, T-2 tetraol | Not reported | (Smoragiewicz et al., 1993) |
| Office | Wallpaper | Satratoxin H | 17 µg/g | (Johanning et al., 1996) |
| Office | Wallpaper, cardboard, wood, plywood, paper-covered gypsum board, mineral wool, paint | Sterigmatocystin, several trichothecenes (e.g., satratoxin G,H, DON, T-2-tetraol), citrine | 0.2 ng/g–11 µg/g | (Tuomi et al., 2000) |
| School or day care centre | Building materials | Macrocyclic trichothecenes (verrucarol type), Trichodermol type | 2–15 ng/cm ² | (Gravesen et al., 1999) |
| Day care centre | Gypsum board | Satratoxin G,H | 17 µg/g | (Andersson et al., 1997) |
| Day care centre | Dust from inside wall and floor | DAS, T-2-toxin, roridin A | 0.02–6 µg/g | (Tuomi et al., 1999) |

DON = deoxynivalenol, DAS = diacetoxyscirpenol

2.3.3 Microbial volatile organic compounds

In studies regarding MVOCs in indoor environments, 10–15 indicator compounds have commonly been analysed in damp buildings. Ström et al. (1994) reported the average concentration of 13 selective MVOCs to be $29 \mu\text{g}/\text{m}^3$ in homes, offices, schools, and day care centres ($n=30$) with water damage or dampness. The corresponding value for the reference buildings was $4 \mu\text{g}/\text{m}^3$ ($n=33$). The sum of various MVOCs has generally remained below $100 \mu\text{g}/\text{m}^3$ in water-damaged schools (Smedje et al., 1996; Morey et al., 1997), but one very high level, $1800 \mu\text{g}/\text{m}^3$, was found in the literature (Morey et al., 1997).

Wilkins et al. (1997) detected 1-butanol, alpha pinene, 3-carene, and limone in the air of mouldy schools and offices. The authors suspected the compounds to be of microbial origin. In the same study, 2-hexanone, 2-heptanone, 2-methylpropanol, 2-methyl-propenal, 3-buten-2-one, 2-methylbutanol, and 5-hexen-2-one were observed in settled dust. MVOCs were also investigated in 50 Canadian homes, most of which were associated with health problems (Miller et al., 1988). Several compounds (e.g., heptane, hexanone, 3-methyl-1-butanol, nonane, 2-butoxyethanol and decanol) were detected in the indoor air of the dwellings.

Many MVOCs are odourous. The odour threshold varies largely depending on the compound, being as low as $0.15\text{--}0.2 \mu\text{g}/\text{m}^3$ for geosmin and rather high for some compounds (e.g., $31\text{--}200 \mu\text{g}/\text{m}^3$ for 3-octanone) (Ruth, 1986; Ström et al., 1994). Higher concentrations of MVOCs were measured in Swedish houses with odour problems than in houses without any complaints, although the authors concluded that the degree of the odour problem was not directly related to the sum of MVOCs in the indoor air (Ström et al., 1994).

2.3.4 Fungal allergens, 1,3-β-D-glucan, ergosterol and extracellular polysaccharides

Studies on fungal **allergens** in the indoor environment are rare. Flückiger et al. (1999) measured airborne *Cladosporium* and *Alternaria* allergen levels of $7\text{--}224 \text{BU}/\text{m}^3$ in homes of allergic patients.

The concentrations of airborne **1,3-β-D-glucan** have been determined in some studies. The levels were observed to be higher in buildings with mould problems and complaints of symptoms ($<110 \text{ng}/\text{m}^3$) than in reference buildings ($<7 \text{ng}/\text{m}^3$) (Rylander et al., 1992; Rylander, 1997b, 1997a, 1998; Rylander et al., 1998; Thorn & Rylander, 1998; Rylander, 1999b). In addition, a 1,3-β-D-glucan concentration of $440\text{--}1000 \mu\text{g}/\text{g}$ has been detected in the settled dust collected from dwellings (Douwes et al., 2000b; Wouters et al., 2000).

Miller and Young (1997) reported **ergosterol** concentrations of $0.01\text{--}194 \text{ng}/\text{m}^3$ in the indoor air of dwellings, and Axelsson et al. (1995) and Saraf et al. (1997) observed $2\text{--}45 \mu\text{g}$ ergosterol/g in house dust. Dales et al. (1997) observed ergosterol concentrations of about $0.001 \text{ng}/\text{m}^3$ in dwellings without water damage and only slightly higher concentrations ($0.002\text{--}0.003 \text{ng}/\text{m}^3$) in dwellings with water or mould damage. Ergosterol concentrations of $0.02\text{--}70 \text{ng}/\text{g}$ have been measured in samples from building materials, being higher in contaminated building materials (Pasanen et al., 1999; Szponar & Larsson, 2000) and

correlating with culturable fungi in house dust (Saraf et al., 1997). The concentrations of **EPS-Aspergillus/Penicillium** in house dust have varied between 40 and 46 000 ng EPS units/g, depending on, for example, the presence of water-damage or the storage time of organic waste in buildings (Douwes et al., 1999; Wouters et al., 2000; Chew et al., 2001).

2.4 Health effects caused by various fungal agents

Fungal exposure has been linked to the development of **occupational diseases** both for work environments with productive fungal sources (e.g., agriculture) and indoor work environments (e.g., offices, schools). In Finland, the causality between the occupational disease and fungal exposure must be proved according to Finnish regulations ("Statute on Occupational Disease," 1988; "Act on Occupational Disease," 1988). The diagnostic criteria for occupational diseases are based on descriptions published elsewhere (Cartier et al., 1989; Rask-Andersen, 1989; Malmberg et al., 1993; O'Donnell, 1995; Reijula, 1998). For example, the main criteria for mould-induced occupational asthma include descriptive symptoms (obstruction or wheezing) at work, positive responses in a specific inhalation challenge test, or reduced peak flow values during a workshift and a history of fungal exposure following additional criteria for levels of specific immunoglobulin (Ig) E antibodies and positive reactions in skin tests (Cartier et al., 1989; Reijula, 1998, 1999). The new cases of occupational diseases caused by fungal exposure in three professional groups during the past five years in Finland are listed in Table 6. The most prevalent diseases include allergic rhinitis, allergic asthma, allergic alveolitis, and ODTS. The prevalence of these diseases has varied year by year, but the number of new cases of ODTS has always been lowest. Fungi have been one of the commonest causative agents of occupational diseases among farmers for a long time. Among teachers and office workers, occupational diseases caused by fungi are new phenomena. (Karjalainen et al., 1996; Karjalainen et al., 1997, 1998; Karjalainen et al., 1999; Karjalainen et al., 2000)

Table 6. New cases of occupational diseases caused by fungal exposure among Finnish farmers, teachers, and office workers in public office buildings during 1995–2000. (Karjalainen et al., 1996; Karjalainen et al., 1997, 1998; Karjalainen et al., 1999; Karjalainen et al., 2000; Karjalainen et al., 2001)

| Year | Incidence of occupational diseases caused by fungal exposure | | |
|-------------|---|-----------------|-----------------------|
| | Farmers | Teachers | Office workers |
| | Number of cases | Number of cases | Number of cases |
| 1995 | 66 | 19 | 25 |
| 1996 | 65 | 29 | 42 |
| 1997 | 68 | 66 | 31 |
| 1998 | 53 | 49 | 12 |
| 1999 | 130 | 30 | 17 |
| 2000 | 42 | 32 | 21 |

Workers are always exposed to a mixture of several physical, chemical, and biological agents in the work environment (Ostrowski, 1999). Biological agents include fungi, bacteria, viruses,

and parasites. In addition, mites, pollen, fragments of plant materials, and human and pet dander are examples of bioaerosols that are often present in the work environment. For the most part, data on the co-effects of these agents are lacking. Even causative agents of fungi are not well understood (Ostrowski, 1999; Rylander, 1999a). Exposure to fungi and their agents can cause infectious, allergenic or toxic effects (Husman, 1996; Crook & Sherwood-Higham, 1997; Reijula, 1999). Non viable spores can also contain agents (e.g., allergenic and toxic components that are possibly related to health outcomes). Therefore, the determination of solely viable fungi does not provide a good estimate for fungal exposure or its related risks (Eduard, 1996; Rylander, 1999a). Health outcomes caused by exposure to microorganisms have been discussed in several, previous Finnish doctoral theses (Reijula, 1985; Koskinen, 1999; Taskinen, 2001). Thus health effects that may be associated with certain fungal agents are presented according to fungal agents in the next eight sections, even if the causality is not yet clear.

Fungal spores. Allergic alveolitis has earlier been considered to develop only as a result of long-term or repeated exposure to high concentrations (10^6 – 10^9 cfu/m³) of spores with a diameter of 1–5 µm. ODTS is defined as a febrile illness associated with chest symptoms which occur in non-sensitized persons 4–8 hours after exposure to organic dust. Differences between the diagnostic criteria of ODTS and allergic alveolitis can be found elsewhere (doPico, 1986; Terho, 1986). ODTS reactions and allergic alveolitis have frequently been reported among farmers (Kotimaa et al., 1984; Lacey & Crook, 1988; Malmberg & Rask-Andersen, 1993; Malmberg et al., 1993; Rask-Andersen, 1995). Exposure levels of airborne culturable fungal spores have typically ranged from 10^6 to 10^8 cfu/m³ or to total spore concentrations of over 10^9 spores/m³ in cases of ODTS in agricultural environments. Lower exposure levels of fungal spores (10^4 – 10^6 spores/m³) may also cause less severe health disorders, such as respiratory and eye irritation (Melbostad & Eduard, 2001). However, recently, both the diseases have also been diagnosed among workers in mouldy buildings, where exposure levels have been much lower than those reported in agricultural environments. (See Table 6.)

A higher prevalence of respiratory infections and cough has been reported for persons in damp buildings with fungal contamination than for those in reference buildings (Andrae et al., 1988; Brunekreef et al., 1989; Koskinen et al., 1995). In their review, Bornehag et al. (2001) found that "dampness" in buildings appears to increase the risk (odd ratio 1.4–2.2) for health effects in the airways (e.g., cough, wheeze and asthma). According to Finnish studies, IgE-mediated allergic rhinitis, asthma, conjunctivitis, or urticaria have been diagnosed for less than 10% of workers who have suffered symptoms in mouldy buildings, and only 1–2% of symptomatic workers have developed hypersensitivity pneumonitis. Nevertheless, over 40% of the exposed persons in these buildings have complained of respiratory or conjunctival symptoms (Reijula, 1996, 1998). Workers' exposure assessment to fungi in the aforementioned studies was based only on the measurements of culturable fungal spores.

Fungal allergens. Over 60 fungal species are known to contain allergens that cause allergic rhinitis, asthma, or allergic conjunctivitis. Exposure to *Alternaria*, *Cladosporium*, *Aspergillus*, *Botrytis*, *Stachybotrys*, *Fusarium*, and *Penicillium* species are frequently associated with the development of allergic diseases (Husman, 1996; Pieckova & Jesenska, 1999; Kurup et al., 2000). Menzies et al. (1998) reported a relationship between respiratory symptoms among office workers and airborne *Alternaria* allergen. The workers with positive skin reactions to

Alternaria were exposed to airborne *Alternaria* spores at their worksites in offices. The estimates for the prevalence of allergy or sensitization to moulds in different populations vary from 3% to 50% (Husman, 1996; Pieckova & Jesenska, 1999; Kurup et al., 2000). On the other hand, recent studies have referred to a protective effect of a childhood farm environment against respiratory allergens (Kilpeläinen et al., 2000; Riedler et al., 2000; Von Ehrenstein et al., 2000). The development of allergic eczema due to airborne mould exposure has been described (Kanny et al., 1996). Several fungi (e.g., several species of *Trichophyton*, *A. fumigatus*, and many yeasts) are dermatophytes that may cause skin infections, especially in persons with compromised immune systems (Gravesen et al., 1994).

Enzymes. The main clinical effects of exposure to fungal enzymes are IgE-mediated responses: respiratory sensitization and asthma (Schweigert et al., 2000). Mild and reversible irritation effects are also linked to prolonged enzyme exposure, depending on enzyme type. For example, proteases are irritating, whereas cellulase, amylase, and lipases are not. Fungal alpha-amylase is an important occupational allergen in bakeries. A positive association between alpha-amylase exposure and work-related respiratory symptoms has been observed (Houba et al., 1996; Houba et al., 1998). Occupational allergic contact urticaria caused by fungal alpha-amylase has also been reported (Kanerva et al., 1997). Occupational IgE sensitization has been observed among workers exposed to phytase and lactase. Furthermore, occupational asthma and IgE sensitization to cellulase in a textile worker have recently been described (Bernstein et al., 1999; Doekes et al., 1999; Kim et al., 1999).

Mycotoxins. According to laboratory experiments, some mycotoxins are carcinogenic, mutagenic, or teratogenic (Wyllie & Morehouse, 1978; WHO, 1990). Liver is a target organ for aflatoxins, while the kidneys are targeted by ochratoxins (Austwick, 1984). The International Agency for Research on Cancer (IARC) has classified aflatoxins B₁, B₂, G₁, and G₂ to carcinogen class 1 (the agent is carcinogenic to humans) and fumonisin B₁ and B₂, fusarin C, and ochratoxin A to class 2B (the agent is possibly carcinogenic to humans) (IARC, 1993). Retrospective studies of Danish feed-processing workers (handling of contaminated maize and peanuts) reported elevated risks of liver and biliary tract cancers in the worker population with 2- to 3-fold risk after a 10-year latency (Olsen et al., 1988; Autrup et al., 1991; Autrup et al., 1993). The exposure to aflatoxin (about 170 mg/day) was concluded to be the most likely explanation for the cancer cases.

Stomachache, diarrhoea, headache, dizziness, and serious health outcomes, such as alimentary toxic aleukia (ATA disease), have been described as a result of eating grains contaminated with *Fusaria* in Russia and China. Trichothecenes may cause haemorrhagic lesions, and severe dermatitis has been reported for a laboratory worker because of an accident with T-2-toxin (Wyllie & Morehouse, 1978; Austwick, 1984; WHO, 1990). Exposure to airborne mycotoxins is also suspected to provoke milder health effects. Many toxins, especially those of *Penicillium* and *A. fumigatus*, have tremorgenic effects (Austwick, 1984; Land et al., 1987). Toxic pulmonary reactions, conjunctivitis, inflammation in mucous membranes of the respiratory system, skin irritation, bleeding from the nose, and leucopenia were reported among farmers who had handled mouldy hay, maize, or straw (Hendry & Cole, 1993; Pieckova & Jesenska, 1999).

Suspensions of mycotoxicosis in indoor environments have been mainly linked to the contamination buildings by *S. chartarum* (Fung et al., 1998). In case studies, flu-like

symptoms, sore throat, diarrhoea, headache, fatigue, dermatitis, generalized malaise, irritation of the skin and respiratory system, and disorders of the central nervous system have been described among occupants in contaminated buildings, in which the presence of trichothecenes (satratoxins G and H) was also verified (Croft et al., 1986; Johanning et al., 1996; Hodgson et al., 1998; Johanning et al., 1999). Infants may be especially vulnerable to fungal toxins: an outbreak of acute pulmonary haemorrhage occurring in infants in the United States has been suspected to be linked to houses contaminated with *S. chartarum* (Etzel et al., 1998; Dearborn et al., 1999)

1,3-β-D-Glucans. 1,3-β-D-glucans may cause several biological effects, primarily through the activation of macrophages (Lacey & Dutkiewicz, 1994). Headache, skin symptoms, and nose and throat irritation have appeared in subjects exposed to aerosolized pure 1,3-β-D-glucan in experimental situations (Rylander, 1998). Similar health effects have also been observed among workers in indoor environments (Rylander, 1998, 1999b). Thorn et al. (1998) studied waste collectors exposed to airborne 1,3-β-D-glucan and found a higher prevalence of reported diarrhoea, congested nose, and unusual tiredness than among the controls. The investigators also found an elevated number of blood lymphocytes in the workers. The response was dose-related to the concentration of airborne 1,3-β-D-glucan at the worksites. Several investigations have shown that exposure to airborne 1,3-β-D-glucan decreases baseline forced expiratory volume in one second (FEV₁), and thus it could be associated with workers' non-specific airways inflammation (Rylander, 1998; Thorn & Rylander, 1998; Rylander, 1999b; Rylander et al., 1999; Mandryk et al., 2000; Rylander & Lin, 2000).

Microbial volatile organic compounds. Many volatile organic compounds (VOCs) are irritants that may cause a burning sensation in the eyes and the upper airways (Molhave, 1985, 1990; Weschler et al., 1990), and thus MVOCs have been suggested to act as causative agents. Pasanen et al. (1998) recently published estimations that irritating symptoms are likely in buildings with microbial contamination when the concentrations of any single MVOC are on the level of hundreds of microgrammes per cubic metre or milligrammes per cubic metre (Pasanen et al., 1998). However, levels of individual MVOCs usually remain on the level of nanogrammes per cubic metre in problem buildings (Ström et al., 1994; Pasanen et al., 1998). In one study, a relationship between the prevalence of asthma among school employees and concentrations of MVOC (< 10 ng/m³ for 2-methyl-iso-borneol and 3-methylfuran, 100 ng/m³ for 2-heptanone and 1-octen-3ol) was noted (Smedje et al. 1996).

Building-related illness (BRI) and building-related symptoms (BRS) have been adopted as diagnosable illnesses with documented physical signs and laboratory findings that are evidently associated with indoor exposure. Even though the sick building syndrome (SBS) has been reported to occur in buildings with poor indoor air quality, such as complaints of odours and dry air, and dampness, with microbial components (like glucans) or some VOCs (Norbäck et al., 1994; Thörn et al., 1996; Li et al., 1997; Wolkoff et al., 1997; ACGIH, 1999), the prevalence of these subjective symptoms also depends greatly on psychosocial factors.

2.5 Methods for assessing fungal exposure

2.5.1 Methods based on spore counting

2.5.1.1 Cultivation methods

So far, **cultivation** methods have been the commonest for fungal exposure assessment. Through the manipulation of growth conditions, such as pH, water activity, nutrients, antibiotics of the substrate, light, temperature, and aeration, the growth of selected fungi can be favoured. Although numerous culture media are available for fungi, two media — 2% malt extract agar (MEA) and dichloran 18% glycerol (DG18) — are recommended for the detection of viable fungi in indoor air. MEA is suitable for fungi that require high water activity for growth and grow rapidly, while the water activity of DG18 is lower and suitable for xerophilic fungi. Other widely used fungal media include potato dextrose agar, rose bengal agar, and Sabourad's agar. The incubation time for fungi typically ranges from 3 to 7 days; however, some xerophilic fungi may require several weeks for mycelial development. Most airborne fungi are mesophilic and grow well at 20–25°C. The incubation temperature can also be used to select certain species, for example, *A. fumigatus* is a thermotolerant species that grows at 37 to 45°C. The detection of thermotolerant fungal species should be considered especially in work environments in which composting or fermentation processes occur (Hurst et al., 1997; Eduard & Heederik, 1998; ACGIH, 1999; Samson, 1999).

Collection. Impactors that collect spores directly onto agar plates are the most frequently used for sampling of airborne fungi. The six-stage cascade impactor (Andersen, 1958) is the most widely used model, and it is almost regarded as a reference instrument. Microorganisms are deposited on the agar plates through the stages separating airborne particles to various size fractions. The 50% cut-off diameter of the stages ranges from 0.6 to 7 µm. The sampler collects particles above 0.8 µm with the efficiency of 95%. The sampler has two modifications, a two-stage impactor and a one-stage version, so-called S6. The two-stage sampler has been observed to underestimate the counts when compared with the six-stage sampler (Jones et al., 1985; Crook & Sherwood-Higham, 1997; Hurst et al., 1997; Eduard & Heederik, 1998; ACGIH, 1999).

Other devices used for collecting viable airborne fungi include slit impactors, spore traps (Burkard), and the Surface Air System (SAS). The SAS and Burkard devices are portable, one-stage, battery-powered impactors which provide agar-filled plates as collection media. The cutpoint is 1.9 µm for the SAS sampler and 2.5 µm for the Burkard sampler. Slit impactors (e.g., Casella) deposit bioaerosols onto an agar surface with a constant airflow rate. Many slit impactors have a moving collection surface that enables temporal discrimination of fungi concentration (Nevalainen et al., 1992; Dillon et al., 1996; Hurst et al., 1997; Eduard & Heederik, 1998).

Traditional impingers (e.g., AGI-30) are not suitable for sampling fungi because many fungal spores are hydrophobic and may be lost as a result of floating to the surface and re-entrance into the exit airflow (ACGIH, 1989). However, in a new impinger, called the biosampler, the

collection of fungi is based both on the impaction and the centrifugal force. In contrast to the traditional impingers, non-evaporating liquids with a high viscosity can be used in the biosampler, which enables long-term sampling. The collection efficiency of the biosampler is about 96% for 1- μm particles if water is used as the collection liquid (Willeke et al., 1998; Lin et al., 1999; Lin et al., 2000).

The “CAMNEA” (collection of airborne microorganisms on Nucleopore filters, estimation and analysis) technique was developed to determine airborne viable and non-viable fungi in highly contaminated environments (Palmgren et al., 1986). First, airborne fungi are collected onto a polycarbonate filter, fungal cells are then suspended from the filter in a buffer solution, and the solution is both cultivated on the culture media via a dilution series and stained for the counting of total spores. The method allows long sampling times (hours). Basically, membrane filters with a pore size of 0.6 μm collect 0.3- μm particles with the efficiency of >95% (Eduard & Heederik, 1998). Even a higher collection efficiency, almost 100% for 1–3- μm particles, can be reached with the same type of filter in a 25-mm cassette with a modified inlet (Hauck et al., 1997; Aizenberg et al., 2000). The advantages of this method include lower wind sensitivity and good filter collection uniformity (lower standard deviation of concentrations) than with the original method. The best recovery has been obtained with a flow rate of 10 l/min.

The Reuter centrifugal air sampler (RCS) and RCS-plus are portable, single-stage, and battery-powered samplers that collect fungi centrifugally onto agar strips. The airstream cannot be measured in the RCS, and its collection efficiency for particles smaller than 3 μm is poor. The RCS-plus sampler loses 33% of 1.8- μm particles and 46% of the 0.7- μm particles. However, the collection efficiency is always affected by several factors (e.g., the presence of aggregates) in field measurements (Nevalainen et al., 1992; Dillon et al., 1996; Hurst et al., 1997; Eduard & Heederik, 1998).

In comparison studies, no significant difference in culturable fungal counts was found between the results of the CAMNEA method and those of the slit sampler at the levels of 10^3 – 10^8 cfu/m³ (Blomquist et al., 1984a). Instead, SAS underestimated culturable counts (range 10–3700 cfu/m³) by approximately 50% when compared with a six-stage impactor and the RCS, which were comparable in occupational settings (e.g., milling flour, feeding animal, laboratory work) (Smid et al., 1989; Dillon et al., 1996; Bellin & Schillinger, 2001).

In conclusion, advantages for cultivation methods include the possibility to identify major fungal species, simple analysing equipment, and a large amount of reference data. However, cultivation methods are slow and always selective, leading therefore to underestimates of total fungal counts and overestimates of the most tolerant species. Furthermore, when these methods are used in highly contaminated environments, many sampling devices (six-stage impactors, SAS, RCS) allow only short collection times (minutes), which do not represent exposure situations well. In addition, many bioaerosol samplers cannot be used as personal samplers (Flannigan, 1997; Pasanen, 2001).

2.5.1.2 Total spore counting

Sampling of total airborne fungal spores is based on impaction onto a glass slide or tape or filtration. Air samples collected with Burkard spore traps are analysed by light microscopy. Staining (e.g., lactophenol cotton blue and basic fuchsin) is commonly used to facilitate the discrimination of spores from debris (Hurst et al., 1997).

With the CAMNEA method (Palmgren et al., 1986), fungal spores are stained with acridine orange for total spore counting and enumerated by epifluorescence microscopy. However, some fungal spore types resist staining or have dark pigmentation that masks fluorescence. New staining methods (e.g., staining with 4,6-diamidino-2-phenylindole instead of acridine orange) have been suggested to improve the counting (Heldal et al., 1996).

The total number of fungal spores can also be estimated by scanning electron microscopy (SEM). In this technique, airborne fungi are collected on polycarbonate filters and a sector (90°) of each filter is gold-coated for the analysis. The microscopic specimen is taken from the middle of the sector, and the number of spores is counted (Eduard et al., 1988; Heikkilä et al., 1988).

Airborne culturable fungi have been previously estimated to comprise about 0.002–25% of total fungal and actinomycete spore concentrations (Lacey & Lacey, 1964; Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Heikkilä et al., 1990; Malmberg et al., 1993; Dutkiewicz et al., 1994; Hanhela et al., 1995; Flannigan, 1997; Louhelainen et al., 1997; Eduard et al., 2001).

Fungal cells can also be enumerated by bright-field or phase-contrast microscopy using a hemocytometer or counting chamber (Hurst et al., 1997).

2.5.2 Identification of fungi

The identification of fungi is mainly based on cultivation, possible isolation of fungal strains on selective media and incubation conditions, and morphological characterization of spores and spore-bearing structures by light microscopy. Identification to the genus level with total spore counting techniques is possible for only a limited number of fungal spore types (Hurst et al., 1997; Eduard & Heederik, 1998; ACGIH, 1999; Samson, 1999).

Molecular biological techniques have recently been developed to identify certain fungal species. Polymerase chain reaction (PCR) is a procedure used to amplify rapidly specific DNA (deoxyribonucleic acid) sequences. This technique and its modifications have been applied to detect, for example, species of *Aspergillus* (e.g., *A. fumigatus*, *A. flavus*, and *A. parasiticus*), *Fusarium* (e.g., *F. sporotrichioides* and *F. culmorum*), *S. chartarum* (Shapira et al., 1996; Hurst et al., 1997; Mule et al., 1997; Land & Must, 1999; Leenders et al., 1999), and yeasts (e.g., *Candida albicans*) (Holmes et al., 1992; Holmes et al., 1994). The PCR primers that amplify rRNA genes (rDNA) from all four major phyla of fungi (Ascomycota, Basidiomycota, Chytridomycota, and Zygomycota) have been published (Borneman & Hartin, 2000).

2.5.3 Methods for metabolic compounds

2.5.3.1 Microbial volatile organic compounds

Active sorbent sampling permits the extraction of low VOC concentrations from large volumes of air. Passive sorbent sampling is another type of collection in which VOCs are diffused through the sampler's internal dead air space and collected on the sorbent. Common sorbents used for both active and passive VOC sampling include activated charcoal, graphited carbon blacks, carbon molecular sieves, silica gels, polymers, such as Tenax TA or GR, Chromosorb, and XAD-4 (Batterman, 1995; Sunesson, 1995). Tenax TA has proved to have the best properties considering the recovery, breakthrough, and standard deviation during sampling or analysis when compared with different sorbents (Sunesson et al., 1995a). However, a long sampling time and repeated (if needed) analysis of VOCs with a low molecular weight is only possible when the air sample is collected on activated charcoal (Ström et al., 1994). Thermal desorption and gas chromatography (GC) with a flame ionization or mass spectrometer detector (FID) (e.g., for Tenax samples) are commonly used to separate and identify VOCs (Kaminski et al., 1974; Batterman, 1995; Sunesson, 1995; Korpi et al., 1998; Fedoruk et al., 1999; Wessen & Hall, 1999).

Many investigators have suggested that MVOCs could be used as tracers of suspected microbial contamination in buildings in which the occupants complain about poor indoor air quality and building-related symptoms (Sunesson, 1995; Wessen et al., 1995; Morey et al., 1997; Wilkins et al., 1997). Some investigators have presented the possibility of identifying fungal species on the basis of MVOC profiles (Börjesson & Kaspersson, 1989; Börjesson & Schnürer, 1990; Wessen et al., 1995; Bjurman et al., 1997; Smedje et al., 1997; Wilkins et al., 1997). However, this possibility seems to be unlikely for environmental settings because the metabolic pathways are common for many microbial species and MVOC profiles depend largely on the availability of nutrients in a substrate (Turner, 1971; Turner & Aldridge, 1983). Furthermore, volatile emissions from numerous outdoor and indoor sources (e.g., traffic, building materials, and human activities) limit the use of MVOCs as tracers of biocontamination in indoor environments (Sunesson, 1995; Wessen et al., 1995). Finally, VOCs that are considered as metabolic products of microorganisms may actually originate from a moist substrate themselves, and microbial growth may merely accelerate their emissions into the air. At high humidities, VOC emissions from sterile constructions have been found to resemble those from contaminated constructions in laboratory experiments (Korpi et al., 1998; Pasanen et al., 1998).

2.5.3.2 Mycotoxins

Ideally, the assessment of mycotoxin exposure in the work environment should be based on measurements of airborne toxins. However, the detection of mycotoxins in air has succeeded only in agricultural environments and with high-volume air samples (Burg et al., 1981, 1982; Ghosh et al., 1997; Selim et al., 1998). Noisy and heavy samplers that allow the collection of

high-volume air samples are not comfortable in indoor environments, and the high flow rate can quickly clean up the air of the space being investigated (Newman-Taylor & Tee, 1990). Therefore, the assessment has been based on analyses of mycotoxins or toxigenic fungi from settled dust or samples of building material (Silas et al., 1987; Johanning et al., 1993; Smoragiewicz et al., 1993; Andersson et al., 1997; Hodgson et al., 1998; Selim et al., 1998; Richard et al., 1999; Tuomi et al., 1999; Tuomi et al., 2000).

The detection of mycotoxins can be based on non-specific screening tests of toxicity (cell culture tests or bioassays) or specific chemical or immunochemical methods for mycotoxins. Cell lines, such as feline foetal lung (FL) cells, insects cells (Logrieco et al., 1996), swine kidney monolayer cells, the rabbit reticulocyte system (Yike et al., 1999), and human B lymphocytes (Logrieco et al., 1996) have been used in cell culture tests. The used bioassays included boar sperm cells (Andersson et al., 1997), tracheal cilia of chicken embryos (Hendry & Cole, 1993; Jesenska & Bernat, 1994), shrimp (*Artemia salina*) (Logrieco et al., 1996), or the rabbit skin irritation test (Pasanen et al., 1993a; Nikulin et al., 1996). The evaluation of cytotoxicity is based on changes in the morphology or density of the cultured cells. For toxicity assays, functional criteria, such as synthesis of protein and DNA, are used (Nikulin, 1999). One of the most widely used screening methods is the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] cytotoxicity test, which is based on the reduction of pale yellow MTT to dark blue MTT-formazan caused by mitochondrial enzymes after the intake of MTT into living mitochondria (Hanelt et al., 1994; Auger et al., 1999; Gareis et al., 1999; Yike et al., 1999). Yike et al. (1999) recently published a new, highly sensitive (400-fold more sensitive to trichothecenes than the MTT test) cytotoxicity assay that is based on the translation of firefly luciferase in a rabbit reticulocyte system.

A variety of chemical methods has been applied to analyse individual mycotoxins or mycotoxin groups including high-performance liquid chromatography (HPLC) (Langseth et al., 1989; Hendry & Cole, 1993; Andersson et al., 1997), gas chromatography with a mass spectrometry detector (GC-MS), or GC with electron-capture detection (ECD) (Rizzo et al., 1986; Hendry & Cole, 1993; Pasanen et al., 1993a), HPLC-MS, HPLC-MS-MS (ion trap mass analyser) (Tuomi et al., 1998; Tuomi et al., 2000), thin-layer chromatography (TLC) (Patterson & Damoglou, 1987; Bosch & Mirocha, 1992; Janardhana et al., 1999), and supercritical fluid chromatography (SFC) (Young & Games, 1993). These analyses usually require complex sample preparation and purification. Immunochemical assays [e.g., enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunofiltration assay (ELIFA)] have also been developed for some toxins (Nikulin et al., 1996; Dietrich, 1999; Janardhana et al., 1999). The ELIFA method has been further modified as an onsite test for trichothecenes (Dietrich, 1999). Molecular biological methods (e.g., PCR) have also been developed to detect toxigenic moulds, like *Fusarium* and *Aspergillus* strains (Shapira et al., 1996; Mule et al., 1997). The detection limits for mycotoxin analyses usually range from microgrammes to picogrammes, depending on the method and toxin.

2.5.4 Fungal specific immunoglobulin G antibodies

High levels of IgG antibodies against microorganisms have been found in the sera of both workers with farmer's lung disease (Grant et al., 1972; Katila & Mäntyjärvi, 1978; Husman et al., 1987; Malmberg et al., 1988) and those suffering from respiratory symptoms (Katila et al., 1986; Eduard et al., 1993), but also in the sera of healthy workers. Thus, in work environments with productive fungal sources, elevated IgG antibody levels against fungi act as a biomarker of heavy exposure to fungi (concentrations $>10^5$ spores/m³ or $>10^4$ cfu/m³) (Wardrop et al., 1977; Malmberg et al., 1985; Eduard et al., 1992; Eduard, 1995). Unlike momentary levels of airborne culturable and total fungi, serum IgG levels have been suggested to reflect long-term exposure. Because the biological half-time of IgG antibodies is several weeks (Spiegelberg, 1974), specific IgG antibody levels against fungi and actinomycetes are related to microbial exposure during the preceding months (Eduard et al., 1992; Eduard, 1995). On the other hand, the serological exposure assessment has been criticized because of difficulties with interpreting individual results, cross-sensitivity between related microbial species leading to false positive responses, and a lack of standardized antigens (and procedures) in analyses of IgG antibodies (Eduard, 1995; Horner et al., 1995). Eduard (1993) concluded that more accurate results are probable when an evaluation is based on antibody levels of a large group and the antigen used in ELISA is prepared from an isolate from the work environment under study.

2.5.5 Other methods

2.5.5.1 Ergosterol

Ergosterol can be detected from material, dust, or air samples (collected by filter sampling) with GC, HPLC or GC-MS (Axelsson et al., 1995; Saraf et al., 1997; Janardhana et al., 1999; Pasanen et al., 1999; Szponar & Larsson, 2000). Ergosterol is stable under dry and cold conditions (Miller & Young, 1997). Szponar and Larsson (2000) reported that the amount of ergosterol in materials did not change much even during a six-week storage period at room temperature. In laboratory experiments, a high correlation between the fungal activity and biomass level at early stages of fungal growth has been detected. At later stages, however, the activity-to-biomass ratio is expected to decline, and no correlation between ergosterol and viable counts has been found (Börjesson & Schnürer, 1990). It has been shown that the amounts of ergosterol in fungi can vary considerably according to species and culture conditions (Schnurer, 1993; Miller & Young, 1997; Pasanen et al., 1999).

2.5.5.2 1,3-beta-D-Glucan

The commonest method used to analyse 1,3-beta-D-glucan is the *Limulus* amoebocyte lysate (LAL) assay that is based on the reactivity of a specific factor (isolated from the amoebocyte of the *Limulus* horseshoe crab) with the 1,3-beta-D-glucan molecule. Basically the same LAL method is also used to determine bacterial endotoxin. The 1,3-beta-D-glucan-specific fraction of LAL that is insensitive to endotoxin was first separated by cation-exchange

chromatography (Kitagawa et al., 1991). Later, the test kit with glucan-specific lysate became commercially available (Rylander et al., 1992; Rylander, 1997a; Alwis et al., 1999). In addition, to increase the specificity of the 1,3-beta-D-glucan analysis, the sample suspension is treated with sodium hydroxide to preserve the structure of 1,3-beta-D-glucan (Thorn & Rylander, 1998). Because the method is highly sensitive (quantities of a few picogrammes can be detected), 1,3-beta-D-glucan can be determined in indoor air samples collected on polycarbonate filters (Rylander, 1997a; Alwis et al., 1999; Rylander, 1999b; Rylander et al., 1999; Thorn et al., 1999; Rylander & Lin, 2000). 1,3-beta-D-Glucan can also be analysed with an inhibition enzyme immunoassay (EIA) technique. Because this method has a higher detection limit than that of the LAL assay, 1,3-beta-D-glucan has been successfully determined from dust samples and air samples with a high volume only in heavily contaminated environments. The method has been stated to be specific, although it is based on polyclonal antibodies (Douwes et al., 1996; Douwes et al., 2000b).

2.5.5.3 Extracellular polysaccharides

EPS, especially EPS of *Aspergillus* and *Penicillium* (EPS-*Asp/Pen*) species in settled dust samples can be analysed with the ELISA inhibition technique, which is based on the assessment of polyclonal antibodies. Cross-reactions between the EPS of *Aspergillus* and *Penicillium* but not between the EPS of *Asp/Pen* and *Cladosporium* have been shown (Douwes et al., 1999; Chew et al., 2001).

2.5.5.4 Fungal allergens

Fungal allergens have been isolated, purified, and identified for *Alternaria alternata* (Horner et al., 1995; Kurup et al., 2000), *A. fumigatus* (Horner et al., 1995; Kurup et al., 2000) and *Cladosporium* (Horner et al., 1995; Kurup et al., 2000). Even some recombinant fungal allergens have been produced (Hemmann et al., 1999; Kurup et al., 2000; Shen et al., 2000; Shen et al., 2001). In addition, the preliminary description of allergenic or antigenic components for some *Aspergillus* (Chou et al., 1999; Kurup et al., 2000), *Penicillium* (Kurup et al., 2000; Shen et al., 2000; Shen et al., 2001), *Stachybotrys* (Raunio et al., 2001) and *Fusarium* species (Verma & Gangal, 1994a; Kurup et al., 2000), as well as those of several yeasts (Horner et al., 1995; Kurup et al., 2000), have been published. Still, more basic research is needed to develop methods with which to measure airborne fungal allergens. Some fungal allergens (e.g., *Cladosporium herbarum*, *A. niger*, *A. fumigatus* and *A. alternata*) have been quantified by radioimmunoassay (RIA) inhibition and by the direct competitive ELISA from air samples collected on filters or in a buffer solution with impingers (Topping et al., 1985; Pratt et al., 1990). The ELISA applications are also used to determine enzymes, like fungal amylase (Houba et al., 1996; Houba et al., 1997; Schweigert et al., 2000).

2.5.6 Evaluation of errors and defects in fungal measurements

Sources of errors and defects have been mainly evaluated for culturable and spore counting methods (Eduard & Aalen, 1988; Eduard et al., 1988; Dillon et al., 1996; Straja & Leonard, 1996; ACGIH, 1999). According to these evaluations both systematic errors and random errors exist. The main systematic errors are as follows:

- 1) Airborne culturable fungi are estimated to comprise about 0.002–25% of the total fungal and actinomycete spore concentrations (Lacey & Lacey, 1964; Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Heikkilä et al., 1990; Malmberg et al., 1993; Dutkiewicz et al., 1994; Hanhela et al., 1995; Flannigan, 1997; Louhelainen et al., 1997). Actinomycetes are difficult to separate from mould spores in the microscopic methods, and therefore actinomycetes are often included in the results.
- 2) The quality of media (e.g., pH, water activity, nutrients, antibiotics of the substrate), as well as growth conditions (e.g., light, temperature), selected for culturing fungi affects the recovery.

Random errors include the following aspects:

- 1) Differences, even small ones, in the composition of the media may lead to large variation in the counts of colony-forming units.
- 2) A sampling period often remains short (minutes) in work environments with productive fungal sources.
- 3) Evaluations of exposure based on stationary sampling may lead to somewhat different conclusions than personal sampling does.
- 4) Samplers and pumps may vary within the error range established by the manufacturers. Sampling in indoor environments does not follow isokinetic sampling (because the air velocity and direction varies, although the velocity usually does not exceed 2 m/s), except for air sampling from ventilation ducts.
- 5) The presence of spore aggregates increases variation in counting processes.
- 6) The transport of samples from the sampling environment to the laboratory may influence the recovery of fungi.
- 7) There may be human errors associated with the sampling and analysis.

Both systematic and random errors must be summed to obtain the total (cumulative) error for the method used.

If the fact that airborne culturable fungi comprise only 0.002–25% of total spore concentrations is considered as a measurement error, the error may be almost 100% in some cases. In regard to other error sources, fluctuations in fungal concentrations over time (and space) are probably always greater than the random sampling errors.

2.6 Reference values for fungal concentrations in indoor environments

So far no health-based guidelines or fungal exposure limit values exist or are likely to be established until more data on dose-response relationships for specific fungal agents and health outcomes are available (Verhoeff & Burge, 1997; ACGIH, 1999). All existent guidelines and recommendations regarding fungi have been given for the indoor environment. They are based on the indoor-to-outdoor ratio and the presence of potentially toxic or unusual fungal species (Rao et al. 1996). The reference values vary greatly between different authorities (governmental or private organizations or investigators) and countries. However, a sort of consensus seems to exist for airborne fungal levels:

1. The indoor air levels of microorganisms should reflect those in the outdoor air.
2. If the indoor-to-outdoor ratio is more than 1 for fungi, it may indicate fungal contamination in the indoor environment and there is a need of more research.
3. Fungal concentrations above 1000 cfu/m³ generally refer to an atypical situation.
4. Fungal concentrations above 10 000 cfu/m³ are very high and indicate heavy contamination.

According to Canadian guidelines, concentrations above 50 cfu/m³ for one fungal species require additional investigations. Fungal levels up to 150 cfu/m³ with a mixture of fungal species are considered normal. The recommendations of the World Health Organization (WHO) correspond to the Canadian guidelines (WHO, 1988). Some other organizations have suggested upper normal limits of 100, 200, 300 or 500 cfu/m³ for fungal concentrations provided that only common indoor air fungi exist in the indoor environment (Rao et al., 1996).

An international group of bioaerosol researchers named fungi that may indicate moisture or health problems when the fungi are detected above a baseline level in indoor air or when isolated from surfaces (Samson et al., 1994). These fungi are the following (* = important toxigenic taxa):

Materials with high water activity (water activity >0.90–0.95): *A. fumigatus*, *Trichoderma*, *Exophiala*, *Stachybotrys**, *Phialophora*, *Fusarium**, *Ulocladium*, yeasts (*Rhodotorula*)

Materials with moderately high water activity (0.90 > water activity > 0.85): *A. versicolor**

Materials with lower water activity (water activity = 0.85): *A. versicolor**, *Eurotium*, *Wallemia*, *Penicillia* (e.g., *P. chrysogenum*, *P. aurantiogriseum**)

The upper normal levels of indoor fungi in Finnish guidelines (Finnish Ministry and Health and Social Affairs, 1997) are based on the study of Reponen et al. (1992). Indoor air fungal concentrations above 500 cfu/m³ in the winter and an indoor-to-outdoor ratio of >1 indicate abnormal fungal sources indoors. The results are applied only to urban and suburban homes. The Finnish guidelines (Finnish Ministry and Health and Social Affairs, 1997) also contain the following additional remarks:

? Fungal concentrations between 100 and 500 cfu/m³ may indicate abnormal fungal levels in indoor environments in winter.

? Fungal concentrations between 100 and 500 cfu/m³ and the presence of unusual fungal species, like *Stachybotrys*, *Fusarium*, *A. versicolor*, and *A. fumigatus*, in winter indicate abnormal fungal sources in indoor environments and a possible health risk.

? If reference samples do not exist, fungal concentrations of >1 000 cfu/cm² on indoor surfaces and >10⁴–10⁵ cfu/g in building materials indicate fungal contamination and a possible health risk.

? Visible mould growth on interior constructions and surfaces is unacceptable and is considered a health risk.

The Finnish guidelines are currently in the process of being updated. The next version will be published in 2002.

According to New York City guidelines, immediate evacuation of all occupants is required if the airborne fungal concentrations exceed 10³–10⁴ cfu/m³ in indoor environments (New York City Department of Health, 1993).

2.7 Fungal exposure assessment

Basics of fungal exposure assessment. The assessment of fungal exposure follows the same basic criteria as the evaluation of any other agents in work environments. The assessment of agents in work environments has mostly been carried out by industrial hygienists because they are trained to identify work-related exposure and assess environmental conditions. The assessment includes the following steps (ACGIH, 1999):

gather information -> formulate hypothesis -> test hypothesis -> make recommendations

First, information on health data of the workers, the work environment, and the agents is needed. Although symptoms seldom verify bioaerosol exposure, they may indicate certain agents that investigators should seek. The investigation should include a walkthrough inspection, information on and visual checking of potential sources of fungal agents, building design and operation (including the heating, ventilation and air conditioning system), and occupant activities. Then, all available information is summarized as a hypothesis that should be tested. A sampling plan (where, when, and how) is often necessary. Environmental samples are primarily collected to recognize suspected sources for agents, identify and quantify the agents, and demonstrate the release of fungi from sources into the air. Samples from the breathing zone (personal samples) of involved persons usually reflect health-based exposure better than stationary samples do, especially in environments with productive fungal sources.

According to the consensus of an international group of bioaerosol researchers (Samson et al., 1994), there is no ideal sampling method for fungal particles in the indoor environment. However, the group concluded that culturable and non-culturable fungal particles are both important in relation to health effects. Filter methods usually fulfil this perspective, although a

specific sampling method should be stated with the aim of a study. The researchers recommended both personal and stationary samples. Furthermore, fungal particles should be collected in duplicate for 6–8 hours when using the filter method (e.g., Nucleopore filters of pore size 0.4 μm), while three to six impactor samples are advisable to cover a corresponding exposure time. In addition, the use of at least two different media, 2% malt extract agar (MEA) and DG18, were recommended for culturable fungi. No recommendations on the detection of fungal agents other than spores exist.

The following step is the data assessment, in which investigators make conclusions on the relevance of human exposure based on environmental observations and measurements, the strength of associations between exposures and existing or eventual diseases or symptoms, and the probability of current or future risks. The main aim of a bioaerosol assessment in workplaces is to characterize biological agents and to evaluate their potential hazards to human health or threats to the environment (Dillon et al., 1996; Crook & Sherwood-Higham, 1997). Finally, the study ends by making recommendations. When the environmental data are used to indicate health risk, investigators should consider evidence for each of the following items: (a) sources of an agent, (b) exposure of a worker to the agent, (c) inhalation of a sufficient dose of the agent to provoke a response, and (d) adverse responses to the exposure (ACGIH, 1999).

Standards. Standard methods do not exist for fungal exposure assessment. Those for chemical agents can be found in European standard (CEN) 689/1995. In principal, instruments for health-relevant aerosol sampling should possess the same sampling efficiency as the human respiratory system. Criteria for health-relevant size fractions of aerosols in the work environment have recently been published by the International Organization for Standardization (ISO) (ISO, 1995) and the Comete European de Normalisation (CEN) (CEN, 1993). Several multi-stage bioaerosol impactors fulfil these criteria well, since single fungal spores are fairly small (typically 2–10 μm) and spores are aspirated with high efficiency (Eduard & Heederik, 1998).

Guidelines. Guidelines exist only for water-damaged buildings. The New York City Department of Health has given guidelines for the assessment and remediation of *S. chartarum* and other fungi in indoor environments (New York City Department of Health, 1993) (www.ci.nyc.ny.us). According to these guidelines, a visual inspection is the most important step in attempts to identify possible fungal contamination. Bulk or surface samples may be needed if the visual inspection is equivocal. Air monitoring may be necessary if a disease that may be associated with fungal exposure has been diagnosed in workers or there are signs that ventilation systems may be contaminated or if the presence of mould is suspected (e.g., musty odours) but cannot be identified by visual inspection or bulk samples (e.g., microbial contamination in hidden locations). Samples from outdoors are always required in air monitoring when abnormal indoor fungal sources are suspected. Remediation and safe instructions have been given according to the extent of the mouldy area (0.3 m^2 , 0.3–0.9 m^2 , or 0.9–3.0 m^2) in constructions. The American Industrial Hygiene Association (AIHA) recommends use of a nonparametric analysis, such as Spearman's rank correlation, to conclude whether the fungal biodiversity is similar or not in the indoor and outdoor environments (Dillon et al., 1996). However, the test has several limitations. For example, poor power is especially apparent with a small number of samples. Thus the number of

outdoor air samples should at least equal the number of samples collected from outside a building (Spicer & Gangloff, 2000).

The Finnish guidelines for fungal exposure assessment and the identification of fungal contamination in indoor environments (Finnish Ministry and Health and Social Affairs, 1997) resemble the New York City guidelines. However, the main aim of the Finnish guidelines has been to recognize mouldy contaminated buildings. Surface and material samples are a first priority when in the recognition of possible fungal sources. Air monitoring is recommended only in the winter, when fungal concentrations are low outdoors. If air monitoring is necessary in other seasons, outdoor air samples must be taken at the time of the investigation. The investigation of a reference building is also strongly recommended. Guidelines for the remediation of mould-damaged buildings and work safety have also been published in Finland (Building Information Foundation RTS, 2000). In these instructions, the remediation should be done in a vacuum if the damaged area is $>0.5 \text{ m}^2$ or the concentration of culturable fungal spores in the materials exceeds 10^4 cfu/g or potential mycotoxins producers are observed.

Currently, the preparation of WHO guidelines for biological agents in the indoor environment and a draft of guidelines for the measurement of airborne microorganisms and endotoxins (prEN 13098) is in progress.

In conclusion, there are general guidelines for assessing microbial exposure and recommendations for the investigation of mouldy buildings, but standards or guidelines for specific agents are missing, although recent knowledge in the literature refers to the fact that the exposure assessment should be based on the measurements of several microbial agents. There is general agreement that both culturable and non-culturable methods are needed in the assessment of fungal exposure. Some guidelines for the sampling of spores have been published, but certainly not enough. Several fungal fragments and the primary, as well as secondary, metabolites produced by fungi exist and may cause or be related to health effects in humans. Several questions arise in relation to the data presented. Are workers exposed to these fungal agents in different work environments and also how can the agents be recognized and measured? Are available methods specific or sensitive enough, and when and where can they be used? Are there differences in exposure assessment strategies between indoor work environments and work environments with productive fungal sources. The purpose of this thesis was to find some preliminary answers to these questions.

III AIMS AND HYPOTHESES OF THE THESIS

The main aim of this thesis was to evaluate the suitability of methods based on cultivation and spore counting and the determination of fungal metabolites and specific IgG antibodies to fungi in assessing exposure in some work environments with and without productive fungal sources.

The aim was divided into the following contributory objectives and hypotheses as follows:

A

To characterize MVOCs produced by selected non-toxigenic and toxigenic fungi under different growth conditions in the laboratory (I, II).

Hypothesis: Some MVOCs are mutual for several fungal species growing on various substrates, and there are associations between the MVOCs and certain mycotoxins in the metabolic pathways.

B

To evaluate the usefulness of MVOC analyses in the detection of fungal contamination and the fungal exposure assessment in regard to the specificity of MVOCs and a degree of exposure in different occupational settings (I, II).

Hypothesis: The detection of several MVOCs indicates fungal contamination, and workers may be exposed to MVOCs in agricultural and indoor environments. Especially in agriculture, general knowledge of the occurrence of volatile compounds would be advantageous because a diversity of volatiles is obvious in many agricultural settings. However, MVOCs may have a minor role in fungal exposure assessment in comparison with other potential fungal agents.

C

To investigate the occurrence of airborne mycotoxins in agricultural environments with two cytotoxicity tests and chemical analyses (III).

Hypothesis: Detectable airborne mycotoxin levels can be measured in agricultural environments, and farmers are occasionally exposed to mycotoxins through inhalation. Cytotoxicity testing is a useful tool to prescreen environmental samples for more expensive and laborious chemical analyses of mycotoxins.

D

To survey farmers' exposure to a xerophilic fungus, *W. sebi*, and potential toxigenic *Fusarium* species with two immunochemical techniques (IV).

Hypothesis: Elevated IgG levels against *Wallemia* and *Fusaria* occur in farmers, and exposure to these fungi can be more significant than has earlier been reported. The ELIFA method has some advantages in antibody measurements, for example, its rapidity and accuracy, in comparison with the traditional ELISA method.

E

Firstly, to evaluate if the inspection and examination of damage in building construction are adequate tools with which to indicate the most urgent remedial needs. Secondly, to evaluate the usefulness of cultivation methods in the assessment of deviant fungal exposure in the air of indoor work environments (in water-damaged buildings) (V).

Hypothesis: It is possible to prioritize remedial actions (e.g., need for repairs or possible evacuation) using walk-through inspections and limited environmental sampling and monitoring. However, cultivation methods may underestimate fungal exposure in water-damaged buildings.

IV MATERIALS AND METHODS

4.1 Laboratory experiments on the production of microbial volatile organic compounds by selected fungal species

The MVOC production of seven fungal strains was investigated under various conditions in the laboratory (Studies I and II). The experimental set-ups are summarized in Table 7. All the fungal strains used were isolated from Finnish agricultural products (grain, bedding materials, and hay). *Fusarium sporotrichioides* and *Penicillium verrucosum* were obtained from the Finnish National Veterinary and Food Research Institute, and the other fungi were isolated at the University of Kuopio (Department of Environmental Sciences). For each experiment, a fresh spore suspension of each fungal strain was prepared in sterile water (10^6 – 10^7 cfu/ml) before the inoculation. The RH was regulated in airtight test chambers ($V=2$ l) with saturated salt solutions (Studies I and II). Sterilized substrate samples (2–20 g) were stabilized at the selected RH before the inoculation of the fungi. The chambers with sterilized substrates (Study I) and clean bedding materials (not used) (Study II) served as controls. There were two chambers containing the same material at each RH level. The incubation temperature of 25°C represented favourable growth conditions for the fungi, and lower temperatures (4–8°C) were used either to stimulate toxin production (Study I) or to simulate winter conditions in horse stables (Study II).

Table 7. Experimental set-ups of Studies I and II for the investigation of the production of microbial volatile organic compounds by different fungal species under various conditions.

| Fungi | Toxigenic or non-toxigenic strain | Substrate | Equilibrium RH of the substrate (%) | Incubation condition | Study |
|---|--|---------------------------------------|-------------------------------------|--|-------|
| <i>Fusarium sporotrichioides</i> | Toxigenic, T-2 producer | Straw, wheat, oats | 84–88, 97–99, 100 | 20–23°C by daylight, 3 days + 6–8°C in the dark, 14 days | I |
| <i>Penicillium verrucosum</i> | ? Toxigenic, ochratoxin A producer ? Non-toxigenic strain | Oats sawn aspen wood, wallpaper | 78–80, 84–85, 97–98 | 20–23°C in the dark, 26 days | I |
| <i>Fusarium poae</i> , <i>Paecilomyces variotii</i> , <i>Penicillium</i> species, <i>Wallemia sebi</i> | Not known | Peat shavings | 98 | 25°C by daylight, 6 days | II |
| <i>Fusarium poae</i> , <i>Paecilomyces variotii</i> , <i>Penicillium</i> species, <i>Wallemia sebi</i> | Not known | Peat shavings | 98 | 4°C in the dark, 22 days | II |

RH = relative humidity of the air

Sampling of volatile compounds. VOCs were collected on Tenax TA resin from the chambers with an airflow rate of 100–160 ml/min for 6–10 minutes. Carbon dioxide levels were measured from the parallel chambers. Clean air was led to the chambers through activated carbon simultaneously with the sampling. In Study I, air samples were taken every second day three times a week using the same Tenax tube to obtain integrated samples over the whole incubation week. In Study II, separate samples were collected on day 4 (Total VOCs), day 6 (MVOCs, 25°C), or day 4 and day 22 (MVOCs, 4°C).

Analysis of volatile compounds. Total VOCs (TVOCs) and selected VOCs (MVOCs) were analysed using a gas chromatograph (Hewlett Packard 5890) with a mass selective detector (GC-MS, Hewlett Packard 5970) attached to a thermal desorption cold trap (TCT) injector. Details of the analysis are described in Study I. TVOCs were analysed by the scanning (SCAN, ions 40–260 amu) mode in Study II, and for the MVOCs the selected ion monitoring (SIM) mode was used in both studies (Studies I and II). The reference compounds (n=8–15, the sources have been reported in Study I) were selected on the basis of suggestions in the literature indicating that they originate from microbial metabolism (Kaminski et al., 1974; Börjesson & Schnürer, 1990; Börjesson et al., 1993; Sunesson, 1995; Sunesson et al., 1996; Bjurman et al., 1997; Korpi et al., 1998) (Table 8). The ratio of the peak areas of the individual VOCs and an internal standard (1-chlorooctane) was compared with the corresponding mass ratio to quantify the VOCs.

Table 8. Reference compounds for selected the volatile organic compounds (potential microbial volatile organic compounds) used in Studies I and II.

| Compound | Study I (SIM mode 3) | Study II, incubation at 4°C (SIM mode 2) | Study II, incubation at 25°C (SIM mode 1) |
|--------------------|----------------------|--|---|
| <i>Ketones:</i> | | | |
| 2-Hexanone | X | X | X |
| 2-Heptanone | X | X | X |
| 3-Octanone | X | X | X |
| <i>Alcohols:</i> | | | |
| 1-Octen-3-ol | X | X | X |
| 3-Octanol | X | X | X |
| 2-Octanol | X | X | X |
| 1-Octanol | X | X | X |
| 2-Ethyl-1-hexanol | Not analysed | Not analysed | X |
| 1-butanol | Not analysed | Not analysed | X |
| 3-methyl-2-butanol | X | Not analysed | Not analysed |
| <i>Terpenes:</i> | | | |
| Alpha pinene | X | Not analysed | X |
| Beta pinene | X | Not analysed | X |
| Camphene | X | Not analysed | X |
| Limonene | X | Not analysed | X |
| <i>Aldehydes:</i> | | | |
| Nonanal | Not analysed | X | X |
| Decanal | Not analysed | Not analysed | X |

SIM = selected ion monitoring

Concentrations of TVOCs and selected VOCs (MVOCs) in the chambers ($\mu\text{g}/\text{m}^3$) (Study II), the relative MVOC proportions of TVOC (%) (Study I), and the emission rates were calculated for individual MVOCs ($\mu\text{g}/(\text{kg h})$, in Study II) and for the sum of the MVOCs (ng/d, in Study I). The sum of the MVOCs (Table 8) in the integrated samples was divided by the number of incubation days in Study I. The concentration of the MVOCs has been multiplied by the airflow rate, which resulted in the mass flow rate, which was divided by the mass of the bedding material used in Study II. If the emission of an individual MVOC was higher in the control chamber than in the corresponding chamber with the inoculated material, the compound was regarded as a volatile impurity of the material or as a general contaminant rather than as an MVOC.

4.2 Study sites and subjects

Horse stable. Fungal concentrations, selected VOCs (MVOCs), and TVOCs were measured in a horse stable with 38 horses as described in detail in Study II. The stable had a mechanical ventilation system (the average airflow was approximately 6000 m^3/h). The floor area of the building was 442 m^2 with a volume of 1348 m^3 . The stable was divided into two equal parts with a partial wall. During the investigations, peat was used as the bedding materials in one part of the stable, and wooden shavings were used in the other part.

Farms. Farmers' exposure to fungi and airborne mycotoxins was studied on eight farms in Study III. On two farms, only grain was cultivated, while both grain culture and cattle breeding were done on the other farms. Details of the farms and weather conditions during the harvest have been presented in Study III (e.g., Table 1, Study III). Each farm had a grain drier, and grain was also milled on most of the farms. Part of the oat harvest was known to be contaminated by fungi on one farm (farm number 3). The other farms were randomly chosen for the study, and no information about the grain quality was obtained in advance.

Schools. The presence of fungi and the needs for repair and other remedial actions were evaluated in water-damaged schools in Study V. Nine schools consisted of ten separate buildings, five of which were built on the site, the other five being prefabricated buildings (two were so-called barrack type buildings). The buildings were built between 1927 and 1986. Water damage had occurred in all of these buildings.

Farmers. Exposure to *Fusaria* and *W. sebi* was investigated among 83 (45 men and 38 women) farmers in Study IV using serological measurements. The farmers (all worked on dairy farms) were divided into the following four groups:

- A) patients with farmer's lung (n=24)
- B) patients with asthma (n=19)
- C) asymptomatic (no diseases diagnosed and no symptoms reported) farmers (n=20) with high serum IgG antibody levels
- D) asymptomatic farmers (n=20) with low serum IgG antibody levels against the farmer's fungal antigen set.

The test set consisted of crude mycelia antigens of *Absidia corymbifera*, *A. fumigatus*, *A. umbrosus*, *Humicola grisea*, *Faenia rectivirgula*, *Penicillium brevicompactum*, *Rhodotorula glutinis*, and *Thermoactinomyces vulgaris* obtained from the Kuopio Regional Institute of Occupational Health. In addition, the sera from printing and office workers (n=16, 2 men and 14 women) who had no agricultural background served as negative controls (group E). The age and sex distributions have been presented in Table 1 in Study IV. The mean age of the studied persons was 43 years. Only non-smokers were primarily selected for study; however, information on smoking habits was not available for all the subjects.

4.3 Sampling and analyses in the field and population studies

4.3.1 Sampling procedures

The sampling protocols are summarized in Table 9 for the study sites. All the samples were collected as stationary samples. In the **horse stable**, two workers fed horses with dry hay and oats three times a day and cleaned the stalls once a day. The workers did not use any respiratory protection. Air samples for culturable fungi and total spores were collected before the workers fed the horses (background period) and during the feeding of the horses in the corridors of the stable. In addition, air samples were taken for fungi during the cleaning of the stalls, where peat or shavings were used as bedding materials. VOC samples were collected from the stalls during the background period and the cleaning of the stalls. The sampling for fungi and VOCs was conducted three times in the winter.

On the **farms**, air samples were collected during the drying and milling of grain and the feeding of cattle (Study III). During the milling, the distance from a sampling site to a place where the farmers controlled the operation of the mill was shorter than 3 metres. On seven farms, grain samples were also studied. Three partly dried oat samples were taken during the drying of grain. The remaining four oat and three oat and barley samples were dry and were collected near storage bins several weeks or months after the drying process. During the drying of grain, the farmers spent about 30% of their time beside the sampling site. In the cow barns, the samples were collected in the middle of the barns. The farmers did not use any respiratory protection.

In the **schools** (Study V), the sampling was conducted after walk-through inspections. Air samples were collected first; then settled dust samples were taken, followed by material samples. The settled dust samples were collected as cumulative air samples in the same classrooms as the air samples. The sampling was performed during normal school work. Thus the classrooms were occasionally occupied during the sampling. In addition, six VOC samples were collected from six school buildings. The stationary samples were taken from the worst water-damaged areas (classes, social rooms and halls).

In the school buildings, dampness-related defects in the constructions were investigated visually, with moisture measurements being taken from surfaces, and by human sensory evaluation (detection of odour). The damaged areas were classified into the following three

categories according to the severity of the moisture problems: 0 (visible moisture damage in a small area, less than 0.5 m², or none, no mouldy odour, and no visible microbial growth), + (visible moisture damage restricted to less than 2 m², no mouldy odour, and no visible microbial growth), and ++ (severe moisture damage, a damaged area over 2 m², mouldy odour, or visible microbial growth).

Table 9. Summary of the sampling procedures used on the study sites.

| Sampling site/study | Air sampling for | | | | Collection of bulk samples for | | |
|-------------------------|---|---|--|---|--|-------------------------------|--|
| | CAMNEA | Cultivation | Selected VOCs/TVOCs analyses | Mycotoxin analyses | Cultivation | Mycotoxin analyses | |
| Horse stable/ II | n=52 Collection onto polycarbonate filters Q=4 l/min t= 60 min V = 240 l h = 1.5 m (<i>additional unpublished data</i>) | Not collected | n= 24 indoors + 3 outdoors collection to Tenax TA Q=100 ml/min t=60 min V=6 l h=1.5 m | Not collected | 2 shaving samples 2 peat samples 1 hay sample 1 oat sample | not collected | |
| Farms/ III | n=32 Collection onto polycarbonate filters Q=2-3 l/min time = 20-60 min volume=60-180 l height=1.5 m | Not collected | Not collected | N=18 Collection of glass fibre filters Q=833 l/min V=16-50 m ³ h=1.2 m | 10 grain samples of 1-2 kg | 10 grain samples of 1-2 kg | |
| Schools/IV | Not collected | N=31 Collection with a six-stage impactor on MEA and TGY t = 10 min V = 200 l h = 1.5 m | N=6 collection to Tenax TA Q=100 ml/min t=106-138 min V=10-14 l h=1.5 m (<i>additional unpublished data</i>) | Not collected | 27 building materials (10 g) from water damaged spots, 31 surface samples (100 cm ²) collected with a sterile swab from dust settled on horizontal surfaces | Not collected | |

VOC = volatile organic compounds, TVOCs = total volatile organic compounds, n = number of samples, t = sampling time, Q = the air flow rate, V = volume of the air sample, h = sampling height

4.3.2 Analyses of fungal agents and exposure

4.3.2.1 Cultivation and identification of fungi

Air samples. Airborne culturable fungal concentrations (Study III and the horse stable) were analysed by the CAMNEA method (Palmgren et al., 1986), in which fungal cells were suspended from the filter to the buffer solution and the solution was cultivated with a dilution series on MEA for mesophilic and thermotolerant fungi and on DG18 for xerophilic fungi. In the schools (Study V) airborne culturable microorganisms were collected with an Andersen six-stage impactor. Mesophilic fungi were cultured on MEA and mesophilic bacteria on tryptone-glucose-yeast (TGY).

Surface samples. After the sampling, the sterile swabs were shaken in the sterile buffer solution and cultured via a dilution series on MEA for mesophilic fungi (V).

Material samples. The samples of grain (Study III) and building materials (Study V), as well as the agricultural materials (grain, hay, peat, and shavings) from the horse stable (10 g of each material) were suspended in dilution water and shaken for 30 minutes. Dilution series were prepared, and suspensions were plated on MEA for mesophilic (all the samples) and thermotolerant (only grain samples) fungi, on DG18 agar for xerophilic fungi (only grain samples), and on TGY agar for actinomycetes (only building material samples). Some grains of each sample were also strewn directly on MEA for fungal identification, because the direct plating is reported to be very effective for grain samples and it gives a reliable indication of the species active in the ecosystem (Samson & van Reenen-Hoekstra, 1998; Dillon et al., 1999; Macher, 2001).

Incubation and identification of fungi. Mesophilic and xerophilic fungi were incubated at 25°C for 7 days, thermotolerant fungi at 40°C for 4 days, and actinomycetes at 25°C for 14 days. The airborne fungal spore concentrations were expressed as colony-forming units per cubic metre (cfu/m³), and the fungal concentrations were recorded as colony-forming units per gramme (cfu/g) for the materials and as colony-forming units per square centimetre (cfu/cm²) for the surface samples. The concentration of viable fungi in the bulk samples over 10 000 cfu/g was considered to indicate active fungal growth (Finnish Ministry and Health and Social Affairs, 1997). The fungi were identified at the genus/species/type level by light microscopy (Samson & van Reenen-Hoekstra, 1998).

4.3.2.2 Total spore counting

The total spore counting was based on the CAMNEA method (Study III and the horse stable) (Palmgren et al., 1986). Part of the suspension used for cultivation was stained with acridine orange, and spores were counted with an epifluorescence microscope without separation of fungal and actinomycete spores (other bacteria were excluded from the counts). The total spore concentration was expressed as spores per cubic metre.

4.3.2.3 Toxicity and mycotoxin analyses

Sample preparation (Study III). Airborne dust samples were extracted from the filters with methanol-water during shaking. Extracts were filtered through folded filter paper, and the extraction was repeated twice. Extracts of each sample were combined, evaporated in a vacuum, and redissolved in acetone-methanol. Each solution was first divided into two fractions, one for the biological toxicity tests and the other for the chemical analyses.

Grain samples (50 g) were homogenized and extracted with methanol-water on a shaker. Extracts were filtered through folded filter paper, and the volumes were adjusted and concentrated in a vacuum. These solutions were washed twice with hexane, and the hexane layers were discarded. The remaining phases were concentrated in a vacuum, and acetonitrile was added. The samples were purified on a charcoal column with acetonitrile. The solutions were evaporated in a vacuum and redissolved in chloroform-methanol. Each sample was then divided into three fractions for the cytotoxicity test, the yeast-cell toxicity test, and the chemical analyses. Solvents were evaporated and the fractions for the cytotoxicity test and the yeast cell test were dissolved in the buffer solution.

Toxins from three *Fusarium* isolates from one oat sample were extracted with methanol-water and purified on charcoal columns as already described. The solutions of each *Fusarium* species were divided into two fractions for the cytotoxicity test and the chemical analyses.

The **cytotoxicity test** (Study III) was carried out with a continuous FL-cell line as previously described in detail (Pasanen et al., 1993a). Each sample extract was diluted on microwell plates, and the plates were observed daily during a seven-day incubation for signs of cytotoxicity, such as dead cells or diminished cell growth. The detection limits (ng/ml) for DON, 3-acetyldeoxynivalenol (3-AcDON), nivalenol (NIV) and T-2-toxin were 183, 2188, 49, and 2.3, respectively.

The **yeast-cell toxicity test** (Study III) was carried out with *Saccharomyces cerevisiae* cells as previously described (Whitehead & Flannigan, 1989). The log-phase culture of the yeast was exposed to the sample extracts during a 24-hour incubation. The difference between the dry mass of the yeast culture and that of the controls, caused possibly by toxins, was measured as absorbance. A sample extract was interpreted as toxic when the decrease in dry mass exceeded 10%. The detection limit for T-2-toxin was 0.1 µg/ml.

Chemical analyses (Study III). Fifteen airborne dust samples (out of 18) were chosen for the trichothecene analyses on the basis of both the occurrence of *Fusaria* in the air or grain samples collected at the same site on the farm and the signs of cytotoxicity in the sample. Trichothecenes were derivatized to their trimethylsilylether derivates and analysed by GC-MS using a SIM system as described Rizzo et al. (1986). The detection limit for DON, AcDON and NIV was 1 µg/kg and 5 µg/kg for T-2-toxin, respectively.

4.3.2.4 Analyses of selected volatile organic compounds (microbial volatile organic compounds) and total volatile organic compounds

The VOC samples from the horse stable (II) were analysed as described in Section 4.1. Potential MVOCs were analysed using SIM mode I in the first and second measurements and SIM mode 2 in the third measurement (Table 8). The concentrations of TVOCs and selected VOCs in the air of the horse stable ($\mu\text{g}/\text{m}^3$) and the rate of selected VOC emissions from bedding materials ($\mu\text{g}/(\text{kg}\cdot\text{h})$) were calculated.

The TVOC samples in the Tenax TA (35/60 mesh) tubes (Perkin-Elmer, Beaconsfield, England) from the schools were desorbed in a Perkin-Elmer ATD 400 (Perkin-Elmer Ltd, Beaconsfield, UK) and analysed with a HP 5890 series II GC, equipped with a HP 5971A mass selective detector and accompanying HP G1034C MS Chemstation (Hewlett-Packard, Palo Alto, California). The detector was operated in the SCAN mode. Desorption was performed at 250°C for 8 minutes. The cold trap operating temperatures were -30°C and 250°C, and the trap was held for 2 minutes. The valve and transferline temperatures were 200°C. The carrier gas was helium at 11.8 psi (pounds per square inch). An Ultra, fused silica capillary column (1.25 m x 0.20 mm inner diameter, 0.33 μm , Hewlett-Packard) was used. The column temperature was held at 40°C for 5 minutes, after which it was raised at the rate of 10°C/min to 220°C, where it was held for 7 minutes.

The main VOCs were identified with the aid of a Wiley version 130K mass-spectral database (Hewlett-Packard) and quantified based on the concentration-dependent peak area of standard compounds. The following VOCs were selected to represent potential MVOCs in the indoor environment (damp buildings) on the basis of previous literature (Batterman, 1995; Sunesson, 1995):

alcohols: 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-octen-3-ol, 2-pentanol
ketones: 2-pentanone, 2-hexanone, 2-heptanone, 3-octanone, 2-octanone, 3-octen-3-one
amino acid derivatives: pyrazin, methylpyrazin, dimethylpyrazin, pyrrole
sulphur compounds: dimethylsulphide, dimethyldisulphide
ethers: 3-methylfuran, anisole, methylanisole, 3-allylanisole, 4-allylanisole
other: terpene derivatives, geosmin.

The concentrations of TVOC in the air ($\mu\text{g}/\text{m}^3$) were calculated. Individual VOCs were examined only qualitatively.

4.3.2.5 Fungal-specific immunoglobulin G antibody measurements

Antigen preparation. Antigen extracts were prepared from *W. sebi*, *F. culmorum*, and *Fusarium avenaceum* (IV) with the same procedure used for the antigen preparation of fungi and *T. vulgaris* in the farmer's antigen set of the Kuopio Regional Institute of Occupational Health. The fungal strains were first grown in mycological peptone broth (*Fusaria*) or of sodium chloride (75 g/l) in the broth (*W. sebi*) under conditions that stimulated sporulation of the fungi. Mycelium and spores were filtered roughly from the broth, sterilized, and washed with phosphate-buffered saline (PBS). The cells in the PBS were disrupted mechanically and

by ultrasonic treatment. The suspensions were centrifuged at +4°C. The supernatants were stored at -70°C and used as crude mycelia antigens in the ELISA and ELIFA at protein concentrations of 1 µg/ml. The protein concentrations of the antigen extracts were determined with the method of Lowry et al. (1951).

The **ELISA** method described in detail in Study IV was performed on microtitre plates coated with the crude antigen extracts of *W. sebi*, *F. culmorum* and *F. avenaceum*. After the incubation, the plates were washed three times with MilliQ-UF water. Serum samples were analysed with two parallel samples at a dilution of 1:100 in 10% foetal bovine serum (FBS) in PBS. After the incubation, the plates were washed twice with PBS containing 0.05% Tween-20, kept in MilliQ-UF water for 2 minutes, and dried. Alkaline phosphatase-conjugated anti-human IgG was added at a dilution of 1:400 and incubated at 37°C for 2 hours. The plates were washed twice and dried. The substrate solution, 0.4 mg/ml p-nitrophenylphosphate in diethanolamine-magnesium chloride buffer, was added and incubated at 37°C for 30 minutes. The enzyme reaction was stopped with 2 M sodium hydroxide, and the absorbance was measured at 405 nm with a spectrophotometer.

The **ELIFA** method described in detail in Study IV was performed by the Easy-Titer™ System according to the protocol recommended by the manufacturer. Immunochemical reactions were produced in a membrane and a microtitre plate was placed at the bottom of the instrument before substrate solution was added. Biotek A nylon membrane (pore size 0.4 µm) was selected for the IgG measurements because of its low absorbance background. The membrane was first moistened, and the flow rate in the system was adjusted with MilliQ-UF water. The membrane was coated by adding the antigens of *W. sebi*, *F. culmorum* and *F. avenaceum* by adsorbing the antigens through the membrane within 5 minutes. The membrane was saturated with 3% bovine serum albumin in PBS. The serum samples were added in duplicate at a dilution of 1:100 in 10% FBS in PBS and absorbed through the membrane within 5 minutes. Alkaline phosphatase-conjugated anti-human IgG was added at a dilution of 1:400. Excess conjugate solution was washed with PBS, after which a microtitre plate was placed in a collection chamber. Then the substrate solution (as earlier described) was absorbed through within 5 minutes. Finally, the enzyme reaction was stopped with 2 M sodium hydroxide, and the absorbance was measured as already described.

After the revision of the absorbance values against an internal control (as described in detail in Study IV), the interpretation of the relative serum IgG antibody levels in different farmers' groups was based on the comparison of the mean absorbances of duplicate measurements in each serum with the mean absorbance of the negative control sera.

4.4 Statistical analysis

An analysis of variance (the Student-Newman-Keuls) was used to compare the IgG levels (absorbances) between the different farmers' groups and between the farmers' groups and the control group in Study IV. Differences in the concentrations of fungi and bacteria in the air, material, and surface samples between classified areas (0, +, ++) were tested with the Kruskal-Wallis one-way analysis of variance in Study V. The P-value of 0.05 was set as a limit for statistically significant difference in both the studies. The Pearson's correlation coefficient was calculated between the estimated variables of the heating, ventilation and air conditioning systems and the concentration of fungi and bacteria in the air, material, and surface samples in Study V.

V RESULTS

5.1 Microbial volatile organic compounds produced by selected fungi under different growth conditions

The selected VOCs (potential MVOCs) detected in the laboratory experiments are listed in Table 10. All of the studied fungi produced 3-octanone, and it was produced on 93% of the culture plates. The other frequently (=71% of the growth media) occurring VOCs were 2-hexanone, 2-heptanone, and 1-octen-3-ol. The production of other VOCs depended more on the fungal species, growth medium, incubation time, temperature, and RH. Part of the selected VOCs (e.g., alpha pinene, beta pinene, camphene, and limonene from shavings) were also present in the emissions of clean materials. The potential MVOCs, 3-octanone, 2-hexanone, and 2-heptanone, detected in laboratory experiments were also recognized in the field studies. (See Section 5.2.2.)

In Study I, the sum of the MVOC emission rates for *F. sporotrichoides* and *P. verrucosum* varied from 0.3 to 15 ng/d. The emission rates for *F. sporotrichoides* were highest for wheat, whereas for *P. verrucosum* wood at any RH levels showed the highest rates. In Study II, the MVOC emission rates produced by four fungal strains on two bedding materials ranged from 0.001 to 0.176 $\mu\text{g}/(\text{kg h})$ at 25°C, whereas the maximum MVOC emission was 0.037 $\mu\text{g}/(\text{kg h})$ at 4°C. The MVOC emissions of all four fungi were always higher from the cultures of shavings than from those of peat. In general, the MVOC levels were low, comprising about 1% of the TVOC concentrations (Study II).

Some relationships were detected between the synthesis of mycotoxins and certain MVOC groups. (See Figure 1 in Study I.) The production of trichothecenes by *F. sporotrichoides* was strongest for oats, while terpenes and ketones were produced slightly more than alcohols. On the contrary, for straw, alcohols were the main MVOCs, and the production of trichothecenes was weak. Likewise, a slight difference in the quality of MVOC production was noted between the toxigenic and non-toxigenic *P. verrucosum* strains, ketones being the dominant MVOCs associated with the growth of the toxigenic strain and the non-toxigenic strain producing mainly alcohols for aspen and wallpaper. However, this difference was not strongly observed when *P. verrocosum* grew on oats.

Table 10. Summary of the microbial volatile organic compounds produced by selected fungal strains on different substrates. (See Study I, Figure 2, and Study II, Tables 1 and 2)

| Compound | <i>Fusarium sporotrichioides</i> (I) | | | <i>Fusarium poae</i> (II) | | <i>Penicillium verrucosum</i> (I) | | | <i>Penicillium species</i> (II) | | <i>Paecilomyces variotii</i> (II) | | <i>Wallemia sebi</i> (II) | |
|---------------------------------|--------------------------------------|-------|------|---------------------------|----------------|-----------------------------------|----------------|----------------|---------------------------------|-----------------------|-----------------------------------|-----------------------|---------------------------|-----------------------|
| | Straw | Wheat | Oats | Peat | Shavings | Oats | Wood | Wall paper | Peat | Shavings ^s | Peat | Shavings ^s | Peat | Shavings ^s |
| <i>Ketones:</i> | | | | | | | | | | | | | | |
| 2-Hexanone | ND | X | X | X ^f | X ^f | X | X | X | X ^f | X ^f | ND | X ^f | X ^f | X ^f |
| 2-Heptanone | ND | ND | X | X | X | X ^d | X | X | X ^f | X ^f | X | X | X ^f | X |
| 3-Octanone | X | X | X | X ^b | X ^b | X | X | X | X ^b | X ^f | X ^b | X ^b | ND | X ^b |
| <i>Alcohols:</i> | | | | | | | | | | | | | | |
| 1-Octen-3-ol | X | X | X | ND | X | X ^d | X | X | ND | ND | X ^b | X ^b | ND | X ^b |
| 3-Octanol | X | ND | ND | ND | ND | X ^e | X ^e | ND | ND | ND | ND | ND | ND | ND |
| 2-Octanol | ND | X | ND | ND | ND | ND | X ^e | ND | ND | ND | ND | ND | ND | ND |
| 1-Octanol | ND | ND | ND | ND | X ^b | X ^e | X ^e | ND | ND | ND | ND | ND | ND | ND |
| 1-Butanol | c) | c) | c) | X ^b | X ^b | c) | c) | c) | a),b) | a),b) | a),b) | a),b) | a),b) | a),b) |
| 3-Methyl-2-butanol | X | X | X | ND | ND | X | ND | ND | a),b) | a),b) | a),b) | a),b) | a),b) | a),b) |
| <i>Terpenes</i> ^{b)} : | | | | | | | | | | | | | | |
| Alpha pinene | X | X | X | X ^b | ME | X | X | X | X ^b | ME | X ^b | ME | X ^b | ME |
| Beta pinene | ND | ND | ND | X ^b | ME | ND | ND | X ^e | X ^b | ME | X ^b | ME | X ^b | ME |
| Camphene | ND | X | ND | X ^b | ME | X ^d | ND | ND | X ^b | ME | ND | ME | X ^b | ME |
| Limonene | ND | X | X | X ^b | ME | X | X | X | X ^b | ME | X ^b | ME | X ^b | ME |

a) not analysed in Study II at 4°C, b) not analysed in Study II at 25°C, c) not analysed in Study I, d) the atoxic strain did not produce, e) the toxic strain did not produce, f) produced only at 25°C, g) produced only at 4°C, ND = not detected, ME = considered mainly as material emissions

5.2 Occurrence of fungal agents in the agricultural environment

5.2.1 Fungal spores and flora

The airborne fungal concentrations and flora measured in the horse stable and on the farms (III) are presented in Table 11. On the farms, the mesophilic and xerophilic fungal concentrations were lowest during grain drying and highest during grain milling and cattle feeding. The thermophilic fungal concentrations were 10–100 times lower than the levels of mesophilic and xerophilic fungi. In the horse stable, the highest fungal concentrations appeared during the feeding of the horses. The concentrations were slightly higher in the part of the stable where peat was used as bedding material. In general, the proportion of mesophilic fungi varied from 0.001% to 38% of the total number of spores on the farms and in the horse stable.

Yeasts and species of *Cladosporium*, *Penicillium*, *Aspergillus* (especially *A. glaucus*, *A. niger*, and *A. fumigatus*), *Paecilomyces*, *Rhizopus*, and *Wallemia* were the commonest fungi in the air samples. *Fusarium* species were present only in about 9% of the air samples, which included about 1% of the culturable fungi during the grain drying, 2% of the fungi during the milling process, and 0.1% of the fungi during the cattle feeding. *W. sebi* was the most frequent xerophilic fungus found during the background period, the feeding of horses, and the cleaning of stalls in the horse stable, as well as during the feeding of cattle on the farms.

The concentrations of culturable fungi in the grain samples are listed in Table 3 (Study III). The concentrations varied from 10^3 to 10^7 cfu/g for the mesophilic and xerophilic fungi, being low (<100 cfu/g) for the thermophilic fungi. The most abundant fungi found in grain were yeasts, *Penicillium* species, *Absidia* species, *Mucor* species, and *Aspergillus fumigatus*. *Fusarium* species were detected in about 22% of the samples when cultured by the dilution method. If grains were strewn directly on MEA, the recovery was 80% of the samples.

The concentrations of culturable mesophilic fungi varied from 10^3 cfu/g (clean shavings) to 10^5 cfu/g (clean peat, used peat and used shavings) in bedding materials and from 10^5 cfu/g (oats) to 10^6 cfu/g (dry hay) in the animal feeds taken from the horse stable. The concentrations of thermophilic fungi were on the level of 10^2 cfu/g (clean shavings), 10^4 cfu/g (clean peat and used shavings), 150 cfu/g (dry hay) and 5 cfu/g (oats). The main fungi were *Penicillium* species, yeasts, *Paecilomyces variotii*, *Fusarium* species, and *W. sebi* in bedding materials and yeasts, and *W. sebi*, *Absidia corymbifera*, *Mucor* species, and *Aspergillus* species in animal feed.

Table 11. Summary of airborne fungal concentrations and flora measured in the agricultural environment and in water-damaged schools.

| Sampling site/ workphase | Total spore concentrations (spores/m ³) × 10 ⁶ | | Culturable spore concentrations (10 ³ cfu/m ³); Geometric mean (range) | | | Main fungi |
|--|---|--------------|---|----------------|------------------|--|
| | Mean | Range | Mesophilic | Xerophilic | Thermophilic | |
| Horse stable, stalls - peat as bedding material • backgound • feeding of horses • cleaning of stalls | 2.8 | 1.5–5.9 | 1.0 (0.1–1.3) | 3.3 (0.06–19) | Not analysed | <i>Wallemia sebi</i> <i>Penicillium</i> species <i>Aspergillus</i> species <i>Aspergillus glaucus</i> <i>Paecilomyces variotii</i> |
| | 7.6 | 1.0–7.6 | 2.0 (0.07–13) | 170 (48–1700) | | |
| | 4.6 | 2.1–6.9 | 3.8 (1.5–14) | 9.8 (3.0–42) | | |
| Horse stable, stalls - shavings as bedding material • backgound • feeding of horses • cleaning of stalls | 1.9 | 0.74–5.4 | 0.6 (0.1–5.3) | 7.4 (1.6–29) | Not analysed | As above |
| | 4.0 | 0.3–4.9 | 7.7 (1.6–46) | 14 (57–490) | | |
| | 1.3 | 0.63–2.6 | 1.0 (0.2–5.2) | 4.7 (1.1–20) | | |
| Horse stable, passages - peat as bedding material • backgound • feeding of horses | 2.5 | 1.2–8.8 | 0.5 (0.06–1.5) | 7.7 (3.8–28) | Not analysed | As above |
| | 14 | 1.7–11.0 | 6.0 (0.7–19) | 170 (71–810) | | |
| | | | | | | |
| Horse stable, passages - shavings as a bedding material • backgound • feeding of horses | 1.6 | 1.2–2.2 | 1.0 (0.5–2.7) | 9.3 (2.2–62) | Not analysed | As above |
| | 6.4 | 2.5–37 | 7.0 (3.7–14) | 110 (44–600) | | |
| | | | | | | |
| Farms (III) • drying of grain • milling of grain • feeding of cattle | 2.0 | 1.0–3.7 | 14 (1.8–150) | 6.8 (1.2–140) | 0.22 (<0.08–3.5) | Yeasts, <i>Cladosporium</i> species, <i>Penicillium</i> species, <i>Aspergillus</i> species, <i>Rhizopus</i> species, <i>W. sebi</i> , <i>Paecilomyces</i> species |
| | 2.7 | 0.28–23 | 68 (4.9–1100) | 64 (8.0–970) | 1.9 (<0.11–3.0) | |
| | 1.7 | 0.87–9.8 | 76 (5.5–3700) | 110 (8.9–5200) | 6.6 (<0.08–67) | |
| Schools (V) • small or no damage • moderate damage • severe and extensive damage | Not analysed | Not analysed | 0.024 (0.05–0.095) 0.097 (0.035–0.78) 0.132 (0.025–0.405) | Not analysed | Not analysed | <i>Penicillium</i> species, non-sporeing isolates, yeasts, <i>Aspergillus versicolor</i> , <i>Aspergillus</i> species |

5.2.2 Volatile organic compounds

The composition of selected VOCs and their concentrations in the horse stable (Study II) are shown in Table 12. Airborne compounds that may have been of microbial origin included 2-hexanone, 2-heptanone, 3-octanone, 3-methylfuran, 3-allylanisole, 2-pentanone, 2-butanone, and camphene. The TVOC concentrations varied from 130 to 1800 $\mu\text{g}/\text{m}^3$ (Table 4 in Study II). The concentrations of potential MVOCs were low, about 0.07–0.31% of the TVOC concentrations. The cleaning of stalls caused no marked difference in the concentrations of TVOC or selected VOCs. The emission rates for single VOCs from bedding materials in the stable varied between 0.224 and 1.975 $\mu\text{g}/(\text{kg h})$, being about 10 times higher than the corresponding rates in the laboratory experiments.

Table 12. Potential microbial volatile organic compounds (MVOCs) detected in the horse stable (Study II) and water-damaged schools (see Section 5.3.2). Different compounds were analysed in the stable and schools because of differences in the fungal species and potential growth substrates.

| Potential MVOC | Range of concentrations ($\mu\text{g}/\text{m}^3$) | |
|---------------------|--|------------------------------|
| | Horse stable | Schools |
| 3-Methylfuran | Not analysed | Detected, but not quantified |
| 3-Allylanisole | Not analysed | Detected, but not quantified |
| 2-Pentanone | Not analysed | Detected, but not quantified |
| 2-Hexanone | <0.1–0.5 | Not detected |
| 2-Heptanone | <0.1–4.6 | Detected, but not quantified |
| 3-Octanone | <0.1–1.5 | Detected, but not quantified |
| 2-Butanone | Not analysed | 0.3–0.6 |
| Camphene | Originated mainly from material emissions | Detected, but not quantified |
| 4-methyl-2-pentanol | Not analysed | 3 |
| Alpha pinene | Originated mainly from material emissions | 1–9 |
| Octanal | Not analysed | 1 |
| Limonene | Originated mainly from material emissions | 0.9–7 |
| Hydroxyacetofenone | Not analysed | 1 |
| 2-Pentylfuran | Not analysed | Detected, but not quantified |

5.2.3 Mycotoxins

None of the 18 air samples collected on the farms during the handling of grain (Study III) was toxic in the cytotoxicity tests. However, chemical analyses revealed low DON concentrations (3 and 20 ng/m^3) in two air samples collected during the milling of grain on two farms. The corresponding grain samples were toxic in the cytotoxicity test and contained DON concentrations of 0.14 mg/kg and 2.3 mg/kg .

Among all the grain samples studied ($n=10$), seven were cytotoxic (Table 3 in Study III), but no toxicity was detected in any samples in the yeast-cell toxicity test. The DON concentrations

ranged from 0.004 to 11 mg/kg in the samples analysed. *Fusarium* species were also identified in these samples. The mouldy, partly dried oat sample (Sample 2 in Table 3) had a high DON concentration (11 mg/kg) and small amounts of AcDON, NIV and T-2-toxins. Three different *Fusarium* species (*F. graminearum*, *F. tricinctum*, and *F. culmorum*) were isolated from this sample, although the pure cultures of *F. graminearum* and *F. tricinctum* did not prove to produce toxins either in the cytotoxicity test or in the chemical analyses under laboratory conditions. However, the *F. culmorum* culture was cytotoxic and produced high quantities of DON (49 mg/kg) and AcDON (3.3 mg/kg) in rice.

5.2.4 Fungal immunoglobulin G antibodies

The serum IgG antibody levels against *W. sebi*, *F. culmorum*, and *F. avenaceum* described by absorbance values are presented on the group level for the study subjects in Table 13 (and also in Table 2 of Study IV). Table 13 shows medians and fractiles for both the methods that the results are more comparable to each other (the ELISA results were not log-normally distributed, but the ELIFA results were). Both methods indicated that the farmer's lung patients and the asymptomatic farmers with high IgG levels against agricultural fungi had the highest IgG levels against the three fungi tested. Correspondingly, the lowest IgG levels were obtained for the controls and asymptomatic farmers with low IgG levels against agricultural fungi (Table 13). Some differences were detected between the methods: The IgG levels measured with the ELIFA for the asthmatic farmers were lower than those of the other groups, while, according to the ELISA results, the IgG levels were mostly higher for the asthmatic farmers than those for the asymptomatic farmers with low IgG levels against agricultural fungi and those of the controls.

Table 13. Medians and 25% and 75% fractiles for the immunoglobulin G (IgG) antibody levels against the three fungi among the farmers and controls (Study IV).

| Study group | Medians and fractiles of fungal specific IgG | | | | | |
|--|--|--|--|--|--|--|
| | <i>Wallemia sebi</i> | | <i>Fusarium culmorum</i> | | <i>Fusarium avenaceum</i> | |
| | ELISA | ELIFA | ELISA | ELIFA | ELISA | ELIFA |
| A) Farmer's lung patients | 1.474 <i>u</i> ₂₅ 0.99 <i>u</i> ₇₅ 2.33 | 1.295 <i>u</i> ₂₅ 1.07 <i>u</i> ₇₅ 1.68 | 1.583 <i>u</i> ₂₅ 1.07 <i>u</i> ₇₅ 2.44 | 1.395 <i>u</i> ₂₅ 1.19 <i>u</i> ₇₅ 1.64 | 1.599 <i>u</i> ₂₅ 0.69 <i>u</i> ₇₅ 2.47 | 1.282 <i>u</i> ₂₅ 0.98 <i>u</i> ₇₅ 1.52 |
| B) Asthmatic farmers | 1.145 <i>u</i> ₂₅ 0.57 <i>u</i> ₇₅ 1.86 | 0.688 <i>u</i> ₂₅ 0.54 <i>u</i> ₇₅ 0.80 | 1.041 <i>u</i> ₂₅ 0.79 <i>u</i> ₇₅ 2.17 | 0.587 <i>u</i> ₂₅ 0.51 <i>u</i> ₇₅ 0.69 | 0.976 <i>u</i> ₂₅ 0.59 <i>u</i> ₇₅ 1.88 | 0.573 <i>u</i> ₂₅ 0.50 <i>u</i> ₇₅ 0.67 |
| C) Asymptomatic farmers with high IgG against agricultural fungi | 1.356 <i>u</i> ₂₅ 1.07 <i>u</i> ₇₅ 1.87 | 0.960 <i>u</i> ₂₅ 0.80 <i>u</i> ₇₅ 1.06 | 1.862 <i>u</i> ₂₅ 1.50 <i>u</i> ₇₅ 2.17 | 0.911 <i>u</i> ₂₅ 0.77 <i>u</i> ₇₅ 1.01 | 1.636 <i>u</i> ₂₅ 1.23 <i>u</i> ₇₅ 2.02 | 0.885 <i>u</i> ₂₅ 0.75 <i>u</i> ₇₅ 0.96 |
| D) Asymptomatic farmers with low IgG against agricultural fungi | 0.957 <i>u</i> ₂₅ 0.41 <i>u</i> ₇₅ 1.35 | 0.776 <i>u</i> ₂₅ 0.66 <i>u</i> ₇₅ 1.07 | 1.282 <i>u</i> ₂₅ 0.55 <i>u</i> ₇₅ 1.96 | 0.783 <i>u</i> ₂₅ 0.62 <i>u</i> ₇₅ 1.04 | 0.839 <i>u</i> ₂₅ 0.44 <i>u</i> ₇₅ 1.57 | 0.759 <i>u</i> ₂₅ 0.60 <i>u</i> ₇₅ 1.09 |
| E) Controls | 0.833 <i>u</i> ₂₅ 0.37 <i>u</i> ₇₅ 1.19 | 0.621 <i>u</i> ₂₅ 0.52 <i>u</i> ₇₅ 0.71 | 1.154 <i>u</i> ₂₅ 0.80 <i>u</i> ₇₅ 1.95 | 0.644 <i>u</i> ₂₅ 0.56 <i>u</i> ₇₅ 0.81 | 0.728 <i>u</i> ₂₅ 0.44 <i>u</i> ₇₅ 1.68 | 0.618 <i>u</i> ₂₅ 0.53 <i>u</i> ₇₅ 0.67 |

ELIFA = enzyme-linked immunofiltration assay, ELISA = enzyme-linked immunosorbent assay

Some statistical differences ($P < 0.05$) were observed for the antibody levels in the ELIFA data. The IgG antibody levels against the three fungal species were significantly higher in the farmer's lung patients than in the other groups. In the ELISA data, significantly higher antibody levels were detected only against *W. sebi* in the farmer's lung patients when they were compared with other groups.

ELIFA was a somewhat more sensitive method than ELISA according to the statistically significant findings. However, ELIFA did not prove to have any clear advantages in practical use when compared with ELISA. In ELIFA, the fluidflow rate was difficult to adjust and maintain constant, and several membrane types had high background levels.

5.3 Occurrence of various fungal agents in the school buildings

5.3.1 Fungal spores and fungal flora

The airborne fungal concentrations and flora in the schools are presented in Table 11, and the bacterial concentrations have been shown in Table 2 in Study V. The fungal concentrations varied between 5 and 780 cfu/m³, and the bacterial concentrations were 10–4600 cfu/m³. The fungal concentrations were significantly associated with the severity of the damage, being highest in the areas with the most extensive water damage. A similar trend was observed for the bacterial counts, although the differences in the counts between various areas were not statistically significant. The geometric means of the actinomycete concentrations were low (5–10 cfu/m³) for the damaged areas, even though some high values (maximum 190 cfu/m³) were occasionally observed.

The microbial concentrations in the material samples (taken from damaged constructions) have been presented in Table 3 in Study V. The fungal concentrations were about four times higher in the most severely damaged areas than in the moderately damaged areas. The bacterial counts were throughout about one order of magnitude higher than the fungal counts. Statistically significant differences were not observed in the concentrations between the damaged areas. The level indicating an active fungal growth (10 000 cfu/g) was exceeded in 21 of the 27 materials samples (78%).

The fungal concentrations in the surface samples varied from 1 to 261 cfu/cm², being about two times higher in the damaged areas than in the non-damaged areas. The bacterial concentrations ranged from 0.2 to 4 200 cfu/cm² in the surface samples. No significant differences in the levels were observed between the areas. The prevalence of actinomycetes was more abundant in the damaged areas than in the non-damaged areas.

Penicillium species, non-sporing isolates, and yeasts were the commonest airborne fungi in all the areas (Table 11). *Aspergillus versicolor* was frequently detected in the air of the damaged areas. Species of *Penicillium*, *Acremonium*, *Aspergillus* (e.g., *Aspergillus versicolor*), *Cladosporium*, yeasts, and non-sporing isolates were the main fungi in the material samples of the damaged areas (Table 4, Study V). In the surface samples, *Aureobasidium*, *Penicillium*

species, *Cladosporium* species, yeasts, non-sporing isolates, and *Alternaria* were commonly found both in the damaged and non-damaged areas. Generally, fungal flora was more abundant and species of *Aspergillus*, *Trichoderma*, *Scopulariopsis*, and *Stachybotrys* were observed more often in the surface samples from the damaged areas than in those from the non-damaged areas.

5.3.2 Volatile organic compounds

The selected VOCs (potential MVOCs) analysed for the schools are presented in Table 12. All the air samples were collected from the worst water-damaged areas. About 83% of the samples contained at least one (maximum 6) of the VOCs analysed. Alpha pinene, limonene, 3-methylfuran, 3-allylanisole, 2-pentylfuran, and 2-butanone occurred the most frequently. The levels of selected VOCs remained below 9 $\mu\text{g}/\text{m}^3$, comprising less than 9% of the corresponding TVOC concentrations (100–270 $\mu\text{g}/\text{m}^3$).

VI DISCUSSION

6.1 Microbial volatile organic compounds as indicators for the detection of fungal contamination and toxigenic fungi

The potential MVOCs, 3-octanone, 2-hexanone, 2-heptanone, and 1-octen-3-ol, detected most frequently in the present laboratory experiments (Studies I and II) have also been reported widely in the literature (Börjesson & Kaspersson, 1989; Börjesson & Schnürer, 1990; Börjesson et al., 1993; Sunesson et al., 1995b; Sunesson et al., 1996; Fischer, 2000), but not necessarily as metabolites of the same fungi or produced on similar substrates as in the present work. 3-Octanone, 2-hexanone, and 2-heptanone were also detected at low concentrations ($<5 \mu\text{g}/\text{m}^3$) in the horse stable (Study II). This result agrees with the findings in the laboratory, where these compounds were produced by all four of the fungal strains studied for peat or shavings or both (Study II). The emission rates of MVOCs were approximately of the same order of magnitude for all the studied fungi on various substrates in the laboratory (II). The rates agree well with those reported earlier in the laboratory studies done for *Aspergillus* species on wheat (Börjesson & Kaspersson, 1989). About 10 times higher emissions of potential MVOCs were, however, estimated to occur in the stable (Study II), possibly because of the abundance and diversity of microbial species and the variations in humidity, substrates, and other growth factors.

In the schools, the commonest individual compounds included 3-methylfuran, 3-allylanisole, 2-pentylfuran, and 2-butanone, as well as alpha pinene and limonene, which, on the other hand, may also have sources other than microbial ones (Sunesson, 1995; Goyer & Lavoie, 2001). The occurrence of 1-octen-3-ol and geosmine with a high affinity for adsorption onto materials has been regarded as an indicator of past microbial growth. Conversely, 3-methylfuran, with a low adsorption affinity, has been suggested to be an indicator of ongoing fungal growth (Wessen et al., 1995). 3-Methyl-furan was one of the most frequently reported potential MVOCs in the water-damaged schools in the present study. Thus it may also represent ongoing fungal growth in building constructions. In addition, 2-pentanone, 2-heptanone, 3-octanone, and camphene were occasionally found in the schools. Earlier, 3-methylfuran, 2-heptanone, and 3-octanone have been reported in water-damaged schools at mean concentrations of $0.02\text{--}1.8 \mu\text{g}/\text{m}^3$ (Smedje et al., 1996; Morey et al., 1997). The levels measured in the schools and horse stable (Study II) in the present studies agreed quite well with the earlier observations of the MVOC concentrations in schools. However, also higher levels of 1-octen-3-ol and 2-octen-1-ol, up to $900 \mu\text{g}/\text{m}^3$, have been reported in water-damaged schools and houses (Ström et al., 1994; Morey et al., 1997).

In the present study, the concentration of any single potential MVOC never exceeded $10 \mu\text{g}/\text{m}^3$ in the horse stable (Study II) or school buildings. However, a comparison of the composition and concentrations of potential MVOCs between the stable and schools in this study, as well as between the results in this study and those in the literature, is difficult because of the differences in the methodology used.

In conclusion, certain MVOCs are produced by several fungi on various substrates and, in principle, the detection of these compounds in the work environment may refer to possible fungal contamination. However, potential MVOCs comprised only 0.07–0.31% of the TVOC content in the horse stable and less than 9% in the schools. Therefore it would be very difficult to evaluate the stage of microbial activity and the degree of fungal contamination by analysing selected VOCs alone. In addition, the compounds (potential MVOCs) may also have several other sources, like moist materials as such, without any microbial contamination (Korpi, 2001).

A connection between the production of terpenes (monoterpenes and sesquiterpenes) and mycotoxins (trichothecenes and aflatoxin) has been presented previously (Zeringue et al., 1993; Jelen et al., 1995). This finding was supported also by the results of Study I. Furthermore, the production of ketones could be linked to ochratoxin production in *P. verrucosum* in Study I. However, it is unlikely that the presence of toxigenic fungi could be recognized on the basis of the VOC profiles in field conditions in that none of the reported terpenes or ketones are specific only for microbial metabolism; instead they may have other possible sources in the environment (e.g., construction materials, wood, cleaning chemicals, or foodstuffs) (Namiesnik et al., 1992; Sollinger et al., 1994; Sunesson, 1995; Etkin, 1996).

6.2 Fungal exposure assessment by determining various fungal agents

6.2.1 Fungal concentration and flora

Generally, the concentrations of airborne culturable fungi (10^2 – 10^7 cfu/m³) and total spores (10^3 – 10^7 spores/m³) were at the same level, and the fungal composition on the farms (Study III) and in the horse stable resembled that reported earlier for agricultural environments (Lacey & Lacey, 1964; Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Heikkilä et al., 1990; Malmberg et al., 1993; Dutkiewicz et al., 1994; Hanhela et al., 1995; Louhelainen et al., 1997; Eduard et al., 2001). The peak fungal concentrations, being even 1000-fold higher than the background levels, were detected during the milling of grain and the feeding of animals. Such high fungal concentrations (10^4 – 10^6 cfu/m³) in these tasks have also been observed in previous studies, in which the evaluation of farmers' exposure was based on sampling at stationary sites (Pasanen et al., 1989; Hanhela et al., 1995; Beard et al., 1996), as also was the case in the present study. Fungal spores were found to be distributed rather evenly within the farm buildings. Consequently, stationary samples probably reflect worker's exposure well. However, comparisons between stationary and personal samples would have been required for a more reliable comprehension of workers' exposure. None of the agricultural workers studied (Studies II and III) used respiratory protection. The workers were exposed to the concentrations measured for several hours a day. Thus there is a risk of adverse health effects (e.g., allergic alveolitis and ODTD) in such work (Kotimaa et al., 1984; Lacey & Crook, 1988; Malmberg & Rask-Andersen, 1993; Malmberg et al., 1993; Weber et al., 1993; Rask-Andersen, 1995; Vogelzang et al., 1999). Preventive actions, like preventing settled dust from becoming airborne, better quality of animal feed, personal protection during short-time critical tasks (e.g., milling of grain, apportioning of feed), would help to decrease airborne fungal levels.

In the agricultural environments, the fungal concentrations were expected to be high during several tasks lasting at least 20 minutes. The CAMNEA method (Palmgren et al., 1986) was chosen for determining the airborne fungal concentrations because it allows long sampling times and the counting of total spores in addition to determining culturable fungal propagules. Only small differences in species composition has been reported, when fungi were collected by a six-stage impactor and the CAMNEA method and the results of fungal flora were compared with the methods (Karlsson & Malmberg, 1989; Rautiala et al., 1995). In addition, it was assumed that identifying the dominant fungal genera was sufficient for an adequate impression of the fungal exposure. When the composition of fungi were compared between the air and material samples, the same fungal genera were found in both types of samples (Studies II and III). The choice of growth media has a significant impact on the recovery of culturable fungi and fungal flora. Along with the use of selective medium for xerophilic fungi, DG18, *W. sebi* was detected to be very common in the agricultural environment, both in the present study (Study III, horse stable) and in the previous study of Hanhela et al. (1995), especially when dry hay was handled. Airborne culturable fungi have been previously estimated to comprise about 0.002–25% of total fungal and actinomycete spore concentrations (Lacey & Lacey, 1964; Kotimaa et al., 1984; Heikkilä et al., 1988; Karlsson & Malmberg, 1989; Pasanen et al., 1989; Heikkilä et al., 1990; Malmberg et al., 1993; Dutkiewicz et al., 1994; Hanhela et al., 1995; Flannigan, 1997; Louhelainen et al., 1997; Eduard et al., 2001), but even a wider variation (0.001–38%) was observed in the present study (Study III). Thus cultivation methods often greatly underestimate the occurrence of fungi in work environments with productive fungal sources. In addition, in highly contaminated work environments, sampling times for the impactors are short (even less than a minute), and, therefore, some other sampling techniques (e.g., the CAMNEA method) are recommended for such settings (Blomquist et al., 1984b; Eduard & Heederik, 1998). However, the detection limit is high for the CAMNEA method, even in measurements of whole workdays, and therefore the method is inappropriate for use in work environments with less contaminated indoor air, such as schools and offices.

The concentrations of airborne culturable fungi in the water-damaged school buildings (Study V) were generally low (geometric mean 132 cfu/m³, range 5–780 cfu/m³) in the present study and were in agreement with the results of previous Finnish studies (Meklin et al., 1996; Taskinen et al., 1997; Taskinen et al., 1999), but lower than those reported abroad (Levetin et al., 1995; Smedje et al., 1996; Norbäck & Smedje, 1997; Bartlett et al., 1999; Würtz et al., 1999b; Scheff et al., 2000). The levels in the schools studied were lower than those reported earlier for water-damaged homes (Nevalainen et al., 1991; Pasanen, 1992a; Pasanen et al., 1992; Reponen et al., 1992; Reponen et al., 1994; Dotterud et al., 1995; Dotterud et al., 1996). The reference values in the Finnish guidelines are especially set up for home environments (Finnish Ministry and Health and Social Affairs, 1997). In Study V, most of the airborne fungal levels, even in damaged school areas, remained below the reference values (94% of the samples < 500 cfu/m³ and 77% < 100 cfu/m³). However, the fungal levels were significantly higher (fivefold) in the damaged than in the non-damaged school areas. Thus Study V suggested that these values may not be applied to indoor environments in general, like schools. The same conclusion was previously presented concerning office environments (Lappalainen et al., 1999). Differences between dwellings and public buildings, like more efficient ventilation, larger rooms, fewer soft furnishing and plants, minor handling of foodstuffs, and the absences of pets in public buildings compared with dwellings, may result in lower airborne levels of fungi (Lehtonen et al., 1993). On the other hand, Study (V) supported the guidelines given for culturable fungal levels (> 10⁴–10⁵ cfu/g) in material

samples, fungal growth being detected in 78% of the material samples taken from damaged constructions in the schools.

Fungal genera, such as *Stachybotrys*, *Acremonium*, and *Trichoderma*, that are considered indicators of indoor fungal sources (Hyvärinen et al., 1993; Reponen et al., 1994; Samson et al., 1994; Koskinen et al., 1995) were only detected in the material and surface samples of the damaged areas, while *Penicillia* were dominant fungi in indoor air in the present and previous studies (Verhoeff et al., 1991; Pasanen, 1992a). Indoors, *Penicillia* may originate from outdoor air (Verhoeff et al., 1991; Pasanen, 1992a), but elevated concentrations of airborne *Penicillia* indicate unusual indoor sources (Hyvärinen et al., 1993). In Study V, *Penicillia* were the commonest fungi in the material samples in the worst damaged areas, and overall their concentrations in the air and materials were higher in the damaged areas than in the non-damaged ones. Therefore, concentrations of the commonest indoor air fungi, like *Penicillia*, are also valuable when conclusions are drawn about abnormal indoor fungal sources.

In addition to air sampling, fungal analyses of building materials, surfaces, or settled dust are often necessary to verify biological contamination and to evaluate fungal exposure (Hurst et al., 1997). Such a situation was also observed in Study V; the settled dust and material samples provided more information about fungal flora (20 fungal genera were identified) than the air samples (15 fungal genera) did. In the schools (Study V), *Stachybotrys* species was not detected at all in the air, but it was found in 6–29% of the material and surface samples. Consistently, on the farms (Study III), *Fusarium* species were observed in about 10% of the air samples, whereas 80% of the grain samples contained *Fusaria*. The use of a selective growth medium with high cellulose content for *Stachybotrys* and the oatmeal agar for *Fusaria* may have yielded better recovery of these fungi (Pasanen et al., 1994; Samson & van Reenen-Hoekstra, 1998; Nikulin, 1999). In addition, a longer incubation time (e.g., 14 days) and possibly a dark-light cycle (for *Fusaria*) might have improved sporulation and thus also the identification of the fungi (Samson & van Reenen-Hoekstra, 1998). Release of *Fusarium* and *Stachybotrys* spores into the air is strongly affected by ambient climatic conditions, and, as large particles, they settle rapidly from the air (von Wahl & Kersten, 1991). This characteristic could also be a reason for the low yield of these fungi in the air in Studies III and V, and it may indicate low airborne exposure levels of these fungi.

Unlike in work environments with productive fungal sources, fungal sources may be difficult to identify in indoor environments (e.g., hidden water damage). Study V showed that a well-trained building inspector can successfully recognize water-related construction defects and possible microbial growth. A thorough inspection is also recommended as the first step in the assessment of fungal exposure (Dillon et al., 1996; ACGIH, 1999; Miller et al., 2000). However, fungal measurements are often needed, for example, if occupational diseases associated with fungi are suspected. In Study V, it was difficult to separate the water-damaged areas from normal ones with the sampling strategy used. Aggressive sampling could be the solution to revealing hidden fungal growth (Kalliokoski et al., 1999). Johanning et al. (1996) and Hodgson et al. (1998) also reported that airborne fungal levels may increase several orders of magnitude in aggressive sampling. On the other hand, such conditions do not reflect the real exposure situation.

6.2.2 Mycotoxins

Type B **trichothecenes**, especially DON and NIV, have commonly been detected in animal feed and human foodstuffs at levels below 4 mg/kg (Jelinek et al., 1989; WHO, 1990; Kim et al., 1993). In Study III, the DON concentrations in the grain samples were at a level (mostly below 0.2 mg/kg) similar to that previously reported for Finnish grain and feed (Karppanen et al., 1985; Rizzo, 1993). Even though a trichothecene risk exists especially for fresh grain, trichothecene concentrations may also increase during and after drying in bins if the airflow in the bins is too low (Langseth et al., 1993). On the other hand, there is also some evidence that trichothecenes may be degraded when grain is stored at 4–5°C for several weeks or months (Scott et al., 1984; Karppanen et al., 1985). Such an occurrence was also found in Study III, in which the DON concentrations decreased in the consecutive samples taken from the same drying bin.

Airborne mycotoxins (aflatoxin concentrations of 0.04–4 800 ng/m³) have been previously reported in agricultural environments in a few studies (Burg et al., 1981, 1982; Kussak, 1995; Ghosh et al., 1997; Selim et al., 1998). Study III showed, for the first time in Finland, that low DON concentrations can be detected during the milling of grain. Low airborne DON levels (below 20 ng/m³) were observed in two air samples collected on two farms. The grain samples taken from these farms contained *Fusaria* and DON (below 2.3 mg/kg), but the fungal spore levels of both the grain (10⁴ cfu/g) and air (<10⁵ cfu/m³) were not particularly high. Thus the occurrence of mycotoxins cannot be predicted reliably merely on the basis of fungal analyses. Study III supported the hypothesis that airborne mycotoxins can be detected in agricultural environments and farmers may be exposed to low mycotoxins through inhalation.

Biological tests are advisable for screening for general toxicity in environmental samples, because they are cheaper, faster, and simpler than laborious chemical analyses for mycotoxins (Hanelt et al., 1994). Cytotoxicity and skin irritation tests (WHO, 1990; Babich & Borenfreund, 1991; Nikulin et al., 1996) are rather sensitive to trichothecenes with high acute toxicity, such as T-2-toxin (LD₅₀ 10.5 mg/kg). However, their sensitivity is much lower to toxins like DON (LD₅₀ 46.0 mg/kg) and NIV (LD₅₀ 38.9 mg/kg), the acute toxicity of which is low (WHO, 1990; Babich & Borenfreund, 1991; Nikulin et al., 1996). This circumstance was also detected in Study III. The cytotoxicity test (FL cell test) yielded negative results for the grain samples with DON concentrations below 30 µg/kg and for all the air samples. It has been previously stated that a grain sample should contain a DON concentration of at least 100 µg/kg for a positive response in the cell toxicity test with a rabbit kidney cell line (Karppanen et al., 1985; Karppanen 1985). The yeast-cell toxicity test was much less sensitive for DON than the FL cell test was; it showed no toxicity for either the grain samples or the air samples. The insensitivity of the yeast cell test for DON has also been observed earlier (Whitehead & Flannigan, 1989).

According to Study III and previous investigations (Burg et al., 1981, 1982; Ghosh et al., 1997; Selim et al., 1998), the use of high-volume air samples (several cubic metres) enables airborne mycotoxins to be detected. The analysis of airborne mycotoxins is useful if materials contain mycotoxins or potential toxigenic fungi or if mycotoxin exposure is suspected to relate to workers' disorders. This principle is appropriate for work environments with or without productive fungal sources. Occupants always have a risk of inhalation exposure to mycotoxins if mycotoxins have been detected in building materials (Silas et al., 1987; Johanning et al., 1993; Andersson et al., 1997; Hodgson et al., 1998; Selim et al., 1998; Tuomi et al., 1999; Tuomi et al., 2000) or settled dust (Smoragiewicz et al., 1993; Richard et al., 1999; Tuomi et al., 1999).

The risk is high especially during remedial actions. However, high volume samplers are not comfortable in indoor environments because they are noisy, heavy, and may quickly clean up the ambient air of the space under investigation. Therefore, special efforts should be directed toward developing further the detection of airborne mycotoxins.

6.2.3 Microbial volatile organic compounds

The occurrence of some VOCs may refer to possible microbial sources in the environment. However, the present studies (Study II and VOCs in schools) showed that potential MVOCs occur at low levels, comprising only 0.07–9% of the TVOC concentrations. Thus it seems that MVOCs do not usually play a major role in exposure to volatile compounds. In addition, Pasanen et al. (1998) and Korpi et al. (1999) concluded that MVOCs at the concentrations detected in water-damaged buildings are unlikely to have a great influence on the prevalence of irritative symptoms among occupants.

6.2.4 Fungal immunoglobulin G antibodies

Although the criteria for the diagnosis of the farmer's lung disease include microbial specific IgG antibodies (Terho, 1986), elevated IgG levels are not directly related to the pathogenesis, but should rather be considered a biomarker of exposure to antigens (Pepys, 1973; Burrell & Rylander, 1981; Starkus et al., 1982; Richersson et al., 1989). Previous Finnish studies indicated that specific IgG antibodies against fungi and actinomycetes can also be detected in the sera of healthy farmers, although the levels are usually lower than those among farmer's lung patients (Katila & Mäntyjärvi, 1978; Ojanen et al., 1980; Katila et al., 1986; Husman et al., 1987; Rautalahti et al., 1990). This was also the case in Study IV, which showed that farmers may be exposed to *Fusarium* species and *W. sebi* to a greater extent than expected according to previous reports based on air sampling and microbiological analyses (Karlsson & Malmberg, 1989; Eduard et al., 1990; Hanhela et al., 1995). Thus it is recommended that *W. sebi* and *Fusaria* antigens be included in the antigen panel when farmers' exposure to microorganisms is evaluated with immunoassays.

Non-specificity is a problem when immunochemical methods are based on polyclonal antibodies. Cross-reactivity has earlier been shown, for example, within different *Fusarium* species (Verma & Gangal, 1994b) and between *Fusarium*, *Aspergillus*, *Cladosporium*, and *Epicoccum* species (Hoffman et al., 1981; Karr et al., 1981; O'Neil et al., 1987; Verma & Gangal, 1994b). Even though only a few studies on the antigenic properties of *W. sebi* have been published, cross-reactivity has already been detected between *W. sebi*, *F. solani*, *E. nigrum*, and *A. fumigatus* (Hoffman et al., 1981; Sakamoto et al., 1989). Similarity between the distributions of the relative IgG antibody levels in Study IV referred to possible cross-reactivity between *F. culmorum* and *F. avenaceum*. On the other hand, it is also possible that the farmers were exposed to various *Fusarium* species.

Some differences in the IgG antibody levels against three of the tested fungi were observed between the data determined with the ELISA and ELIFA methods. However, a direct comparison was not possible because the absorbance data on the control group analysed with the ELIFA were log-normally distributed, while the distribution was not log-normal for the ELISA data. This difference particularly affected the results of the relative IgG antibody levels and hampered the interpretation of the results. The ELIFA method is an active technique in which antigen and antibody are forced near each other during the filtration, whereas, in the ELISA method, they can freely catch each other under immobilized conditions. This difference in the binding process of antibodies with antigens might be a reason for the differently distributed data between the methods.

The determination of fungal-specific IgG antibodies has proved to be useful in the assessment of heavy exposure to fungi on the group level (Eduard et al., 1992; Eduard, 1995). However, the interpretation of individual results is problematic because of a high variation in the antibody production among humans (Eduard, 1995; Reiman et al., 1998). IgG antibody measurements have also been applied to moisture- and mould-damaged buildings as a biomarker of long-term fungal exposure. It has been concluded that environmental monitoring of fungi gives information on the building at the time of the measurements, whereas IgG antibodies reflect long-term integrated exposure in the total environment, including the workplace and the home (Hyvärinen et al., 1999). Because airborne fungal levels are not distinctly higher in water-damaged buildings than in the living environment in general, IgG measurements may not necessarily reveal abnormal fungal exposure in water-damaged buildings (Taskinen, 2001).

6.3 Discussion on fungal exposure assessment in the work environment

Study V indicated that visual inspection of damage in buildings, the detection of odours, and moisture measurements of surfaces enable priorities to be set for repair and remedial measures in water-damaged buildings. This inspection is also recommended as the first step for fungal exposure assessments in water-damaged buildings (Dillon et al., 1996; ACGIH, 1999; Miller et al., 2000). The inspection of the damage of constructions also aids the choice of appropriate sites for fungal measurements of air, material, and settled dust samples. The knowledge of both counts and flora are valuable, if culturable methods are used. Fungal exposure assessments based merely on the measurements of airborne culturable fungi would underestimate the real exposure to fungi and other agents associated with them. Therefore, both environmental monitoring and building inspections are needed. Worker's exposure to fungi should always be determined with measurable methods when an occupational disease is suspected, even if the damage is extensive and fungal contamination is obvious in a visual inspection.

The identification of fungal species is often important in the exposure assessment. The knowledge of associations of some fungal species with certain diseases and occupational environments is increasing continuously. Although thousands of fungal species are found in nature, less than 100 species are responsible for all human diseases caused by fungi (Glazer & Rose, 2002). Woodworkers exposed via inhalation to wood dust contaminated with *Alternaria* species may develop hypersensitivity pneumonitis, asthma, or allergic rhinitis. Several *Aspergillus* species are associated with hypersensitivity diseases (e.g., extrinsic allergic alveolitis, allergic bronchopulmonary aspergillosis, baker's asthma, and allergic sinusitis), infections (invasive aspergillosis and aspergilloma), mycotoxicosis, and aflatoxin-induced liver cancer. Farmers, sawmill workers, waste handling workers, and greenhouse workers, among many other groups, may be exposed to *Aspergillus* species. *Cladosporium* species may cause asthma, allergic rhinoconjunctivitis, and hypersensitivity pneumonitis. Agricultural workers and occupants of water-damaged buildings are at risk of exposure to *Cladosporium*. (Glazer & Rose, 2002) Many opportunistic fungi may cause infections in the environments in which patients with low resisting power are treated, like in hospitals. In such environments, fungal species must always be carefully identified (Anderson et al., 1996; Fridkin et al., 1996; Willinger & Aspöck, 1998). There are several other examples.

Recently differences in induction of cytotoxicity have been reported between different fungal species often detected in mouldy buildings (Murtoniemi et al., 2001), which may emphasize the role of fungal identification in the future. However, so far dose-response relationships are mainly unknown for fungal agents and health outcomes, although peak values have been suggested to be important in the development of ODS (Malmberg et al., 1993). Furthermore, exposure levels of airborne fungi exceeding 10^6 cfu/m³ is reported to relate to high prevalence of respiratory symptoms and allergic alveolitis (Kotimaa et al., 1984; Lacey & Crook, 1988; Eduard, 1993; Malmberg & Rask-Andersen, 1993; Malmberg et al., 1993; Weber et al., 1993; Rask-Andersen, 1995; Vogelzang et al., 1999). Therefore, in addition to flora, the fungal counts are important to determine in the fungal exposure assessment.

Fungal sources and critical work tasks regarding fungal exposure are usually easy to recognise in working environments with productive fungal sources. However, more attention should be paid to sampling strategies and representative measurements. Fungal concentrations during both a background period and peak concentrations during critical workphases are usually measured. Study III indicated that analyses of culturable and non-culturable fungal spores, as well as, in some cases, mycotoxins, are valuable for environments with productive fungal sources in order to assess fungal exposure in the agricultural environment. On the other hand, the prevalence of selected VOCs (potential MVOCs) was not associated with certain fungal species, and the presence of mycotoxins was not connected with airborne fungal concentrations in the present studies (II, III, V). In addition, results of IgG antibody levels and microbiological measurements from environmental samples may lead to different conclusions about fungal exposure. Therefore, none of the methods discussed in this thesis are, by themselves, adequate for fungal exposure assessment.

At present, it is not clear which fungal components should be measured in the assessment of fungal exposure. Several reviews have concluded that assessing fungal exposure using only cultivation methods is not satisfactory because culturable fungi comprise only a small portion of the total or potentially allergenic or toxigenic fungal units in the air (Crook & Sherwood-Higham, 1997; Flannigan, 1997; Eduard & Heederik, 1998; Pasanen, 2001). Some interesting new collection methods for fungi have been recently presented. The biosampler, a modified impinger, enables long-term sampling (Willeke et al., 1998; Lin et al., 1999; Lin et al., 2000). Collection into liquid provides good recovery of different fungal species (Willeke et al., 1998; Lin et al., 1999; Lin et al., 2000). The personal nasal air sampler, a sampler to be worn inside the nostrils of a subject, was developed to assess personal exposure. It is designed to catch inhalable particles larger than a cut-off diameter of about 5 μm . However, it has also been used to determine individual exposure to ambient allergens, and also analyses of fungal agents other than spore counts are possible (Graham et al., 2000). Molecular techniques may complement current methods and provide noteworthy alternatives for detecting certain fungi. The development of methods to detect fungal allergens in environmental samples has recently advanced quickly (Bush et al., 1999; Hemmann et al., 1999; Pasanen, 2001). However, the determination of fungal allergens is only a small part of fungal exposure assessment because allergic diseases associated with fungal exposure are believed to be a minor problem (Reijula, 1996). More data on possible relationships between glucans and symptoms and diseases related to exposures in indoor air are also expected in the future (Rylander, 1998, 1999b; Rylander & Lin, 2000). In conclusion, it seems more likely that no single method is appropriate for assessing fungal exposure; multiple methods are required.

VII CONCLUSIONS

A

Several volatile compounds, like 1-octen-3-ol, 2-heptanone, 2-hexanone, and 3-octanone, mainly originate from fungal metabolism but are not specific for certain fungal species, genera, or growth conditions (e.g., substrate). Some relationships exist between the production of trichothecenes and volatile terpenes and also between the formation of ochratoxins and volatile ketones.

B

Some potential MVOCs (e.g., 2-heptanone and 3-octanone) can occur in work environments (e.g., in water-damaged buildings and agriculture). However, reliable evaluation of a stage of microbial activity or the existence of abnormal fungal growth or toxigenic fungi in work environments is not possible on the basis of VOC analyses because VOCs regarded as potential MVOCs may also have other sources and their concentrations are very low when compared with those of other volatile compounds. Although workers may be exposed to VOCs of microbial origin in their work environments, exposure levels are very low, and, therefore, potential MVOCs have a minor role in fungal exposure assessment.

C

Airborne trichothecenes (low DON concentrations, ng/m³) were detected for the first time in a Finnish agricultural environment in this study, though the occurrence of mycotoxins, for example, in Finnish grain material samples has earlier been reported many times. The finding proves that where material contaminated with mycotoxin is being handled there is the potential for it to be made airborne and potentially to be inhaled by exposed workers. However, the quantities present in the air may be below the threshold of sensitivity for assays such as cytotoxicity tests. Therefore, high volume samples are recommended to collect sufficient airborne material for detection and chemical methods need to be developed to improve sensitivity of detection. Overall, the cytotoxicity tests chosen for these assays were of limited value; greater sensitivity may have been achieved with an FL cell line (even in analysis of material samples). The yeast-cell toxicity test proved to be of no value in the analysis of mycotoxins because the detection limit was very high. Although measuring airborne mycotoxins is difficult, efforts to detect them are recommended to increase knowledge on exposure levels and possible health effects, where materials containing mycotoxins are being handled. It is equally important to consider the potential for chronic symptoms as well as recognised occupational disease.

D

Farmers are generally exposed to *W. sebi* and *Fusarium* species. Significantly high IgG antibodies to *W. sebi* and *Fusarium* were observed in farmer's lung patients. Thus *W. sebi* and *Fusarium* species should be included as a part of the methodology (e.g., antigen panels for antibody measurements) when the fungal exposure of farmers is being estimated. The preliminary studies of ELIFA and ELISA did not prove clear advantages or disadvantages for either of the methods; more comparative data are needed.

E

A visual inspection of damaged areas in buildings linked with moisture measurements from surfaces and constructions is usually an adequate measure with which to prioritize remedial actions, and fungal analyses are not necessary. The assessment of fungal exposure in indoor environments should be based both on inspections of a building and on fungal measurements. Cultivation methods may underestimate airborne fungal levels, but they are still useful for identifying fungal composition.

According to the methods studied in this thesis, it is advisable to measure culturable and non-culturable (total) fungi, IgG antibody levels, and, in some cases, mycotoxins when workers' exposure to fungi is being assessed in environments with productive fungal sources, like agriculture. None of these methods is adequate alone for thorough fungal exposure assessment. These methods are, however, difficult to apply to indoor environments, and therefore the development of new methods for indoor investigations should be emphasized. The independence of culturability and long sampling times with good recovery for fungi are essential aspects in the development work.

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