

# **Biological Agents in Indoor Environments**

## **Assessment of Health Risks**

Work conducted by a WHO Expert Group between 2000-2003

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## Preface

The earliest publication of WHO addressed mycotoxins in No.11 of the Environmental Health Criteria Series in 1979, updated for selected mycotoxins in 1990 in Environmental Health Criteria No. 105. In 1990 WHO also published a report on a WHO meeting entitled 'Indoor air quality: biological contaminants'.

After the publication of the second edition of the 'Air Quality Guidelines for Europe' and the first globally applicable 'Guidelines for Air Quality' in 2000 the idea emerged to develop guidelines or guidance for biological agents in the indoor environment. After initial deliberations between WHO and various experts in January 2000 eight background papers on the health aspects of exposure to fungi, mould spores, mycotoxins, endotoxins, house dust mite allergens, and microbial volatile organic compounds were commissioned, including also interactions between biological agents and air pollutants and practical considerations in remediation of infected houses. These background papers were discussed in a meeting of the WHO Steering Committee which was convened back to back to the Healthy Building 2000 conference, in Espoo, Finland on 4 August 2000. A report of the meeting was published in late 2000.

As a primary objective of a guidance document the Steering Committee identified the provision of information to decision makers and building operators on biological agents present in non-manufacturing indoor environments. The focus of the document was envisaged to be on the:

- a) Protection of building occupants from potential adverse health effects from biological agents; and
- b) Provision of advice to decision makers and building operators on management of biological agents in indoor environments.

It was the consensus of the Steering Committee that mould spores (funga; fungi); bacteria; mites; animal allergens; cockroach allergens; pollen; and microbial toxins including endotoxins, MVOCs, etc., metabolites and fragments should be considered in terms of evaluation of exposure; health risk evaluation; recommended guideline value; relation to building moisture; and practical aspects of management and control.

The Steering Committee agreed that the guideline document should recognise the limits of current science and provide, for many biological agents, only general qualitative advice, with minimal numerical exposure-response relationships and health-based guideline values. There was also consensus that practical management of biological agents in indoor air is an important issue worldwide. The 'guidelines' were envisaged to provide general advice on management of biological agents based on experience mainly in developed countries, but the general principles should hopefully be applicable for buildings world-wide including those in developing countries. The 'guidelines' were also envisaged to clarify research needs and emphasise the need to harmonise biological agents indicators in future epidemiological studies.

The Steering Committee also finalised the outline of the guideline document, identified the contributors and reviewers of the various chapters, and recommended the participation of important agencies in the United States and Canada.

The first draft of the 'guideline' document was completed mid of 2001 and sent to the reviewers. After receiving the reviewers' comments WHO convened an expert meeting in Berlin, 22-26 April 2002, to which all contributing experts were invited.

This meeting was convened although it was recognized that not all the required or desired data were yet available. The meeting was held in collaboration with the United States Environmental Protection Agency, Washington DC and the National Institute for Occupational Health and Safety, Morgantown, WV.

In this meeting the objective of the Expert Group was to consider the state of knowledge about exposure-response relationships of and the potential derivation of guideline values for biological agents in the indoor environment, with respect to the impact of these agents on the health and well-being of building occupants. It was felt, however, that there was only rudimentary information available on the quantitative exposure-response relationships for biological agents, which would not allow the derivation of appropriate guideline values to protect the whole population or at least the most susceptible groups from the impact of biological agents in the indoor environment. The document summarized the knowledge in the area of biological agents indoors as of 2002, described the principles of their assessment and control, and also presented a list of recommendations that can be drawn on the basis of the current understanding.

Editorial meetings were held during the Indoor Air 2002 conference, 1-4 July 2002, Monterey and in a meeting in Sosnowiec, 5-8 January 2003.

The 'guideline' document was intended to provide a clear stand of WHO with respect to the biological agents issue and a meaningful procedure for exposure and epidemiological studies which would be of value to the international scientific community. Harmonisation of biological agent indicators and monitoring and instrumental procedures would be beneficial for world-wide inter-comparison of study results and optimal spending of spare funds. However, the work of the Steering Committee proved that deriving to a set of recommendations acceptable to the whole group was a very challenging task, considering the many gaps in knowledge still available in this field. This was causing delays in finalization of some of the document's sections.

In the mean time, the development of health-based guidelines for indoor air quality was recommended to WHO by the working group for Global Update of WHO Guidelines for Air Quality, in 2005. The planning meeting for this task, organized in Bonn in October 2006, recommended addressing indoor air quality issues under three sub categories of which one was dampness and mould in indoor air, and was closely related to the document in progress.

At that point, it was decided that the parts of the document on biological factors in indoor air which were completed, including the chapters that provided a general introduction to biological agents in indoor environments, and those that discussed the health effects of biological agents, as well as assessment methods and interactions of biological agents with other air pollutants, would be useful supplementary material to the guidelines on dampness and mould. Professors Lidia Morawska and Aino Nevalainen (members of the Steering Committee for the document) were entrusted a role of bringing the chapters together, ensuring that the literature references were up to date and that all the necessary editorial corrections were conducted. This document is a product of this work, and it is made available as a technical report supplementing scientific evidence for WHO guidelines on dampness and mould.

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## Acronyms for Biological Agents

ABPA	Allergic Bronchopulmonary Aspergillosis
ACGIH	American Conference of Governmental Industrial Hygienists
Ag-3	Allergen of <i>Aspergillus fumigatus</i> , nowadays Asp f 1
Ag-10	Allergen of <i>Aspergillus fumigatus</i>
Ag-40	Allergen of <i>Aspergillus fumigatus</i>
AGI-30	All-Glass Impinger
ASHRAE	American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc. (Atlanta, GA)
ASTM	American Society for Testing and Materials
A <sub>w</sub>	Water activity
BA	Biological Agent
BAP	Biological Aerosol Particles
BCYE	Buffered Charcoal Yeasts Extract
Bet v 1	Allergen of <i>Betula verrucosa</i> , the major allergen of birch pollen
BHR	Histamine challenge test
Bla g 1	Allergen of <i>Blattella germanica</i> , cockroach allergen
Bla g 2	Allergen of <i>Blattella germanica</i> , the major allergen of cockroach
Bla g 4	Allergen of <i>Blattella germanica</i> , cockroach allergen
BRI	Building Related Illness
BRS	Building Related Symptoms
BSA	Bovine serum albumin
BW	Body Weight
Can f 1	Allergen of <i>Canis familiaris</i> , the major allergen of dog
CAMNEA	Collection of Airborne Micro-Organisms of Nuclepore Filters, Estimation and Analyses
CCIE	Crossed Radioelectrophoresis
CD	Cluster Determinant or Cluster of Differentiation
CDC	Center for Disease Control and Prevention
CEN	European Committee for Standardization
CFU	Colony Forming Unit
Cla h 1	Allergen of <i>Cladosporium herbarum</i>
Cla h 2	Allergen of <i>Cladosporium herbarum</i>
CMD	Cyclopedic Medical Dictionary
CNS	Central Nervous System
CO	Carbon Monoxide
CO <sub>2</sub>	Carbon Dioxide
COPD	Chronic Obstructive Pulmonary Disease
CRIE	Crossed Radioimmunoelectrophoresis
D <sub>50</sub>	Cut-off size 50%
Der p 1	Allergen of <i>Dermatophagoides pteronyssinus</i> , the major allergen of the house dust mite species
Der p 2	Allergen of <i>Dermatophagoides pteronyssinus</i>
Der f 1	Allergen of <i>Dermatophagoides farinae</i> , the major allergen of the house dust mite species
Der f 2	Allergen of <i>Dermatophagoides farinae</i>
Der f 3	Allergen of <i>Dermatophagoides farinae</i>
Der m 2	Allergen of <i>Dermatophagoides microceras</i>

DG 18	Dichloran 18% glycerol agar
DNA	Deoxyribonucleic acid
DNPH	2,4, Dinitrophenylhydrazine
DON	Deoxynivalenol
EAA	Extrinsic Allergic Alveolitis
ECA	European Concerted Action
ECRHS	European Community Respiratory Health Survey
EHC	Environmental Health Criteria
EIA	Enzyme-linked Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
EMPAT	Environmental Microbiology Proficiency Analytical Testing
EQA	External Quality Assessment
EQC	External Quality Control
ERH	Equilibrium Relative Humidity
ETS	Environmental Tobacco Smoke
EU	Endotoxin Unit
Fel d 1	Allergen of <i>Felis domesticus</i> , the major allergen of cat
FEF <sub>25-75</sub>	Forced Expiratory Flow – interquartile range
FEP	Free Erythrocyte Protoporphyrin
FEV <sub>1</sub>	Forced Expiratory Volume in first second of expiration
FVC	Forced Vital Capacity
GC	Gas Chromatography
GC-FID	Gas Chromatography with Flame Ionization Detector
GC-MS	Gas Chromatography with Mass Selective Detector
HC	Hydrocarbons
HEPA	High Efficiency Particulate Air
HP	Hypersensitivity Pneumonitis
HPLC	High Pressure Liquid Chromatography
HPLC-DAD	High Pressure Liquid Chromatography - Diode Array Detection
HSE	Health and Safety Executive
HVAC	Heating, Ventilation and Air Conditioning System
IARC	International Agency for Research on Cancer IgE
IAQ	Indoor Air Quality
IGAP	International Global Aerosol Program
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgG4	Immunoglobulin G, subclass 4
IICRC	Institute of Inspection Cleaning & Restoration Certification
IL-1	Interleukin-1
IL-1 $\beta$	Interleukin-1 $\beta$
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
I/O	Indoor/Outdoor
IPM	Integrated Pest Management
IQC	Internal Quality Control
IRM	Intranasal Air Sampler
ISIAQ	International Society of Indoor Air Quality
ISO	International Standards Organization

IU	International Unit for allergen extracts
KU/L	Kilounits per litre
LAL	Limulus Amebocyte Lysate Test
LD50	Lethal Dose 50%, median lethal dose
LOAEL	Lowest-Observed-Adverse-Effect-Level
LOEL	Lowest-Observed-Effect-Level MDF
LPS	Lipopolysaccharide
MAb	Monoclonal antibody
MCBS	Multiple Chemical and Biological Sensitivity
MDF	Medium Density Fibre board
MEA	Malt Extract Agar
MPO	Myeloperoxidase
µm	micrometer
µg	microgram
MVOC	Microbial Volatile Organic Compound
MZ	Monozygotic
N6	Six-stage cascade impactor
N-95	A type of respirator
NAS	National Academy of Science
NIOSH	National Institute of Occupational Safe and Health
NO	Nitrogen monoxide
NO <sub>2</sub>	Nitrogen dioxide
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NYC	New York City
O <sub>3</sub>	Ozone
ODTS	Organic Dust Toxic Syndrome
OEL	Occupational Exposure Limit
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PEF	Peak Expiratory Flow
PEFR	Peak Expiratory Flow Rate
PM	Particulate Matter with no regard to size of particles
PM <sub>10</sub>	Concentration of particles with aerodynamic particle diameters of less than 10 micrometers.
PM <sub>2.5</sub>	Concentration of particles with aerodynamic particle diameters of less than 2.5 micrometers.
PPE	Personal Protective Equipment
QA/QC	Quality Assurance/Quality Control
QAP	Quality Assurance Programme
RAST	Radioallergosorbent test
RCS	Reuter Centrifugal Sampler
RH	Relative Humidity
RIA	Radioimmunoassay
RIE	Radioimmunoelectrophoresis
RLV	Residential Limit Value
RNA	Ribonucleic acid
SAS	Surface Air Sampler
SBS	Sick Building Syndrome
SIGN	Scottish Intercollegiate Guidelines



SO <sub>2</sub>	Sulfur dioxide
SOPM	Standard Operating Procedure Manual
SPM	Soot Particle Mixture
SPT	Skin Prick Test
SQ	Standard Quality Unit for pollen allergens
T-2	T-2 toxin, a Fusarium toxin
Th1	Helper T 1 lymphocyte
Th2	Helper T 2 lymphocyte
TSA	Tryptone Soya Agar
TC	Tolerable concentration
TI	Tolerable intake
TNF- $\alpha$	Tumor necrosis factor-alpha
TVOC	Total Volatile Organic Compounds
TYGA	Tryptone Yeast extract Glucose Agar
UFFI	Urea Formaldehyde Foam Insulation
ULPA	Ultra Low Penetrating Air
UR	Unit Risk
USEPA	US Environmental Protection Agency
V 8	V8-juice agar
VOC	Volatile Organic Compounds
WHO	World Health Organization
UV	Ultraviolet

## Glossary

Absorption affinity	A tendency of a mixture of gases or vapours to be bound or absorbed on to solid surfaces (Penguin 1990).
Absolute humidity	The amount of water vapour actually present in unit quantity of a gas, generally expressed as mass of water vapour per unit volume of gas + water vapour
Acaricide	A group of pesticides to kill house dust mites.
Actinomycetes	Actinomycetaceae: A genus of bacteria that contain gram-positive staining filaments. These bacteria produce both mycelium and spores and can cause various diseases in humans.
Actinobacteria	A group of Gram-positive bacteria with high G+C ratio.
Activation of complement	The process that stimulates non-functional white blood cells to assume their role in the immune response.
Adverse effect	Change in morphology, physiology, growth, development or life span of an organism exposed to air pollution, which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences (WHO 1994).
Aeroallergen	Allergen suspended in air.
Aerosol	A suspension in a gaseous medium of solid particles, liquid particles or solid and liquid particles having a negligible falling velocity (ISO 1994).
Aflatoxin	Aflatoxin is a mycotoxin produced by <i>Aspergillus flavus</i> , <i>A. parasiticus</i> and some uncommon species. It is found on maize and groundnuts as well as a wide variety of stored products. It is an IARC class 1 human carcinogen and among the most potent genotoxic carcinogens known. There is a JECFA tolerable daily intake based on a considerable toxicology database. Excess exposure occurs in countries where people consume a large percentage of their calories as groundnuts or sometimes maize.
Agar	a substance from certain red algae used to make culture media into gels which micro-organisms can liquefy (Ainsworth & Bosby 1995).

Air exchange rate	Volume of air in a room that is exchanged during a time span.
Airway obstruction	Partial or complete blockage of the breathing tubes to the lungs. Obstruction of the airway can be due to different causes including foreign bodies, allergic reactions, infections, anatomical abnormalities and trauma.
Airway permeability	Capability of allowing the passage of air through the natural passageway for air to and from the lungs (CMD 1997).
Airway responsiveness	Bronchoconstrictor response to a wide variety of non-specific and allergic stimuli. Airway hyper-responsiveness is an exaggerated bronchoconstrictor response to these agents (see bronchial hyper-responsiveness).
Algae	Nonparasitic plants without roots, stems or leaves; they contain chlorophyll and vary in size from microscopic forms to moss, living in moist places. (CMD 1997).
Alkaline	pH >7
Allergen	Any substance that causes manifestations of allergy. Among common allergens are inhalants, foods, drugs, infectious agents, contactants, and physical agents (CMD 1997).
Allergenic	Producing allergy (CMD 1997)
Allergic	Pertaining to, sensitive to, or caused by an allergen (CMD 1997).
Allergic alveolitis	Inflammation of the alveoli, caused by an allergen (CMD 1997).
Allergic bronchopulmonary	Inflammation of the bronchioles and surrounding interstitial tissue in patients with atopic or inherited asthma exposed to <i>Aspergillus fumigatus</i> (CMD 1997).
Aspergillosis	A wide variety of diseases caused by fungi of the genus <i>Aspergillus</i> .
Allergy	An acquired, abnormal immune response to a substance that does not normally cause a reaction (CMD 1997).
Alveolar	Pertaining to an alveolus (CMD 1997).
Alveolus	Air sac of the lungs (CMD 1997).

Amoeba	A minute, one-celled protozoan of the genera <i>Amoeba</i> , etc found in soil and water (CMD 1997).
Antibody	Any oft he complex of glycoproteins produced by B lymphocytes in response to the presence of an antigen (CMD 1997).
Antigen	A protein to which antibodies are raised but not implicated in a disease.
Antigenic	Capable of causing the production of an antibody (CMD 1997).
Arthropod	A member of invertebrate animals marked by bilateral symmetry; a hard; jointed exoskeleton, segmented bodies; and jointed paired appendages (CMD 1997).
Ascomycetes	One of the two large groupings of higher fungi in which the sexual spores are produced and contained within an ascus or sac. Most fungi historically classified as Deuteromycetes are the asexual forms of Ascomycetes.
Aspergillosis	Aspergillus infection in the tissues or on any mucous surface (bronchi, lungs, aural canal, skin, membranes of the eye, nose, or urethra); marked by inflammatory granulomatous lesions (CMD 1997).
Aspergillus	A genus of Ascomycetes fungi, including several mould species, some of which are pathogenic (CMD 1997).
Asthma	A disease caused by increased responsiveness of the tracheobronchial tree to various stimuli, which results in paroxysmal constriction of the bronchial airways (CMD 1997). Also see paroxysm.
Atherogenic	Pertaining to the formation of degenerated or thickened walls of the larger arteries, marked by cholesterol-lipid-calcium deposits (CMD 1997).
Atopic	Pertaining to atopy.
Atopy	A type I hypersensitivity or allergic reaction, for which there is genetic predisposition (CMD 1997).
Attributable risk	In population health, the percentage of a disease burden assigned to a given exposure.
Bacteria	A group of microorganisms all of which lack a distinct nuclear membrane and have a cell wall of unique

	composition. Most bacteria are unicellular; the cells may be spherical (coccus), rod-shaped (bacillus), spiral (Spirillum), comma-shaped (Vibrio) or corkscrew-shaped (Spirochaete).
B-cell	Immunoglobuline producing cell
Basidiomycetes	One of the four major classes of true fungi of the division Eumycetes, which includes toadstools, rusts, smuts, mushrooms, and tree fungi. This type of fungus is distinguished by sexual spores (basidiospores) that form on a specialized structure. B. cause plant diseases, and their toxins may be lethal to humans when eaten. Basidiospores have been implicated as a cause of allergic asthma (CMD 1997; MWMD 1995).
Beta-glucan, $\beta$ -glucan	$\beta(1,3)$ -glucans are glucose polymers with variable molecular weight and degree of branching that may appear in various conformations i.e. triple helix, single helix or random coil structures of which the triple helix appears to be the predominant form (Williams, 1997).
Biliary	Pertaining to a thick, viscid, bitter-tasting fluid, secreted by the liver (CMD 1997).
Birth cohort study	Method of analytical epidemiology; groups of newborns classified on the basis of the presence or absence of exposure to a particular factor and then followed for a specific period of time to determine the development of disease in each exposure group.
Bioactive compound	Compound affecting living tissues (CMD 1997).
Bioaerosol	Suspended particle of biological origin.
Bioassay	The determination of the strength of a drug or substance by comparing its effect on a live animal or an isolated organ preparation with that of a standard preparation (Ref).
Biocide	A substance, esp. a pesticide or an antibiotic that kills living organisms (CMD 1997).
Biodegradable	Capable of being broken down into innocuous products by the action of living things (such as microorganisms).
Biodeterioration	The process of being broken down into innocuous products by the action of living things.

Biofilm	A structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface (CMD 1997).
Biomarker	Any parameter that can be used to measure an interaction between a biological system and an environment agent, which may be chemical, physical or biological (WHO 1993).
Biomass	The mass (weight) of cells of an organism living or dead.
Biotic	Of or relating to life (Webster 1994).
Box method	A box is being placed for 3-6 months at a height of 1.5-2.5 m in the room for sampling of dust.
Bronchi	The two main branches leading from the trachea to the lungs, providing a passageway for air (CMD 1997).
Bronchial hyper-responsiveness	A state characterised by easily triggered bronchospasm (contraction of the bronchioles or small airways).
Bronchiectasis	Chronic dilatation of a bronchus or bronchi, with a secondary infection that usually involves the lower portion of the lungs (CMD 1997).
Bronchiole	One of the smaller subdivisions of the bronchial tube (CMD 1997).
Bronchiolitis	Inflammation of the bronchioles (CMD 1997).
Bronchitis	Inflammation of the mucous membrane of the bronchial airways (CMD 1997)
Bronchoalveolar lavage	The removal of secretion, cells and proteins from the lower respiratory tract by insertion of a sterile, saline solution in the lung through a fiberoptic bronchoscope (CMD 1997).
Bronchoconstriction	Constriction of the bronchial tubes (CMD 1997).
Bronchodilator	A drug that expands the bronchial tubes by relaxing bronchial muscle (CMD 1997).
Bronchoscopic lavage	Ssee 'Bronchoalveolar lavage'
Bronchoscopy	Examination of the bronchi through a bronchoscope (an endoscope designe to pass through the trachea for visual inspection of the tracheobronchial tree (CMD 1997).

Brownian diffusion	Brownian diffusion i.e. molecular diffusion, which is independent of any convection associated the air itself. Small particles in still or smooth airflows are seen to move randomly due to collisions with the surface of the particles of gas molecules, which are in thermal motion. (Mark D 1998)
Building Envelope	The building envelope is collectively the external shell of a building including the exterior wall system, fenestration/windows, roof, and below ground/grade surfaces in contact with the soil.
Building related illness	Illness related to indoor exposures to biological agents (e.g. fungi, bacteria), biological and chemical substances (e.g. endotoxins, mycotoxins, radon, carbon monoxide, formaldehyde) which is experienced by some people working or living in a particular building and it does not disappear after leaving it.
By-product	Or Side-product. A compound formed during a chemical reaction or a metabolic pathway at the same time as the main product (Oxford 1996)
Capillary movement	The movement of a liquid along the surface of a solid caused by the attraction of molecules of the liquid to the molecules of the solid.
Carbonyl compounds	The $C = O$ group including aldehydes and ketones. (Penguin 1990)
Carbohydrate	One of a group of chemical substances, including sugars, glycogens, starches, dextrans, and celluloses, that contain only carbon, oxygen, and hydrogen (CMD 1997).
Carcinogen	Any substance or agent that produces cancer or increases the risk for developing cancer in humans and animals (CMD 1997).
Carcinogenicity	The production of cancer, equivalent to carcinogenesis (CMD 1997)
Cardiovascular	Pertaining to the heart and blood vessels (CMD 1997).
CD4	A protein on the surface of CD8 cells that normally helps the body's immune system combat disease (CMD 1997).
CD8	A type of white blood cell that is involved in fighting certain types of infection (CMD 1997).

CD14	A Glycosylphosphatidyl-anchored glycoprotein (encoded on chromosome 5), CD14 is now recognized to serve as a receptor for the LPS- “LPS-binding protein” complexes.
CD4/CD8 ratio	An indicator of the overall level of immune suppression or damage done by HIV. The lower the CD4/CD8 ratio, the worse the damage. The CD4/CD8 ratio is rarely less than 1.0 in HIV negative individuals.
CD14 receptor	Glycosylphosphatidyl-anchored glycoprotein (encoded on chromosome 5), CD14 is now recognized to serve as a receptor for the LPS- “LPS-binding protein” complexes.
Cell lysis	Destruction of cells, e.g. Hemolysis (hemo-lysis) is the destruction of red blood cells with the release of hemoglobin
Cell-mediated type IV allergic reaction	Delayed reaction, in which the symptoms appear hours, or even days, after contact with the substance.
Chemical marker	Specific chemical only present in the agent, which can be used for determination of the agent.
Chemotype	Chemotype is a strain of a toxigenic fungus that produces a distinct pattern of secondary metabolites [see secondary metabolites]. OR Strain of a fungal species that produces a distinct pattern of secondary metabolites different from an other group(s) of the species [see secondary metabolites].
Chronic bronchitis	Bronchitis marked by increased mucus secretion by the tracheobronchial tree. The productive cough is present for at least three months of two consecutive years (CMD 1997).
Chronic obstructive pulmonary disease (COPD)	A disease process that decreases the ability of the lungs to perform ventilation. Diagnostic criteria include a history of persistent dyspnea on exertion, with or without chronic cough, and less than half of normal predicted maximum breathing capacity. Diseases that cause this condition are chronic bronchitis, pulmonary emphysema, chronic asthma, and chronic bronchiolitis (CMD 1997).
Citrinin	A toxin isolated from <i>Penicillium citrinum</i> , <i>P. verrucosum</i> , <i>P. expansum</i> and <i>Monascus ruber</i> . It is



	nephrotoxic with relatively low potency and is immunomodulatory in mice.
Coarse particles	Particles with mean aerodynamic diameters > 2.5 µm.
Cockroach	A common insect of the order Orthoptera that infests homes, food-handling and storage places (CMD 1997).
Colony forming unit	Unit of growth of microorganisms in a culture; usually considered to have grown from a single organism (CMD 1997).
Commissioning	The formal plan describing the design, operation, and maintenance of a building. Commissioning may involve plans for drying the building in the case of a flood or decontaminating a building after a sewage backflow.
Condensation	The change of the physical state of aggregation (or simply state) of matter from gaseous phase into liquid phase.
Confounder	An extraneous factor changing the magnitude or direction in epidemiological studies of the association between two variables. For example, smoking in epidemiological studies is often a confounder for the association between e.g. microbial exposure and airway symptoms.
Conidium (plural conidia)	An asexual spore of mold (Hyphomycetes), produced on the external surface of mycelium, not in a sporangium.
Conjunctivitis	Inflammation of the mucous membrane that lines the eyelids and is reflected into the eyeballs (CMD 1997).
Contagious bacterium	A bacterium that easily transmits a disease from host to host by casual cutaneous contact or respiratory droplets (adapted from CMD 1997).
Control sample	A standard against which observations or conclusions may be checked to establish their validity (adapted from CMD 1997).
Corynebacteria	A family of bacteria, which are rod shaped, gram positive, and nonmotile. The most important is <i>Corynebacterium diphtheriae</i> , pathogenic in humans (CMD 1997).
Culture medium/plate	Medium with nutrients where microorganisms can grown, usually solidified with agar (a carbon hydrate)

Cumulative effect	Effect, increasing by successive additional exposures; the total effect is usually greater than the sum of all the additions (adapted from CMD 1997).
Cumulative exposure	Sequence of successive exposures.
Cumulative rate of sensitivity	Increase per unit time of sensitivity.
Cytotoxicity	The quality of being toxic to cells.
Dehumidification	Reduction of humidity.
Deoxynivalenol	Deoxynivalenol is a trichothecene [sesquiterpene-derived molecule] mycotoxin produced by <i>Fusarium graminearum</i> and <i>F. culmorum</i> . Deoxynivalenol is a potent neurotoxin and immunotoxin and there is a JECFA tolerable daily intake based on a considerable toxicology database. The toxin is common in wheat and maize and a large percentage of the world population has excess exposure.
Derivatization	Chemical modification of a component to stabilise or make a specific component easier to measure.
Dermatitis	Inflammation of the skin, evidenced by itching, redness, and various skin lesions (CMD 1997).
Der p 1/Der p 2	Allergens of house dust mites
Deuteromycetes	See 'Ascomycetes'
Diffusion	Net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion.
Disease	A pathological condition of the body that presents a group of clinical signs, symptoms, and laboratory findings peculiar to it and setting the condition apart as an abnormal entity differing from other normal or pathological condition (CMD 1997).
DNA adduct	A piece of DNA covalently bonded to a (cancer-causing) chemical.
Dose-response relationship	Describes the change in effect on an organism caused by differing levels of exposure (or doses) to a stressor (usually a chemical or biological substance).

Dust	Small solid particles, conventionally taken as those particles below 75 µm in diameter, which settle out under their own weight but which may remain suspended for some time (ISO 1994). National standards may be more specific and include particle diameters or a definition in terms of a sieve of specified aperture. Dust occurs in the atmosphere both naturally and as a result of the activities of man (Willeke 1993).
Eczema	A form of dermatitis or inflammation of the epidermis.
Effect	Change in morphology, physiology, growth, development of life span of an organism exposed to air pollution. It might be either an adverse effect or an alteration, which is not distinguishable from the range of a target variable observed in not exposed organisms of the same species (WHO 1994c).
Endotoxigenesis	Diseased condition resulting from endotoxin poisoning
Endotoxin	A lipopolysaccharide (linkage of molecules of lipids with polysaccharides) that is part of the cell wall of gram-negative bacteria (CMD 1997).
Entomophilous	For fungi - spores are distributed by insects. For pollen – see ‘Insect-pollinated’.
Environmental Tobacco Smoke (ETS)	ETS is a complex mixture of over 4000 compounds generated by the combustion of tobacco products. These include over 40 known or suspected human carcinogens, such as 4-aminobiphenyls, 2-naphthylamine, benzene, nickel, and a variety of PAH and N-nitrosamines. A number of irritants, such as ammonia, nitrogen oxides, sulphur dioxide, various aldehydes, and cardiovascular toxicants, such as carbon monoxide and nicotine are also present (WHO 2000).
Enzyme	An organic catalyst (protein) produced by living cells, but capable of acting outside cells, that changes the rate of chemical reactions without needing an external energy source of being changed themselves (CMD 1997).
Enzyme immunoassay	An assay to detect certain agents (often high molecular weight proteins) by using specific animal or human antibodies. Enzyme labelled antibodies (in combination with a suitable substrate) are used to produce the final signal (often a colour reaction)

Eosinophil	A type of granulocytic white blood cell characterised by a polymorphic nucleus and cytoplasmic granules that stain with eosin (rose-coloured dye) or other acid stains (CMD 1997).
Eosinophil infiltration	Infiltration of eosinophils (type of granulocytes with strong IgE affinity that play an important role in e.g. the immune response in allergic asthma).
Eosinophilia	An unusual number of eosinophils in the blood (CMD 1997)
Epithelial cells	The cells that form the outer surface of the body and line the body cavities and the principal tubes and passageways leading to the exterior. These cells from the secreting portion of the glands and their ducts (CMD 1997).
Equilibrium relative humidity	A term used in microbiological publications rather than water activity ( $A_w$ ). $ERH = A_w \times 100$ (in %)
Ergosterol	Ergosterol is the primary membrane sterol of higher fungi and a few species of marine algae. Ergosterol measurements are widely used as surrogates for total fungal biomass in natural substrata including in particulate air samples.
Erythrocyte	A mature red blood cell (CMD 1997).
Erythrocyte sedimentation rate	The rate at which red blood cells precipitate in a period of 1 hour.
Ethiological agents	Causal agents.
Exposure	Exposure to a chemical is the contact of that chemical with the outer boundary of the human body. The outer boundary of the human body is the skin and the openings into the body such as the mouth, the nostrils, and punctures and lesions in the skin (WHO 1999).
Exposure assessment	Quantitative or qualitative evaluation of the contact of a chemical with the outer boundary of the human body, which includes consideration of the intensity, frequency and duration of contact, the route of exposure (e.g. dermal, oral or respiratory), rates (chemical intake or uptake rates), the resulting amount that actually crosses the boundary (a dose), and the amount absorbed (internal dose) (WHO 1999).

Exposure limit	Values of exposure (concentration, duration, uptake, dose) in the indoor environment that should not be exceeded in order to protect the health of building occupants. Dietrich S
Exposure route	Exposure pathway by inhalation, ingestion and/or dermal contact.
Extrinsic allergic alveolitis	See 'Allergic alveolitis'
Fc-receptor	A protein found on the surface of certain cells, including macrophages, neutrophils, and mast cells, that contribute to the protective functions of the immune system.
Fibrotic	Marked by or pertaining to abnormal formation of fibrous tissues (CMD 1997).
Filariasis	A disease caused by the presence in the lymph vessels of the parasitic nematode worms <i>Wuchereria bancrofti</i> , transmitted by various mosquitos, and leading to inflammation and blocking of lymph vessels.
Fine particles	Particles with aerodynamic diameters between 0.1 and 2.5 micrometers.
Forced expiratory Volume (FEV)	The volume of air that can be expired after a full inspiration. The expiration is done as quickly as possible and the volume measured at precise times; at ½, 1, 2 and 3 seconds. This provides valuable information concerning the ability to expel air from the lungs (CMD 1997).
Fuminosin	Fumonisin is a mycotoxin found in maize produced by <i>Fusarium verticillioides</i> . It is a potent nephrotoxin but causes a wide array of diseases in various animals because it inhibits ceramide synthetase. It is a weak complete carcinogen but a potent cancer promoter. There is a JECFA tolerable daily intake based on a considerable toxicology database. Excess exposure occurs in countries where people consume a large percentage of their calories as maize.
Fumitremorgen	A metabolite of <i>Aspergillus fumigatus</i> and some <i>Penicillium</i> species that induces tremors in mice when injected ip.

Function	The act of carrying on or performing a special activity. Normal function is the normal action of an organ. Abnormal activity or the failure of an organ to perform its activity is the basis of disease or disease processes (CMD 1997).
Fungal volatiles	See 'Microbial volatile compounds'
Fungi	The kingdom of organisms that includes yeasts, moulds, and mushrooms. Fungi grow as single cells, as in yeast, or as multi-cellular filamentous colonies, as in moulds and mushrooms. (CMD 1997).
Genotoxic	Toxic to the genetic material in cells (CMD 1997).
Gliotoxin	A toxin produced by <i>Aspergillus fumigatus</i> which is a potent immunosuppressant. The toxin is known to be produced in vivo during the development of <i>A. fumigatus</i> -caused aspergillosis. Not known to be found in the spores of <i>A. fumigatus</i> .
Glycoproteins	Compound composed of both a protein and a carbohydrate joined by a covalent bond. Glycoproteins are common in mammalian tissues. The attached carbohydrate may have several effects: it may help the protein to fold in the proper geometry, stabilize the protein, affect physical properties such as solubility or viscosity, helps it to orient correctly in a membrane, or make it recognizable to another biochemical or cell.
Gram-negative bacteria	Losing the crystal violet stain and taking the colour of the red counterstain in Gram's method of staining bacteria (CMD 1997).
Gram-positive bacteria	Retaining the colour of the crystal violet stain in Gram's method of staining bacteria (CMD 1997).
Group 1 allergens	Acidic or basic glycoproteins found in all grasses, which elicits a specific IgE response in about 90% of grass pollen-allergic patients.
Guideline	Any kind of recommendation or guidance on the protection of human beings or receptors in the environment from the adverse effects of air pollutants. As such, it is not restricted to a numerical value but might also be expressed in a different way, for example as exposure-response information or as a unit risk estimate (WHO 1998).

Guideline value	A particular form of a guideline. It has a numerical value expressed either as a concentration in ambient air, a tolerable intake, or as a deposition level, which is linked to an averaging time (WHO 1998). In the case of human health, the guideline value defines a concentration below which the risk for the occurrence of adverse effects is negligibly low. It does, however, not guarantee the absolute exclusion of effects at concentrations at or below the guideline value.
Haemosiderosis	The accumulation of abnormal amounts of hemosiderin (an iron-storing complex) in the tissues.
Hay fever	A type I hypersensitivity reaction or allergic disease of the mucous membranes of the nose and upper air passages induced by external irritation. Causes inflammation, watery discharge from the eyes, headache, and asthmatic symptoms (adapted from CMD 1997).
Health (WHO definition)	A state of complete physical, mental and social well-being, not merely the absence of disease and infirmity (WHO 1948)
Hemosiderin-laden macrophages	Hemosiderin: An iron-containing pigment derived from hemoglobin from the disintegration of red blood cells (CMD 1997).
Hepatitis	A group of disease characterised by injury to the liver as a result of the presence of inflammatory cells in the tissue of the organ.
High efficiency particulate air (HEPA)	A term used to indicate that a filter is at least 99.97% efficient at removing particles $\geq 0.3$ micrometers from the air stream.
Histamine challenge test	Histamine is a chemical found in the body which can cause narrowing of the airways. By measuring the maximum airflow using an electronic spirometer, one can estimate how narrow the airways have become in response to various doses of Histamine.
House dust mites	A type of mite, <i>Dermatophagoides pteronyssinus</i> or <i>D. farinae</i> , that ingests shed human skin cells (CMD 1997).
Humidifier fever	A flu-like illness marked by fever, headache, chills, muscle aches, and fatigue. Often a result of exposure to various bacteria, endotoxins and fungi found in humidifier reservoirs, air-conditioners, and aquaria.

Hypersensitivity pneumonitis	Immunologically induced inflammation of the lungs of a susceptible host caused by repeated inhalation of a variety of substances including dust, moulds and other fungi from sources such as cheese, vegetables, bark of trees, detergents and contaminated humidification systems. In the acute stage, symptoms include cough, fever, chills, malaise and shortness of breath. In the subacute and chronic forms, the onset of symptoms is gradual and prolonged (CMD 1997).
Hypha (plural hyphae)	An individual fungal thread or filament of connected cells; the thread that represents the individual parts of the fungal body.
Idiopathic pulmonary hemorrhage	An abnormal, severe discharge of blood in the lungs without clear pathogenesis (adapted from CMD 1997).
Illness	The state of being sick (CMD 1997).
Immune function	Function of being protected from or resistant to a disease or infection by a pathogenic organism as a result of the development of antibodies or cell-mediated immunity (CMD 1997).
Immune dysfunction	Malfunction of the immune system.
Immune system	The lymphatic tissues, organs, and physiological processes that identify an antigen as abnormal or foreign and prevent it from harming the body.
Immune tolerance	The process by which the immune system does not attack an antigen.
Immunoassay	Measuring the protein and protein-bound molecules that are concerned with the reaction of an antigen with its specific antibody (CMD 1997). ?Determination of specific components using antibodies against that specific component.?
Immunochemical techniques	See 'Immunoassay'
Immunocompromise(d)	Having an immune system incapable of reacting to pathogens or tissue damage (CMD 1997).
Immunogenic	Capable of inducing an immune response (CMD 1997).



Immunoglobulin	One of a family of closely related though not identical proteins capable of acting as antibodies, abbreviation Ig (CMD 1997).
Immunoglobulin A	The principal immunoglobulin in external gland secretions such as respiratory and intestinal mucin (mucus glycoprotein), saliva, and tears (CMD 1997).
Immunoglobulin E	An immunoglobulin that attaches to mast cells in the respiratory and intestinal tracts and plays a major role in allergic reactions, abbreviation IgE (CMD 1997).
Immunoglobulin E-mediated allergy	An allergic reaction provoked by re-exposure to a specific type of antigen referred to as an allergen
Immunoglobulin G	The principal immunoglobulin in human serum, important in producing immunity in the infant before birth, abbreviation IgG (CMD 1997).
Immunological marker	Measurable and quantifiable biological parameters (e.g. specific enzyme concentration, specific hormone concentration, specific gene phenotype distribution in a population, presence of biological substances) which serve as indices for health- and physiology-related assessments, such as disease risk, disease diagnosis, metabolic processes, epidemiologic studies etc.
Immunomodulation	Any change in immune system parameters above or below normal values.
Immunosuppression	Involves an act that reduces the activation or efficacy of the immune system.
Incidence	The frequency of occurrence of any event or condition over a period of time and in relation to the population in which it occurs.
Inertial impaction	Aerosol particles larger than 3 $\mu\text{m}$ in a gas stream may possess sufficient inertia, so that when their trajectories intercept collecting surface, the surface retains the particles for example by adhesive forces.
Inflammation	The non-specific immune response that occurs in reaction to any type of bodily injury (CMD 1997).
Influenza	An acute, contagious respiratory infection characterized by the sudden onset of fever, chills, headache, tenderness or pain in the muscles, and sometimes absolute exhaustion (CMD 1997).

Insect-pollinated	Insect-pollinated, entomophilous. Fertilisation of the female flower happens with pollen carried by insects visiting it.
Inspirable	Capable of being inspired or drawn into the lungs.
Interleukin	Circulating proteins produced by various cells (macrophages, T-cells, epithelial cells etc) that play a major role in orchestrating various immune responses including both humoral and cellular immune responses.
Intervention	One or more actions taken in order to modify an effect (CMD 1997).
<i>In vitro</i>	An in vitro test is one done in the laboratory, usually involving isolated tissue, organ, or cell preparations.
<i>In vivo</i>	In the living body or organism.
Irritant	A substance that causes irritation or tends to produce inflammation (MWMD 1995).
Irritation/Irritative effect	A condition of irritability, soreness, roughness, or inflammation of a bodily part, e.g. airways and skin (MWMD 1995).
Ischemic	Pertaining to a local and temporary deficiency of blood supply due to obstruction of the circulation to a part (CMD 1997).
Laryngitis	Inflammation of the larynx.
Legionnaires' disease	A severe, often fatal disease characterized by pneumonia, dry cough, tenderness or pain in the muscles, and sometimes gastro-intestinal symptoms (CMD 1997).
Leukotriene	Any of a group of metabolites of arachidonic acid (an essential fatty acid, formed from unsaturated acids of plants and present in peanuts) that functions as a chemical mediator of inflammation (CMD 1997).
Limulus amebocyte lysate test	Endotoxin test for human injectable drugs (including biological products), animal injectable drugs, and medical devices.
Lipid / Lipid A	The lipid portion of endotoxin that is responsible for its toxicity by binding to lipopolysaccharide binding protein (LBP) and inducing the inflammatory cascade.

Lipopolysaccharides	Long chain endotoxin; the most common form in gram-negative bacteria.
Lower respiratory symptom	Symptom in the lower respiratory tract (i.e. the respiratory tract from trachea to bronchioles).
Lowest-observed-adverse-effect level	Lowest concentration or amount of a substance, found by observation or experiment, which causes an adverse effect (WHO 1994).
Lowest-observed-effect level	Lowest concentration or amount of a substance, found by observation or experiment, which causes an effect.
Lung biopsy	The obtaining of a representative lung tissue sample for microscopic examination, usually to establish a diagnosis.
Lung cancer	Cancer that may appear in the trachea, air sacs and other lung tubes. It may appear as an ulcer in the windpipe, as a nodule or small flattened lump, or on the surface blocking air tubes. It may extend into the lymphatic and blood vessels (CMD 1997).
Lung diffusion capacity	Determination how well oxygen passes from the air sacs of the lungs into the blood
Lung function	The working of the lung as described by spirometric parameters such as forced expiratory volume in 1 sec.
Pulmonary lavage	Washing of the lungs with repeated injections of water.
Surfactant	A surface-active agent that lowers surface tension (e.g. oils and various forms of detergents).
Lymphocyte	A cell found in blood and lymphatic tissue (CMD 1997).
Macrophage	A monocyte that has left the circulation and settled and matured in a tissue.
Macrocylic trichothecenes	Trichothecene [see deoxynivalenol] with a macrocylic (large circle) group attached to the core part of the trichothecene molecule.
Malaise	Discomfort, uneasiness, or indisposition, often indicative of infection (CMD 1997).
Mesophilic	Preferring moderate temperatures.

Metacholine test	See 'Histamine Challenge Test' (except it uses metacholine instead of histamine).
Microbial organic volatile compounds	A large group of gaseous compounds that are produced in the metabolism of micro-organisms mainly as by-products.
Mildew	Fungus growth on surfaces, causing colour and structural deviation.
Mites	See 'House dust mite'
Monoclonal antibody	A type of antibody derived from hybridoma cells (cells produced by the fusion of antibody-producing and multiple myeloma (tumor) cells).
Mononuclear cells	Cells having one nucleus.
Morbidity	The number of sick persons or cases of disease in relationship to a specific population (CMD 1997).
Morphological	Pertaining to the science of structure and form of organisms without regard of function (CMD 1997).
Mortality	The death rate; the ratio of the number of deaths to a given population (CMD 1997).
Myalgia	Tenderness or pain in the muscles; muscular rheumatism.
Mycelium	A mass of hyphae; not in the form of large spore producing parts such as mushroom.
Myeloperoxidase	An enzyme produced by neutrophils that plays a role in killing microbes.
Mycotoxicosis	Disease either caused by toxins on moulds or produced by moulds.
Mycotoxin	A toxin produced by an organism of the fungus kingdom, which includes mushrooms, molds and yeasts.
Th0	T-cells that have not yet developed into T-helper 1 (Th1) or Th2 cells.
Nasal instillation	To administer a fluid to the nasal cavity.
Neurological symptoms	Symptoms of the nervous system.
Neurotoxicity	Capable of harming nerve tissue (CMD 1997).

Neutrophil	A granular white blood cell (CMD 1997).
Neutrophilia	An increase in neutrophils (type of granulocytes that play a role in e.g. non allergic airway inflammation caused by irritants such as endotoxin)
O-antigen	Complex polysaccharides, often containing unusual monosaccharides, which occur on the surfaces of gram-negative bacteria, uniquely marking each strain.
Ochratoxin	A mycotoxin produced by <i>Penicillium verrucosum</i> and a number of species of <i>Aspergillus</i> including <i>A. ochraceous</i> and <i>A. carbonarius</i> among others. Is a potent renal toxin. There is a JECFA tolerable daily intake based on a large toxicology database. There remain questions as to whether ochratoxin is a human carcinogen.
Odds ratio	The ratio of the number of exposed persons with effects and the number of unexposed persons with the same health effects.
Organic dust toxic syndrome	A non-allergenic, non-infectious, respiratory disorder caused by inhalation of organic dusts and foreign materials contained therein, including bacteria, mould, and fungi. The most important sources are cotton dust; grain dust; and exposure to mouldy hay.
Osmotic tension	Osmotic tension refers to the concentration of solutes in solution as they affect the growth of microorganisms. Honey has very high osmotic tension and pure water has no osmotic tension.
Particle	Small discrete mass of solid or liquid matter (ISO 1994).
Particle aerodynamic diameter	Diameter of a sphere of density 1 g/cm <sup>3</sup> with the same terminal velocity due to gravitational force in calm air as the particle, under the prevailing conditions of temperature, pressure and relative humidity (ISO 1995).
Particle size distribution	The distribution of equivalent diameters of particles in a sample or the proportion of particles for which the equivalent diameter lies between defined limits (Willeke and Baron 1993).
Particulate burden	Human exposure concerning airborne particles

Passive exposure	Being subjected to an action or influence by an external agency (exposure) without being active in causing the exposure.
Pathogen	A microorganism or substance capable of producing a disease.
Pathogenesis	The origin and development of a disease.
Pathogenic	Productive of disease
Pathomechanism	The mechanism of producing a disease.
Pathophysiology	The study of how normal physiological processes are altered by disease.
Patulin	Patulin is a metabolite of several species of <i>Penicillium</i> and some other fungi of modest toxicity.
Peptidyl transferase activity	Peptidyl transferase is a key ribosomal enzyme that is one of the biochemical targets for trichothecene mycotoxins.
Personal protective equipment	Term referring to respiratory protection equipment (respirators), full-body covering (full body disposable coveralls), head covering, eye protection, gloves, and shoe covers used by workers removing visible fungal growth and other biological agent hazards.
Phagocytosis	Ingestion and digestion of bacteria and particles by cells such as leukocytes and macrophages.
Pharyngitis	Inflammation of the passageway for air from the nasal cavity to the larynx (CMD 1997).
Phylloplane fungi	Fungi that live on the surfaces of leaves, e.g. <i>Cladosporium cladosporioides</i> and <i>Alternaria alternata</i> .
Plenum	A space in a HVAC system where the pressure of the air in the enclosed space is greater than or less than that of the outdoor air. For example, the fan plenum, the cooling coil plenum, etc.
Pneumonia	An inflammation of the alveoli, interstitial tissue, and bronchioles of the lungs due to infection by bacteria, viruses, or other pathogenic organisms, or to irritation by chemicals or other agents (CMD 1997).

Pneumonitis	Inflammation of the lung, usually due to hypersensitivity (allergic) reactions to organic dust, such as wheat or other grains, or chemicals (CMD 1997).
Pollen grain	Pollen grain is the reproductive body of plants, which is produced by the male part for the purpose of fertilisation of the female flowers.
Polysaccharide	Long chain of monosaccharides (sugars) linked by glycosidic bonds
Porous	Permeable by liquid or gas.
Prevalence	The number of cases of a disease present in a specified population at a given time.
Primary metabolism	Primary metabolism refers to the basic pathways that covert sugar to energy and supply precursors from the citric acid cycle and amino acid pathways for more complex cell compounds.
Pro-inflammatory compounds	Compounds favouring inflammation.
Propagules	In mycology, propagule refers to the fact that some fungi can form new colonies from different types of spores, sclerotia [resting structures] and mycelial fragments.
Prospective study	A study where the individuals of the sample are followed up from the beginning until its termination
Protozoa	A group of microscopic single-celled organisms. Disease-causing protozoa include <i>Plasmodium</i> , <i>Leishmania</i> , and <i>Trypanosoma</i> and can cause malaria, kala-azar and sleeping sickness, respectively.
Pulmonary fibrosis	Formation of scar tissue in the connective tissue framework of the lungs folloing inflammation or pulmonary disease.
Relative Humidity	A term used to describe the amount of water vapor that exists in a gaseous mixture of air and water.
Respiration	The act of breathing during which the lungs are provided with air (inhaling) and carbon dioxide is removed (exhaling) (CMD 1997).
Respirator	A device designed to protect the wearer from inhaling harmful dusts, fumes, vapors and/or gases.

Respiratory	Pertaining to respiration (CMD 1997).
Retrospective study	A study in which the individuals of a sample or their records are investigated after they have experienced the disease or condition.
Rhinitis	Inflammation of the mucous membrane of the nose. Symptoms include nasal congestion, thin watery discharge from the nose, sneezing and itching of the nose (CMD 1997).
Rhino-conjunctivitis	Rhinitis and inflammation of the mucous membrane that lines the eyelids and is reflected onto the eyeball
Rhino-sinusitis	An inflammation of the paranasal sinuses, which may or may not be as a result of infection, from bacterial, fungal, viral, allergic or autoimmune issues
Roridin	A macrocyclic trichothecene produced by <i>Stachybotrys chartarum</i> and <i>Myrothecium roridum</i> .
Sampling	The collection of a representative portion for analysis and testing (WHO 1980). <i>Continuous sampling</i> is sampling, without interruptions, throughout an operation or for a predetermined time. <i>Grab sampling</i> or <i>spot sampling</i> is the taking of a sample in a very short time (ISO 1994).
Satratoxin	A macrocyclic trichothecene produced by <i>Stachybotrys chartarum</i> .
Secondary metabolites	As it applies to fungi, a secondary metabolite is a compound produced after one or more nutrients become limited on a cellular basis. The genetic ability to produce secondary metabolites including mycotoxins is particular to given species. Fungi that produce mycotoxins as defined here are called "toxigenic" fungi.
Sequence	The order or occurrence of a series of related events.
Settled dust	Dust settling on surfaces from the air
Scratch test	Placement of an appropriate dilution of a test material, suspected of being an allergen, in a lightly scratched area of the skin. If the material is an allergen, a wheal (a more or less round and evanescent elevation of the skin, white in the centre with a pale-red periphery, accompanied by itching) will develop within 15 minutes.



Sensitisation	A condition of being made sensitive to a specific substance (i.e. antigen) such as a protein or pollen.
Sensory irritation	A physiological reaction caused by a stimulation of trigeminal nerve endings in the mucosal membranes of the upper respiratory tract by inhaled chemicals evoking a burning sensation of the nasal passages and eyes, inducing coughing from laryngeal stimulation and inhibiting respiration (Alarie Y. CRC Critical Reviews in Toxicology 1973;2:299-363).
Serotypes	The kind of microorganism as characterized by serologic typing (testing for recognizable antigens on the surface of the microorganism).
Sick building syndrome	Specific symptoms with unspecified aetiology which are experienced by a proportion of people working or living in a particular building and disappear after leaving it.
Sinusitis	Inflammation of a cavity within a bone, especially the nasal cavities.
Skin (prick) test	Any test in which a suspected allergen or sensitizer is applied to the skin to detect an individual's sensitivity to specific allergens. The most commonly used tests are the intradermal test, the prick or puncture test, and the scratch test.
Socio-economic status	The combined social and economic level of individuals or groups. Such classification is useful in studying the relationship of income and living conditions to the prevalence and incidence of various diseases. Although socio-economic status is related to income, education, profession, or some combination of these, it is difficult to determine the relative importance of each in influencing mortality and morbidity; and to explain why socio-economic status operates to influence health.
Sorbent	Or Adsorbent. A solid medium or substance upon whose surface the process of adsorption occurs, i.e. molecules, atoms, or ions are attached to the surface of the sorbent (Penguin 1990).
Spectrophotometry	An estimation of colouring matter in a solution (CMD 1997).
Spore/Spora	A small, usually single-celled reproductive body that is highly resistant to desiccation and heat and is capable of

growing into a new organism, produced especially by certain bacteria, fungi, algae, and nonflowering plants.

Standard	A level of an air pollutant, e.g. a concentration or a deposition value, which is adopted by a regulatory authority as enforceable. Unlike a guideline value, a number of elements in addition to the effect-based level and the averaging time must be specified in the formulation of a standard. These elements include the measurement strategy, data handling procedures, statistics used to derive, from measurements, the value to be compared with the standard. The numerical value of a standard may also include the permitted number of exceedings (WHO 1998).
Sterigmatocystin	A toxin produced by a number of fungi including some <i>Chaetomium</i> species but mainly from <i>Aspergillus versicolor</i> . This compound is a precursor to aflatoxin [see aflatoxin] and is an IARC 2A carcinogen.
Susceptibility	Having little resistance to a disease or foreign agent.
Symptom	Any perceptible change in the body or its functions that indicates the kind or phases of disease (CMD 1997).
Synergistic Effect/Synergism	An action of two or more agents or organs working with each other to produce a more than additive effect.
Systemic	Pertaining to a whole body rather than to one of its parts (Synonym: somatic).
Thermal gradient	The rate of temperature change with distance.
Thermophilic	Preferring or thriving best at high temperatures.
Thoracic	Pertaining to the chest or thorax
Toxoplasmosis	A disease caused by infection with the protozoan <i>Toxoplasma gondii</i> .
Trachea	A cylindrical tube from the larynx to the primary bronchi (CMD 1997).
Ultrafine particles	Particles with aerodynamic diameters < 0.1 micrometer.
Urticaria	A vascular reaction of the skin characterized by a sudden general eruption of pale evanescent wheals or papules, which are associated with severe itching. This condition may be caused by contact with an external irritant such

	as the nettle, physical agents, foods, insect bites, serum sickness, pollens, drugs, or neurogenic factors.
Vapour barrier	Any material, typically a plastic or foil sheet, that resists diffusion of moisture through wall, ceiling and floor assemblies of buildings.
Viable organisms	An organism able to live outside a host (CMD 1997)
Vital capacity	The volume of air that can be quickly and forcibly breathed out (CMD 1997).
Volatile organic compound	A group of chemicals the boiling-point of which ranges from 50-100 °C to 240-260 °C (WHO 1989)
Water activity	The ratio of the vapour pressure of water in a substrate to the water pressure of pure water at the same temperature and pressure. It is a measure of the amount of water available for microbial growth. Water activity is expressed as a fraction $\leq 1$ .
Water content	Or moisture content. The total amount of water in a substrate, i.e. a sum of free water and water bound in different compounds and pores.
Wheeze	A continuous musical sound caused by narrowing of the space of a respiratory passageway (CMD 1997).
Wind-pollinated	Wind-pollinated, anemophilous. Fertilisation of the female flower happens with airborne pollen. Most allergologically important pollen types are carried by wind.
Wood decay fungi	Fungi that are capable of causing damage to wood such that its structural properties are affected. These include white rot fungi - species that degrade lignin and cellulose and brown rot fungi - species that degrade cellulose. Some fungi cause "soft rot" which result in limited degradation of cellulose.
Xerophilic	Fungi that grow under conditions of low water content.
Zearalenone	Zearalenone is a polyketide [derived from acetate] produced by <i>Fusarium graminearum</i> and <i>F. culmorum</i> and some other fusaria mainly on maize but also on wheat. Zearalenone is a potent estrogen and resulting in reproductive toxicity in humans and animals.

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# 1. Introduction to Biological Agents

This document summarizes the present knowledge in the area of biological agents<sup>1\*</sup> indoors and describes the principles of their assessment and control. It focuses on “non-industrial, non-agricultural, indoor built environments”. Not all environments that go into definition of “indoor space” as opposed to outdoor environments, are covered in this document because of several reasons. The following types of indoor environments were excluded:

- (1) Specialized environments for which there are either current or anticipated recommendations being developed by other authorities, for example transportation vehicles or hospitals;
- (2) Manufacturing environments, for which recommendations or regulations have been or should be developed by agencies such as the ILO (International Labour Organization), which have expertise in those areas; and
- (3) Indoor environments connected with agricultural enterprises (barns, silos, animal facilities, et cetera) because they involve a more complex and specialized range of biological exposures that cannot necessarily be prevented by the recommendations described in this document.

The document is organized as follows:

Chapter 2 introduces the basic facts of biological agents in indoor environments. The discussion includes information on indoor environment characteristics (including humidity, dampness and moisture), the type, size and sources of biological agents, as well as a description of the major groups of bioaerosols found in indoor environments.

In Chapter 3, the state of knowledge on health effects due to key biological agents, including moulds, bacteria, dust mites, pollen, cockroaches and microbial, toxins are presented. This chapter includes discussions regarding susceptibility, as well as presenting evidence from epidemiological studies.

Chapter 4 relates to the assessment of biological agents, including identifying source emissions and modelling biological agent concentrations. It also presents requirements with respect to monitoring equipment and measurement procedures, and guidance on applicable methods with respect to evaluation objectives, as well as information on quality assurance, data analysis and interpretation.

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<sup>1</sup> The use of the term “biological agent” or “biological contaminant” in this chapter refers to the presence of contaminants such as micro organisms, dust mites, cockroaches, allergens from pets etc. This chapter does not refer to deliberately introduced biological warfare agents such as those causing anthrax and smallpox.

In Chapter 5, information on the impact of air pollution on the release of biological agents to the air is provided, as well as information on the interactions between non-biological air pollutants and biological agents affect the fate of biological agents in the air. Results from clinical observations are also summarized, which are a valuable source of information from the non-epidemiological perspective.

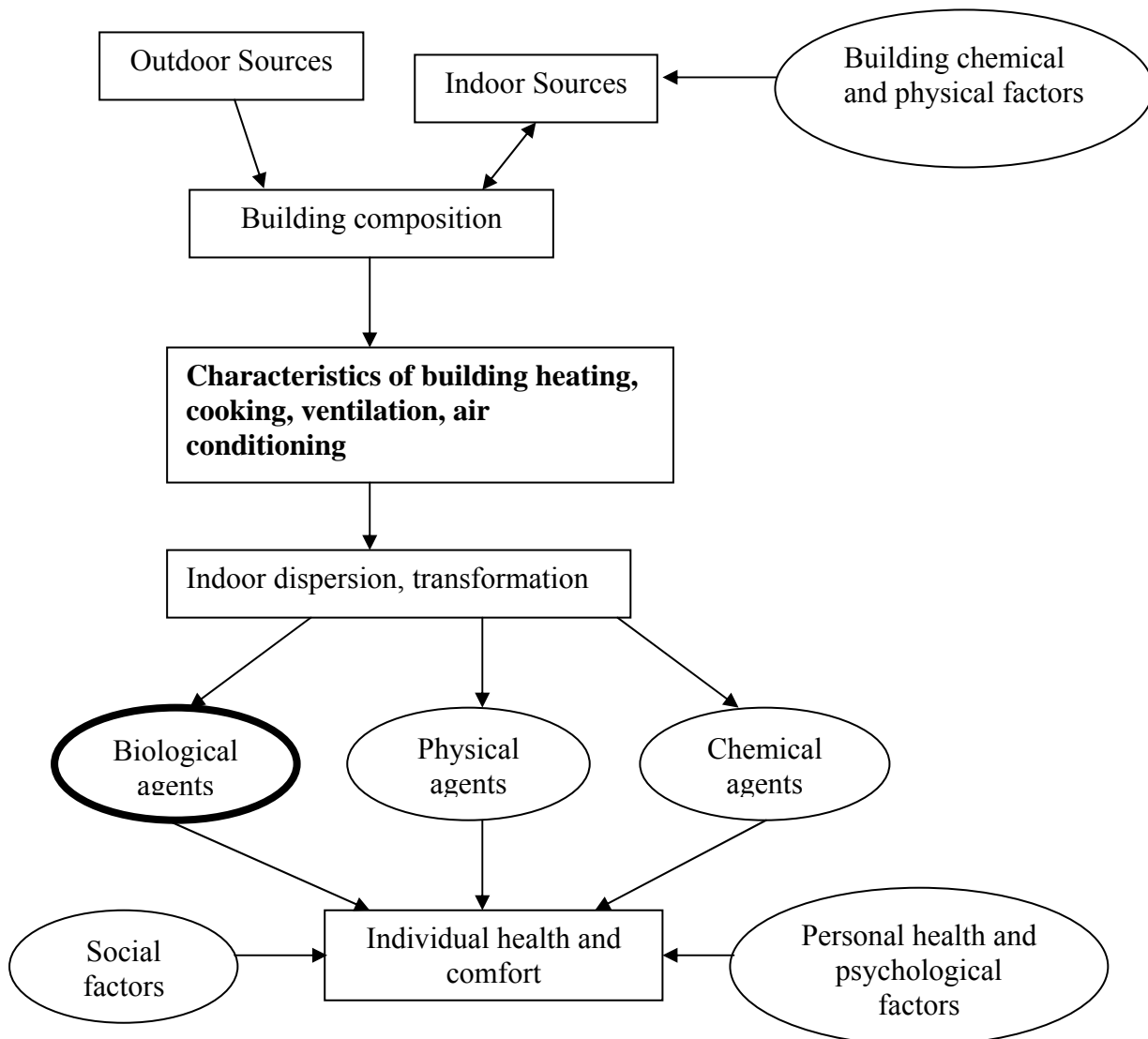
Finally, Chapter 6 presents information for health care professionals with respect to the potential health effects from biological agents in the indoor environment, as well as providing overall conclusions and recommendations.

## 2. Biological Agents in Indoor Environments

### 2.1 Building and Indoor Environment Characteristics

People spend a remarkable part of their time indoors, and hence the quality of the indoor environment contributes to their environmental health conditions. Several factors determine the quality of the indoor environment and its influence on human health. These factors include building-related aspects including climate, culture, social values and technical issues. Different physical, chemical and biological agents affect the quality of the indoor environment and the health and comfort of the building occupants. The interplay of these factors is presented in Figure 2.1.

Figure 2.1 The role of biological agents in the indoor environment.



The indoor environment as depicted in Figure 2.1 is very complex, and the various elements indeed have effects on each other. Not all the factors and the interactions of different phenomena are well known. There is a substantial amount of knowledge on physical and chemical agents and their health effects in indoor environments (e.g. WHO 2000a; b; Maroni et al., 1995). The role of biological agents is less clearly understood. This is partly due to their complex nature which leads to substantial methodological difficulties in exposure assessment. However, the biological agents of indoor environments are associated with a number of health effects among the occupants although the exact causal relationships are poorly known for many of them.

A specific characteristics to many biological agents is the fact that, being living organisms, they are able to increase their concentrations indoors. Thus, even if the original source of a microorganism or other biological agent would be outdoors, it may find an amplification site in the indoor environment and thus create an indoor source of its spores, metabolic products and other pollutants into the indoor air. The diversity of the microbial species and other biological agents in indoor environments is large. However, apart from some exceptions, such as house dust mites or pets as sources of allergens, the relative importance to health of individual biological agents in indoor environments is still to be resolved.

Biological agents are a heterogeneous group of particles or chemicals of microbial, animal or plant origin. They have numerous sources, some of which can be considered normal, temporary sources and part of the everyday use of the building (e.g., Flannigan 2001; Lehtonen et al., 1993), and some of which are clearly harmful and therefore need to be specifically controlled (ACGIH 1999). There are always some types of biological particles present in the indoor environment. For example, many bacteria that belong to the normal microflora of the human skin are continuously emitted into the immediate surroundings of humans (Noble, 1976).

Biological contaminants occur as particles or chemicals, and they share many characteristics with the non-biological particle and chemical pollutants of the indoor air. However, they also differ from non-biological pollutants by their sources, amplification, emission characteristics, by their effects on humans and their control strategies. Therefore it is important to focus in this document on the biological part of indoor environment pollution separately, and provide the criteria for good indoor air quality taking into account the specific features of these pollutants.

## **2.2 Humidity, dampness and moisture in buildings**

Biological contamination of indoor environments is essentially regulated by humidity and moisture conditions, i.e., by different forms of water. Water is used in practically all buildings. In dwellings, water is used daily in cooking and washing. Running water is present in the plumbing and sewage network, and liquid water is also present in the drip pans of air cooling system and in various humidification systems. Water may also end up onto the interior surfaces or into the building structures in an undesired way, either via leakage of liquid water or through condensation of water vapour. This may cause damage to the materials and support microbial growth on surfaces, often seen as



visible mould. Excess humidity or moisture can also contribute to development of sources of allergens such as dust mites or cockroaches.

Water present in vapour form in the air is described as humidity. The indoor air humidity is mainly regulated by the outdoor conditions, but there also are intramural sources of humidity, mainly the occupants and their activities, such as washing and cooking (Maroni et al., 1995). The humidity of air is expressed with the term “relative humidity” (RH). Relative humidity is the percentage of the water vapour present in the air of the quantity of water vapour, which would saturate at the temperature in question. Thus, the amount of water vapour that air can hold depends on the temperature. Cold air can hold much less water vapour than warm or hot air. For indoor conditions, in practice, this means that when cold intake air is heated into the human comfort range, its relative humidity may drastically decrease, and when warm and humid outdoor air is conditioned to this range, considerable amounts of water will be removed from the air.

The range of relative humidities that is perceived comfortable by human beings is around 25-60% as specified by design conditions in air-conditioned buildings. For humid climates, a relative humidity of 70% is commonly considered acceptable. Above the comfort range of the given temperature, the air is perceived as damp. Dampness may lead to condensation on surfaces or structures. Dampness of indoor air may be a result of weather conditions, of insufficient ventilation that fails to remove the moisture produced by the occupants, or of faults in the building structures, such as plumbing failures or poorly insulated details. Serious dampness and moisture problems may also result from acute incidents such as floods, fire fighting and hurricanes.

The presence of water is a critical factor for most biological contaminants, regulating their survival and proliferation. The other essential factors for the growth or amplification of biological agents are nutrition and temperature. For the growth of microbes, i.e., fungi and bacteria, the nutrient and temperature needs are usually met anywhere in the building. House dust and building materials provide enough nutrition (Cruz, 2002; Percival et al. 2000), and temperatures over freezing point are usually sufficient for environmental microorganisms. Although many man-made building materials such as concrete or ceramic materials are poor sources of nutrients, this does not prevent the growth once the material has been wetted. In fact, many microbial habitats in nature have very low concentrations of nutrients (Fry, 1990), and nutrient limitations may rather select the organisms that tolerate the conditions in question than retard microbial growth (Gooday, 1988). The growth may well take place even if optimal growth conditions are not met. The growth may be slow in temperatures lower than the optimal growth temperature, but taking into account the lifespan of a building, microbial communities have plenty of time to develop, once the moisture requirements are met. From the microbiological point of view, buildings are not natural amplification sites for microorganisms but rather one of the extreme environments where, once the moisture control of the building fails, a number of environmental microbes may find new ecological niches to be filled. Microbial growth is usually the first phase of biological deterioration processes, which tend to proceed from the most simple organisms into more complex ecosystems. Thus, what seems to be visible mold, may in fact include both various fungi and bacteria, protozoa, nematodes and insects (Yli-Pirilä et al., 2004).

House dust mites are dependent of the ambient humidity, since they can only amplify at humidities >50% RH (van Strien et al., 1994). They use e.g. human skin scales as nutrition, and proliferate in mattresses, bedding, upholstery, carpets and other such microenvironments. At relative humidities <70%, they feed sparingly and produce little faecal material and associated allergen (Arlian, 1999). While house dust mites are typically associated with bedrooms and living rooms, cockroaches are typical contaminants of bathrooms and kitchens. They are often found in environments where food is handled but where the sanitation practices are inadequate (Rose, 1999). In contrast to mites, cockroaches tolerate low ambient humidity and are able to actively search for water.

In case there is a technical fault that allows water accumulation into materials or structures, one may use the term moisture or water damage. This may be due to leaks in roof, windows or plumbing; inadequate insulation that leads to condensation; misplaced vapour barriers, rising damp from soil or other such fault in the moisture control of the building (Lstiburek and Carmody, 1994). Acute events such as floods or fire fighting may moisten the structures thoroughly. Constant or repeating moistening of materials may lead to microbial growth, chemical deterioration and undesired increase of dampness in the immediate surroundings of the damage, which in part increases the risk of many types of other biological contamination, such as rot fungi, insects and mites. Although the general phenomenon of moisture damage in a material and associated microbial growth is well known and often easily recognized as damp spots and visible mold, the microbial ecology of building materials is so far poorly understood compared to many natural microbial habitats. However, different building materials have different characteristics in the way they become microbially contaminated (Hyvärinen et al., 2002). Thus, they may also show different characteristics as sources of indoor pollution, and eventually, human exposure to the pollutants. The factors that regulate the growth of organisms on different materials, as well as the microbial communities and interactions involved are an area of future research.

There is an extensive literature describing adverse health effects associated with dampness and moisture damage of a building. The association is well shown and extensively reviewed by e.g., Peat et al. (1998) and Bornehag et al. (2001, 2004), see also Chapter 3 of this document. The phenomenon of indoor dampness has already previously been recognized as a cause of adverse health effects by WHO working groups (WHO 1988,1990). However, the causal relationships between the exposing biological agents and the observed adverse health effects, as well as the mechanisms of most health effects are still insufficiently known. Although the details of the chain of events from dampness or moisture damage to harmful exposures and health effects are not yet well known, it is a general consensus that a healthy building should not have problems with dampness or moisture control (IOM 2000, IOM 2004). Therefore, prevention of undesired biological contamination is essentially based on good moisture control of the indoor environment and the structures of the building.

## 2.3 Biological agents and their sources in indoor environments

Biological agents are defined as agents or micro-fragments from plant or animal matter or from microorganisms. Whole microorganisms that are either viable or dead are also included in this definition. Airborne particulate matter of biological origin is often referred to collectively as bioaerosol. Biological agents include filamentous fungi and yeasts, fungal spores and allergens, bacteria and bacterial spores, microbial toxins and other secondary metabolites; biologically active components of microbial cells (e.g. bacterial endotoxin and peptidoglycans; fungal  $\beta(1,3)$ -glucans), pollens and their allergens, mites and their allergens, pet and insect allergens, microbial extracellular polysaccharides and volatile organic compounds (MVOCs), algae, protozoa, helminthes and viruses (Wanner et al. 1993). Biological agents are a large and heterogeneous group of contaminants. They can be classified in many ways, e.g., according to the health effects they may cause, according to the types of organisms they are representing, or by the sources from which they originate.

Many bacteria, fungi, viruses and protozoa may be infectious to humans, i.e., they may cause a specific infectious disease. Infectious agents are usually transmitted from human to human, but in some cases, they may be transmitted from animals; this transmission called as zoonoses, or from environmental sources, such as contaminated water. Although infectious agents are included in the definition of biological agents given above, they will not be covered in this report, with the exception of *Legionella* bacteria. The reasons for excluding the infectious agents are: agents causing infectious diseases have rarely any indoor sources other than the occupants or their pets, and the focus of this report is on the indoor environment; risk assessment for these agents is very different from other biological agents, particularly for the indoor environment; and only limited experience is available about their behaviour in indoor environments.

The *Legionella* species that cause legionellosis (*Legionnaires'* disease and Pontiac fever) are an exception among the infectious agents described above, since these bacteria are not transmitted from human to human. Instead, the agents of legionellosis and legionellosis epidemics are in most cases associated with indoor sources (Fields et al. 2002).

Allergens are a group of agents that may cause a specific IgE-mediated reaction in humans. Thus, this grouping is based on the type of effect. Biological agents that have allergenic properties may originate from plant, animal or microbial material. Plant fragments, pollen and their fragments are known allergens of outdoor air that may also be transported into the indoor environment. Other agents include allergenic material from animals, such as cat, dog, birds, rodents, and other pets; house dust mites; vermin such as mice, rats and cockroaches. Allergenic fungi include species of e.g., *Cladosporium* and *Alternaria*. Some of the allergens are well known and defined for their sources and occurrence indoors, for example mite allergens Der p 1 and Der f 1 (IOM, 2000). Specific sensitization of building occupants to such agents can be clinically verified.

Besides allergenic properties, biological agents may show other kinds of potential health effects. Fungal spores and bacterial cells have inflammatory and cytotoxic properties (Huttunen et al., 2003). Among the cellular components of microbes, bacterial endotoxins that are cell wall components of gram negative bacteria and

fungal 1,3- $\beta$ -glucans that are present in all fungal cells, have inflammatory properties (see Chapter 3) which differs from allergenic action. Microbes and their structural components can have both outdoor and indoor sources. For bacteria, humans are an important source in indoor environments.

Both fungi and bacteria produce metabolic products while growing; among such products are volatile metabolites and secondary metabolites, many of which have toxic properties. These products are resulting from microbial growth, the most important indoor sources are the amplification sites of the microorganisms, i.e., moist, damp or wet sites within the building.

Other biological agents possibly present in indoor environment are parasites, amoebae and other protozoa, nematodes, algae, insects and any biological debris or organic dust. The indoor exposures and health importance of these agents have not, however, been studied, and thus their specific relevance as exposing biological agents in indoor environments cannot be assessed.

Most biological agents originate from both outdoor sources and a variety of indoor sources, and the relative importance or interactions of outdoor and indoor exposures has not been well characterized. Indoor sources of biological agents include both the building and its occupants and their activities. The building envelope and structures, the systems of the building function such as plumbing and HVAC system, the furniture and decoration as well as the users of the building and their activities may act as sources of biological agents. Table 2.1 shows the most important sources of the essential biological agents.

Table 2.1. Main sources of the most important biological agents in indoor environments. (The assessed source strength of each source of an agent is marked with four-class scale: 0 = not-significant source; x low source strength; xx moderate source strength; xxx high source strength).

Source	Bacteria	Fungi	Plant/pollen fragments	Non-fungal & non-pollen allergen	Other
Outdoor	xx	xxx	xxx	x	x
Humans	xxx	0	0	0	x
Microbial growth on moist or flood-damaged materials, or in HVAC systems	xx	xxx	0	x	x
Microbial growth in humidifier water	xx	xx	0	0	x
Pets	xx*	x**	0	xxx	x**
House plants	x	x	x	0	x
House dust	x	x	x	xxx	x

\*Endotoxin – dogs; \*\* Carriers?

Any biological agent can be perceived as contamination of the indoor environment, that is, as an undesired presence of the agent. However, no environment is sterile or free of biological agents unless vigorously kept so, as in specific clean rooms for pharmaceutical or high-tech production facilities. The presence of many biological agents in low levels can be regarded as a common and “normal” phenomenon. Problems may arise when their levels are increased due to the indoor amplification of the agent, or due to their accumulation because of failure in the basic removal processes such as cleaning and ventilation. Management procedures may then become necessary, see Chapter 6.

## **2.4 Aspects of the exposure assessment to biological agents in the indoor environment**

Exposure is defined by as contact over time and space of a biological, chemical or physical agent with the outer part of the human body, such as the skin, mouth or nostrils (WHO 2000). Quantitative assessment of exposure to environmental agents is usually a challenging task, but it is an important part of a health risk assessment. Exposure assessment is especially complicated for biological agents, which are not single compounds but complex mixtures of molecules with various biological activities. Some of the factors contributing to the complexity of the exposure assessment of biological agents are discussed in the following section. For description of the sampling and analysis methods and techniques, see Chapter 4.

Apart from the infectious agents, the effects of which are specific infections, or allergens, which may cause a specific immunoglobulin E-mediated reaction, the health effects of many biological indoor pollutants are non-specific in their nature. The causative relationships between the biological agents and the health effects are not sufficiently known, nor are the interactions between the different agents. The exposures to biological agents in indoor environments consist of simultaneous exposure to a large number of agents, many of them occurring in extremely low concentrations. All these exposing agents may interplay and contribute to the individual's health and perceived well-being. For example, non-specific irritation and stress symptoms may be caused by irritating chemicals in an individual previously sensitized to pollen (Jantunen, 2001). Due to the diversity and complexity of the biological agents, and the insufficiently known causal relationships with health, the exposure assessment focused solely on an individual component may not reflect all relevant aspects of exposure.

All the assessment methods are selective for their ability to express the exposure to biological agents both qualitatively and quantitatively. Sampling methods vary for their effectiveness to collect particles of various sizes, thus being selective for the particle size distribution of the collected sample. Depending on the collecting techniques, various agents may be affected for their viability or biological activity. There may also be technical limitations with the length of the sampling time, thus resulting in insufficient coverage of the timely variation of the agent. The detection and analysis phase is also selective. For example, counting the total number of fungal

spores can be made with a microscope, but only a part of fungi can be identified based on the appearance of spores. Better identification capacity is obtained by culturable methods, where fungal spores or fragments are cultured into colonies that are more easily identifiable. However, also culture media are selective, and thus the culturing methods detect only a minor part of airborne fungi. Microbial products such as volatile organic compounds (MVOC) or microbial toxins are detected using methods of chemical analysis, allergens are determined with immunochemical methods, and endotoxins and glucans with a bioassay. Thus, no single sampling and analysis method can cover the whole range of biological agents indoors.

The concentrations of biological agents in indoor air vary greatly in time and space even within the same indoor environment (Hunter et al., 1988, Nevalainen et al., 1992; Flannigan 2001; Hyvärinen et al., 2001). Outdoor sources and weather conditions have an important impact on indoor concentration, as well as the ventilation system of the building, the use of the building and occupant activities (Flannigan, 2001). Geographic and climatic differences have a major impact on the outdoor concentrations (Mullins, 2001). All of these factors affect the distribution of concentrations of biological agents in the indoor environments. Thus, exposure assessment should always include a large enough number of samples or a time-integrated sample to cover these variations. Personal exposure to biological particles has been shown to be higher than the levels measured with stationary sampling; probably caused by the resuspension of settled dust due to the movements of the individual (Toivola et al., 2002). Thus, stationary measurements of an agent may not accurately reflect the actual exposure of an individual.

Exposure assessments should be relevant to the health outcome of interest, and take into account the various sources of biological agents in relation to individual contact and activity. The methods for sampling and analyses have not been standardized, and a variety of methods are in current use. Since different sampling and analysis methods actually capture and detect agents of different size distributions, different quantities and qualities, the results obtained with different methods are not directly comparable. Thus, the exposure data obtained with different methods can only be compared with caution (ACGIH 1999; Baron and Willeke 2001). The many limitations of the present assessment methodology compromise the possibilities to determine quantitative guidelines for the biological agents. Methods to sample and analyse biological agents in indoor environments are described in more detail in Chapter 4.

Even though the knowledge of the essential factors involved in the environmental exposure assessment as part of risk assessment is far from complete, assessment and quantifying the biological agents must be done with the means available. The reported studies have usually not been designed to show the comprehensive distributions of the concentrations, but in most studies, the data is from a limited number of short time samples. As discussed above, the values are strongly associated with the method used. Many studies report total counts of fungal spores or bacteria without information of the microbial species. Such an approach may be useful in identification of an intramural source, i.e., a microbial amplification site in the building (ACGIH, 1999, Miller 2001; Hyvärinen, 2002). However, there is little evidence in the literature that the total counts of airborne fungi alone would correlate with any health effects (Verhoeff and Burge 1997; ACGIH 1999; Hyvärinen et al., 2001). Thus, sampling

and assessment of “total” microbial counts without further identification of the microbial flora may be of limited use in the risk assessment of biological agents.

The information about the health relevance of the airborne concentration of any single fungal type is equally scarce, but the identification of the fungal types in the samples is usually essential to interpret the findings. The mycoflora of indoor and outdoor environments have a large diversity, influenced by climatic, geographical and many other factors. Therefore, interpretation of fungal data should always be done with sufficient knowledge on the conditions of the sampling site and time (Dillon et al., 1996; ACGIH, 1999).

## **2.5 Description of the major groups of biological agents in indoor environments**

In the following section, each major group of biological agents in indoor environments is given a concise description, followed by an exposure evaluation within the framework of the limitations in the present knowledge and methodology in exposure assessment as discussed above.

### **2.5.1 Fungi in indoor environments**

The building-associated fungi consist of filamentous microfungi (moulds) and yeasts. The most common fungal genera occurring in indoor environments are *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria* and yeasts (Miller et al. 1988, Hyvärinen et al 1993, Verhoeff et al 1994b, Pasanen, 1992 Kuo et al., 1994). These genera are also the most frequently occurring fungi in outdoor air, which is usually a major source of building-related fungi (Miller, 1992). The total concentrations of fungi are usually higher outdoors than indoors. Outdoor concentrations have a strong seasonal variation, resulting in lower concentrations in winter than in frost-free periods. This has an impact on indoor air fungi as well. The phenomenon is especially notable in cold climates (Mullins, 2001; ACGIH, 1999; Reponen et al. 1992).

The concentrations of fungi in indoor environments show significant spatial and temporal variation (Hyvärinen et al. 2001). Spatial variation inside the buildings is caused e.g., by local sources of biological agents in certain parts of the building, but also varying activities and varying ventilation rates in different rooms may contribute to this variation. Temporal variation probably results from variation in air exchange rates, which affects the rate of removal of biological agents. The source strength in each location is also affected by the activities of the occupants, by vibrations of the building frame and other such factors.

While indoor fungi have normal sources such as outdoor air, most concern has been focused on cases where fungi may grow and amplify in indoor environments. This phenomenon, generally referred as mold growth, results from moistening of materials and structures, caused by different technical reasons as discussed in section 2.2. Spores of fungi are present everywhere, and they are able to germinate wherever there

is water available. As discussed above (section 2.2), the nutritional needs and environmental conditions that are needed to enable microbial growth, are usually met in buildings, once there is moisture available. The most xerophilic (dryness-tolerant) fungi are able to germinate once the moisture content of the material exceeds the water activity ( $a_w$ ) level 0.7, which corresponds to relative humidity of air RH 70% in equilibrium situation. Along increasing moisture level, more fungal types are able to grow, and as a result, a visibly moldy material usually contains a number of fungal genera and species.

The concentrations of fungi and bacteria in indoor air vary and are affected by many factors, such as climate and season, building type and use, and intramural sources. Examples of reported concentrations of viable indoor fungi from various regions are listed in the Tables 2.2 and 2.3. Table 2.2 shows examples of concentrations that have been observed in domestic environments, and Table 2.3 those from public buildings. The reported studies vary in their aims, design and methodology, as well as the way the results are reported and therefore, the results between different studies cannot be directly compared. As a general rule, the concentrations of viable fungi in indoor environments have a range from  $10^1$  to  $10^4$  CFU/m<sup>3</sup> (colony forming units per cubic meter of air), the variation being a result of the factors discussed above (section 2.4). Outdoor concentrations may exceed these levels by 1-2 orders of magnitude (Mullins, 2001).



Table 2.2 Examples of airborne fungi and bacteria concentrations in domestic environments from different regions as reported in the literature. Due to various study designs, the home characteristics vary from study to study.

Country	Characteristics	Total Bacteria (CFU/m <sup>3</sup> )	Total fungi (CFU/m <sup>3</sup> )	Reference
Poland	Healthy homes n=27	<sup>δ</sup> 1021	<sup>δ</sup> Summer: 225 <sup>δ</sup> Winter: 59	(Pastuszka et al. 2000) <sup>a</sup>
	Moldy homes n=43	<sup>δ</sup> 980	<sup>δ</sup> Summer: 834 <sup>δ</sup> Winter: 256	
Poland	Homes	<sup>δ</sup> 854 <sup>ε</sup> 90-3445	<sup>δ</sup> 189 <sup>ε</sup> 2-1997	(Gorny 1999)
Netherlands	Homes		<sup>ε</sup> 125-1425	(Beaumont et al. 1985 )
German	Homes(n=7) with asthmatic children		<sup>ε</sup> 100-1000	(Senkpiel et al. 1996)
Sweden	Homes (n=88)		<sup>ε</sup> 100-8800	(Bjornsson et al. 1995) <sup>c</sup>
Japan	Homes		<sup>ε</sup> 13-3750	(Takahashi 1997 ) <sup>c</sup>
Taiwan	Homes (n=93)		<sup>δ</sup> 411-659	(Li et al. 1995 ) <sup>c</sup>
Taiwan	Homes (n=6)		<sup>δ</sup> 1288 <sup>ε</sup> 951-1760	(Li et al. 1994 ) <sup>c</sup>
	Homes (n=12)		<sup>δ</sup> 1950 <sup>ε</sup> 1508-2502	
Taiwan	Homes Spring Summer Fall Winter		<sup>δ</sup> 299 <sup>δ</sup> 1698 <sup>δ</sup> 287 <sup>δ</sup> 63	(Kuo et al. 1994) <sup>a</sup>
Taiwan	Homes Spring Summer Fall Winter		<sup>μ</sup> 11,096 <sup>μ</sup> 6,798 <sup>μ</sup> 11,083 <sup>μ</sup> 20,551	(Su et al. 2001) <sup>c</sup>
Taiwan	<b>Homes(n=155)</b> Winter Summer		<sup>δ</sup> 9,672 <sup>δ</sup> 4,380	(Su et al. 2001) <sup>c</sup>
USA	Homes (n=1000)		<sup>μ</sup> MEA: 1033 <sup>μ</sup> DG18: 846	(Ren et al. 2001) <sup>c</sup>
USA	Home (n=44)	<sup>μ</sup> 1258 <sup>ε</sup> 220-4006	<sup>μ</sup> 2190 <sup>ε</sup> 0-48760	(Ross et al. 2000) <sup>c</sup>
USA	Home(n=147)		<sup>ε</sup> 1993-7063	(Johanning et al. 1999) <sup>c</sup>
USA	Home		<sup>ε</sup> 8-6030	(DeKoster et al. 1995 ) <sup>c</sup>
USA	Home	<sup>π</sup> 98	<sup>π</sup> 198	(Macher et al. 1991)
USA	Home (n=68)		<sup>ε</sup> 36-5,984	(Kozak et al. 1979)
USA	Homes		<sup>ε</sup> 50-1,500	(Strachan et al. 1990 )
Mexico	Home		<sup>ε</sup> 0-2087	(Garcia et al. 1995) <sup>a</sup>
Australia	Home (n=485)		<sup>ε</sup> 37-7,619 <sup>π</sup> 549	(Dharmage et al. 1999) <sup>b</sup>
Australia	Homes		<sup>ε</sup> 20-54,749 <sup>π</sup> 812	(Garrett et al. 1998 )

<sup>a</sup> SDOS <sup>b</sup>EBSCO <sup>c</sup> MEDLINE

<sup>δ</sup> Geometric Mean <sup>ε</sup> Range <sup>μ</sup> Mean <sup>π</sup> Median

Table 2.3 Examples of concentrations of airborne bacteria and fungi in public indoor environments as reported in the literature.

Type of Building	Country	Total Bacteria (CFU/m <sup>3</sup> )	Total fungi (CFU/m <sup>3</sup> )	Reference
<b>Office</b>	France	<sup>μ</sup> 447	<sup>μ</sup> 113	(Parat et al. 1997) <sup>a</sup>
	Poland	<sup>δ</sup> 295	<sup>δ</sup> Summer: 245 <sup>δ</sup> Winter: 49	(Pastuszka et al. 2000) <sup>a</sup>
	European		<sup>μ</sup> 2476	(Klanova 2000)
	Hong Kong	<sup>μ</sup> 444	<sup>μ</sup> 530	(Law et al. 2001) <sup>a</sup>
	USA		<sup>δ</sup> 21-140	(Burge et al. 2000) <sup>c</sup>
	USA		<sup>δ</sup> 110 <sup>δ</sup> 42	(Schillinger et al. 1999) <sup>b</sup>
<b>School</b>	Finland		<sup>ε</sup> 7-100	(Haverinen et al. 1999) <sup>b</sup>
	Finland	<sup>δ</sup> Area 0: 256 <sup>δ</sup> Area +: 457 <sup>δ</sup> Area++: 539	<sup>δ</sup> Area 0:24 <sup>δ</sup> Area +: 97 <sup>δ</sup> Area ++: 132	(Lappalainen et al. 2001) <sup>a</sup>
	Taiwan		<sup>δ</sup> Win: 9,730 <sup>δ</sup> Sum: 3,565	(Su et al. 2001) <sup>c</sup>
	USA		<sup>μ</sup> 1,124 <sup>μ</sup> 130 <sup>μ</sup> 352 <sup>μ</sup> 1,119	(Levetin et al. 1995) <sup>a</sup>
<b>Hospital</b>	Italy		<sup>ε</sup> 0-489	(Rolandi et al. 1998) <sup>a</sup>
	India		<sup>μ</sup> 755	(Vittal. et al. 1995) <sup>a</sup>
<b>Library</b>	India		<sup>μ</sup> 1456	(Vittal et al. 1995) <sup>a</sup>
<b>Underground station</b>	Italy		4648 colony	(Picco et al. 2000) <sup>a</sup>
<b>Shopping mall</b>	Hong Kong	<sup>ε</sup> 0-2200		(Li et al. 2001) <sup>a</sup>
<b>Rural bakery</b>	India		<sup>ε</sup> 65-2061	(Adhikari et al. 2000) <sup>c</sup>

<sup>a</sup> SDOS <sup>b</sup>EBSCO <sup>c</sup> MEDLINE

<sup>δ</sup> Geometric Mean <sup>ε</sup> Range <sup>μ</sup> Mean <sup>π</sup> Median

There are many difficulties in airborne sampling of biological agents (discussed in detail in Chapter 4) that may compromise the viability of the microbes or that result from short time sampling. Therefore, indoor fungi have often been determined from house dust samples. These are samples of settled dust that have been vacuumed or otherwise collected from surfaces. Fungi are a common constituent of house dust, and gravitational settling is one of the mechanisms by which fungal spores are removed from the air. A summary of the observed concentrations of fungi in house dust

samples is given in Table 2.4. As seen from the Table 2.4, fungal concentrations in house dust have large variations. At present, there is little information on the relevance to health of these concentrations.

Table 2.4 Examples of culturable microbial concentrations in dust samples from indoor environments in different regions

Type of building	Country	Sample sources / sample size	Total fungi (CFU/g)	Total bacteria (CFU/g)	Reference
<b>Homes</b>	Belgium	Living room (Carpet)	<sup>δ</sup> 135,586		(Beguin et al. 1996) <sup>a</sup>
	Belgium	Bedroom carpet	<sup>δ</sup> 119,462		(Beguin et al. 1996) <sup>a</sup>
	Belgium	Mattress	<sup>ε</sup> 1,000-70,000,000 <sup>π</sup> 30,000		(Beguin 1995) <sup>a</sup>
	Netherlands	<b>Living room floor</b>			(Chew et al. 2001) <sup>c</sup>
		Fall	<sup>δ</sup> 60,729		
		Winter	<sup>δ</sup> 10,226		
	Netherlands	Spring	<sup>δ</sup> 84,016		(Verhoeff et al. 1994) <sup>c</sup>
		<b>Bedroom</b>			
		Fall	<sup>δ</sup> 71,280		
	Netherlands	<b>Living room floor and mattress</b>			(Dales et al. 1998)
		Carpeted floors	<sup>δ</sup> 12880		
			<sup>δ</sup> 3530		
		Smooth floors	<sup>δ</sup> 6760		
	Poland	Mattresses			(Horak et al. 1996)
		Bed	<sup>μ</sup> 1.6 x 10 <sup>4</sup>	<sup>μ</sup> 1.6 x 10 <sup>6</sup>	
<b>Office</b>	Canada	<b>Child's Bedroom</b>			(Dales et al. 1997)
		Less contamination	<sup>δ</sup> 4.6×10 <sup>3</sup>		
		High contamination	<sup>δ</sup> 5.4×10 <sup>3</sup>		(Dharmage et al. 1999) <sup>b</sup>
	Canada		<sup>δ</sup> 255×10 <sup>3</sup>		
	Australia	Bedroom floor			(Takatori 2001) <sup>e</sup>
	Japan		<sup>μ</sup> 10 <sup>4</sup> -10 <sup>6</sup>		
<b>Classroom</b>	Belgium	Carpet	<sup>δ</sup> 40,703		(Beguin et al. 1996) <sup>a</sup>
	Belgium	Carpet	<sup>δ</sup> 84,080		(Beguin et al. 1996) <sup>a</sup>

<sup>a</sup> SDOS <sup>b</sup>EBSCO <sup>c</sup> MEDLINE <sup>e</sup>regional report

<sup>δ</sup> Geometric Mean <sup>ε</sup> Range <sup>μ</sup> Mean <sup>π</sup> Median

As mentioned above, fungal spores are generally present everywhere, but each micro environment provides different growth conditions. Thus, the environment selects the fungal types that are able to grow in that particular site. Moistened building materials favor various fungal species, depending on their nutritional content, pH and other characteristics. Fungal genera that typically grow on moisture damaged building materials include *Stachybotrys*, *Chaetomium*, *Fusarium*, *Trichoderma* and

*Paecilomyces* (Cruz 2002). Often associated with moldy building materials are also *Aspergillus versicolor*, *Aspergillus fumigatus*, *Eurotium*, *Exophiala*, *Phialophora*, *Ulocladium*, *Wallemia* and yeasts (e.g., *Rhodotorula*) (Samson et al., 1994), and *Acremonium*, *Aureobasidium*, *Cladosporium*, *Oidiodendron*, *Tritirachium* and *Sphaeropsidales*-group (Hyvärinen et al., 2002). These fungi are typically cosmopolitan in distribution and, broadly, the species found are similar in modern style buildings regardless of geographical location. Although these fungi can be mentioned as examples of organisms typically growing on moisture-damaged building materials, the diversity of fungal species that can be found in various samples from indoor environments is more extensive.

The diversity of the mycobiota in indoor environments is illustrated in Table 2.5, where the fungal genera and species reported from indoor air, house dust or from unspecified indoor environments are listed. As can be seen from the Table, there are a number of fungal genera that have often been reported, while the majority of the less frequent types have been observed only sporadically. Certain genera, such as *Penicillium* and *Aspergillus*, consist of numerous species, which adds to the extensive diversity of indoor fungi.

Table 2.5. Fungal genera and species found in indoor air, dust or in unspecified indoor environments as reported in the literature. The numbers refer to the reference at the bottom of the table.

Fungal genera and species	Air	Dust	Indoor
<i>Absidia</i>	12		
<i>Absidia corymbifera</i>	32		10,11
<i>Acremonium</i>	5,7,12,22,24,30,32,37	19	10
<i>Acremonium strictum</i>	9,28,32,33		11
<i>Acrodontium</i>	12		10
<i>Acrodontium salmoneum</i>	32		
<i>Alternaria</i>	5,6,12,22,29,32,35,37	2,8,25	
<i>Alternaria alternata</i>	28,31,32,33,34	4,8,12,31,34	10,11
<i>Alternaria tenuissima</i>	32		
<i>Arthrinium</i>	12	34	10
<i>Arthrinium phaeospermum</i>	32		
<i>A. state of Apiospora montagnei</i>	32		
<i>Ascomycetes</i>	37		
<i>Aspergillus</i>	3,5,6,7,12,14,15,17,20,22,24,27,30,32,33,34,35,36,37	19,31,34	10
<i>Aspergillus candidus</i>	32	34	11
<i>Aspergillus clavatus</i>	24,32		
<i>Aspergillus flavipes</i>			11
<i>Aspergillus flavus</i>	6,24,32,33,35	19	11
<i>Aspergillus fumigatus</i>	6,9,12,16,26,28,31,32,33,35, 37	2,19,31,34	11
<i>Aspergillus glaucus</i>	12,32,33,37	19	
<i>Aspergillus nidulans</i>	32,33	19	11
<i>Aspergillus niger</i>	6,9,20,25,28,31,32,33,34,37	2,8,19,25,31,34	11

<i>Aspergillus niveus</i>			11
<i>Aspergillus ochraceus</i>	32,34	2,19,23,25, 34	11
<i>Aspergillus penicilloides</i>		12	11
<i>Aspergillus repens</i>	12		
<i>Aspergillus restrictus</i>	32	2,12	11
<i>Aspergillus sclerotiorum</i>	32		
<i>Aspergillus sydowii</i>	27,32	19,25	11
<i>Aspergillus tamaris</i>	32		
<i>Aspergillus terreus</i>	33,37		11
<i>Aspergillus ustus</i>	28,32	19	11
<i>Aspergillus versicolor</i>	9,16,18,20,27,28,29,30,31,32,33,34,35,36,37	2,4,8,19,34	11
<i>Aureobasidium</i>	5,6,12,17,25,37	2	
<i>Aureobasidium pullulans</i>	12,28,31,32,33	4,8,12,34	10,11
Basidiomycete	22,34,37	34	
Basidiomycete <i>Ganoderma</i>	35		
<i>Beauveria</i>	12		
<i>Beauveria alba</i>	32		
<i>Bipolaris</i>	22		
<i>Blastobotrys</i>	12,32		
<i>Blastobotrys aristata</i>	32		
<i>Botryosporium</i>	7,12,32		10
<i>Botryotrichum</i>	9,12,32		
<i>Botryotrichum piluliferum</i>	32		
<i>Botrytis</i>	5,12,17,37		
<i>Botrytis cinerea</i>	31,32,33	8	10,11
<i>Chaetomium</i>	9,12,14,32,33,37		
<i>Chaetomium aureum</i>	28		
<i>Chaetomium globosum</i>			10,11
<i>Cephalosporium</i>			10
<i>Chromelosporium</i>	12		
<i>Chromelosporium fulvum</i>	32		
<i>Chrysonilia</i>	37		10
<i>Chrysonilia sitophila</i>			11
<i>Chrysosporium</i>	12,32,37		
<i>Chrysosporium keratinophilum</i>	32		
<i>Cladobotryum</i>	12,32		
<i>Cladosporium</i>	3,5,6,7,12,14,17,18,20,22,24,25,27,29,32,33,37	2,19,25,31, 34	
<i>Cladosporium cladosporioides</i>	12,28,31,32,33,34,35	4,8,31,34	10,11
<i>Cladosporium herbarum</i>	9,12,28,31,32,33,35	8,23,34	10,11
<i>Cladosporium macrocarpum</i>	32,35		
<i>Cladosporium sphaerospermum</i>	12,28,32,33,35	8	10,11
<i>Cochliobolus lunatus</i>	33		
<i>Cunninghamella elegans</i>	32,33		
<i>Curvularia</i>	12,32	19	10
<i>Curvularia aureum</i>			11
<i>Curvularia inaequalis</i>		34	
<i>Curvularia lunata</i>			11

<i>Dactylaria</i>	32		
<i>Dichobotrys</i>	12		
<i>Dichobotrys abundans</i>	32		
<i>Dicyma ampullifera</i>	32		
<i>Doratomyces</i>	12,33		10
<i>Doratomyces stemonitis</i>	32		
<i>Drechslera</i>	12,32		
<i>Emericella nidulans</i>			10,11
<i>Epicoccum</i>	5,12,22,29		
<i>Epicoccum nigrum</i>	28	8,19	10,11
<i>Epicoccum purpurascens</i>	32,33		
<i>Eurotium spp.</i>	16,24,26,27,37	2	
<i>Eurotium amstelodami</i>			10,11
<i>Eurotium chevalieri</i>			11
<i>Eurotium herbariorum</i>		4,8	10
<i>Eurotium repens</i>		12	
<i>Eurotium rubrum</i>			10
<i>Exophiala</i>	12,16,32,37		
<i>Exophiala jeanselmei</i> group			10,11
<i>Fusarium</i>	5,12,16,26,32,33,37	2,19	
<i>Fusarium culmorum</i>	33	8	10,11
<i>Fusarium moniliforme</i>	33		11
<i>Fusarium solani</i>	32		11
<i>Geomyces</i>	5,12,37		
<i>Geomyces pannorum</i>	32,33		10
<i>Geotrichum</i>	12,37		
<i>Geotrichum candidum</i>	32,33,34	34	
<i>Gliocephalis</i>	37		
<i>Gliocladium</i>			10,11
<i>Gliocladium roseum</i>	32,33		
<i>Gliomastix</i>	12,32		
<i>Gliomastix luzulae</i>	32		
<i>Gliomastix murorum</i>			11
<i>Gonatobotrys</i>	37		10
<i>Gonatorrhodiella</i>	37		
<i>Graphium</i>	32,37		10
<i>Harzia</i>			10
<i>Helminthosporium</i>	5		
<i>Humicola</i>	37		10
<i>Hyalodendron</i>	37		
Members of the <i>Mucorales</i>	6		
<i>Microsporum</i>			10
<i>Monascus</i>			10
<i>Monilia sitophila</i>		34	
<i>Monocillium</i>	37		
<i>Monodictys</i>	12		
<i>Monodictys asperospora</i>	32		
<i>Monodictys castaneae</i>	32		

<i>Monodictys levis</i>	32		
<i>Mortierella</i>	12		10
<i>Mortierella ramanniana</i>	32		
<i>Mucor</i>	5,12,22,32,33,34,37	8,31,34	
<i>Mucor hiemalis</i>			11
<i>Mucor mucedo</i>			11
<i>Mucor plumbeus</i>	32,33		10,11
<i>Mucor racemosus</i>	33		11
<i>Mycelia sterilia</i>	5,9,12,17,22,25,30,32,35,36, 37	19,34	
<i>Myxotrichum</i>	12,32		
<i>Nectria inventa</i>	33		
<i>Nigrospora</i>			10
<i>Oedocephalum</i>	7,32,37		10
<i>Oidiodendron</i>	5,12,37		
<i>Oidiodendron cerealis</i>	32		
<i>Oidiodendron griseum</i>	33		
<i>Oidiodendron rhodogenum</i>	33		
<i>Oidiodendron tenuissimum</i>			10,11
<i>Olpitrichum</i>	37		
<i>Ovularia</i>			10
<i>Ovulariopsis</i>	33,37		
<i>Paecilomyces</i>	12,17,22,37	34	
<i>Paecilomyces lilacinus</i>	32		
<i>Paecilomyces variotii</i>	28,32,33	34	10,11
<i>Papulaspora</i>	33		10
<i>Penconia</i>		34	
<i>Penicillium</i>	3,5,6,7,9,12,14,15,17,18,20,22,24, 25,27,29,30,32,33,34,35,36,37	1,2,19,23,25,3 1,34	10
<i>Penicillium adametzioides</i>	33		
<i>Penicillium aurantiogriseum</i>	28,32	8,34	11
<i>Penicillium brevicompactum</i>	28,32,33,34,35	4,8,34	11
<i>Penicillium camembertii</i>	32		
<i>Penicillium chrysogenum</i>	28,31,32,33,34,35	8,12,34	11
<i>Penicillium citrinum</i>	32,33,35		11
<i>Penicillium commune</i>	28		11
<i>Penicillium corylophilum</i>	32,33,35		11
<i>Penicillium corymbiferum</i>	33		
<i>Penicillium crustosum</i>	32		11
<i>Penicillium cyclopium</i>	33,35		
<i>Penicillium decumbens</i>	34	34	
<i>Penicillium digitatum</i>	32,34	34	
<i>Penicillium echinulatum</i>	32		
<i>Penicillium expansum</i>	28,32,33,35		11
<i>Penicillium fellutinum</i>	34,35	34	
<i>Penicillium frequentas</i>	33		
<i>Penicillium glabrum</i>	24	8	11
<i>Penicillium griseofulvum</i>	32		
<i>Penicillium italicum</i>	32,33		
<i>Penicillium janthinellum</i>	34	34	

<i>Penicillium lividum</i>	34	34	
<i>Penicillium minioluteum</i>	33		
<i>Penicillium nigricans</i>	33		
<i>Penicillium olsonii</i>	32		11
<i>Penicillium polonicum</i>	31		
<i>Penicillium purpurecsens</i>	32		
<i>Penicillium purpurogenum</i>		34	
<i>Penicillium roqueforti</i>			11
<i>Penicillium rubrum</i>	35		
<i>Penicillium rugulosum</i>	34	34	11
<i>Penicillium simplicissimum</i>		34	
<i>Penicillium solitum</i>			11
<i>Penicillium spinulosum</i>	32,33,35		11
<i>Penicillium vinaceum</i>	33		
<i>Penicillium viridicatum</i>	28,32,33,34,35	31,34	11
<i>Penicillium variabile</i>			11
<i>Pestalotia</i>			10
<i>Phialocephala</i>	33		
<i>Phialophora</i>	12,15,16,32,33,37		10
<i>Phialophora fastigiata</i>			11
<i>Phialophora melinii</i>		34	
<i>Phialophora olivacea</i>	32		
<i>Phoma</i>	5,9,12,32,37	8,19,34	10
<i>Phoma glomerata</i>			11
<i>Phoma herbarum</i>	33		
<i>Phoma macrostoma</i>			11
<i>Pithomyces</i>	12,22		10
<i>Pithomyces chartarum</i>	28,32		
<i>Pleiochaeta</i>	12,32		
<i>Pleospora herbarum</i>	33		
<i>Polyscytalum</i>	37		
<i>Pyronema</i>			10
<i>Rhinocladiella</i>	12,22,33,37		
<i>Rhinocladiella atrovirens</i>	32		
<i>Rhizomucor pusillus</i>	32		10
<i>Rhizopus</i>	12,32,37	8,31,34	
<i>Rhizopus microsporus</i>	32		
<i>Rhizopus stolonifer</i>	32,33		10,11
<i>Scedosporium</i>	12,32		
<i>Scopulariopsis</i>	5,9,12,22,30,32,33,37		
<i>Scopulariopsis asperula</i>	32		
<i>Scopulariopsis brevicaulis</i>	32,33	8	10,11
<i>Scopulariopsis brumptii</i>	32		
<i>Scopulariopsis candida</i>	32		11
<i>Scopulariopsis chartarum</i>	32		
<i>Scopulariopsis fusca</i>	32		10,11
<i>Serpula</i>			10
<i>Sistotrema</i>	5		10



<i>Sistotrema brinkmanii</i>	33		11
<i>Sordaria</i>			10
<i>Sphaeropsidales</i> -group	37		
<i>Sporothrix</i>	5,12,32,33		
<i>Sporotrichum</i>	12,32		
<i>Stachybotrys</i>	7,12,16,37		
<i>Stachybotrys atra</i>	32,33,35	34	
<i>Stachybotrys arum</i>			11
<i>Stachybotrys chartarum</i>	13,14,20,21,28	19	10
<i>Stemphylium</i>	12		10
<i>Stemphylium botryosum</i>	31,32		
<i>Stilbella</i>			10
<i>Syncephalastrum racemosum</i>			11
<i>Talaromyces thermophilus</i>	32		
<i>Tamnidium elegans</i>	32,33	34	
<i>Thermomyces lanuginosus</i>	32		
<i>Trichoderma</i>	5,12,16,20,27,29,30,32,37	2	
<i>Trichoderma harzianum</i>	28		10,11
<i>Trichoderma viride</i>	21,31,33,34,35	31,34	10,11
<i>Trichocladium</i>			10
<i>Trichophyton</i>	12		
<i>Trichophyton terrestre</i>	32		
<i>Trichothecium</i>	12		10
<i>Trichothecium roseum</i>	32		
<i>Trichurus</i>			10
<i>Tritirachium</i>	22,37		10
<i>Tritirachium oryzae</i>	32	23	
<i>Ulocladium</i>	5,12,22,26,31,32,33,37	23,31	
<i>Ulocladium atrum</i>	32		
<i>Ulocladium botrytis</i>	32,34	31,34	
<i>Ulocladium chartarum</i>	28,32,33	4,8	10,11
<i>Ulocladium consortiale</i>	33		
<i>Verticillium</i>	5,12,32,33		10
<i>Verticillium dahliae</i>	32		
<i>Verticillium lamellicola</i>	32		
<i>Verticillium lecanii</i>			11
<i>Verticillium tenerum</i>	32		
<i>Verticladiella</i>		34	
<i>Wallemia</i>	5,12,16,26,37		
<i>Wallemia sebi</i>	27,32	2,4,8	10,11
White-rot Basidiomycetes	5		
Unknown	22,25		
Yeasts	5,6,7,12,14,15,17,25,28,30,32,33,34,36,37	1,4,8,19,34	
<i>Candida</i>	6		
<i>Cryptococcus</i>		8	
<i>Cryptococcus albidus</i>			10,11
<i>Cryptococcus laurentii</i>			10,11
<i>Rhodotorula</i>	6,16,37	8,19,25	

<i>Rhodotorula glutinis</i>			10,11
<i>Rhodotorula minuta</i>			10,11
<i>Rhodotorula mucilaginosa</i>			10,11
<i>Torula</i>	5,33		
<i>Torula herbarum</i>	33	34	
<i>Torulopsis</i>	6		

References for the Table: 1. Flannigan et al., 1994; 2. Takatori et al., 1994; 3. Cole et al., 1994; 4. Verhoeff et al., 1994; 5. Hunter and Lea, 1994; 6. Mouilleseaux and Squinazi, 1994; 7. Nevalainen et al., 1994; 8. Hoekstra et al., 1994; 9. Heinemann et al., 1994; 10. Samson and Hoekstra, 1994; 11. Samson, 1999; 12. Nolard, 1999; 13. Etzel and Dearborn, 1999; 14. Craner, 1999; 15. Hyvärinen et al., 1999; 16. Taskinen et al., 1999; 17. Bartlett et al., 1999; 18. Carlson and Quraishi, 1999; 19. Hodgson and Scott, 1999; 20. Hung, 1999; 21. Robertson, 1999; 22. Jenkins and Sierck, 1999; 23. Salkinoja-Salonen et al., 1999; 24. Gqaleni et al., 1999; 25. Fedoruk et al., 1999; 26. Toivola et al., 1999; 27. Morey and Sawyer, 1999; 28. Rand, 1999; 29. McGuinness and McGuinness, 1999; 30. Lappalainen et al., 1999; 31. Gravesen et al., 1994; 32. Beguin and Nolard, 1994; 33. Hunter et al., 1988; 34. Miller et al., 1988; 35. Flannigan et al., 1991; 36. Lappalainen, 2002; 37. Hyvärinen, 2002.

The changes in building practices, building materials, use of buildings and other lifestyle aspects have modified the indoor environments in the industrialized countries during the last 20-30 years. This has had its impact on the indoor environments as microbial habitats. As an example, the petroleum crisis in the 1970ies increased the efforts to insulate the buildings more efficiently. This was often done without proper ventilation or in an erroneous way, and it led to condensation in the structures and subsequent mould growth. A parallel change in lifestyle with frequent showers and washing has increased the moisture load in dwellings, and therefore, increased the risk of moisture damage and mold growth.

The growth of molds in indoor environments is mainly associated with occupants' health, but the damage mold is causing to the building structures is usually repairable to a reasonable cost and effort. Another type of microbial problems associated with building dampness are the rot fungi that destroy the mechanical firmness of wooden structures. In Europe, the basidiomycete *Serpula lacrymans* (previously *Merulius*), the dry rot fungus, is a considerable problem (Jennings and Bravery, 1991), while *Meruliporia incrassata* is a more common dry rot fungus in the US (Burdall, 1991). Rot fungi can be considered primarily problems of building structure, but it should be noted that moisture or dampness problems that lead to growth of rot fungi, may simultaneously result in mold growth in other structural parts of the building.

### **2.5.2 Fungal components and products**

Airborne fungal particles consist of spores, spore fragments and mycelial fragments. These contain various components and products, e.g., fungal allergens,  $\beta$ -glucans, ergosterol, and secondary metabolites including mycotoxins. Volatile end products of

fungal metabolism, MVOC (microbial volatile organic compounds) appear as gaseous compounds in indoor air or adsorbed in materials.

### **2.5.2.1 Allergens**

All fungal spores are potentially allergenic although only few fungal allergens have been characterized (IOM, 2000). Allergenic properties are variable and differ even among different species within the same fungal genus. Many fungal allergens appear to be glycoproteins, but also enzymes, such as enolase and proteinases, belong to allergens in most fungi (Kurup et al. 2000). Strain-to-strain and batch-to-batch variations occur in the processing of allergenic extracts, and therefore, the allergenic extracts used in the clinical diagnosis of fungal sensitivity have large variability and are often unpredictable in their biological activity (IOM, 1993, Kurup et al., 2000).

The best known fungal allergens are those of *Alternaria alternata*, *Cladosporium herbarum* and the opportunistic pathogen *Aspergillus fumigatus*. The major allergenic fraction of *A. alternata* is heterogeneous and can induce allergic reactions at low concentrations in sensitized individuals. Several studies have shown the complexity and variability in allergens among different *Alternaria* strains. Investigators have characterized 32 antigens and 19 different allergens (Horner et al., 1995). Alt a 1 (31 kDa) has been reported to be the most frequently recognized allergen binding to IgE of more than 80% of asthmatic patients with *Alternaria* allergy (Kurup et al., 2000).

Although *Cladosporium herbarum* is less common in outdoor air than *C. cladosporioides* and *C. sphaerospermum*, it has been studied extensively. Approximately 60 antigens have been reported from extracts. Aukrust et al. (1985) isolated two allergens, Cla h 1 (13 kDa) known as Antigen 32 and Cla h 2 (23 kDa) known as Antigen 54, from *Cladosporium herbarum*. Both allergens have been found to vary widely from strain to strain. Variability in composition is demonstrated by the finding of a 0% to 100% range in allergen content among 10 isolates of *Cladosporium* (Kurup et al., 2000).

Wallenbeck et al. (1984) were able to isolate two clinically important allergens of *A. fumigatus*, Ag-10 and Ag-40. Longbottom (1986) similarly uncovered the major allergen Ag-3. About 20 allergens from *A. fumigatus* have been purified and characterized by now. An extensive cross-reactivity within different *Aspergillus* species and between *Aspergillus* and *Penicillium* species have been reported. E.g. similar serine proteinases (33-34 kDa) with sequence homology have been identified from various species of *Penicillium* and *Aspergillus* (Kurup et al., 2000). Several recombinant allergens from *A. alternata*, *C. herbarum*, and *A. fumigatus* have been identified and purified so far. In a long run this trend will give better opportunities to control the cross-reactivity in immunoassays.

### **Exposure Evaluation**

There is little data on exposure levels to fungal allergens in indoor environments. However, since all fungi are potentially allergenic, the fungal exposures invariably mean potential exposures to allergens.

### 2.5.2.2 $\beta(1\rightarrow3)$ -glucans

$\beta(1\rightarrow3)$ -glucans are structural components of fungal cells. Chemically they are glucose polymers with variable molecular weight and degree of branching that may appear in various conformations i.e. triple helix, single helix or random coil structures of which the triple helix appears to be the predominant form.  $\beta(1\rightarrow3)$ -glucans originate from a large variety of sources, including most fungi, some bacteria, most higher plants and many lower plants. They are water insoluble structural cell wall components of these organisms, but may also be found in extracellular secretions of microbial origin. In the fungal cell wall,  $\beta(1\rightarrow3)$ -glucans are linked to proteins, lipids and carbohydrates such as mannan and chitin, and they contain  $\beta(1\rightarrow6)$ -glucan side-branches which may connect with adjacent  $\beta(1\rightarrow3)$ -glucan polymers. Glucans may account for up to 60% of the dry weight of the cell wall of fungi (Klis, 1994). The  $\beta(1\rightarrow3)$ -glucan content of fungal cell walls has been reported to be relatively independent of growth conditions (Rylander, 1997a).

### Exposure evaluation

In Table 2.6, examples of reported concentrations of  $(1\rightarrow3)$ - $\beta$ -D-glucan and other fungal components are presented.

Table 2.6 Examples of concentrations of  $(1\rightarrow3)$ - $\beta$ -D-glucan and other fungal indicators in indoor environments from different regions

Type of building	Country	Sample sources / sample size	$\beta(1\rightarrow3)$ glucan ( $\mu\text{g/g}$ dust)	$\beta(1\rightarrow3)$ glucan ( $\text{ng/m}^3$ air)	Ergosterol ( $\text{ng/m}^3$ air)	Reference
<b>Homes</b>	Australia	Bedroom floor	$^{\epsilon}0\text{--}61.5$ $^{\pi}3.8$			(Dharmage et al. 1999) <sup>b</sup>
	Netherlands	Living room floor	$^{\delta}$ Fall 1,720 $^{\delta}$ Winter 1,784 $^{\delta}$ Spring 1,551			(Chew et al. 2001) <sup>c</sup>
			$^{\delta}$ Fall 2,050			
	Netherlands		23,000			(Wouters et al. 2000)
	Netherlands	Living room	$^{\delta}$ 1,293			(Douwes et al. 1999)
		Bedroom	$^{\delta}$ 1,286			
		Kitchen	$^{\delta}$ 1,168			
		Mattress	$^{\delta}$ 757			
	Sweden	airborne		$^{\epsilon}0\text{--}19$		(Thorn et al. 1998)
	Canada	Airbone Dust			$^{\epsilon}0.01\text{--}194$	(Miller et al. 1997)
<b><u>School</u></b>	Sweden	airborne		$^{\epsilon}0\text{--}6.9$ $^{\pi}2.9$		(Rylander et al. 1998)
<b>Day-care centre</b>	Sweden	airborne		Before renovation: 11.4 After renovation: 1.2		(Rylander 1997)

<sup>a</sup> SDOS <sup>b</sup>EBSCO <sup>c</sup> MEDLINE <sup>e</sup>regional report

<sup>δ</sup> Geometric Mean <sup>ε</sup> Range <sup>μ</sup> Mean <sup>π</sup> Median

Methods used to analyse  $\beta(1\rightarrow3)$ -glucans in settled or airborne dust samples have not been standardized and therefore the results are not well comparable across studies. In the Netherlands and Germany, mean  $\beta(1\rightarrow3)$ -glucans levels in house dust determined with a specific enzyme immunoassay were around 1,000 - 2,000  $\mu\text{g/g}$  dust and 500 - 1,000  $\mu\text{g/m}^2$  (Douwes et al., 1996, 2000; Gehring et al., 2001). Samples were taken in homes that were not selected based on mould problems. No airborne samples were taken in those studies.

### **2.5.2.3 Ergosterol**

Ergosterol is the principal sterol present in membranes of hyphae and spores of filamentous fungi and thus provides a chemical marker for fungal biomass. Ergosterol content in spores varies somewhat between different fungal species (Pasanen et al., 1999, Miller and Young, 1997). However, it is not a good indicator for yeasts which being non-filamentous, contain less ergosterol (Pasanen et al. 1999).

In house dust, ergosterol concentrations have been reported to be around at  $<62 \mu\text{g/g}$  dust (Axelsson et al. 1995, Miller et al. 1988, Dharmage et al. 1999b) and the correlation with the counts of viable fungi has been good (Miller et al. 1988). Miller and Young (1997) observed ergosterol in the air in range of  $0.01\text{-}194 \text{ ng/m}^3$ , while mean ergosterol values of  $200\text{-}3000 \text{ ng/m}^3$  were reported by Dales et al. (1997). Ergosterol was detectable only in about 5-25% of the air samples in these studies. The ergosterol concentrations have been higher in damaged materials ( $0.01\text{-}0.15 \text{ ng/g}$ ) than in dry materials samples (nd) (Szponar and Larsson 2000) and correlated with concentrations of viable fungi (Pasanen et al. 1999).

Ergosterol concentrations in house dust have been found to vary seasonally, being highest in winter (Dharmage et al., 1999), while the variation is the opposite for culturable fungi. Ergosterol levels in dust have been associated with visible or reported mold and fitted old carpets, with pets and infrequent airing (Dharmage et al., 1999). However, in the study of Dales et al. (1997), ergosterol level in dust or in the air were not associated with visible mold areas or reported mold and water damage. While ergosterol has been suggested to estimate fungal exposure, its relationship to the observed health effects are still obscure (Szponar and Larsson 2001).

### **2.5.2.4 Microbial toxins**

Many of the fungal types growing on moistened building materials are able to produce toxic metabolites called mycotoxins. Mycotoxins are non-volatile secondary metabolites with various chemical structures including polyketides, terpenes and indoles. Among the most important toxigenic fungi that are observed in indoor environments are *Stachybotrys chartarum*, *Aspergillus versicolor*, and species of the genera *Chaetomium*, *Fusarium*, *Penicillium*, and *Trichoderma* (Cruz 2002). Toxin production is a genetic characteristics of an organism that varies between the species of the same genus, and even between the strains of the same species. The toxin

producing genes may be expressed in various environmental conditions or in competitive situations between different species.

The spores of many species of moulds have been shown to contain mixtures of mycotoxins. The concentrations of toxins in spores of a number of moulds have been 1-650 µg/g. These moulds include *Fusarium graminearum* (deoxynivalenol, or DON), *F. sporotrichioides* (T-2 toxin), *F. moniliforme* (fumonisin), *Stachybotrys chartarum* (satratoxins), *Penicillium expansum* (citrinin), *P. chrysogenum* (roquefortine C), *P. brevicompactum* (mycophenolic acid), *Aspergillus versicolor* (sterigmatocystin), *A. flavus/parasiticus* (aflatoxins), and *A. fumigatus* (fumitremorgen B, verrucologen) (Flannigan and Miller, 1994).

Mycotoxins include several families of compounds, some of which overlap across fungal species, and some of which are species-specific. The production of various metabolites depends on the growth conditions, e.g., on pH, osmotic tension and sometimes temperature. It is known that morphologically similar strains of toxigenic *Fusarium* and *Aspergillus* as well as *Stachybotrys chartarum*, have important differences in metabolite pattern (Jarvis et al., 1998, Nielsen, 2002).

When toxigenic fungi grow on building materials, they produce one or more of the metabolites for which they have the genetic potential. It has been demonstrated that *Stachybotrys chartarum* produced a number of different mycotoxins when growing on materials in buildings, and that the quantities of toxic metabolites produced in this fungi was significantly higher than in the other fungi also typically growing on building materials (Nielsen et al., 1998, 1999). Only 35 % of the isolates investigated produced the highly toxic satratoxins but in the rest, spiriocyclic drimanes were detected. Both chemotypes appear to occur together (Nielsen 2002), so both families of toxins are typically found. When growing on water damaged building materials some metabolites from *Penicillium chrysogenum* were detected. *A. versicolor* produced sterigmatocystin on wallboard and on a moisture damaged carpet (Engelhart et al., 2002). *P. brevicompactum* produced mycophenolic acid, *P. polonicum* the tremorgenic verrucosidin and *Chaetomium globosum* large quantities of the toxic chaetoglobosins when growing on water damaged materials (Nielsen, 2002). Sterigmatocystin is an IARC 2A carcinogen that is genotoxic. Satratoxin is a tricothecene that affects membrane function and inhibits peptidyl transferase. The toxicity of the other compounds measured on wallboards is not clearly known.

## Exposure Evaluation

Toxic metabolites from *Stachybotrys* have been found in air samples where satratoxins dominated. In addition, several spiriocyclic drimanes have been detected in air (Nielsen 2002). Although the presence of many mycotoxins have been shown in the indoor air, the human exposure to mycotoxins is not yet well documented. Due to the acute toxicity of many microbial toxins, information about exposure levels to toxins in the indoor environments would be urgently needed. At this point, however, the information is too scarce for the evaluation of the inhalable toxin exposures.

#### **2.5.2.5 Microbial volatile organic compounds (MVOC)**

Some fungi produce soluble and volatile metabolites that are responsible for the typical mold odor. The ecological importance of these compounds is probably in their toxicity to microbial competitors. For example, fungal volatile organic compounds (VOCs) inhibit the growth of plant pathogenic and wood decay fungi. VOCs from fungi are also known to attract insects that are essential in transporting fungi to new food sources.

The fungi that occur in buildings produce roughly the same mass of volatile compounds per unit weight of fungal growth. The volatiles produced comprise a mixture of compounds that are common to many species and are also common industrial chemicals. In addition, fungi produce compounds that are genus or species specific. These differences have not been adequately studied.

Interest in the use MVOC as indicators of microbial contamination arose in the food-processing industry in the 1970's, when fungal and bacterial odours were suggested as a tool to detect spoilage in grain, meat and fish (Miller et al. 1973; Lee et al. 1979; Dainty et al. 1989; Börjesson et al. 1992; Wilkins and Scholl 1989). Later, MVOC analyses and profiles were applied to the taxonomy research to identify and separate microbial (mainly fungal) species or strains (Zeringue et al., 1993; Larsen and Frisvad, 1995a; Larsen and Frisvad, 1995b; Jelen et al. 1995; Fischer et al. 1999; Wilkins et al. 2000). MVOC were applied to indoor air environments for the first time in the 1980's (Ström et al. 1994; Bayer and Crow, 1994; Wessén and Schoeps, 1996; Morey et al., 1997; Wilkins et al., 1997).

The possibility of detecting indoor fungal and actinomycete growth in interior surfaces without opening building structures was presented by analyzing MVOCs. Additionally, gaseous MVOCs may pass through vapour barriers (Ström et al., 1994, Wessén and Hall 1999). Though MVOC mainly indicate the active phase of microbial contamination, certain MVOC were suggested to refer to old microbial growth because of their high absorption affinity to building materials (Wessén et al., 1995).

So far, more than 200 compounds have been recognised as MVOC in the laboratory studies (Wilkins and Larsen 1995). The variety of MVOC profiles obtained in the studies depends on microbial species and strain, growth conditions (substrate, nutrients, pH, moisture content, temperature) and the analytical method used (Sprecher and Hanssen 1982, Börjesson et al., 1992, Börjesson et al., 1993, Bjurman and Kristensson, 1992, Rivers et al., 1992, Zeringue et al., 1993, Larsen and Frisvad, 1995, Sunesson et al., 1997, Pasanen et al., 1996, Korpi et al., 1997, Wilkins et al., 2000). The majority of the studies has been carried out with pure cultures of fungi and actinomycetes. In a few studies, MVOC production of mixed cultures on building materials have been made (Ezeonu et al., 1994, Wessén et al., 1995). Approximately 80 compounds have been identified from the growth of *Penicillium*, *Aspergillus*, *Cladosporium*, *Paecilomyces*, *Stachybotrys*, *Trichoderma*, *Acremonium*, *Serpula*, *Chaetomium*, *Poria*, *Coniophora*, and *Streptomyces* on various building materials (Ezeonu et al., 1994, Wessén et al., 1995, Sunesson et al., 1997, Wessén and Schoeps 1996, Korpi et al., 1998, Korpi et al., 1999, Wilkins et al., 2000).

The following compounds have been reported from contaminated building materials:

- Hydrocarbons: e.g., dimethylhexadiene, 1-heptene, 1-methoxy-3-methylbutane, 1,3-octadiene, pentane, benzene, 1(1,1-dimethylethyl)-4-ethylbenzene, methyl benzene, 1-methoxy-4-(1-methylethyl)-benzene, xylene, anisole, 3-methylanisole
- Alcohols: e.g., 1-butanol, 2-butanol, ethanol, 2-ethyl-1-hexanol, 2-heptanol, 2-hexanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, 2-nonanol, 2-octanol, 1-octen-3-ol, 1-pentanol, 3-pentanol, 2-propanol
- Ketones: e.g., acetone, 2-butanone, 2-heptanone, 2-hexanone, 3-methyl-2-butanone, 3-methyl-2-pentanone, 2-nonanone, 2-pentanone, 3-pentanone, 2-octanone, 3-octanone
- Aldehydes: e.g., acrolein, decanal, formaldehyde, octanal
- Esters: e.g., methyl acetate, methyl-2-methylpropionate, propyl acetate
- Terpenoid (isoprenoid) compounds: e.g., monoterpenes (pinenes, limonene), sesquiterpenes and their corresponding alcohols, geosmin
- Miscellaneous compounds: e.g., 2,5-dimethylfuran, 3-methylfuran, 2,3,5-trimethylfuran, dimethyldisulphide, dimethyltrisulphide

## **Exposure Evaluation**

It is difficult to make a summary of MVOC that would occur in buildings with microbial contamination. In addition, any individual MVOC cannot be related to a certain microbial species, because the same MVOC may be produced by different microorganisms; even by both fungi and bacteria. Mold odor has been associated with building-related symptoms, but in such cases, the exposure has only been observed as perceived odor, and the concentrations of odorous metabolites have not been measured. Concentrations and profiles of indoor MVOC vary extensively. At present, there is insufficient knowledge if the observed concentrations of MVOC may have causal connections with the health effects.

### **2.5.2.6 Fungal fragments**

The presence of airborne fragments is clearly documented with pollen exposures, as the onset of seasonal allergies is shown to start several weeks before the respective pollen grains are detected in the air (Rantio-Lehtimäki et al., 1994). In contrast, the role of fragments in fungal exposures has not been sufficiently recognized. The reason for this may be that fine and ultrafine fragment particles cannot be detected with traditional bioaerosol sampling and analysis methods. However, previous reports on mycelium pieces (that were large enough to be detected by light microscopy) indicate the possibility of the presence of fragments. Li and Kendrick (1995) showed that the concentrations of fungal fragments in indoor air can reach an average level of 29 to 146 pieces per m<sup>3</sup>, i.e., approximately 6% of all fungal propagules indoors. Madelin and Madelin (1995) reported that pieces of mycelium are often blown away from contaminated surfaces, and some of these remain viable and capable of initiating new growth. It is also possible that the fragments are pieces of spores and fruiting bodies



or are formed through nucleation from secondary metabolites of fungi such as semi-volatile organic compounds.

Laboratory experiments on the release of fungal propagules show that during the release process from contaminated building material, simultaneously with spores, particles significantly smaller than the single spore size are aerosolized. The measurements of optical size distributions reveal high concentration of fungal fragments in the submicrometer size range. The released fragments consistently outnumber the spores (up to 320 times) and can exceed  $6 \times 10^5$  particles per  $\text{cm}^2$  (Górny et al., 2002).

Some researchers have compared the allergic response of spore and mycelial extracts and have found that both of them share common allergens but their reactivity varies and, in some cases comparing extracts, the intensity of the reaction from mycelium can exceed those obtained from the comparable spore extract studies (Aukrust et al., 1985, Fadel et al., 1986 and 1992).

Recent studies using ELISA tests performed with monoclonal antibodies produced against *Aspergillus* and *Penicillium* fungal species reveal that the fungal fragment and spore suspensions both share common antigens and have immunological reactivity (Górny et al., 2002).

Similarly to fungal fragments, some actinomycete colonies, which produce aerial mycelium (e.g., *Streptomyces albus*) and can grow on building material, are also able to release small fragments into the indoor air (Górny et al., 2002a).

### **2.5.3 Bacteria in the indoor environments**

Building-associated bacteria are mostly the common saprophytic bacteria of the normal human skin, mouth and nose that are emitted into the indoor air (Noble et al., 1976), or those originating from outdoor air (Nevalainen et al., 1991). Pets may also be an important source or carriers of bacteria, since their presence is associated with airborne endotoxins (Park et al. 2000). Common bacteria of indoor environments are also heterotrophic bacteria that grow in the water reservoirs or moist sites of the building, such as bathroom sinks (Finch et al. 1978). Specifically, legionellae and non-tuberculous environmental mycobacteria occur in biofilms of water pipelines or in water reservoirs of cooling systems. Streptomycetes and other actinobacteria, *Bacillus* species and many other bacteria may grow in wetted building materials together with fungi (Andersson et al., 1997). Bacterial agents include bacterial cells and spores, bacterial endotoxin which is a structural component of all gram-negative bacteria, peptidoglycans, which is another structural component of bacteria, bacterial MVOC (microbial volatile organic compounds emitted from bacterial growth) and exotoxins and other secondary metabolites produced by bacteria growing on moist building materials (Peltola et al., 2001).

Some bacteria are always present in indoor environments, where they can be found on surfaces, in house dust, in tap water and in indoor air. Bacteria may become harmful exposure agents in situations where they are able to grow or accumulate in the indoor environment, i.e., if there is water available for their growth, or if they accumulate on

surfaces and house dust due to inadequate cleaning. The seriousness of the health risk depends on the bacterium, intensity of exposure and the individual factors of the exposed person. Most of these harmful exposures are preventable.

There are three main sources of bacteria in the indoor environment: humans, animals and indoor bacterial growth. In addition, outdoor air contains bacteria from soil and vegetation, and these bacteria are transported into indoor air via infiltration, ventilation and on people's clothing as well as by pets and pests. Most outdoor air bacteria are harmless environmental organisms, and pathogens are seldom spread via such routes. The filter of a mechanical ventilation system removes most bacteria from the intake air and further losses occur as the air travels through ventilation ductwork, therefore, the contribution of outdoor air bacteria typically is minimal in a well-maintained building with mechanical ventilation. In cases where water accumulates into the ventilation duct by leak or condensation, bacteria and other microorganisms may easily start to grow and thus create an intramural bacterial source.

Humans are an important source of indoor bacteria. The upmost layer of the normal human skin is continuously renewed, and skin scales containing bacteria are shed into their environment (Sciple et al., 1967). Some bacteria also originate from the human airways while talking, coughing or sneezing. The number of airborne bacteria is often highly associated with the number of humans present, and it can be used as a measure of occupant density and the efficiency of ventilation, similarly to CO<sub>2</sub> measurements. Bacterial genera that typically originate from humans are micrococci, staphylococci, streptococci and corynebacteria (Nevalainen, 1989). The human shedding of bacteria is usually an ordinary phenomenon that is not directly harmful to health.

Pets are another source of indoor bacteria. In studies of indoor endotoxin, which is a marker of gram-negative bacteria, the presence of dogs and cats has been among the main predictors of endotoxin levels in house dust (Park et al., 2001; Bischof et al., 2002). Other predictors are presence of vermin such as mice, and infrequent cleaning, which indicate poor hygienic conditions in the home (Bischof et al., 2002). Storage of organic household waste indoors also increases bacterial contamination in the indoor environment (Wouters et al., 2000; Senkpiel et al., 1996).

Like fungi, bacterial growth takes places everywhere where water is present. In indoor environments, one may find standing water in the drip pans of air-cooling devices, freezer or refrigerator, in humidifiers and on wet surfaces of bathrooms and kitchen (Ojima et al., 2002, Rusin et al., 1998). Water may also originate from leaks in plumbing or roof, or be a result of condensation or harsh rain or snowing (Lstiburek and Carmody, 1994). Standing water is a potential source of bacteria, and once disturbed, bacteria may become airborne. Bacterial growth in water is usually dominated by Gram-negative bacteria, and consequently, their unusual occurrence in indoor air, whether measured as viable bacteria, chemical markers, or as endotoxin, is an indication of a wet site or excess humidity in the indoor environment (Otten and Burge, 1999).

Due to different problems in moisture control of the building, water intrusion into building materials by condensation or capillary movement is also possible, even if no standing water is visible. Such moisture accumulation results in bacterial growth, usually in parallel with fungi. The microbial growth associated with building

dampness or other moisture problems is usually considered a "mould problem", but in most mouldy materials where fungal growth is detected, bacterial growth is also seen (Hyvärinen et al., 2002). However, the roles of different microbes as causative agents of the reported health effects are still poorly known. There is evidence that many fungi and bacteria produce toxic substances and malodorous volatile compounds while growing on building materials (Sunesson et al., 1997, Nielsen 2002, Andersson et al., 1998, Roponen et al., 2001). Fungi and bacteria have complicated interactions when growing together (Whipps, 2001), which may regulate their growth, toxin production and other secondary metabolism, but these interactions on building materials are so far poorly known.

As a summary, outdoor air bacteria and those originating from humans are considered fairly harmless agents of the indoor air, and they can be controlled with ordinary ventilation and cleaning routines. The third source, bacteria actively growing or accumulating in the indoor environment, may cause health effects, and may require specific actions to control the growth and prevent the exposures.

### **Exposure Evaluation**

Concentrations of viable bacteria in indoor environments are usually within the range  $10^1$ - $10^3$  CFU/m<sup>3</sup> in homes, and  $10^2$  CFU/m<sup>3</sup> in offices (Pastuszka et al., 2000; Gorny, 1999). A rough estimation might be that a concentration  $<1000$  CFU/m<sup>3</sup> can be regarded as "low" and that of  $>5000$  CFU/m<sup>3</sup> can be regarded as "high" (Reponen et al., 1992). These numbers reflect mainly the crowdedness of the room and the efficiency of the ventilation, and thus are a measure of the hygienic quality of the indoor air. However, it should be emphasized that the number concentrations of airborne bacteria are closely associated with the sampling and analysis method used, and therefore the concentrations between different studies can only be roughly compared. The aspects of exposure evaluation and sampling and analysis methods are further discussed in Chapter 4 of this document. The health importance of the various environmental or human-derived, non-infectious bacteria is not known, hence the measurements of total viable bacteria in indoor air have little predictive value for any specific health effects among the occupants.

Examples of concentrations of airborne bacteria from various regions reported in the literature are shown in Tables 2.2 and 2.3, which also show corresponding examples of fungal concentrations. The data available for the concentrations of airborne indoor bacteria are mainly descriptive, and there is too little data available to make good assessment of the distributions of the concentrations and factors affecting them.

Characterizing the bacterial flora in the sample may give additional information about the sources of the bacteria present. If Gram positive cocci and corynebacteria, the common bacteria of the human skin, are the dominating group, this indicates human sources (Otten and Burge, 1999). Gram-negative bacteria are not common on skin, but they are typical water bacteria. Hence, their abundant presence in the indoor air may indicate standing water or moist material with active bacterial growth. The presence of gram-negative bacteria can also be shown as endotoxin content of air (see 2.5.4.1). Gram-positive exo- and endospore-forming bacteria *Streptomyces* and *Bacillus* are not typically shed by humans but they may grow on moist building materials and their spores may survive the absence of water, and therefore, their

presence in indoor air at concentrations higher than found outdoors may be an indication of an intramural source, i.e., microbial growth. The presence of fecal bacteria, such as *Enterobacter* species may indicate a sewage leakage (Otten and Burge, 1999) and the presence of actinomycetes may indicate the presence of microbial growth in building material (Nevalainen et al., 1991). On the other hand, in agricultural environments they belong to the normal flora. Thus, the interpretation of airborne measurements for bacteria is mainly qualitative but may support the conclusions made on the need of interventions to eliminate microbial sources and thus improve the quality of indoor air.

In environmental habitats of buildings, a large diversity of bacterial species are growing, and based on the present knowledge, it is not possible to rank the non-infectious species for their possible importance as exposing agents. However, there are two groups of bacteria that have special importance as indoor contaminants, i.e., *Legionella* and actinomycetes. As they differ from the majority of the bacteria of the indoor environment in their ecology and potential to cause health effects, they are presented separately in their own paragraphs.

#### **2.5.3.1 Legionella**

*Legionella* spp. are gram negative environmental bacteria that have found a remarkable ecological niche in man made water systems, especially in those serving air conditioning systems for heat transfer (HSE, 1994). While legionellae are fairly common in natural waters, occurring there in low numbers, they appear to benefit from the nutritionally richer conditions of various man-made water systems, where they often grow intracellularly in protozoa within biofilms. *Legionella* reproduction is especially high at temperatures between 20 and 45° C. This danger does not exist in cold or very hot water. The exposure to the bacteria may occur via the emissions from cooling towers, showers or other aerosolization process, and a mechanical air distribution system may effectively spread the bacterium even within a building.

#### **2.5.3.2 Streptomyces**

Streptomyces are gram positive, spore-forming actinobacteria that are typical soil organisms, giving organic soil and potato cellar their typical odor. They are metabolically extremely active organisms being able to synthesize thousands of bioactive compounds (Anderson and Wellington, 2001). Mesophilic streptomyces have been found in the indoor air of moisture-damaged buildings, and since they do not belong to the indoor normal flora of urban environments, their presence is regarded as an indicator of a building-related moisture problem (Samson et al., 1994). They often grow in mould-damaged building materials together with fungi, favoring especially ceramic materials and gypsum board (Hyvärinen et al., 2002).

Streptomyces may also produce toxins; one of the identified toxins being valinomycin (Andersson et al., 1998). The cytotoxic and/or pro-inflammatory properties of the *Streptomyces* spores are not dependent of their viability (Hirvonen et al., 1997), but depend on the substrate on which they are growing (Hirvonen et al.,

2001; Roponen et al., 2001). Experimental studies on microbial growth on building materials have included materials such as gypsum board, wood, concrete and mineral wool, and the varying toxicity and bioactivity may be caused by production of secondary metabolites while growing on those materials. In addition to streptomycetes, another group of actinobacteria, *Nocardioopsis spp.*, has been shown to occur in indoor environments and to have toxic potential (Peltola et al., 2001). Although less attention has been paid to non infectious bacteria indoors than on fungi, there is accumulating evidence about their importance as indoor air contaminants (Huttunen et al., 2003). However, human data is so far limited and only preliminary evidence of connection of bacterial exposures and health effects is available. Therefore, more studies are required before any proper risk assessment is possible.

#### **2.5.4 Bacterial components**

While there are practically no studies focusing on the health importance of total viable airborne bacteria in indoor environments, the endotoxins of gram negative bacteria are among the best known biological agents in the indoor environments. Endotoxin has been a focus of attention due to its well known inflammatory potential (Hornef et al., 2002), and it has also been considered a marker of bacterial biomass in epidemiological studies. The other essential structural component of bacterial cells, peptidoglycans, have been studied much less, and their role as exposing agents is not clear.

##### **2.5.4.1 Endotoxins**

Endotoxins are integral components of the outer membrane of gram-negative bacteria and are composed of proteins, lipids, and lipopolysaccharides. The term “endotoxin” refers to the toxin as present in the bacterial cell wall and, therefore, exposure to endotoxins also means exposure to bacterial proteins and other cell materials. Thus, although purified LPS can reproduce most of the biologic effects of endotoxin, it is not correct to assume that this term is always preferable to the term endotoxin in defining biologic responses to this bacterial component. However, the terms 'endotoxins' and 'lipopolysaccharides' are often used interchangeably in the scientific literature. In the environment airborne endotoxins are usually associated with dust particles or aqueous aerosol with a broad size distribution.

LPS contains three parts: O-antigen (sugar-colitose- amino acids), the core oligosaccharide (sugar/polysaccharide) and the Lipid A (a series of long-chain phosphorylated lipopolysaccharides). O-antigen provides molecular recognition and lipid A portion is responsible for the toxicity including the potent fever-inducing properties of endotoxin. The polysaccharide part of the molecule accounts for the solubility in water. Endotoxin is heat stable. There is material variability in the lipid A structure among bacterial species and serotypes.

## **Determinants of indoor endotoxin levels**

Since endotoxins are a cell wall component of all gram negative bacteria, the determinants of endotoxin levels are close to those of gram negative bacteria. The bacteria present in outdoor air are mainly Gram-negative species (Fuzzi et al., 1997). The contribution of bacteria to fine particle load of outdoor air may be high, particularly after fog or rain events. Bacteria and yeast particles increased 100 times during fog versus clear air, rising to 47,000/m<sup>3</sup> at 10 °C. Temperature change from 5 to 10 °C increased bacterial concentrations during fog events 10-fold (Fuzzi et al., 1997). Endotoxin in outdoor air samples shows circa-annual variations with highest concentrations in summer and lowest during the cold season (Park et al., 2000). One source of endotoxin indoors is from fine particle ingress from outdoor air.

Another source of endotoxin is pets, as mentioned with bacteria. Park et al. (2001) found that the presence of dogs were associated with increased dust endotoxin concentrations in settled dust. Gehring et al. (2001) and Bischof et al. (2002) in Germany also found that endotoxin concentrations were higher with cats and dogs present. These studies showed that endotoxin was higher in old buildings, with longer duration of living in the apartment, low ventilation rates, and poor housekeeping. The study by Bischof et al (2002) showed a negative association of endotoxin levels with ventilation.

Seasonal variation of endotoxin concentration in house dust has been described by Rizzo et al., (1997). Instead, outdoor and indoor temperature or relative humidity were not associated with endotoxin exposure (Bischof et al. 2002). Predictors of airborne endotoxin in homes have been investigated by Park et al. (2000). Consistent with the endotoxin load of house dust, airborne endotoxin is associated to “current dog in home“, “any sign of mice“ and “mould/mildew in bedroom“. As expected, higher concentrations of “living room dust endotoxin“ was an important predictor of high airborne endotoxin.

## **Exposure Evaluation**

In the literature, endotoxin concentrations are often expressed as Endotoxin Units per g of dust or per cubic meter of air. However, many publications also present concentrations in ng/m<sup>3</sup>. In this report, endotoxin levels are described in the units given in the original publications. One ng of endotoxin is approximately 10 EU, but the exact factor is dependent on the endotoxin standard used in the laboratory analysis.

Reported values of endotoxin concentrations in indoor environments are shown in Table 2.7.

Table 2.7 Levels of Endotoxin exposure in different indoor environments

Type of building	Country	Sample sources / sample size	Endotoxin (EU/mg)	Endotoxin (EU/m <sup>2</sup> )	Reference
<b>Dust samples</b>					
<b><u>Homes</u></b>	U.S.A.	Bedroom bed	δ43.50		(Park et al. 2000)
		Bedroom floor	δ76.80		
		Kitchen room floor	δ105.40		
	U.S.A.	Bedroom floor	δ63.00		(Park et al. 2001)
		Children's beddings	δ50.00		
		Family room floor	δ79.00		
		Kitchen room floor	δ100.00		
	U.S.A.	Family room floor	δ96.00		(Park et al. 2001)
	Netherlands	Living room floor	δ10.92-13.85	δ2082-2550	( Douwes et al. 2000)
		Bedroom floor	δ6.91-9.56	δ2128-2850	
		Children's mattresses	δ3.98-7.61	δ1202-3402	
	Netherlands	<b>Living room floor</b>	δ4.36	δ950.00	(Wouters et al. 2000)
		No organic bin	δ7.77	δ2443.00	
		Organic waste bin	δ7.85	δ662.00	
		<b>Kitchen room floor</b>	δ19.17	δ2276.00	
		No organic bin			
		Organic waste bin			
	Germany	<b>Living room floor</b>	δ1.52 (ng/mg)	δ1246.00(ng/m <sup>2</sup> )	(Heinrich et al. 2001)
		□without pets and vermin	δ2.20 (ng/mg)	δ2267.00 (ng/m <sup>2</sup> )	
		□with pets or vermin			
	Germany	Living room floor	π2.20 (ng/mg)		(Gehring et al. 2001)
	Belgium		0.120-20.00 (ng/mg)		(Michel et al. 1991)
	Belgium		δ1860.00(ng /mg)		(Michel et al. 1996)
	Taiwan	<b>Children's mattresses</b>	δ45.18 (ng/mg)		(Su et al. 2001)
			δ17.96 (ng/mg)		
		Spring	δ50.25 (ng/mg)		
		Summer	δ18.84 (ng/mg)		
		Fall			
		Winter			
Type of building	Country	Sample sources / sample size	Endotoxin		Reference
<b>Air samples</b>					
<b><u>Homes</u></b>	Taiwan		δ65 (pg /m <sup>3</sup> )		(Wan et al. 1999)
	USA	Bedroom	δ0.64 (EU/m <sup>3</sup> )		(Park et al. 2000)
	U.S.A.	Living room	δ0.77 (EU/m <sup>3</sup> )		(Park et al. 2001)

<b><u>Daycare centers</u></b>	Taiwan	--	$\delta$ <b>157</b> (pg /m <sup>3</sup> )	(Wan et al. 1999)
<b>Office buildings</b>	Taiwan		$\delta$ <b>58</b> (pg /m <sup>3</sup> )	(Wan et al. 1999)
<b>Office buildings</b>	USA		<b>0.5-3.0</b> (EU/m <sup>3</sup> )	(Reynolds et al. 2001)
	USA		$\delta$ <b>0.24</b> (EU/m <sup>3</sup> )	(Hines et al. 2000)

$\delta$  Geometric Mean    $\epsilon$  Range    $\mu$  Mean    $\pi$  Median

The endotoxin concentrations of house dust in non-industrial indoor spaces vary between 100-120,000 ng/g in dwellings with mean concentrations of 1,860 ng/g or geometric means of 77 to 80 EU/mg or in office buildings, 2 – 340,000 ng/g , or mean concentration of 59 EU/mg (Michel et al., 1991, Michel et al., 1996, Bischof et al., 2002). A study by Park et al. (2000) that measured airborne endotoxin in 15 homes reported a mean airborne endotoxin level of 0.64 EU/m<sup>3</sup> and a mean dust endotoxin level between 44-105 EU/mg.

As these studies suggest, homes are characterised by high inter-building variability of house-dust-bound-endotoxin. Endotoxin exposures in domestic environments are illustrated in Figure 2.2. It can be seen from the Figure that endotoxin levels in dwellings are much lower than those in the industrial environments such as agriculture, textile industries and paper mills, where endotoxin levels typically range from several tens to thousands EU/m<sup>3</sup>.

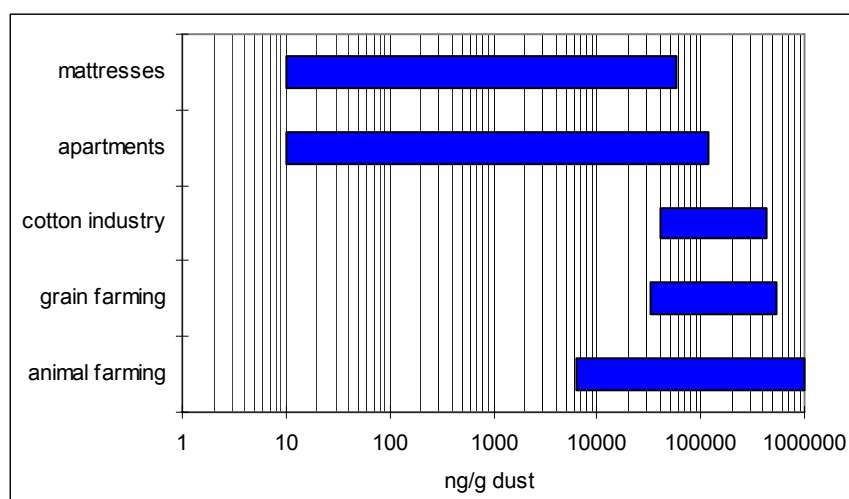


Figure 2.2 Endotoxin levels in settled dust of occupational and home environments.

#### **2.5.4.2 Peptidoglycans**

Peptidoglycans are cell wall components of all bacteria but are most prevalent in the cell wall of gram-positive bacteria. They have been shown to have pro-inflammatory properties that resemble those of endotoxins. Peptidoglycans, determined as their



chemical marker muramic acid, were found in hospital and home air conditioner filters (Fox and Rosario, 1994). However, relationships between environmental peptidoglycan exposure and respiratory health has to date not been studied, and therefore its role as an exposing agent in indoor environments is unclear.

#### **2.5.4.3 House Dust Mites**

House dust mites are arachnids, distantly related to ticks and spiders. The major allergen produced by the house dust mite *Dermatophagoides pteronyssinus* was isolated in 1980 and called *Der p1*. *Der p1* was later established to be a protease present in high amounts in fecal pellets of mites (NAS, 2000). A second allergen called *Der p2*, is also present but less often measured. There are in addition many minor epitopes from dust mite feces that cross-react with other allergens from other taxa. Another house dust mite species, *D. farinae* produces as its major allergen *Der f1*.

The concentrations of house dust mite allergens that are detected in air are “low, highly variable and often undetectable by conventional assays in the absence of disturbance” (IOM, 2000). The allergen that becomes airborne after disturbance, for example, during bed-making, is associated with particles > 5 microns and falls rapidly after the disturbance is over. However, modern personal exposure tools have demonstrated that exposure occurs even while sleeping and the allergen is carried on fecal particles, flakes and amorphous particles with aerodynamic diameters less than their maximum dimensions (O’Meara and Tovey, 2000).

### **Exposure Evaluation**

The amount of house dust mites can be estimated with determination of the main allergens of house dust mites, *Der f1* and *Der p1*, or the nitrogenous excretory product of mites, guanine, in the dust. The allergen measurements are usually performed with ELISA technique and the amount of guanine is determined with a semi-quantitative colorimetric test (Björnsson et al. 1995).

The mite allergen levels (*Der p1* and *Der f1*) in house dust vary typically between <0.02-20 :g/g (van Strien et al. 1994, Custovic et al. 1999, Su et al. 2001). However, high concentrations 60-200 :g/g can be found, especially in mattresses. House dust mites are known to need high levels of indoor air humidity (RH>50%) and a warm temperature is favorable for them (van Strien 1994), but such associations are not always found (Su et al. 2001). The median concentrations of mite allergens did not associate with reported mold and symptoms or asthma (van Strien et al., 1994, Dales and Miller, 1999). Even though house dust mite allergens are found in dust, it is possible that no allergens will be found in the air (Custovic et al., 1999)

House dust mite allergens are found virtually in all homes in moderate climate (van Strien et al., 1994), but less frequently in a cold climate. This low frequency of house dust mites in the Scandinavian climate was supported by a Swedish study, where

house dust mites were found relatively infrequently, in only 13% of homes (Björnsson et al., 1995). Their presence was, however, associated with prevalence of asthma.

In Table 2.8, examples of reported levels of various allergens in indoor environments are shown.

Table 2.8 Allergens exposure in indoor environments

Type of building	Country	Location	Der p1	Der f1	Der p2	Fel d1	Can f1	Reference
Homes	German	Carpet dust samples	$\pi$ 0.23			$\pi$ 0.065		(Wahn et al. 1997)
Homes	German	Carpet dust Mattress	$\pi$ 0.18-0.48 $\pi$ 5.60			$\pi$ 0.03-0.06 $\pi$ 0.1		(Lau et al. 2000)
Homes	German	<u>Mattress dust</u> 133 atopics 55 non-atopics	$\pi$ 0.6 $\pi$ 0.4					(Lau et al. 1989)
Homes	Germany	Living room floor	$\pi$ 0.16	$\pi$ 0.42		$\pi$ 0.24		[(Gehring et al. 2001)]
Homes	German, Denmark	<u>Child's mattress</u> Atopic children Non-atopic children	$\pi$ 0.60 $\pi$ 0.40	$\pi$ 0.68 $\pi$ 0.45				(Lau et al. 1989)
Homes	Netherlands	Bedroom floor Living room floor Mattress surface	$\delta$ 2.37 $\delta$ 2.20 $\delta$ 5.08					(van Strien et al. 1994)
Homes	Netherlands	<u>Case (Children with respiratory symptoms)</u> Living room floor Child's bedroom floor Child's mattress	$\delta$ 2.04 $\delta$ 1.96 $\delta$ 4.64					(Verhoeff et al. 1994)
Homes	Netherlands	<u>Control (Children without respiratory symptoms)</u> Living room floor Child's bedroom floor Child's mattress	$\delta$ 2.76 $\delta$ 2.48 $\delta$ 5.59					
Homes	Netherlands	<b>Living room floor</b> No organic bin Organic waste bin	$\delta$ 0.69 $\delta$ 0.72			$\delta$ 0.90 $\delta$ 0.67		(Wouters et al. 2000)
Homes	Netherlands	<b>Kitchen room floor</b> No organic bin Organic waste bin	$\delta$ 0.44 $\delta$ 0.48			$\delta$ 0.22 $\delta$ 0.26		
Homes	Netherlands	Living room floor Bedroom floor Children's mattresses	$\delta$ 0.11-0.15 $\delta$ 0.19-0.34 $\delta$ 0.96-1.48					(Douwes et al. 2000)
Homes	Netherlands	Living room floor Bedroom floor Children's mattresses	$\delta$ 7.20 $\delta$ 2.40 $\delta$ 4.10					(Douwes et al. 1999)

<b>Home</b>	Netherlands	<u>Infants of non-atopic parents</u>	$\delta$ 0.54-5.84				(van Strien et al. 1995)
		Living room floor	$\delta$ 1.14-7.93				
		Parental bedroom floor	$\delta$ 0.79-3.63				
		Child's bedroom floor	$\delta$ 3.33				
		Child's mattress surface					
		<u>Infants of atopic mothers</u>	$\delta$ 0.25-3.79				
		Living room floor	$\delta$ 0.64-4.51				
		Parental bedroom floor	$\delta$ 0.24-2.56				
		Child's bedroom floor	$\delta$ 1.61				
		Child's mattress surface					
<b>Homes</b>	Sweden	Child's mattress, floor of bedroom, living room, kitchen, bathroom, and laundry room	$\pi$ 0.04		$\pi$ 0.63	$\pi$ 0.80	(Warner et al. 1996)
<b>Homes</b>	Sweden	Floor	$\delta$ 0.02-1.94				(Munir et al. 1993)
<b>Homes</b>	Italy	Living room floor	$\delta$ 0.15	$\delta$ 0.83			(Moscato et al. 2000)
		Mattresses	$\delta$ 0.34	$\delta$ 7.76			
<b>Homes</b>	England	Bedroom floor	$\delta$ 4.30				(Sporik et al. 1990)
		Living room floor	$\delta$ 2.40				
		Mattress dust	$\delta$ 18.40				
<b>Homes</b>	England and USA	Bedding (pillows and quilt)	$\pi$ 4.40				(Custovic et al. 1996)
		Bedroom carpet	$\pi$ 1.20				
		Mattress	$\pi$ 3.60				
<b>Homes</b>	USA	Bedding	$\delta$ 11.39				(Smith et al. 1985)
		Bedroom floor	$\delta$ 5.79				
		TV room floor	$\delta$ 7.98				
		TV room furniture	$\delta$ 11.33				
<b>Homes</b>	Taiwan	<u>Children's mattresses</u>					(Su et al. 2001)
		Spring	$\delta$ 3.78	$\delta$ 1.41			
		Summer	$\delta$ 5.61	$\delta$ 1.49			
		Fall	$\delta$ 9.11	$\delta$ 1.96			
		Winter	$\delta$ 10.15	$\delta$ 3.32			
<b>Homes</b>	Australia	<u>Sydney</u>					(Marks et al. 1995)
		Bedding	$\delta$ 38.90				
		Bedroom floor	$\delta$ 22.40				
		Living room floor	$\delta$ 13.70				
<b>Homes</b>	New Zealand	<u>Busselton</u>	$\delta$ 14.90				(Wickens et al. 1997)
		Bedding	$\delta$ 11.20				
		Living room floor					
		Beds	$\delta$ 46.60				
<b>Homes</b>	New Zealand	Living room floor	$\delta$ 25.50				(Wickens et al. 2001)
<b>Public building</b>	New Zealand	Living room floor	$\delta$ 40.00				(Wickens et al. 1997)
<b>Public building</b>	New Zealand	Floor	$\delta$ 0.58				(Wickens et al. 1997)
		Carpet floor	$\delta$ 0.95				

$\delta$  Geometric Mean  $\epsilon$  Range  $\mu$  Mean  $\pi$  Media

### **2.5.6 Pollen**

Plants are either insect-pollinated or wind-pollinated. Reproductive success is maximised especially in wind-pollinated plants by the release of large amounts of pollen. For instance, a pine tree can produce tens of billions of pollen grains per year. The size of a single pollen grain varies between 10 and 200 µm in diameter. In many countries, as many as 10-15 % of the population are sensitised to the proteins carried by these particles. The allergenic proteins are found inside the grains, in the pollen walls, but also in other parts of the allergenic plant. Due to the huge production of pollen allergens in nature, air both indoors and outdoors contains a mixture of intact and empty pollen grains, small pollen fragments and other plant-derived protein containing particles. Sedimentation rates of these particles vary depending on their size, shape, and surface features but are generally high in indoor environments. Therefore indoor horizontal surfaces are reservoirs of pollen allergens.

### **Exposure Evaluation**

Penetration transfer mechanisms for bioaerosols from outdoors include infiltration, from mechanical ventilation and transport on clothing (Yli-Panula and Rantio-Lehtimäki, 1994; 1995).

Pollen exposure occurs outdoors but also indoors. High pollen allergen concentrations in indoor settled dust have been reported in several studies (Yli-Panula and Rantio-Lehtimäki, 1995; Fahlbusch et al., 2000). The smallest particles are resuspended in the air, and they have the tendency to remain buoyant for extended periods after vacuuming and other house cleaning operations. Vacuum cleaners without HEPA- or other fine particle filters actually increase the airborne concentration especially in the smallest particle size classes.

In Swedish school and office rooms, Holmquist and Vesterberg (1999) found substantial amounts of airborne pollen allergens, predominantly in particles with smaller diameters than the intact pollen grains. Settled pollen grains in dry conditions retain their allergenic activity until the next pollen season. Yli-Panula and Ahlholm (1998) incubated birch pollen at room temperature for up to 12 months and observed minimal changes in allergenicity.

In general, bringing flowering twigs of allergenic plants such as willow or birch indoors can cause a substantial increase in pollen concentration and allergens in the air.

## **2.6 The sizes of bioaerosol particles**

Most biological agents are in particulate form and, by that, present major challenges when attempting to determine their fate and dynamics in the air and deposition in the respiratory tract. In any case, the particle size is a critical characteristics of particles that contributes to the exposure of humans to these particles. Biological aerosols (or bioaerosols) may be described as naturally occurring or an artificially generated

collection of biological particles diffused in the air or in another gaseous phase (Dimmick and Akers, 1969; Lacey and Dutkiewicz, 1994). With regard to these definitions, the bioaerosols, as particles, can be classified by their sizes. A broad size classification of biological agents is presented in Figure 2.3. For comparison, majority of particles in terms of number (as opposite to mass) originating from motor vehicle emissions are below 1  $\mu\text{m}$ , while particles resulting from mineral and material processing are in the range from a few to tens of micrometers.

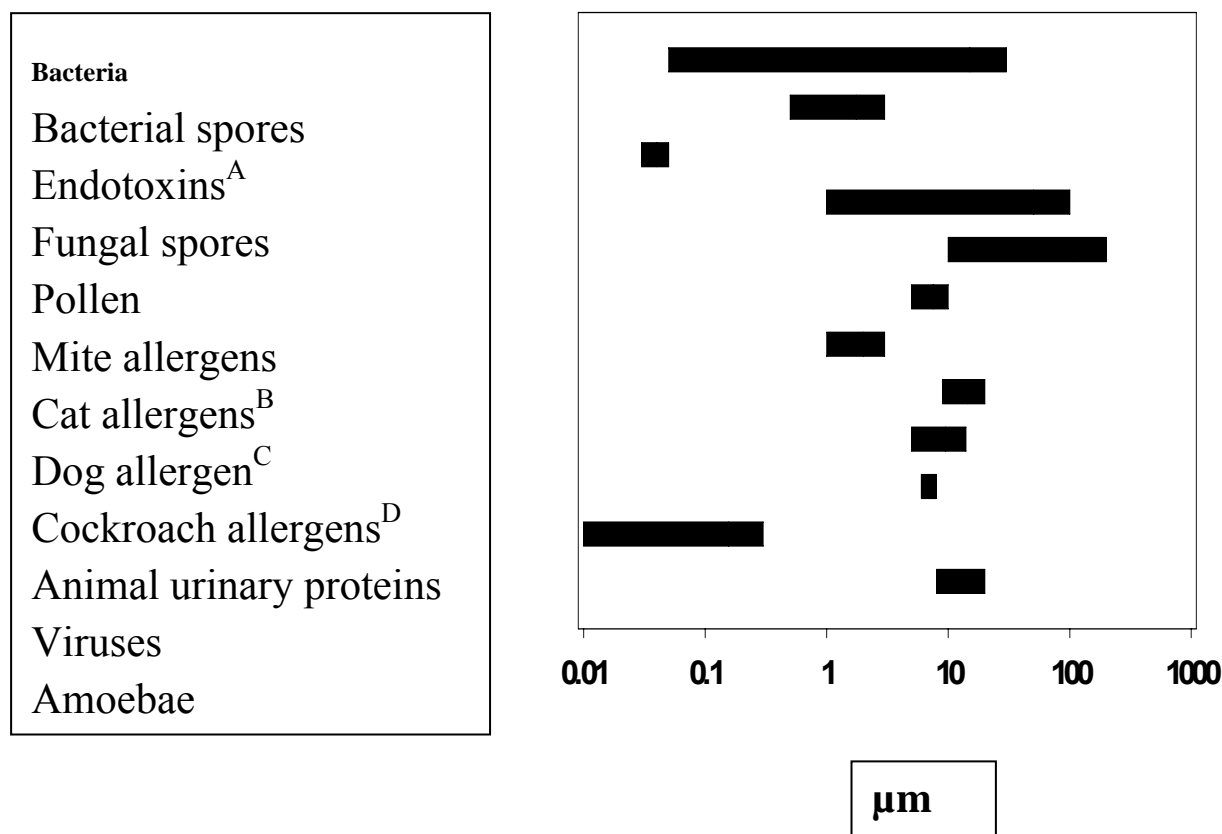


Figure 2.3 Sizes of biological particles (After: Dutkiewicz et al., 1992; ACGIH, 1999; Baron and Willeke, 2001).

- A) – sizes of endotoxin vessels; in the air endotoxins are usually found in the fraction of fine particles, below 5  $\mu\text{m}$
- B) – are found in saliva and skin dander; transported in the air as small particles, below 2.5  $\mu\text{m}$
- C) – are carried with large particles
- D) – are carried on particles bigger than 5  $\mu\text{m}$  in size

Bioaerosols can occur in the air as single cells or aggregates, as fragments of cells or spores, as endotoxins, exotoxins, enzymes, glucans, mycotoxins, or animal protein particles, or as agglomerates or conglomerations, e.g., endotoxins, allergens, animal urinary proteins or as water or saliva droplets, so called “droplet nuclei”, which usually contain no more than one microbe per particle.

For biological particles, dust often acts as their carrier (Dutkiewicz et al., 1988). In this way, both allergens, microbes and other agents are transferred. For example, inorganic dust can serve as transporting medium for bacterial cells dispersed in the air. The effectiveness of this transfer process depends on the sizes of both transporting and transported particles. Small combustion particles cannot transport whole cells of bacteria or fungi, but can become a good conveyor for e.g., bacterial endotoxins (Górny et al., 1999).

As shown in Figure 2.3, bioaerosols present in indoor air are of various sizes. Examples of the aerodynamic diameters of some bacteria and fungal spores are listed in Table 2.9.

Table 2.9. Physical dimensions and aerodynamic diameters of selected microorganisms.\*

Microorganism	Aerodynamic diameter (µm)	
Bacteria:		
<i>Bacillus subtilis</i> var. <i>niger</i>	0.9	
<i>Thermoactinomyces vulgaris</i>	0.6	
<i>Streptomyces albus</i>	1.2	
<i>Pseudomonas fluorescens</i>	0.8	
<i>Micrococcus luteus</i>	1.0	
Fungi:		
<i>Aspergillus flavus</i>	3.6	
<i>Aspergillus fumigatus</i>	2.1	
<i>Aspergillus versicolor</i>	2.4	
<i>Cladosporium cladosporioides</i>	1.8	
<i>Paecilomyces varioti</i>	2.6	
<i>Penicillium chrysogenum</i>	2.8	
<i>Penicillium brevicompactum</i>	2.3	
<i>Penicillium melinii</i>	3.1	
<i>Stachybotrys chartarum</i>	5.6	
<i>Scopulariopsis brevicaulis</i>	5.3	

\* After: Madelin and Johnson, 1992; Reponen et al., 2001

The degree of hydration of spores, a consequence of the prevailing relative humidity, affects bioaerosol particle size ranges (Madelin and Johnson, 1992). If a particle is hygroscopic, its size can dramatically increase in the human airways, where the

relative humidity of the air approaches 100%. For bioaerosol particles ranging from 0.1 to 10  $\mu\text{m}$  in diameter, the greatest change in the respiratory deposition due to hygroscopic size changes occurs in the particle size range of 0.5-2.0  $\mu\text{m}$  (Reponen et al., 1996). This may have a significant impact on the actual exposure or inhaled dose of the particles.

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### 3. Health effects of biological agents in the indoor environment

#### 3.1 Introduction

Exposures to biological agents in the indoor environment have been suggested to be associated with a wide range of health effects with major public health impact, including airway inflammation, allergies, neurological effects, and acute toxic effects. These categories of health effects are not isolated events, may partially overlap and may be caused by the same exposures. Indoor exposures to viruses, bacteria, fungi and other infectious agents may cause infectious diseases but these will - with the exception of legionnaires disease - not be discussed in this report as explained in the introduction. Some biological agents e.g. viruses and mycotoxins have been proposed to play a role in cancer causation in *industrial and agricultural occupational environment*, however, there is no evidence that *indoor* exposures are involved. Therefore cancer will not be discussed in this chapter.

Respiratory symptoms are probably among the most important health effects associated with indoor bioaerosol exposure. Asthma has received most attention by far, however, other respiratory conditions such as toxic pneumonitis, hypersensitivity pneumonitis (HP), and chronic bronchitis and chronic airflow obstruction (described in more detail below) may have to be considered as potential health outcomes as well. In addition to adverse health effects some protective effects of microbial exposure on atopy and atopic conditions has also been suggested. In this chapter we will first briefly describe some of the basic concepts of the most important health effects. The reader is referred to medical and immunology textbooks for a more extensive review of these diseases. Secondly, we will briefly discuss susceptibility issues and interactions between indoor exposures. We then consider the epidemiological data linking dampness and exposure to biological agents with health outcomes. In addition, where appropriate we will review case reports. The main focus of this chapter will be on respiratory symptoms and asthma.

#### Basic descriptions and concepts of health effects associated with dampness and biological agents

It is important to make a distinction between primary and secondary causation (i.e. development versus exacerbation of pre-existing disease). *Exacerbation* may be caused by exposures that do not necessarily cause *the development* of the disease, or, they may be caused by the same exposures but at different levels. Most studies reviewed in the current chapter (with exception of some birth cohort studies focusing on indoor allergen exposure and atopy and asthma) were of a cross-sectional nature thus hampering assessment of primary causation. In those cases where information is available on primary causation we will mention that explicitly in the text.

Secondly, for each of the conditions it is important to stress that none are exclusively associated with indoor exposures to biological agents. Symptoms or diseases can also be caused (i.e. primary and secondary causation) by exposures to a number of non-

biological agents either in the indoor or outdoor environment (cigarette smoke, air pollution, occupational chemicals etc). In addition, these symptoms or diseases may be caused by biological agents in the *outdoor* environment.

Finally, it is important to differentiate between symptoms and diseases on the basis of the underlying mechanisms and exposures. Most respiratory health effects associated with indoor exposures to biological agents (asthma, toxic pneumonitis, HP, bronchitis etc) result from airway inflammation. Based on the underlying inflammatory mechanisms and subsequent symptoms, a distinction between allergic and non-allergic respiratory diseases can be made. Non-allergic respiratory symptoms reflect a non-immune-specific airway inflammation, whereas allergic respiratory symptoms reflect an immune-specific inflammation in which various antibodies (IgE, IgG) can play a major role in the inflammatory response. This distinction is particularly important in the case of asthma and rhinitis, conditions that may be induced both by allergic (IgE mediated; see below) and non-allergic immune responses. As a consequence asthma and rhinitis may thus also be caused both by allergenic and non-allergenic exposures.

### **Atopy**

Atopy is defined as IgE-mediated sensitisation to “common allergens” such as house dust mite, pet, pollen, grasses, trees, weeds, cockroach and various food allergens. Atopy is usually assessed by using skin prick tests or specific serum IgE measures of sensitisation to common allergens. However, a positive test only indicates that a subject is sensitised against a common allergen but does not constitute proof that symptoms experienced by the subject are caused by allergic (IgE) mediated mechanisms. Depending on the definition, 20-40% of the people living in affluent countries are atopic. Atopy is strongly associated with (allergic) asthma. Whether atopy should be considered a health effect or only an intermediate of allergic diseases (such as allergic asthma and rhinitis; see below) is a subject of debate.

### **Asthma**

Asthma is a heterogeneous chronic inflammatory disorder of the airways involving airflow limitation that is at least partly reversible and which results in recurrent episodes of symptoms such as wheezing, breathlessness, chest tightness and cough. The inflammation also causes an associated increase in airway responsiveness to a variety of stimuli. *Allergic asthma* represents asthma with an underlying atopic airway inflammation involving IgE sensitisation and eosinophils whereas *non-allergic asthma* is characterised by non-atopic airway inflammation (not involving IgE and eosinophils). In occupational medicine it has since long been recognised that a substantial proportion of work-related asthma is non-allergic. A recent systematic review of population-based studies suggested that the proportion of asthma cases that are attributable to atopy was less than one-half in the general population (Pearce et al., 1999; Douwes et al., 2002) indicating that non-allergic asthma may at least be as important as non-allergic asthma.

### **Rhinitis**

Rhinitis is caused by allergic or non-allergic inflammation in the upper respiratory tract mucosae. Usually the conjunctival mucosa is also affected. Typical symptoms of

rhinitis include sneezing, rhinorrhea, and/or nasal congestion (runny and/or stuffy nose) often accompanied by tears, itchy eyes, and red and swollen eyelids (conjunctivitis). Episodic symptoms are the hallmark of allergic rhinitis (e.g. “hay fever”). Rhinitis is associated with asthma, although they are separate conditions and the overlap between the two conditions is not very great (ISAAC, 1998).

### **Bronchial hyperresponsiveness**

Bronchial hyperresponsiveness is an exaggerated bronchoconstrictor response to a wide variety of non-specific and allergic stimuli. As previously mentioned, asthmatics may develop a non-specific bronchial hyperresponsiveness (BHR), and it has been suggested that asthma should be defined as symptomatic BHR (BHR can be assessed by histamine, methacholine, or saline challenges). However, although BHR is clearly related to asthma, and may be involved in many of the pathways by which variable airflow obstruction may occur, variable airflow obstruction may occur independently of BHR, and vice versa (Pearce et al, 1998). Thus, they remain separate phenomena both of which usually involve inflammation of the airways (Chung, 1986). In fact, available evidence from random population surveys indicates that BHR (or the combination of BHR and symptoms) is inferior to symptom questionnaires when compared with the “gold standard” of a careful clinical diagnosis of asthma (Pekkanen and Pearce, 1999).

### **Organic dust toxic syndrome**

Organic dust toxic syndrome (ODTS) describes a condition that is characterized by an acute febrile *non-allergic* illness, neutrophilic airway inflammation, shivering, dry cough, chest tightness, dyspnea, headache, muscle and joint pains, fatigue, nausea and general malaise (Donham and Rylander, 1986; Von Essen et al, 1990a, Rylander 1997). The symptoms resemble those of influenza, but symptoms usually disappear the following day after initial exposure. Whether indoor exposures play a role is currently not clear.

### **Irritation, non-specific symptoms and Sick Building Syndrome**

Many non-specific, but temporal complaints have been described by various investigators of health effects in association with indoor moisture and mould exposure. Effects may be related to irritant-toxic effects of bacteria and fungi and /or their structural components causing localized and systemic non-specific inflammatory effect. The symptoms include: irritation of the skin and mucous membrane, eye or nasal itching and dryness, prolonged sore throat, hoarseness, dry cough, chest discomfort, as well as nausea, vomiting, headaches, joint pain, and low grade fever. Usually most of those complaints subside rapidly with removal from the exposure. Episodes of nonspecific health complaints in indoor workplaces (sometimes called sick building syndrome or nonspecific building-related symptoms), not attributable to specific recognized disease or exposures, have been commonly reported in recent decades (Mendell, 1993). In the 1990's the concept of “sick building syndrome” (SBS) was well established in the literature. The term had been coined in a World Health Organization Working group report in 1983 (World Health Organization, 1983). Various definitions of SBS have been used (Jaakkola, 1998), but generally included are irritation of eyes, nose and throat, headache, and lack of concentration.



These symptoms are tightly tied to being in the building. The role of biological exposures play in SBS is not yet understood.

### **Hypersensitivity pneumonitis (HP)**

Among the most severe, although less common in indoor air situations is the pulmonary disease called hypersensitivity pneumonitis (HP) which is used synonymously with extrinsic allergic alveolitis (EAA) or “farmers lung” or “pigeon breeder’s lung”. The etiology of the disease is associated with exposure to fungi and thermophilic actinomycetes (spore-forming bacteria). HP is a generic term used to describe a serious pulmonary condition with delayed febrile systemic symptoms, manifested by an influx of inflammatory cells to the lung parenchyma and the formation of granulomas there (Curtis and Schuyler, 1994). Symptoms characteristic for HP are very similar to those described for organic dust toxic syndrome (ODTS; see above) although they are different diseases with different underlying immunological mechanisms. HP is more serious and in its chronic stage may lead to permanent lung damage and work disability (Pickering and Newman Taylor, 1994) in contrast to ODTS which is reversible. The underlying immunological mechanisms of HP are complex and only partly understood but both allergic and non-allergic immunological responses are believed to play a role (Salvaggio, 1997).

### **Chronic bronchitis**

Chronic bronchitis is defined as cough with phlegm (productive cough) for at least 3 months per year for 2 years or more (American Thoracic Society, 1962). Chronic bronchitis may be present with or without airway obstruction. The underlying mechanism is an increase in mucus secreting glands and alterations in the characteristics of the mucus itself (Parkes, 1994). Airway inflammation is present and an increased number of neutrophils can be observed (Von Essen et al., 1990b). Subjects with chronic bronchitis may also have symptoms such as dyspnea, chest tightness, and wheezing and there is thus a considerable overlap in symptoms between allergic asthma, non-allergic asthma and bronchitis (Schenker et al., 1998). Chronic bronchitis occurs rarely in children and the prevalence increases in older age groups. Smoking is one of the most important causes of chronic bronchitis. However, biological agents (e.g. endotoxin and  $\beta$ -glucan) may play a role as well, as has been demonstrated in the occupational environment (Simpson et al., 1998; Mandryk et al., 2000). Whether indoor exposures play a role is currently not clear.

### **Chronic airflow obstruction**

Several studies have shown associations between chronic airflow obstruction as measured by spirometry (particularly FEV1) and organic dust exposures in industrial and agricultural occupational populations (Kennedy et al., 1987; Heederik et al 1991; Rylander and Bergstrom, 1993; Smid et al., 1992; Zejda et al., 1994; Schwartz et al., 1995). Associations were particularly strong for endotoxin exposure. Again, as mentioned for chronic bronchitis it is currently not clear whether indoor exposures to biological agents may also cause chronic airflow obstruction.

### **Neurological effects**

Biological agents have been suggested to be associated with symptoms such as headache, fatigue and forgetfulness which may have a neurological basis. However, not much is known about the potential influence of biological agents and their association with neurological effects.

**3.2 Remarks on susceptibility**

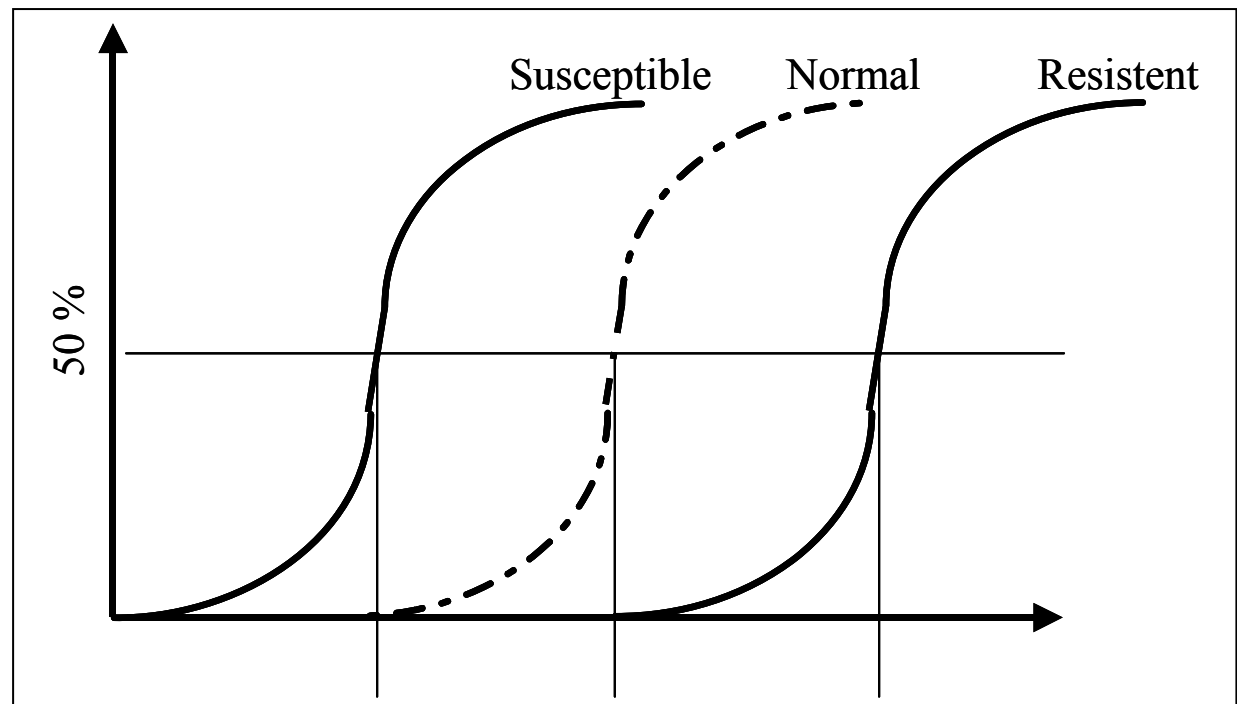
Biological pollutants in indoor air comprise irritants, allergens, infectious agents and carcinogens. Susceptibility towards an environmental agent is either genetic like alpha-1-antitrypsin or acquired like allergy towards an allergen present in the environment (Sigsgaard et al., 1994). Furthermore, humans are experiencing different states of physiological balance during their lifetime, rendering susceptibility towards different agents during the life. Table 3.1 shows the factors that affect human susceptibility towards non-infectious effects of biological agents.

**Table 3.1.** Host factors influencing susceptibility

Age
Gender
Genetic factors
Atopy
Allergy
Asthma and other respiratory diseases
Smoking

Susceptibility is defined as an increased reaction towards an exposure, resulting in a reaction at lower dose or concentration compared to the rest of the population. This is illustrated in Figure 3.1. As is seen from the Figure, theoretically all persons in the susceptible group might elicit symptoms before any person from the rest of the population is experiencing any adverse effects. The resistant group would still at relative high levels of exposure have no adverse effects. This resistant subgroup of people has often been studied in occupational health studies as the “survivor population” at some particular industry. In the living environment, all individuals must be able to breathe and feel comfortable. Therefore, the target of primary

prevention in environmental medicine is the susceptible group since the rest of the population is well covered by the standards that are set for the most susceptible group. However, even in environmental medicine, very small proportions of the population, like severe asthmatics, will have to be treated as a special group. From an economical standpoint it may be impossible to implement measures that would bring the exposure under the lower limit for adverse effects of these susceptible individuals.



**Figure 3.1:** Theoretical dose response relationships for susceptible, normal and resistant groups

### 3.3 Age groups with increased susceptibility

#### Children

Small children breathe more than adults in relation to their body weight. In consequence, uptake of airborne biological agents may be higher in small children. In addition, the immune apparatus of children is maturing, providing them less defence against biological exposures. Therefore, allergic sensitisation through exposure to biological agents might be facilitated as compared to adults' exposure. Environmental tobacco smoke (ETS) might even aggravate allergic sensitisation since small children are susceptible to ETS exposure (Gurkan et al., 2000). Mycotoxins present in the environment will also have a greater effect on children as compared to adults (Dietert et al., 2000).

#### Elderly

Among elderly people (a group which is rapidly increasing world-wide), it is well known that the rate of tissue-repair is decreasing together with a decrease in the metabolic rate. This means that the elderly will be more susceptible to the effect of cellular damage caused by e.g. mycotoxins. Also the efficiency of the defence mechanisms of the airways, i.e. the macrophages and ciliary function is declining with increased age (McCafferty et al., 1995).

## **Gender**

Females have been shown to be more sensitive to many environmental irritants, including volatile chemicals and volatile microbial products (Cometto Muniz and Cain, 1993, Shusterman, 2001). This may indicate that females will rate the quality of the environment lower at an earlier stage than their male colleagues. Whether there is a gender difference in sensitization towards allergens is still under debate. In one study boys had more positive skin prick tests to indoor allergens (Sears et al., 1993), but in another study, sensitisation towards indoor allergens was more frequent in girls (Schwartz and Weiss, 1995).

## **Genetic factors**

There is considerable variability between individuals in the response to inhaled allergens, but it is not known why certain people develop asthma when challenged with environmental agents and others remain healthy. Recent investigations have implicated genetic background as an important susceptibility factor for the pathogenesis of asthma as well as responsiveness to environmental agents including allergens, endotoxin, and ozone. The site where an irritant or allergen is deposited is a major determinant for the mucosal reaction towards an environmental contaminant. The literature on this topic, however, is scarce. The immunocompetent cells reside in greatest number in the peripheral airways and the clearance is much slower in these segments of the lung epithelium. Hence more peripheral deposition will have a tendency to increase the severity of the response. Persons with a genetically lower  $\alpha_1$ -antitrypsin concentration in the blood have been shown to be more vulnerable towards contaminants in the air. In a study of cotton workers exposed to organic material including LPS, an increased risk of respiratory symptoms among the MZ heterozygous was found, after control for other factors, i.e. smoking, sex and exposure (Sigsgaard et al., 1994).

Further, polymorphisms in the gene for toll-like receptor 4 (TLR4) have been identified as important determinants of responsiveness to endotoxin in mice and humans. Sarpong et al. (2001) have designed a study to address the hypothesis that a polymorphism in the gene for TLR4 (Tlr4) also determines susceptibility to interaction between O<sub>3</sub> and inhaled recombinant cockroach allergen in mice.

## **Atopy and allergy**

Atopy may be another factor effecting individuals' susceptibility towards the contaminants of indoor environment. This was shown in the Danish town hall study by Skov et al. (1992). Persons with allergy have are in risk of having symptoms if allergens like cat allergen Fel d1 are brought into their immediate surroundings

(Smedje et al. 1997). Likewise they might react with antibody formation towards allergens encountered in the environment like e.g. mould spores.

## **Asthma**

Asthma is a growing problem, and one of the major causes of admission of children to hospital in major cities in the United States (Griffiths et al., 1995). Among adults there is also an increasing prevalence of asthma. People with moderate to severe asthma will react adversely towards biological and other contaminants in the indoor environment, due to a non-specific bronchial hyperresponsiveness. Michel et al. (1991, 1996) has shown that the severity of asthma and need of medication in house dust mite allergic patients is closely related to the amount of endotoxin in house-dust in a dose dependent manner.

### **3.4 Modification of health effects of indoor biological agents by other exposures**

Exposure to mixtures of contaminants may result in changes in the health effects of indoor biological agents, which reflect synergistic or antagonistic actions between specific contaminants (Schlesinger, 1995).

Tobacco smoke exposure increases the effect of allergens on allergic sensitisation (Moerloose et al., 2006). Tobacco smoke also induces inflammation in the airways and a decrease in ciliary beat frequency due to the effect of nicotine on the epithelial cells. There is evidence for synergism between different indoor environmental exposures. The combination of exposure to environmental tobacco smoke, damp housing and high level of Fel d 1 ( $>8 \mu\text{g/g}$ ) resulted in a very high risk of sensitisation to cats (odds ratio of 42) (Lindfors et al., 1999). Data from the European Community Respiratory Health Survey, was analysed to determine the association of smoking with total IgE levels and with sensitization to 3 common environmental allergens. The results from 13,002 randomly selected young adults living in the areas served by 34 centers in 14 countries showed that the geometric mean total IgE level was higher in smokers compared with lifetime nonsmokers. Current smokers were at an increased risk of sensitization to house dust mite allergen (OR, 1.13; 95% confidence interval [CI], 1.02-1.26) but a decreased risk of sensitization to grass (OR, 0.76; 95% CI, 0.67-0.88) and cat allergens (OR, 0.69; 95% CI, 0.59-0.80) (Jarvis et al., 1999). A recent Canadian study found an interactive effect of smoking and household pets on asthma incidence in females but not in males (Chen et al., 2002).

Pollen allergens interact with other sources of fine particles in polluted air, which could cause the allergens to become concentrated in polluted air and to trigger asthma attacks (Knox et al., 1997). As for the interaction between biological and non-biological agents, airborne particles such as diesel exhaust particles have an adjuvant activity for IgE antibody and cytokine production (Takafuji et al., 1989; Fujimaki et al., 1995). Intranasal instillation of ragweed allergens with diesel exhaust particles to

allergic patient significantly increased allergen-specific IgE production and enhanced Th2 – type cytokine mRNA expression compared to those of allergic patients instilled with ragweed alone (Diaz-Sanchez et al., 1997).

Both allergens and viruses have been known to worsen asthma symptoms. A study in Britain demonstrated that allergen exposure and viral infections may act synergistically to trigger exacerbations in adults (Green et al., 2002).

### **3.5 Evidence of the health effects of dampness and fungal contamination**

There is substantial epidemiological evidence that dampness, excess humidity or moisture and water damage of buildings are strongly associated with adverse health effects of the occupants. The two reports by the American Institute of Medicine on asthma “Clearing the Air: Asthma and Indoor Air Exposures” and dampness “Damp Indoor Spaces and Health” had the following conclusions: that damp conditions are associated with both the development of asthma and the exacerbation of existing asthma; that the factors related to dampness that lead to the development and exacerbation of asthma are not yet known but probably relate to dust mite and fungal allergens; that there is sufficient evidence of an association between fungi or moulds and the exacerbation of existing asthma; and that there is insufficient evidence to determine whether or not an association exists between fungi or moulds and the development of asthma (IOM, 2000, 2004). The mechanisms underlying the health effects of exposure to fungal components is not well understood and both IgE-mediated allergy, other immunologic responses, non-specific inflammatory responses are all possible. For example, although many of the reported symptoms mimic allergic symptoms, only a few percent of the exposed individuals actually develop allergies against moulds (Taskinen et al., 1997; Immonen et al., 2001).

Most studies reporting the epidemiological findings have been cross-sectional questionnaire studies where both health effects and assessment of water damage and mould contamination were reported by the study participants or the visual environmental assessments were carried out by the study investigators. Since 1982, a large number of studies have been conducted on the association of dampness, mould and respiratory health in Europe and North America in residential housing. Studies in the USA and Canada have involved the largest numbers of individuals. A study of the respiratory health of 4,600 children from six cities in the northeast USA demonstrated that the presence of mould and dampness in the homes were correlated to several respiratory symptoms as well as a number of non-respiratory symptoms. The effect was of similar dimension to parental smoking (Brunekreef et al., 1989). Two studies involving 15,000 children and 18,000 adults from 30 communities in Canada came to similar conclusions. The authors concluded that a non-allergenic mechanism was involved. A dose-effect was also seen in that more visible mould yielded more symptoms. Overall the mould contamination was associated with a 50% increase in asthma and a 60% increase in upper respiratory disease (Dales et al., 1991a; 1991b). A large European study (Zock et al., 2002) including 38 study centres worldwide and 19,000 adults concluded that indoor mould growth has an adverse effect on adult asthma. Reported mold exposure in the last year was associated with asthma

symptoms and bronchial responsiveness (OR range, 1.14-1.44). This effect was homogeneous among centers and stronger in subjects sensitized to *Cladosporium* species. As in the Canadian studies, the authors concluded that both allergic and non-allergic mould-related effects were involved in the health outcomes. There is some evidence that exposure to environmental moulds may play a role in asthma-related mortality (Targonski et al., 1995). The data are reviewed e.g., in Health Canada (2004) and Koskinen et al. (1999). The effects of fungal contamination of housing on health remain when adjusted for socioeconomic factors, pets, household smokers, endotoxins and dust mites (Dales and Miller, 1999).

Similar health effects have been reported both among children (e.g., Dales et al. 1991a) and adults (e.g., Dales et al. 1991b, Brunekreef et al., 1992, Pirhonen et al., 1996). The literature has been reviewed by Peat et al. (1998) and more recently, two interdisciplinary reviews concerning dampness in buildings and health effects were published by Bornehag et al. (2001, 2004). The reviews show that dampness in buildings appears to increase the risk for health effects in the airways, such as cough, wheeze and asthma, with relative risks in the range of OR 1.4 –2.2. As the authors point out, the causal agents for health effects related to the dampness as well as the mechanisms of the health effects are not known. A dose-response relationship between the extent of water damage in the home and health findings has been reported (Haverinen et al., 2001). Although studies have mainly been on homes, similar relationships have been reported from children's day care centers (Koskinen et al., 1997), and schools (Smedje et al 1997, Meklin et al. 2002).

Studies of indoor non-industrial work environments have documented health effects in relation to dampness and signs of fungal contamination. In a case report, an outbreak of respiratory diseases was described among workers at a water-damaged building in Finland (Seuri et al. 2000). In the United States, occupants of a water-damaged, mouldy office building had excess pulmonary disease (asthma and HP) and mucous membrane irritation as compared to occupants of an undamaged building. There was also increased risk for respiratory symptoms in occupants of the water-damaged building who had longer tenure, closer proximity to water-damaged material, and jobs requiring disruption of settled dusts. Symptom rates just prior to re-occupancy of the building after remediation were lower than those reported before evacuation and remained lower some months and re-occupancy (Jarvis and Morey 2001).

In recent occupational studies in the United States, positive associations with indications of a dose-response effect were found between indexes of water-damage and mould as evaluated by industrial hygienists, and reported respiratory symptoms which improved when away from the building (Cox-Ganser et al. 2002; Park et al. 2002).

### **3.5.1 Building-related hypersensitivity pneumonitis**

Building-related hypersensitivity pneumonitis (HP) or extrinsic allergic alveolitis (EAA) is a rare disease. Cases of HP have been associated with fungal contamination in office buildings (Jarvis and Morey, 2001; Kreiss et al., 1989). When the disease

occurs in clusters among building occupants, cause and effect are easy to establish. HP is often serious and accompanied by objective abnormalities on physical examination, chest radiograph, pulmonary function tests, bronchoscopic lavage and biopsy. Outbreaks have occurred in settings with contaminated water-spray humidification systems, contaminated air conditioning systems, and ductwork; water leaks and sewage in large buildings; and secondary contamination of building contents (Kreiss et al., 1989). In these outbreaks, building-related HP and asthma have both been found to occur in the building occupants.

### **3.5.2 Fungal components and health effects**

A major barrier in determining the attributable risk of indoor moulds in relation to population health is lack of knowledge of their allergens (IOM, 2000). The list of fungi common in the indoor environment bears little relationship to the list of fungi for which there are characterized allergens. An example of this is the finding of Japanese researchers that in asthmatic patients, 10-18 % reacted to the indoor mould *Wallemia sebi* (Sakamoto et al., 1989). The use of high water activity media in most studies prevented its detection in many studies of fungi in house dust. Several research groups have identified antigens from *Stachybotrys chartarum* (Barnes et al., 2002, Raunio et al., 2001).

Sensitization to the most common outdoor and indoor fungal species like *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium spp.* is associated with allergic respiratory disease, especially asthma. However, to date, there is very little evidence that supports an important role for type I allergy to fungi in respiratory disease. One reason may be that the most potent allergenic proteins that have been identified as ‘major allergens’ in *in vitro*-produced mould extracts are not the same as those that subjects are actually exposed to in the indoor environment. This may potentially explain the non-detectable fungal allergen levels in many house dust samples and the lack of positive skin prick test or IgE results in asthmatics.



### **3.5.3 $\beta(1\rightarrow3)$ -glucans and health effects**

#### **Animal studies**

Two studies in which guinea pigs were experimentally exposed to an aerosol of  $\beta(1\rightarrow3)$ -glucan indicated no neutrophilia in the airways after a single exposure, as is normally observed after endotoxin exposure (Fogelmark et al., 2001). A combined aerosol exposure to  $\beta(1\rightarrow3)$ -glucan and endotoxin showed that the inflammatory response to the endotoxin exposure was reduced by  $\beta(1\rightarrow3)$ -glucans in a dose-related fashion. Repeated exposures for a period of 5 weeks to an aerosol of  $\beta(1\rightarrow3)$ -glucans, endotoxin or a combination of both resulted in an increase in inflammatory cells in the airways of exposed animals. The combined exposure resulted in an even larger increase in inflammatory cells than for the two compounds separately. Thus, it may be, that in order to mediate symptomatology, glucans need to act in combination with other etiologic agents such as endotoxin or allergens (see below).

One study in mice showed that pre-exposure to inhaled  $\beta(1\rightarrow3)$ -glucans enhanced ovalbumin induced IgE antibody responses and airway antigen-specific allergic reactions including eosinophil infiltration (Wan et al., 1999). In addition, the same authors observed that spleen cells derived from mice pre-exposed to  $\beta(1\rightarrow3)$ -glucans produced significantly higher amounts of IL-4 (IL-4 stimulates B-cells to IgE production and naïve Th0 cells to differentiate toward a Th2 state. Interestingly, a study in guinea pigs that were exposed daily to an aerosol of  $\beta(1\rightarrow3)$ -glucans for a period of five weeks showed an increase in eosinophil numbers in the airways of exposed animals (Fogelmark et al., 2001), confirming the previous study to a certain extent. Thus, the results of animal experiments regarding a potential adjuvant effect of  $\beta(1\rightarrow3)$ -glucan are not yet clear.

#### **Field and human challenge studies**

It has been suggested that  $\beta(1\rightarrow3)$ -glucans play a role in bio-aerosol induced inflammatory responses and resulting respiratory symptoms. However, only a limited number of field or experimental exposure studies are available. Several small field studies in Sweden have been performed in the home environment, a day care centre and among house hold waste collectors and paper mill workers, suggesting a relation with respiratory symptoms and airway inflammation in exposed individuals (Rylander et al., 1997, Thorn et al., 1998, Rylander et al., 1999). A study in Taiwan showed a positive association between  $\beta(1\rightarrow3)$ -glucan exposure and lethargy/fatigue across three populations (i.e. 40 day care centre employees, 69 office employees and 22 residents from the same area) (Wan et al., 1999). One study in The Netherlands showed an association between peak flow variability and  $\beta(1\rightarrow3)$ -glucan levels in house dust among young children (n=159) with respiratory symptoms (Douwes et al., 2000), also after adjusting for potential confounders and other exposures (pets, endotoxin, and house dust mite allergen). Although the studies described above appear to indicate that  $\beta(1\rightarrow3)$ -glucan exposure is involved in the development of respiratory illnesses, it is important to acknowledge that most studies were small, not

always appropriately controlled for other potential causal exposures and/or had weaknesses in the design, thus hampering a valid assessment of the exact role of  $\beta(1\rightarrow3)$ -glucan. Therefore, further and larger studies are needed to assess whether  $\beta(1\rightarrow3)$ -glucans are involved in respiratory health problems in the indoor and occupational environment.

#### **3.5.4 Mycotoxins and health effects**

Mycotoxin exposure and related adverse health effects in indoor environments are gaining more attention although their link is still considered controversial. Many of the observed clinical findings in patients with a known history of toxic fungal exposure cannot be explained with allergy or simple irritant effects. A wide variety of symptoms have been reported. These include respiratory tract bleeding, cough, rhinitis, conjunctivitis, pharyngitis, burning/irritation sensation of the skin or mucus membranes of the nose, flu-like relation with mild temperature elevations among others (Johanning, 1996).

Reviews of the literature in 2001 and 2003 on fungi and human health in the indoor environment with a focus on mycotoxins led to the conclusion that the evidence for inhalational disease from mycotoxin exposure in residential and office settings is so far weak (Burge, 2001, Kuhn and Ghannoum, 2003).

Case reports on pulmonary haemosiderosis in children in relation to fungal exposure began with pediatric pulmonologists at the Rainbow Babies and Children's Hospital, Cleveland, USA, recognizing that there had been an unusually high number of cases of idiopathic pulmonary haemosiderosis between January 1993 and December 1994. Ten cases were diagnosed during this time period compared to 3 cases in the previous ten years (Dearborn et al., 1999). A hypothesis was made of an association between acute idiopathic pulmonary hemorrhage in children and exposure to potent mycotoxins produced by *Stachybotrys chartarum* or other fungi growing in moist household environments (Etzel et al., 1998; Jarvis et al., 1998). However, the evidence was not considered sufficient to show the association between household fungi and AIPH (Jarvis et al., 1998) or any inferences regarding causality.

In another case, *Stachybotrys* was isolated from the bronchoalveolar lavage fluid of a child with pulmonary hemorrhage. *Stachybotrys* was also recovered from his water-damaged home. The patient recovered completely after his immediate removal from the environment and subsequent cleaning of his home (Elidemir et al., 1999). Another case of an infant with pulmonary hemorrhage was reported where air sampling of the residence revealed significantly elevated total spore counts in the patient's bedroom and in the attic. *Aspergillus/Penicillium* species were predominant. *Stachybotrys* spores were found in the air sampled in the patient's bedroom, as well as from surfaces sampled in the patient's closet and the attic ceiling. Additionally, a small patch of *Stachybotrys*-contaminated area in the closet ceiling was sent for mycotoxin analysis. This material proved to be highly toxigenic (Flappan et al., 1999). A 40-day-old male infant had been exposed to fungi for a discrete 2-week period followed by acute exposure to environmental tobacco smoke prior to development of a life-threatening pulmonary hemorrhage. Two fungi associated with mycotoxin production were cultured from surface samples collected in the residence: *Penicillium* (possibly *Penicillium purpurogenum*) and a *Trichoderma* species. *Stachybotrys atra* was not

isolated from air or surface samples. Environmental tobacco smoke exposure occurred over a discrete several-hour period prior to onset of the acute pulmonary hemorrhage (Novotny and Dixit 2000).

Two studies of health effects in occupants of water-damaged buildings with *Penicillium*, *Aspergillus* and *Stachybotrys* spp contamination did not show direct evidence of a relationship between the health outcomes and mycotoxin exposure, but interpreted that the results at least implied the potential for such an association (Johanning et al. 1996; Hodgson et al., 1998).

Immunomodulation has also been reported in some studies. Johanning et al. (1996) reported a lower CD4/CD8 ratios in the group of employees exposed to *Stachybotrys chartarum* compared to a control group. Forty-three of 49 New York employees exposed to satratoxin H producing- *Stachybotrys* in an office building (>100,000 viable spores/cm<sup>2</sup> in sheetrock walls) underwent a health examination. Complaints involved the general areas of respiratory, fatigue, central nervous system, and skin and eye irritation. Immunoglobulin E specific for *Stachybotrys* was detected in four subjects.

### **Animal studies using mould spores**

In animal models, intra tracheal installation of high volumes of spores from toxin-producing strains of *Stachybotrys* have resulted in massive lung damage and acute lethality (Nikulin et al., 1997). In mice, low exposures of *Stachybotrys* spores or pure satratoxin, but not *Cladosporium* spores, affect lung surfactant production (Mason et al., 1998) and the ultrastructure of type 2 lung cells (Rand et al., 2002). In rats, spores of *Stachybotrys*, but again not by *Cladosporium* spores, affected other aspects of lung biology; the differential effects of *Stachybotrys* spores was almost eliminated in the case of methanol-extracted spores (Rao et al., 2000). In indoor air settings, spore counts as high as those modeled in the above studies are rarely detected, since *Stachybotrys* produces spores in a slimy mass that become airborne only when dried.

*Stachybotrys* conidia instilled into the lungs of neonate Sprague-Dawley rats resulted in extensive haemorrhage with an LD50 of 10<sup>5</sup>/g BW and growth of surviving animals was impaired in a dose-dependent fashion. Histology of the lungs revealed fresh haemorrhage, hemosiderin-laden macrophages and evidence of inflammation including thickened alveolar septa infiltrated by lymphocytes and mononuclear cells and intra-alveolar macrophages. None of these effects were observed in animals treated with ethanol to remove the toxins (Yike et al. 2002).

Studies of the effects of fungal spores on rat pulmonary alveolar macrophage cells have shown variability that may be mediated by spore-borne toxins. Spores of five species of *Aspergillus*, *Penicillium spinulosum* and *Cladosporium cladosporioides* resulted in the release of leukotriene B<sub>4</sub>, increased super-oxide anion production and activation of complement. Several species of *Aspergillus* inhibited LPS-stimulated IL-1 whereas the other species had no effect (Sorenson et al., 1994). This implies the presence of toxic factors in some fungal species (Sorenson and Lewis, 1996).

### **Studies using pure toxins**

Studies of experimental inhalation exposures to the trichothecenes have been done in mice, rats, guinea pigs and swine. In general, the LD50 was typically one order of

magnitude less by inhalation compared to systemic administration (Creasia et al., 1990).

When in experimental trials Fisher 344 rats and Swiss mice were exposed to aflatoxin B1 by inhalation, Fc-receptor mediated phagocytosis was attenuated by exposure, and the effect persisted for up to two weeks after exposure. Whistar rats exposed to aerosols of killed conidia of *A. fumigatus* on which aflatoxin had been deposited showed dose dependent lung lesions of varying severity, with signs of immune dysfunction (Jakab et al., 1994).

### 3.6 Microbial volatile organic compounds and health effects

It is known that inhalation exposure to fungal spores and mycelial fragments can result in health effects of various kinds. It is difficult to contemplate situations where people would be exposed to fungal volatiles in the absence of exposure to spores. None the less, fungal volatiles have been suggested as possible contributors to adverse health effects e.g. by causing nasal irritation (Fischer and Dott, 2003).

While the toxicity of fungal volatiles based on individual LD50 values in animal studies is generally low, this does not address the toxicity of mixtures of these fungal volatiles. It is known that the volatiles of *Trichoderma viride* and related species are highly toxic to other fungi. In general, compounds that are toxic to fungi are also toxic to human and animal cells. Volatiles from indoor fungi affected ciliary beat frequency, and inflammatory mediators were released by the cells (Pieckova and Kunova, 2002). All of these data are suggestive that where microbiological growth is large in an enclosed space, the volatiles may contribute in some way to the nature of the complaints and or the objective health effects (Korpi et al., 1999).

Fungal volatile concentrations may be an appreciable fraction of the total volatile organic compound burden in residential housing albeit when fungal growth is essentially visible (Korpi et al., 1998). Furthermore, fungal volatiles can pass through a plastic vapour barrier and therefore, even growth occurring outside the occupied space could contribute to volatiles inside the occupied space. Hence volatiles may explain occupant complaints under some circumstances in the absence of meaningful concentrations of spores in the space (Pasanen et al., 1998).

Complaints of unpleasant odours are often reported in buildings with microbial contamination (Smedje et al. 1996). MVOC have musty, earthy, mushroom-like, and/or fruity smells, and they have been assumed to be responsible for certain types of odours in problem buildings. Many factors affect MVOC levels indoors and the human sensation of odours varies. The odour thresholds vary at least by a factor of  $10^7$  for individual MVOC (Hau and Connell 1998). These compounds are detected in problem buildings at the ng/m<sup>3</sup> to the mg/m<sup>3</sup> level (Dewey et al., 1995).

Sensation of unpleasant odours as such may lead to stress responses and unspecific somatic symptoms, e.g., headache, fatigue, dizziness, and nausea. In epidemiological and case studies, the presence of MVOC or musty, earthy odours has been associated with the prevalence of general symptoms, as well as eye, nose, and throat irritation, and even asthma (Jaakkola et al., 1993; Ruotsalainen et al., 1995; Wessén and Schoeps, 2000). However, there is no clear evidence on the causality between MVOC at the concentrations usually detected in buildings and irritation responses or possible

mechanisms for health effects caused by MVOC. Considering the thresholds for sensory irritation of individual MVOC or MVOC mixtures reported in the literature (in a range of  $10^1$  -  $10^6$  mg/m<sup>3</sup>), sensory irritation could be expected when the concentration of single MVOC in the indoor air approach a level of hundreds of µg/m<sup>3</sup> or mg/m<sup>3</sup> (Cometto-Muniz and Cain 1994, Pasanen et al., 1998, Korpi et al., 1999). The recent ACGIH Bioaerosols Committee publication (Macher et al., 1999) could find only suggestive evidence that MVOCs contribute in a material way to the health effects in mouldy buildings. Resolution of their possible contribution is not currently possible because of inadequate methods of exposure characterization.

### **3.7 Bacteria and health effects**

In a Swedish case-control study, asthma related symptoms were significantly associated not only with house dust mite exposure but also with exposure to bacteria, assessed with a non-viable microscopic counting method (Björnsson et al., 1995). However, to date, with the exception of specific bacteria (*Legionella* and streptomycetes) and specific bacterial agents such as bacterial endotoxin, little information is available regarding health effects of indoor exposures to bacteria.

#### **3.7.1 Specific bacteria and bacterial agents**

##### **3.7.1.1 Legionella**

Legionellae were found during an intense search for the agent that caused several fatal cases of pneumonia after a veteran conference in Philadelphia 1976 (CDC 1977). Since then sporadic cases or outbreaks of legionellosis have been repeatedly reported in hospitals and in association with cooling towers (Castellani Pastoris et al., 1997; Sabria et al., 2006, Formica et al., 2000).

The exact infective doses of different species and strains of *Legionella* remain unknown. A theoretical infectivity level for humans of 1000 CFU/l have been suggested, but no data has been shown relating to this concentration. Such concentrations are commonly detected in hot and cooling water systems, and occasionally even in water systems treated with generally efficient prevention methods. In hospitals and other critical environments, however, even such low concentrations may lead to infections. European guidelines focusing on the prevention of legionella infections, including acceptable limits for legionella counts in different water systems, are under preparation and probably available since 2002.

##### **3.7.1.2 Streptomycetes**

*Streptomyces* spores are potent inducers of inflammatory responses both in mouse and human cell lines (Hirvonen et al., 1997a, Jussila et al., 1999, Huttunen et al., 2002), and in mouse lungs *in vivo* (Jussila et al., 2001). Even low doses of the spores of *S. californicus* can activate both adaptive and non-adaptive mechanisms of host defense in mouse lungs after repeated airway exposure (Jussila et al., 2003). It has been shown that spores of streptomycetes are more potent than spores of fungi to induce production of proinflammatory mediators in cell cultures *in vitro* (Huttunen et al., 2003). Moreover, both the bacteria and fungi trigger production of proinflammatory mediators at lower concentrations than needed for cytotoxicity indicating that

inflammation may be the primary response in lungs. These experimental results are supported by clinical data showing that the same inflammatory mediators can be detected in the nasal lavage fluid of individuals exposed to a mouldy indoor environment including also spores of streptomyces (Hirvonen et al., 1999). Thus, the correlation found between *in vitro* and *in vivo* data supports the view that streptomyces may play a role in the cascade of events leading to adverse health effects in the occupants of moisture-damaged buildings.

### **3.7.1.3 Endotoxins Health Effects**

Endotoxins have remarkable biological potential and they probably have a role as both an exposing agents leading to adverse health effects and as a protective agent against allergy.

#### **Challenge studies**

There are studies in which workers and naive healthy subjects were experimentally exposed to endotoxin-containing cotton dust (Rylander et al., 1985, Castellan et al., 1987). These studies showed similar health effects including fever, malaise, chest tightness, and breathing difficulties that were clearly associated with the level of endotoxin exposure but not or only poorly with the level of dust exposure. The larger of the two studies (Castellan et al, 1987) provides a good quantitative basis for the assessment of a no effect level. A no effect level for endotoxin exposure based on a 0% FEV1 decrease was calculated to be 9 ng/m<sup>3</sup>. An 'effective concentration' required for 50% of the exposed population to achieve a 5 % or larger decrease in FEV1 (EC50) was calculated to be approximately 100 ng/m<sup>3</sup>. EC50 for a 10% decrease in FEV1 was suggested at 1 µg/m<sup>3</sup>.

#### **Indoor studies**

Several studies (Michel et al., 1991, Douwes et al., 2000, Thorne et al., 2005, Michel et al., 1996, Park et al., 2001) have shown that endotoxin in house dust is associated with exacerbations of pre-existing asthma in children and adults (see table 3.2.). One cross-sectional study performed in Belgium showed that in 69 adult asthma patients domestic endotoxin levels were significantly correlated with a decrease in FEV1 and an increase in symptoms and daily need for oral and inhaled asthma medication (Michel et al., 1996). Douwes et al., (2000) have shown that endotoxin levels in house dust were positively associated with peak flow variability in asthmatic children but not in non-asthmatic children. After adjusting for the presence of pets in the home (which was significantly associated with high levels of endotoxin) the association for endotoxin disappeared. Interestingly, dust mite allergen levels were not associated with symptoms or lung function in these studies. A recent birth cohort study in 499 infants with a familial predisposition to asthma or allergy showed that early indoor endotoxin exposure was associated with an increased risk of repeated wheeze during the first year of life rather than a decreased risk (RR=1.6, 95% CI=1.03-2.38) (Park et al., 2001). Another study on 1,884 infants showed that the risk of wheezing during the first year was increased with higher endotoxin exposure (OR=1.60 95% CI=1.10-2.20) (Gehring et al., 2001). During the first 6 months of life eczema was significantly decreased by

endotoxin exposure in dust of the mothers' mattress (OR=0.5 95% CI=0.28–0.88) whereas the risk was increased for respiratory infections (OR=1.69, 95% CI=1.25–2.28). For the entire first year of life these associations attenuated. In a study of employees in a health care facility, endotoxin in air was associated with lower respiratory symptoms after controlling for personal and home factors (Cox-Ganser et al., 2002).

Although studies in the indoor environment have suggested adverse health effects associated with endotoxin levels in house dust, it has recently been suggested that exposure to environmental endotoxin in infancy might protect against the development of atopy and asthma (Martinez and Holt, 1999; Von Mutius et al., 2000; Liu and Leung, 2000; Gereda et al., 2000). It was proposed that bacterial endotoxin drives the response of the immune system – which is known to be strongly skewed in an atopic Th2 direction during foetal and perinatal life – into a Th1 direction and away from its tendency to develop atopic immune responses (Martinez and Holt, 1999; Holt et al., 1997; Martinez, 1999). However, to date only one small study in 61 infants has produced direct in-vivo evidence that endotoxin exposure may protect against the development of atopy by enhancing Th1 responses. Finally, one study in ovalbumin sensitised rats demonstrated that airborne endotoxin exposure early in the sensitisation process protected against the development of specific ovalbumin IgE antibodies (Tulic et al., 2000). However, in complete contrast, another study showed that mice pre-exposed to airborne endotoxin enhanced ovalbumin IgE sensitization (Wan et al., 2000).

Table 3.2: Overview of epidemiological studies indicating adverse respiratory effects related to indoor endotoxin exposure (adopted from Douwes et al., 2002).

Ref.	Population	N	Exposure*	Health effect
Michel et al., 1991	Adult asthmatic patients	28	2.59 ng/mg	Decline in FEV1 and FEV1/FVC Increase in asthma medication and symptoms
Michel et al., 1996	Adult asthma (40)/rhinitis (29) patients	69	1.78 ng/mg	Decline in FEV1, and FEV1/FVC Increase in asthma medication and symptoms
Rizzo et al., 1997	Children (50% with asthma)	20	1-100 EU/mg	Increase in asthma medication and symptoms in asthmatic children
Douwes et al., 2000	Children (50% with airway symptoms)	148	24.9 EU/mg	Increased PEF variability in atopic children with asthma symptoms†
Park et al., 2001†	Infants	499	100 EU/mg	Increased prevalence of wheeze during first year of life
Gehring et al., 2001	Infants	1,884	2.9 EU/mg	Increased prevalences of wheeze and respiratory infections Decreased risk of eczema

\* exposure is expressed as the mean exposure (or range of (mean) exposures if no overall means is given) in ng or endotoxin units per m<sup>3</sup> or per mg of house dust; one endotoxin unit is approximately 0.1 ng (the exact conversion factor varies depending on the source of endotoxin for calibration).

† Longitudinal study (all other studies were cross sectional studies)

## Office buildings

As noted by Mendell (1993), building cleanliness in relation to sick building syndrome (SBS) is a factor that emerges from several published studies. Total particulate burdens have been shown to be a strong predictor of SBS symptoms. In mechanically-ventilated office buildings, particulate burdens comprise ash, mineral fibres, endotoxin and fungi (Molhave et al., 2000).

A number of recent studies have shown variation in the concentrations of endotoxin and fungi in office and residential dusts (see Chapter 2), although these are somewhat confounded by analytical problems (Milton, 1999). Chamber studies involving people exposed to such dusts collected in office environments indicate exposure-dependent neurological symptoms. Dust exposures resulted in increased perception of poor air quality, increased odor intensity, eye irritation, “heavy head” symptoms, feeling of increased perspiration, increased general irritation and mood changes. The NOEL was ca. 140  $\mu\text{g}/\text{m}^3$  (Molhave et al., 2000). The symptoms were similar to those reported from some studies on the effects of MVOCs (see 3.1.2.4). However, causal agents were not identified, and the role of endotoxin is unknown.

#### **3.7.1.4 Peptidoglycans and health effects**

Relationships between environmental peptidoglycan exposure and respiratory health has to date not been studied, and therefore its role in indoor induced respiratory health effects is not clear.



### **3.7.2 Possible protective effects of microbial exposure**

In recent years the “hygiene hypothesis” has shifted attention from the adverse health effects to the potential beneficial effects of microbial agents (Martinez and Holt., 1999). This postulates that growing up in a more hygienic environment may enhance atopic (Th2) immune responses (Holt et al., 1997; Martinez, 1999). The “hygiene hypothesis” has been prompted by the results of several epidemiological studies showing that overcrowding and unhygienic conditions were associated with a lower prevalence of atopy, eczema and hay-fever although the evidence for asthma is less consistent (Strachan 1989, 1997). Number of siblings (and especially older siblings) and attendance of day care centres were determined to be particularly protective (Krämer et al., 1999; Ball et al., 2000). An increase in infections (Martinez, 1994) and exposure to certain microbial agents (e.g. bacterial endotoxin; Douwes et al., 2002) early in life has been proposed as an explanation for these findings. Some animal and in vitro work (Tulic et al., 2000) and one small population study (Gerreda et al., 2000) appear to support the theory that endotoxin exposure may protect from developing atopy and allergic asthma. In addition, several studies in farmers’ children have shown that growing up on a farm protects against atopy and asthma (Braun-Fahrlander et al., 1999; Riedler et al., 2001). The reasons for this are not established but high endotoxin exposures in this environment were suggested to play a role (Von Mutius et al., 2000). However, only few studies have addressed this important area and results are inconclusive.

### ***3.8 Dust mites: Epidemiological studies in indoor environments***

The majority of the initial epidemiological studies related to house dust mite allergens and asthma. This was due in part to mites being the most important source of allergens in temperate climates and partly because two site mAb ELISA to Group 1 mite allergens was the first to be developed.

Before addressing the relevance of allergen exposure in the development and maintenance of asthma symptoms, and allergen avoidance in the prevention and treatment of atopic diseases, it is important to establish the relationship between IgE mediated sensitisation to common inhalant allergens and the disease.

Epidemiological studies have consistently shown that more patients with asthma are skin test positive to house dust mites, as compared with the general population. An increased prevalence of immediate hypersensitivity to dust mites in patients with asthma has been demonstrated in many different countries including, Europe (Di Berardino et al., 1987, Omenaas et al., 1996; Kuehr et al., 1995), USA (Squillace et al., 1997; Burrows et al., 1989), South America (Arruda et al., 1991), New Zealand (Burrows et al., 1992, 1995; Sears et al., 1993), Australia (Peat et al., 1996), Asia (Leung et al., 1997) and Africa (Cookson et al., 1975; Commey et al., 1973), making the relationship between mites and asthma a world-wide problem. A high prevalence of mite allergy among patients with asthma compared to control subjects has been reported in most studies, whilst negative studies came from the areas of low mite exposure.

Amongst middle class children on the east coast of the USA sensitisation to mite allergens is the single strongest risk factor for asthma (Squillace et al., 1997). In an area with low mite allergen exposure (Sweden) a close association is reported between mite sensitisation and asthma (OR 4.9) (Wickman et al., 1993). In an ethnic Chinese population, living in three different areas of south-east Asia (Hong Kong, Kota Kinabalu and San Bu) sensitisation to aeroallergens was a significant risk factor for asthma, with adjusted odds ratios for mite sensitisation being 1.9, 5.8 and 5.6, respectively (Leung et al., 1997).

Higher levels of exposure in more westernised communities may be above the threshold for the development of airway reactivity, and thus responsible for the higher asthma prevalence (e.g. prevalence of wheeze or asthma in Hong Kong was 11.6%, compared to 1.9% in San Bu (Leung et al., 1997)). The characteristics of exposure strongly influence the pattern of sensitisation and disease expression in different areas, indicating that it is important to understand the local climatic and housing conditions in order to identify the principal causes of asthma in any particular area.

The reviewed evidence strongly suggests that being sensitised to dust mites is a risk factor for asthma.

### **3.8.1 Mite allergen exposure and allergic sensitisation**

A number of reports from different parts of the world have demonstrated a dose-response relationship between allergen exposure at home and sensitisation. In a prospective study of German school children, skin prick tests to inhalant allergens were performed at twelve monthly intervals over a period of 2 years and levels of Der p 1 were measured in mattresses (Kuehr et al., 1994). Exposure to levels of Der p 1 > 9 µg/g conferred a risk of definite sensitisation in the whole population. However for children already sensitised to other inhalant allergen a significant risk of sensitisation was posed by exposure to levels of Der p 1 > 2 µg/g. The authors proposed this level as the minimum avoidance level for primary prevention in children with sensitisation to other inhalant allergens.

Although the mite allergen concentrations have been found to be similar in the beds of both atopic and non-atopic children, the levels in mite allergic children were 25 fold higher (Lau et al., 1989). In atopic children exposed to Group 1 allergen levels > 2 µg/g, the odds ratio for the risk of developing sensitisation to mite allergens was 5 (Lau et al., 1989). Furthermore, population studies conducted in 6 different climatic regions of New South Wales, Australia with a broad range of mean Der p 1 levels, the percentage of children sensitised to house dust mite in each region increased with increasing mean levels of Der p 1 (Peat et al., 1996).

The hypothesis of a cause and effect relationship between exposure to dust mite allergens and sensitisation, was tested in a study comparing the prevalence of asthma and positive skin test to mites in subjects living in the Alps and those living at sea level (Charpin et al., 1988). Briancon in the French Alps (altitude 1,326 m) is the highest city in Europe, with mean annual absolute outdoor humidity of 5.7 g/kg, thus making it unfavourable for mite population growth. Martigues (near Marseilles), on the contrary, is located on the French Mediterranean coast and has a mean annual absolute outdoor humidity of 9.3 g/kg, which is conducive for mite growth. The prevalence of mite allergy in a random sample of 18 to 65 years old adults was found to be fourfold higher in those living in Martigues (44.5%) compared to Briancon

(10%). In a later study conducted by the same authors, a similar pattern of sensitisation was found in schoolchildren where the prevalence of skin test sensitivity to mite allergens was found to be 16.7% in Martigues compared with 4.1% in Briançon (Charpin et al., 1991). House dust mite allergen level in mattresses was found to be much lower in the Alps (0.36 µg/g of dust) than at sea level (15.8 µg/g of dust).

In the German Multicentre Atopy Study new-born babies were recruited in 5 German cities (Wahn et al., 1997) and the influence of exposure to indoor allergens was studied prospectively in a high risk group and a random group of babies. The number of children who were sensitised to mite was small at the first birthday, but increased by the 3 years of age, with cumulative rates of sensitisation being 0.5%, 1.9% and 3.8% in the first 3 years of life. Children who were sensitised to dust mite had significantly higher level of mite allergen in their homes than those in non-sensitised group (868 ng/g vs 210 ng/g). Furthermore, the quantitative relationship between exposure and sensitisation showed different patterns when children were stratified by family history of atopy. In those with positive family history, exposure below 750 ng Group 1 allergen/g dust was found to be sufficient to result in 3% sensitisation rate, whilst for the same outcome in terms of sensitisation, an exposure of up to 25,000 ng/g was necessary in children with negative family history. Other studies of children at high risk have reported very low levels of sensitisation to mite allergen when exposures are maintained at low levels (Munir et al., 1997). One of 86 high risk children becoming sensitised over the first 5 years, at levels of exposure of 0.12 µg Group 1 mite allergen/g of dust during the first year of life, 0.05 µg/g at 5 years of age; the highest level of mite allergens recorded during the study was only 3.5 µg/g (Munir et al., 1997).

### **3.8.2 Mite allergen exposure and development of asthma**

Countries with regions, which differ in asthma prevalence and allergen exposure, provide an excellent opportunity to investigate the relationship between allergen exposure and asthma. A series of epidemiological studies compared regions in Australia (Peat et al., 1993, 1994, 1996) reported differing asthma admission rates for children (suggesting a difference in prevalence), and differing climatic conditions (suggesting a difference in exposure to mite allergens). A study of children from 6 regions of New South Wales with a range of levels of mite allergens has provided further evidence on the role of exposure to mite allergens in childhood asthma (Peat et al., 1996). A large random sample of school children between the ages of 8 and 11 years were assessed for the presence of respiratory symptoms, non-specific BHR (histamine challenge test) and sensitisation to common allergens. A random group of 60-80 children in each region were also assessed for exposure to Der p 1. Exposure to mite allergens was much higher in humid coastal regions (Lismore, Belmont and Sydney) than in the drier inland regions (Moree, Wagga Wagga and Broken Hill). In regions with high mite allergen exposure, more children were skin test positive to mites. Furthermore, those who were sensitised to house dust mites were at significant risk for having current asthma (defined as both the presence of wheeze within previous year and increased BHR), and the magnitude of risk increased with increasing exposure. After adjusting for sensitisation to other allergens, the risk of mite sensitised children having asthma approximately doubled with every doubling of Der p 1 level (Peat et al., 1996). Although the prevalence of sensitisation to cat in the

above study was low (~5-6%), being sensitised represented a significant risk for having asthma in four out of the six regions studied. The risk of asthma in children being sensitised to cats as compared to being sensitised to mites was much lower in the regions with high mite allergen exposure, but similar in the regions with relatively low Der p 1 levels. This would imply that either cat allergen is less "allergenic" than mite allergens, or, that exposure to cat allergen is, relatively speaking, lower than the exposure to mites.

Although epidemiological studies have shown a good association between indoor allergen exposure and sensitization only few studies have shown a clear association between allergen exposure and asthma. Pearce et al., (2000) reviewed the epidemiological evidence on asthma and allergen exposure i.e. are allergens *the major primary cause* of asthma. They concluded that the currently available evidence does not support this notion. The combined evidence from cross sectional studies was weak with weighted averages of the population attributable risks in children of 4% for Der p1, 11% for Fel d1 (cat), -4% for Bla g2 (cockroach), and 6% for Can f1 (dog). The two currently available longitudinal birth cohort studies with data on allergen exposures found no association between early dust mite allergen exposure and asthma measured at the age of 7 years. In the German Multicentre Allergy Study levels of mite and cat allergen in early life remained strongly related to respective specific sensitisation at age 3 to 7 years (Lau et al., 2000) (at 7 years, this effect was stronger for mite than for cat allergen), confirming the trends observed in earlier life (i.e. at 1 and 3 years of age) (Wahn et al., 1997). At 7 years of age, sensitisation to indoor allergens was strongly associated with wheezing, doctor's diagnosis of asthma and higher bronchial responsiveness (Lau et al., 2000). However, there was no dose-response relationship between allergen exposure (either early, during the first year of life, or later at the ages of 4 and 5 years) and any measure of asthma/wheeze at 7. This may suggest that different factors may be important in the development of atopy from those involved in the development of asthma. The authors suggest that rather than indoor allergens inducing asthma, the reverse may be true: i.e. that a capacity to produce IgE responses to allergens and develop sensitisation is a characteristic of asthmatics.

In the review on environmental predictors of allergic disease, von Mutius suggested that the mechanisms inducing asthma may not be susceptible to changes in allergen levels, and that the capacity to mount a strong IgE response to indoor allergens may be a feature of asthmatic subjects (von Mutius et al., 2000). Whilst this possibility cannot be discounted, this hypothesis is not supported by experience in another area - i.e. occupational asthma. Of the subjects who develop occupational asthma to some of the high molecular weight agents, it is previously atopic individuals who are at particular risk and in many instances a dose response relationship is demonstrable between exposure and the development of sensitisation and clinical disease. Aside from the possibility that allergen exposure per se does not cause asthma, existing studies might have suffered from exposure misclassification and unmeasured or unknown confounding from other inhalation exposures indoors such as fine particles, ozone and/or pro-inflammatory compounds such as endotoxin or glucan.

A number of studies have indicated that concurrent exposure to endotoxin and dust mite allergen increases the clinical response (Michel et al., 1991, 1996). This and other studies of endotoxin in housing suggest that the response to dust mite in some of

the epidemiological studies reviewed above require further consideration in the light that endotoxin is common in house dust.

### **3.9 Other allergens**

#### **3.9.1 Cat and dog allergens**

The importance of exposure and sensitisation to the allergens of domestic pets (namely cats and dogs) is highlighted in a study in Los Alamos, New Mexico, USA. The town is at high altitude (~2200 m) in a dry area and is one of the highest, economically stable towns in the USA (Sporik et al., 1995). The prevalence of asthma, pattern of sensitisation and the exposure to indoor allergens in a group of school children aged 12 to 14 years was reported (Sporik et al., 1995). Mite allergen concentrations were very low, but the striking characteristic of Los Alamos was the very high number of households (77%) with a pet (cat or dog) usually kept indoors. Asthma in this population was again associated with sensitisation to indoor allergens, but the pattern of sensitisation was different than in previously described studies where immediate hypersensitivity to dust mites was associated strongest with asthma. In Los Alamos the pattern of sensitisation reflected exposure to local allergens. The strongest associated risk for the current asthma was cat sensitisation. The low prevalence of mite and cockroach sensitisation reflected low levels of exposure.

In a further study of the same population it was found that the concentration of both cat and dog allergens were high in almost all homes with pets but also in a significant proportion of the houses without pets, and the combination of sensitisation and increased exposure for both cat and dog showed a strong correlation with asthma (Ingram et al., 1995). Sixty seven percent of asthmatic children were sensitised to dog and 62% to cat. These results strongly suggest that in areas with high levels of cat and dog allergen in homes, asthma will be associated with sensitisation to these allergens. Cats and dogs were reported to be the sensitizing agents most closely associated with asthma and airway reactivity in other areas of the world with high proportion of pet ownership, but low mite allergen levels (e.g. Sweden (Plaschke et al., 1999)). However, as discussed in the previous section on house dust mite allergens (see above) the population attributable risks related to pet allergen exposure was low in children (Pearce et al., 2000).

#### **3.9.2 Pet ownership, sensitisation and atopic disease**

This is a major area of controversy highlighted in recent years. Different investigators have published intriguing and often conflicting data on the effect of pet ownership in early life on the subsequent development of sensitisation and atopic disease. Some studies found that exposure to cats and dogs in early infancy was associated with specific IgE sensitisation and allergic disease later in childhood. Others however reported the opposite finding – i.e., a protective effect (Hesselmar et al., 1999; Sporik et al., 1999).

In Sweden the prevalence of respiratory allergy is significantly lower in children who were exposed to pets during the first year of life (Hesselmar et al., 1999). Questionnaire data were supported by the finding of lower prevalence of positive skin tests to cat in children exposed to cat during the first year of life. It can be speculated that early exposure to high levels of pet allergens could induce a form of immune

tolerance. There is some data for other allergens that supports this view, for example, it has been reported that children born during the tree pollen season are less likely to develop allergic rhinitis, positive skin test or high specific IgE to tree pollen at the age 12-15 years (Nilsson et al., 1997). In contrast, a strong positive association between the concentration of cat allergens in floor dust and sensitisation both to cat and dog has been reported (Lindfors et al., 1999). There was a strong suggestion for the synergism between different indoor environmental exposures, and the combination of exposure to environmental tobacco smoke, damp housing and high level of Fel d 1 ( $>8 \mu\text{g/g}$ ) resulted in a very high risk of sensitisation to cats (odds ratio of 42) (Lindfors et al., 1999). In a study from New Zealand, there was a modest association between asthma and the presence of cat in home both in early life and currently (7-9 years), compared to owning a cat in just one of these times or not owning a cat at all (Wickens et al., 2002).

In a recent study from Japan, the prevalence of sensitisation to cat dander amongst patients with asthma progressively increased as the patients grew older, up to the age of 16 years (suggesting the importance of cumulative exposure) (Ichikawa et al., 1999). Overall, 34% of patients were sensitised to cats, but did not keep them in their homes. These patients must have been sensitised due to passive exposure to cat allergen in their homes or in other places. Further intriguing findings on the prevalence of sensitisation to cats were reported from Tristan da Cunha (Chan-Yeung et al., 1999). In this unique island with a high prevalence of asthma, cats were exterminated in 1974 because of toxoplasmosis, but 20% of the 282 islanders who took part in the study were skin test positive to cat 1993 (i.e. almost 20 years after cats had been eliminated of the island).

The Stage II of the ECRHS reported a strong protective effect of pet ownership in childhood on adult atopy (Svanes et al., 1999; Roost et al., 1999). The finding that this effect was similar in subjects with and without parental allergy could suggest that this was not due to the lower proportion of dog ownership in families with allergies. A recent UK study has demonstrated that the prevalence of sensitization to cat is decreased in the lowest and highest cat allergen exposure groups (Custovic et al., 2001). Although several studies have suggested that early contact with pets may prevent the development of atopy and asthma this should be interpreted with caution, since avoidance behaviour (removal of pets in the families with sensitized and/or symptomatic children) may have contributed to this inverse association.

A study in The Netherlands in 3,000 primary school children showed that current pet ownership was inversely associated with cat, dog, indoor and outdoor allergen sensitisation (Anyo et al., 2002). An inverse association was also found for hay fever and rhinitis but not for BHR, wheeze and asthma. Odds ratios associated with past pet ownership were generally above unity, and significant for asthma. These results suggest that the inverse association was partly due to removal of pets. This study also showed that pet ownership in the first two years of life only appeared to be protective against pollen sensitisation. The majority of studies, which investigated the relationship between pet ownership in early life and subsequent sensitisation and atopic disease, were cross-sectional and retrospective. In a prospective study a strong dose-response relationship between exposure to cat allergen and specific sensitisation during the first three years of life (Wahn et al., 1997) and to a lesser degree by the age of 7 years has been demonstrated (Lau et al., 2000). In contrast, in another prospective birth-cohort study it was found that exposure to 2 or more dogs or cats in the first year of life was associated with a lower prevalence of allergic sensitisation at age 6 to 7

years regardless of exposure to dogs and cats at age 6 years (Ownby et al., 2002). The inverse relationship was present for both indoor and outdoor allergens.

How can we explain this potential protective effect of pet ownership seen in some studies? A recent important study has suggested that many children who are exposed to high level of cat allergen made a modified Th2 response characterised by the presence of IgG4 antibody to cat proteins without becoming allergic (i.e., no IgE response), which could be regarded as a form of tolerance (Platts-Mills et al., 2001). The production of IgG and IgG4 antibodies without IgE may explain reported decreased risk of asthma in children living in homes with cats. Alternatively, increased exposures to bacterial endotoxin may play a role since it is known that pets in the home are associated with higher endotoxin levels in house dust (von Mutius et al., 2000). Endotoxin exposure early in life has been hypothesised to protect from development of atopy and asthma (Martinez et al., 1999; Gereda et al., 2000). In other parts of the world (Guinea-Bissau and Nepal) it has been shown that pigs and cattle in the home are associated with less atopy (Shaheen et al., 1996; Melsom et al., 2001). This is in line with observations that animal contact in farmers' children may confer protection (Riedler et al., 2001), and as hypothesised for the farmers' children, increased endotoxin exposures may play a role (von Mutius et al., 2000).

Is it possible to explain these apparently irreconcilable differences between various studies? Cat allergen is ubiquitous, and exposure outside the domestic environment may lead to specific IgE responses (Perzanowski et al., 1999). The recent report from the ECRHS has indirectly confirmed the potential importance of passive exposure, finding a significant correlation between the community prevalence of cat ownership and community prevalence of sensitisation to cats, respiratory symptoms, physician-diagnosed asthma and current asthma medication in 35 centres from 16 different countries (Roost et al., 1999). If cumulative exposure to allergens is important for the development of sensitisation (and symptoms), then clear-cut differences regarding pet ownership are likely to be seen only in early life (Custovic et al., 2001). The cumulative passive exposure of non-pet owners in homes without pets and public places may induce sensitisation in later childhood and adolescence, confounding the effect of pet ownership. Furthermore, those exposed to very high levels of cat allergen may initially mount an IgE response (high cumulative exposure in early life), which as a consequence of continuous high exposure may be replaced by a modified Th2 response as a form of tolerance.

### **3.9.3 Cockroach allergens**

Cockroaches are the common source of indoor allergen in some parts of the world, particularly in the homes of patients of lower socio-economic status (Kang et al., 1979, Menon et al., 1991, de Blay et al., 1997). The majority of data on cockroaches comes from the USA. Although there are over 50 species of cockroaches in the US, only a few of those occur indoors, the most common being *Blattella Germanica* (German cockroach) and *Periplaneta Americana* (American Cockroach). The antigenic relationship between different species is not well understood. The significance of sensitisation to cockroach allergens in the UK is as yet unknown (preliminary results suggest that in children in some parts of inner city London it is as high as 11%; Christina Lyczinska, personal communication).

The precise source of cockroach allergens has not been fully identified, but significant progress has been made in determining their molecular structure. A full sequence of

several allergens is now available. Immune responses to cockroach allergens have not been extensively studied. Some preliminary data suggest that monoclonal-antibody-purified Bla g 2 and recombinant Bla g 4 elicit T-cell responses in cockroach allergic patients with asthma (Chapman et al., 1997).

A recent study in the US suggested that in the inner city areas with a high proportion of cockroach-infested houses, sensitisation to cockroach allergens was common, highlighting the importance of these allergens as a potential risk factor for asthma (Kang, 1976). A high prevalence of cockroach sensitisation (up to 70%) was found among asthmatic patients in several U.S. cities, including Chicago, Boston, Detroit, Washington, New Orleans, Atlanta, Shreveport, Tampa and Kansas City (Kang et al., 1979; Hulett and Dockhorn, 1979; Menon et al., 1991). Cockroaches have been suggested to be an important cause of asthma in the other parts of the world, including south-east Asia, Central America, India, South Africa, and most recently Europe (Lan et al., 1988; Tandon et al., 1990; de Blay et al., 1997). In Strasbourg cockroach sensitive patients with asthma were found to have high levels of cockroach allergens in their homes (de Blay et al., 1997). Cockroach sensitisation is not confined to inner-city populations but occurs wherever substandard housing or apartment buildings sustain cockroach infestation. Interestingly, house dust mite and cockroach allergens have distinct and opposite associations with socio-economic factors and population density (Leaderer et al., 2002). Nevertheless, levels of exposure determined by house dust analysis are important determinants of sensitisation to both mite and cockroach allergens.

#### **3.9.4 Pollen Health effects**

Pollen allergens can cause rhinitis (hay fever), breathing disorders and asthma and irritation symptoms of eyes, ears and skin. The allergenic proteins are stable, water-soluble compounds able to penetrate mucosal defense mechanisms and gain access to sites controlled by the immune system. The individual's genetic background is important in determining whether exposure will trigger an allergic i.e. IgE-mediated response.

Determining reaction threshold concentrations for pollen allergens in humans is technically difficult. Viander and Koivikko (1978) reported symptoms in 16 % of untreated birch allergic patients when outdoor birch pollen count exceeded 10/m<sup>3</sup> of air, and 89.5 % had symptoms, when the pollen count exceeded 80/m<sup>3</sup>. On the basis of this study threshold values for other pollen types have been calculated using their respective protein quantity (Rantio-Lehtimäki et al., 1991). The threshold quantity of allergen may be significantly lower in persistent or continuous exposure.

There are individual differences in the effective concentrations needed for sensitisation, but generally the most sensitive birch pollen allergic persons experience symptoms at concentrations of 500 SQ units (=standard quality unit, ALK, Copenhagen)/m<sup>3</sup> which approximately corresponds to the protein content of one birch pollen grain (Schäppi et al., 1997). In indoor environments, these concentrations are easily exceeded when settled dust becomes airborne through human activities. Up to 400 SQ units of birch allergen were found in an undisturbed situation in a school in Sweden (Holmquist and Vesterberg, 1999). Up to 2800 SQ units of birch and 200 of



grass allergen were obtained in house dust studies in Finland (Yli-Panula and Rantio-Lehtimäki, 1995; Yli-Panula et al., 1997).

### **3.10 Conclusions and recommendations**

Dampness and water-damage in buildings is associated with adverse health effects. But, the role of non-infectious microorganisms and their components in indoor-environment-related diseases is currently only poorly understood. The relative lack of knowledge regarding the role of microorganisms in the development and exacerbation of those diseases is largely due to the lack of valid quantitative exposure assessment methods. Therefore it is important to develop better exposure assessment methods. Also, it is not clear which specific microbial agents primarily account for the presumed health effects and which subpopulations are most susceptible. Thus in addition to better exposure methods - preferably directed at measuring the relevant pathogenic substances - there is also a great need to develop methods to further define susceptible populations. Tables 3.3 and 3.4 summarize evidence for health effects of biological agents (table 3.3), and indicate whether sufficient methods to measure them are available (table 3.4).

Table 3.3 Satisfactory evidence for health effects of dampness and biological agents in the indoor environment

AGENT	SENSITIZATION	EXACCEBATION OF ASTHMA	DEVELOP ASTHMA	DEVELOP ASTHMA	HP	SINUSITIS	NEUROLOGICAL	INFECTION	RESPIRATORY SYMPTOMS	ADVERSE HEALTH	ADVERSE HEALTH
DAMPNESS		S		S	S				S		S
<b>FUNGI</b>											
FUNGAL SPORES	S	S	S		S	S		S*		S	S
ALLERGENS										S	S
GLUCAN										S	S
TOXINS										S	
MVOC										S	
<b>BACTERIA</b>											
G- BACTERIA										S*	S*
LEGIONELLA								S	S	S	S
ENDOTOXIN		S								S	
G+BACTERIA											
ACTINOMYCETE					S						
S											
<b>OTHER</b>											
<b>ALLERGENS</b>											
MITES (DER P/F)	S	S	S	S						S	S
ANIMALS	S	S	S		S	S	S	S		S	S
COCKROACHES	S	S	S		S	S	S	S		S	S
POLLEN	S	S	S							S	S

\* for some species

Table 3.4 Satisfactory methods to measure exposure to biological agents indoors

AGENT	BIOMARKERS FOR FOR BUILDING FOR HEALTH INVESTIGATI		
DAMPNESS			
FUNGI			
ALLERGENS			
GLUCAN		S	
TOXINS			
MVOC		S	
SPORES		S	
(TOTAL)			
SPORES		S	
(IDENTIFIED)			
ERGOSTEROL		S	S
BACTERIA			
TOTAL			
BACTERIA			
LEGIONELLA	S	S	
ENDOTOXIN		S	S
TOXINS			
G+BACTERIA			
ACTINOMYCE		S	
TES			
OTHER			
ALLERGENS			
MITES (DER	S	S	S
P/F)			
ANIMALS	S*	S*	S*
COCKROACHE	S*	S*	S*
S			
POLLEN	S*	S	

\* for some species

### **Inhalation of fungal spores**

There is satisfactory evidence that exposure to fungal spores in outdoor air result in disease in animals and humans. There is satisfactory evidence that the fungi occurring indoors result in disease in animals and humans. There is limited evidence that the relative risks of disease due to fungi are higher indoors. It is not known whether this is because of intrinsic factors (e.g. co-occurrence of fungal glucans or low molecular weight toxins in spores) or extrinsic factors (e.g. co-occurrence of endotoxin). There is also evidence from epidemiology studies of an association of mould with increased rates of upper respiratory disease.

There are no good methods for quantitatively determining the degree of exposure to fungi in air, the health effects of fungi that can be found indoor and outdoor air are not the same and there is a large variation in individual sensitivities. Based on the current evidence it is not yet possible to recommend a safe level of any fungal spore type that occurs in the built environment.

International regulatory practice for natural contaminants such as ionizing radiation and natural toxins including mycotoxins is to reduce exposure to "as low as can be reasonably achieved". The conditions that permit fungal growth on building materials should be prevented and mould growth that is detected should be removed under safe conditions. There is evidence from several studies that the hazard posed by fungal growth in buildings is proportional to the extent of mould growth.

### **Fungal allergens**

There is satisfactory evidence that fungal allergens result in disease in humans and animals. Because of inadequate knowledge of the allergens of building associated fungi it has not been possible to determine exactly the attributable risk for asthma resulting from fungi in the built environment. Estimates of the maximum attributable risk range to 20% (Dekker et al., 1991, Horner et al. 1995, Kurup et al. 2000). It is not feasible on a routine basis to measure fungal allergens in air. No guidelines based on fungal allergens occurring in residential environments are currently feasible.

### **Fungal $\beta(1\rightarrow3)$ -glucan**

There is satisfactory evidence that  $\beta(13)$ -glucan causes disease in animals. The evidence is insufficient to suggest a role for  $\beta(1\rightarrow3)$ -glucans in the development of airway diseases or the development of symptoms from field and controlled challenge studies. It is feasible to measure fungal glucans in indoor air although there remain methodological uncertainties. Based on the current evidence it is not yet possible to recommend a safe level of  $\beta(1\rightarrow3)$ -glucan exposure.

### **Mycotoxins occurring in buildings**

Although a number of fungi that produce potent toxins occur on building materials and there is satisfactory evidence that their toxins occur on those materials, animal studies are available only for one fungus. There is satisfactory evidence that trichothecene toxins from *Stachybotrys chartarum*, which have been measured in the air from the built environment, cause disease in animals via the lung. There is evidence that neonate animals are much more sensitive to these effects via the lung. The mechanisms that result in harmful effects of trichothecenes apply to humans. There is no direct evidence available from human tissues that such damage has occurred from building exposures although there are suggestive data. It is not feasible

to measure fungal toxins in air on a routine basis. Based on the current evidence it is not yet possible to recommend a safe level of any fungal toxin that occurs in the built environment.

### **Bacteria and endotoxin**

Excepting *Legionella*, building-associated bacteria whether Gram-positive or Gram-negative have not been associated with large effects on population health. The gram-negative bacterial compound endotoxin has been associated with increased response to allergens and with various non-specific respiratory symptoms and wheeze. However, the evidence for this is insufficient since only few indoor studies have included endotoxin measurements.

At this time, knowledge about indoor air bacteria is not sufficient to establish any numerical guideline values. The main reasons why such values cannot be developed are:

- The total number of bacteria is a result of bacteria emitted from different sources. The potential health importance of these bacteria differs enormously, and the total count is not a valid measure of the hazardous exposure;
- The relationship between airborne bacteria and health effects are poorly known. The importance of cellular components or metabolic products of bacteria are not sufficiently understood.
- No good sampling methods are available to quantitatively assess indoor bacterial exposure.

Also, based on the current evidence it is not yet possible to recommend a safe level in the built environment for endotoxin.

### **Mite and pet allergens**

There is sufficient evidence that exposure to mite or pet allergen can cause specific sensitisation. Exposure can also exacerbate pre-existing asthma in mite or pet allergic subjects. However, it is not clear whether mite and pet allergens cause asthma. Early exposure to cats and dogs may in fact protect from developing asthma. However, the evidence is mixed.

Exposure guidelines should be aimed at preventing sensitisation. However, it is not possible, on the basis of the available evidence, to provide exposure guidelines in relationship to clinical disease except to recommend that exposure levels of Der P1 are kept to a minimum by adopting the recommended control measures. In terms of children who are 'at risk' (those with atopic parents) of sensitisation, levels of Der P1 should be maintained at less than 2 µg/g of dust.

At present the evidence of the relationship between exposure to pet allergen and the development of sensitisation and asthma is contradictory, both low and high levels of exposure to pet allergen offering apparent protection. Therefore, based on the current evidence it is not yet possible to recommend a safe level in the built environment for pet allergens.

### **Cockroach allergen**

Cockroach allergen exposure is associated with sensitisation and there is evidence that exposure exacerbates asthma in cockroach allergic subjects. However, it is not clear whether cockroach allergen exposure causes asthma. Exposure to cockroach allergen should at all times be minimised.

### **3.10.1 Research needs**

#### **Fungi**

Fungi cause allergic, toxic and infectious disease but the nature and extent of the problem arising from living or working in damp buildings is not clearly known. With current technologies for assessing exposure it remains difficult or impossible to determine causality and attributable risk.

With this in mind, the working group identified the following research needs:

#### ***Exposure assessment***

There are several methods available for helping to identify buildings with fungal damage. None of the currently available methods for documenting fungi in buildings have much value in assessment of personal exposure. Culturable methods are of limited use, particularly in epidemiological studies. Non-culturable methods to assess fungal exposure appear to be more promising but only limited experience with those methods in field studies is available. Methods are needed that provide information on exposure at the genus level, and for some specific well known pathogenic or toxic moulds (e.g. *Stachybotrys chatarum*) information at the species level is required. Methods should preferably be based on fungal antigens or some close proxy.

The high number and reactivity of the fungal fragments, which could be released from contaminated surfaces into the indoor air, is striking, suggesting that these may significantly influence the health of exposed individuals. This factor has been overlooked so far in studies evaluating indoor air quality. The high number of small particles being immunologically reactive and penetrating into the human respiratory tract can potentially be the cause for adverse health effects and can, at least in part, be responsible for unexplained cases of respiratory symptoms in damp buildings. Thus, future studies in mould problem buildings should include the measurement of fungal fragments in addition to intact spores.

#### ***Population studies on fungi in homes and allergic and non-allergic disease***

Prospective longitudinal and intervention studies are needed on the association between residential exposures to building-associated fungi and asthma to determine the attributable risks. Both allergic and non-allergic pathways leading to the development of asthma should be investigated since it is not clear whether fungal exposure may cause asthma through allergic or non-allergic mechanisms. Such studies cannot be effective until exposure assessment methods are improved.

#### ***Biomarkers for fungal allergens, glucan and toxins***

Particularly for case investigations, biomarkers for exposure to fungal antigens and other metabolites are critically necessary. Animal studies can provide evidence to take public health actions and to determine whether the mechanisms of action of those toxins applies to humans. Circumscribing symptoms, conditions or injuries ascribed to toxins requires direct evidence that those toxins have affected human tissue obtained from exposed individuals.

### ***Defining susceptible populations***

Exposures to building-associated fungi results in discomfort and increased relative risks for several diseases for the general population. There is evidence that children, particularly infants, have greater susceptibilities to such exposures. A long list of medical conditions have demonstrated or suspected increased relative risks to diseases from exposure to building-associated fungi compared to the general population. More information is required to address this issue. In addition specific markers of susceptibility should be developed (e.g. genetic polymorphisms for microbial receptors such as toll-like receptors and CD 14) to allow sub group analyses on susceptible populations in epidemiological studies.

### ***Neurological symptoms***

Several case reports and some cross-sectional studies have reported neurological symptoms in association with exposure to building-related fungi. There are few clinical data to support such observations, namely limited chamber studies where headache was a symptom associated with ( $\beta$ (1,3)-glucan) exposure but not with exposure to endotoxin.

Epidemiological, clinical and mechanistic studies are needed to resolve this association.

### **Bacteria and endotoxin**

Studies are needed on the contribution of bacteria (including the streptomycetes) and endotoxin to disease and symptom burdens in residential and non-industrial workplace environments. Studies should include assessment of personal versus area exposure. Further studies of allergen-endotoxin interactions are needed and the potential for endotoxin-specific biomarkers of exposure needs to be explored in animal studies. In addition, the potential protective role of early life exposures of endotoxin needs further study.

### **Building-associated mites**

Although it has often been suggested that building-exposures to *Dermatophagoides pteronyssinus* and *D. farinea* are associated with asthma, better characterized prospective studies are needed to define the attributable risks involved. In addition, in some countries, other species of mites might be important and these deserve more studies. Interaction between allergen and irritant exposures (such as endotoxin) should be further studied.

### **Pets**

The role of pet exposure in early life on the development of asthma is unclear. Evidence for both inducing as well as protective effects have been presented. Therefore prospective studies are needed including extensive immunological characterization of subjects to clarify underlying mechanisms of these potential protective effects.

### **Dampness**

Building dampness has a major effect on the population health of occupants of similar dimension to cigarette smoking indoors. Public policy measures to reduce dampness in the indoor environment should be pursued.

Epidemiological and clinical studies covering an array of health outcomes including psychological problems are associated with living or working in damp buildings.

While the majority of the population health effects have been to date related to dust mites, fungi and endotoxin, other factors including the accumulation of outdoor-sourced fine particulate matter, combustion spillage and the accumulation of metals, particularly lead, undoubtedly contribute to the array of health consequences observed.

Greater emphasis on the psycho-social aspects of the responses associated with living or working in a damp environment is needed.

More residential and non-residential building and health studies are needed outside Europe and North America.

Methods are needed to permit "building dampness" to be rigorously quantified for the purposes of public health interventions and research studies. There is an urgent need for health-based intervention studies to determine which changes in damp buildings result in improvements in health.

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## **4. Assessment of Biological Agents in Indoor Environments**

### **4.1 General aspects of sampling and analyses of biological agents**

Small quantities of most biological agents can be found practically everywhere as part of our normal environment. Fungi, bacteria and other biological agents occur in nature, often in connection with soil, plants and animals. It is totally normal to have some bacteria and fungi in indoor air, on different surfaces and materials. However, as soon as biological agents start to accumulate or amplify in some part of the building and their amount exceeds the normal background levels, they may become contaminants of the indoor environment and may even pose a health risk to the occupants of the building. The general function of the assessment program is to find out if the levels or concentrations of the biological agents in question are above what is considered normal in the given conditions. The prerequisite for a successful assessment is that the background concentrations are reasonably well known (ACGIH 1999, AIHA 1996).

Since the biological agents of the indoor environment consist of numerous different agents, each of which needs a specific technique for detection and quantification, no single sampling and analysis technique covers all the agents of interest. Which methods and measurement strategies are relevant, depends largely on the purpose of the assessment and on the available resources of the investigator. The environmental measurements are usually a part of a larger investigation, which may also include health studies, measurements of the physical and chemical quality of the indoor air, and technical investigations of the building. All these investigations may be costly and labour intensive, and therefore, a careful overall study plan including a thought-through sampling strategy is necessary to achieve the maximum benefit from the results of each measurement.

### **4.2 Need for environmental sampling and measurements**

Sampling for biological pollutants can be used in individual building investigations to confirm the visual or odour observations or to verify the results of technical measurements, e.g., on moisture content of materials or the performance of the ventilation system. Sampling the indoor air, which usually is time consuming and labour intensive, should be planned on the basis of the results of the observational walk-through. All studies on biological pollution should be connected with the other building investigations, e.g. testing the building envelope for pathways of water or rain entry, in order to make correct conclusions about the sources and causes of

contamination and to end up with correct interventions or repair measures (Haverinen et al., 1999).

Sampling for biological agents may be needed for monitoring purposes of indoor air quality, for exposure assessment in health risk evaluation, for identifying a pollution source (e.g., moisture damage) associated with complaints or suspicions of degraded indoor air quality, establishing concentration levels or for other study purposes of the characteristics and behaviour of indoor air pollutants. Most of the biological indoor air pollutants occur as airborne particles, designated as bioaerosols, and thus the physical principles of the sample collection from air are similar to non-microbial agents. Further analysis of the sample that focuses on the biological characteristics of the measured pollutant may use several different techniques depending on the purpose of the sampling. (ACGIH 1999; AIHA 1996; Reponen et al., 2001).

### **4.3 Developing a sampling strategy for biological agents**

Before the actual sampling, it is necessary to plan a sampling strategy to assure that the measurements will give an answer to the question they are used for, and if sampling is actually necessary. In cases where microbial (mould) damage is visible and evident, a conclusion about the need of remediation can be made without any major sampling and analysis program. Sometimes the costs of a well designed and performed sampling and analysis campaign may turn out to be higher than actual remediation, and a consideration about the priorities is necessary. The sampling strategy should be based on consideration of the following items:

- What kind of samples will be taken: surface, bulk, or air samples?
- How many samples will be taken to represent the situations or areas of interest, and to cover the needs of quality assurance?
- What are the locations of sampling?
- What is the timing of the sampling, e.g., before or during the occupation of the building, before and after switching on the ventilation, or during a certain activity?
- What kind of control samples will be needed, e.g., if swab samples are taken from surfaces that appear microbially damaged, are control samples from undamaged sites needed for comparison?
- What is the price of sampling and analyses compared with the price of remediation steps?
- Do occupants have adverse health effects likely associated with the building; if so, is the sampling protocol adequate to suggest “unusual” bioaerosol exposure?



Usually samples are taken during the ordinary conditions and use of the building or the room. In some cases of building investigations, where the goal of the sampling is to find a source of contamination, agitated dust sampling or other manipulative techniques may be applied. The needed effect can be obtained by mechanically disturbing the structure in question. However, the agitation procedure cannot be easily made in a reproducible manner, and therefore it is not suitable for larger studies than individual building investigations. In a similar manner, samples to find “hidden mold” within a structure can also be taken e.g., by sucking air from inside the wall structure.

Assessment of exposure to biological agents is usually made in connection with health studies, which may consist of a questionnaire, together with clinical studies of an individual patient or group of individuals. The health studies may be large population studies or focus on the occupants of an individual building. Thus, environmental sampling may be part of an extensive study program consisting of a number of buildings, or to focus on finding a link between an agent and symptoms or health complaints in a particular building. In large studies, the sampling strategy aims at finding out the distribution of the concentrations of the measured agent, but in individual building investigations, the goal is to assess the hygienic quality of indoor air. It may be enough to find out if the concentration of the biological agent differs from background concentrations, or if the concentration is higher in some rooms compared to other rooms of the building. Identifying a source of pollution may additionally include focused bulk sampling of house dust, water, contaminated surfaces or materials.

Not only the concentrations of biological material are of interest, but also the composition of the sample is important. In sampling of airborne microbes, the presence of unusual species of fungi or bacteria may reveal an unusual source. Control samples from outdoor air are needed to verify the concentrations and species of fungi, bacteria or other agents that are normally present in outdoor air. To make each sampling effort and its results useful, the purpose of the sampling must be defined before any samples are taken. For example, if the purpose of the sampling is to find out whether the ventilation system is a source of fungal contamination, air samples both before and after the HVAC device is turned on should be taken. To verify the existence of fungal growth within the duct, surface swab or cellotape samples from the suspected site and a control site may be relevant. For experienced researchers, control samples may not always be necessary if the variation and distribution of the background values is well known for the parameter in question.

Samples may be taken by an individual resident, a health official, a consultant or a researcher. However, as a general rule of thumb, sampling of biological agents should be made by an experienced investigator, who is aware of the principles of sampling, analysis and interpretation of the results. After the sampling strategy has been thought

through and a sampling plan developed, the methods for the sampling and analyses can then be selected. The most common sampling techniques and analyses are described below (4.5). For more details of each analysis techniques, one should consult the handbooks and other literature. A more detailed discussion on sampling strategy and the various approaches for different situations is included in practical field guides for assessment of biological agents, e.g., by ACGIH (1999) and AIHA (1996).

#### **4.4 Statistics in study design and interpretation of the results**

Investigations of indoor biological agents require considerations of an appropriate statistical approach to be used in the study design and interpretation of the results. The standard approach in any experimental study is to first decide on the significance level and statistical power desired for the study and then determine the number of samples necessary to achieve this power. Such sample size determinations are best made using preliminary experimental data or information culled from the relevant literature.

Once the data are collected, the initial analytical intent is to describe the value measured in terms of its central tendency, either the mean or median and its variability, either the standard deviation or standard error in the case of the mean, or the range or inter-quartile range in the case of the median. Confidence intervals could also be included in the presentation. The experimental study may also be designed to explain the relationship between the outcome of interest and other factors that are present. These might include investigating associations between different pollutants, environmental factors or health effects, or studying spatial or temporal variability of the pollutant concentrations. In these instances, more advanced statistical approaches such as data reduction, multivariate analyses or time series analyses will need to be used. More detailed discussion of statistical aspects of study design, sample size determination and data analyses and interpretation is beyond the scope of this document and the reader is referred to the relevant publications (Mendenhall and Sincich 1995; Manly 2001; Cohen 1977; Altman 1991; Gardner and Altman 1989).

Before deciding on a statistical approach for investigations of biological agents, however, the purpose of the investigations should be carefully reviewed, and the type of outcomes expected from the study identified, as these will determine the required level of sophistication in study design and statistical approaches used. Not all types of investigations require the same degree of comprehensiveness nor would the same statistical methods be used in every case.

In some cases of problem identification, a single sample showing the excess presence of a specific biological agent (for example *Stachybotrys*) may be sufficient to confirm the hypothesis of the existence of the problem and the need for remediation. Similarly, a single sample collected from an area damaged by water and showing the presence of specific biological agents could be the basis for undertaking appropriate control steps without the need for more extensive measurements of concentration of the biological agent in the air. It should be stressed, however, that the absence of the specific agent in a single sample does not support the conclusion that the problem does not exist. Sampling at different times and/or different location of the building, may still reveal its presence in the building.

It is important to realize that there often exists a significant spatial and temporal variation in concentration levels of biological agents, up to orders of magnitude, not only between different buildings in the same area but also between different parts of the same building (Hyvärinen et al., 2001; ACGIH 1999). Under certain conditions a specific agent may not be detected at all, while changed conditions of for example moisture or temperature could reveal very high concentration of the same agent. The variation may result from a number of factors including: biological cycle of the agent investigated, variation in temperature, humidity and other environmental conditions, air exchange rate, etc.

For some types of investigations, for example epidemiological population studies it may be necessary to establish the baseline of concentration of the biological agents and their variability. Such a study would require that the measurements be conducted in a sufficiently large number of houses and for sufficiently long time, or for sufficiently long time in representative locations of an individual building. Advanced statistical methods would be required for the design, sampling considerations and data analyses and interpretation of the results of such studies. However, in case of building remediation, identification of the degree of damage or source identification, such complex statistical approaches may not be required and may result in unnecessary over sampling and inflating of the cost of the study.

In summary, the degree of the comprehensiveness of the investigations depends on the purpose of the investigations and the outcomes defined, and based on these appropriate statistical approaches need to be identified. In some cases a *yes/no* answer (presence/absence of the pollutant) may be sufficient, in other cases an identification of a range of concentrations existing could fulfill the requirement of the investigations, while other studies would require a more stringent research design and complex statistical analyses to provide scientifically valid answers.

## **4.5 Overview of sampling methods**

Sampling as its simplest may be cutting out a piece of carpet or wallpaper that appears to be damaged by mould, which is sent to a laboratory for microbial analysis, or the sampling may be part of an extensive study programme including tens or hundreds of samples. All details of the sampling methods, strategies or analyses do not apply to all purposes. The principles presented below should help the investigator to select an optimal approach for the purpose in question. Detailed information about the methods and measurements of biological agents can be found in handbooks (Cox and Wathes, 1995; Dillon et al., 1996; ACGIH, 1999; Reponen et al., 2001).

### **4.5.1 Surface sampling**

A sample taken from a surface is appropriate for many purposes. Sometimes a verification of microbial growth and subsequent identification of the fungal species is needed as the surface in question looks damaged. The degree of microbial contamination on different surfaces within a room, between rooms or between the different floors of the building may be compared in order to find the source of contamination. Surfaces of bedding, carpet, rug or floor can also be sampled. Surface sampling may be divided into three main types of samples:

- The most common method is using a swab to collect dust or biomass from a certain area, e.g., of 10 cm<sup>2</sup>, for determination of viable organisms or for biochemical analyses (e.g., endotoxin). In case of determinations of viable microbes, the sample should be kept in a sterile buffer and cool temperature until analyzed.
- Vacuum sampling of dust or biomass on a filter.
- Using a contact agar plate (Rodac plate) which can be pressed onto a suspected microbially infested surface and then cultured in the laboratory (Gravesen et al., 1986). An experienced mycologist may also take a contact sample with a piece of sticky tape, and analyze it with direct microscopy.

### **4.5.2 Sampling of materials**

A sample of material that is visibly mouldy or otherwise deteriorated can be taken to ensure whether the observed changes in appearance are caused by microbial growth. Determination of the microbial species in question provides additional information about the damage. A material sample can be taken with a knife, scissors or tweezers, or other tools that are appropriate to the purpose. The device or tool should be sterile to avoid cross contamination. Tools that heat the sampled material, for example a drill, must not be used since the heat easily destroys the microbes. Usually a sample

of 10 g is enough for a microbiological analysis. Sterile gloves should be worn when taking the sample, to avoid bacterial contamination of the sample from hands. The sample is put into a plastic bag, Petri dish or box, closed and marked, and transported into the laboratory as soon as possible, preferably within 24 hours. Desiccant can be added to the sample bag in case the material is damp. A box is a preferable transportation container in cases where direct microscopy is to be performed, as the sample can be squeezed in the plastic bag, making it more difficult to see the fragile fungal structures such as conidiophores.

#### **4.5.3 Sampling of dust**

House dust consists of both settled airborne particles and of larger particles that may have derived from humans, animals, textiles, papers, cooking and other activities or handling of materials, and of particles originating from outdoors. House dust samples, although heterogeneous by origin, can be considered a long-term integrated sample of the dust of the indoor environment in question. The mass of dust that is needed for an analysis is dependent on the agent. The result is usually given per gram of dust, thus the original amount of the sample is not necessarily critical. To obtain comparable results and to minimize the biasing effect of the heterogeneity of the dust, the sampling protocol should follow the same quality assurance system in each case. For a comprehensive review of sampling methods for surface dust in buildings, the reader is directed to Schneider (2003).

House dust samples can be taken from beds, mattresses, carpets or floors for mites or allergens (Dreborg et al., 1995, Dharmage et al., 2001), for analyses of viable microbes (Wickman et al., 1993; ACGIH 1999), of microbial components such as ergosterol (Saraf et al., 1999), 1,3-beta glucans and endotoxin (Douwes et al., 2000), or microbial products such as mycotoxins (Nielsen 2002). Samples are taken with a vacuum cleaner into a clean bag, or on filters with a special device that can be used in connection with a vacuum cleaner (Dillon et al. 1996; Macher, 2001). Large particles can be removed by sieving (sterile screen), thus minimizing dust heterogeneity. Another method for sampling settled airborne dust is the box-sedimentation where dust is collected during several months at a height of e.g., 2 m, to avoid collection of the largest particles (Würtz et al. 2002). This collection method is in the development stage and needs further validation.

#### **4.5.4 Air sampling**

In studies linking measured levels of biological agents and adverse health effects, usually little correlation has been found between these two (Bornehag et al., 2001). One of the most important reasons for this observation may be the large variation of airborne concentrations over time combined with the often short sampling times (Hyvärinen et al, 2001; ACGIH 1999). Another reason is probably the insufficient knowledge of the actual causal agents of the health effects, i.e., the problems to identify and thus determine these specific agents. On the other hand, air sampling focuses directly on the particle or gaseous material that people actually inhale, and thus is a more direct way to assess the human inhalation exposures than sampling settled dust, surfaces or building material. Even though the results of air sampling do not necessarily correlate with the observed health effects in epidemiological studies (Verhoeff and Burge, 1997; Bornehag et al., 2001), air sampling is an important tool in building investigations. Its results may give good indications of abnormal conditions in the indoor environments, and it may be very useful in finding a source of contamination within the building.

Most airborne biological agents are particles, and therefore the sampling devices for biological aerosols are based on the same physical principles that apply to any particulate material. However, the biological properties of bioaerosol particles set specific demands to the collection techniques as well as to the analysis phase. Prior to selecting a sampler for a particular application, considerations should be given to a number of criteria, such as collection efficiency, particle size range, maintenance of microbial viability, optimal sampling time, compatibility with the analytical methods, suitability to the field conditions, and cost of analyses. In this document, a concise overview on the principles and characteristics of air samplers is given. More detailed presentations of the theoretical and practical aspects of bioaerosol sampling and sampling devices in indoor environments can be found in handbooks, e.g., Morawska and Salthammer (2003); Buttner (2002); Reponen et al. (2001); Willeke and Macher (1999) and Cox and Whates (1995), and in many comprehensive reviews on bioaerosol sampling (Nevalainen et al., 1992; Henningson and Ahlberg, 1994; Griffiths and DeCosemo, 1994; AIHA 1996; Pasanen, 2001).

##### **4.5.4.1 Impactors**

Impactors are particle collection devices that use the inertial impaction of the particle as the collection mechanism (Reponen et al., 2001). Inertia is closely dependent on

the particle size. Impaction may take place either on agar medium or onto an adhesive surface, and utilizing the different inertia of particles with different sizes, the device may be designed to fractionate the particles according to their size, called cascade impactors. An example of a cascade impactor is the Andersen 6-stage impactor that collects particles directly on culture media (Andersen, 1958). Among one-stage impactors are Andersen 1-stage impactor, SAS-impactor and centrifugal impactors, collecting on culture media, and the different applications of Burkard samplers that may use either culture media, microscopic slide or well plate as collection surface (Reponen et al., 2001). Impactors are the most commonly used samplers for bioaerosol collection. The collection efficiency depends on the particle inertial properties such as size, density, and velocity, and on the impactor physical parameters such as inlet-nozzle dimensions and airflow paths. The effective concentration range for the majority of agar-plate impactors is considered to be below  $10^4$  CFU/m<sup>3</sup> (Cox and Wathes, 1995).

Cut-off size of the sampling device is a concept that expresses the efficiency with which a device is collecting airborne particles of certain size. The efficiency is usually expressed as a 50% cut off size. For example, a device that has a 50% cut-off size of 2.1 µm, means that this device collects approximately 50% of the airborne particles of this size. The efficiency is higher for larger particles and lower for smaller particles. Impactors have usually a low cut-off size, some even 0.6 µm (Willeke and Macher, 1999; Reponen et al., 2001), which means that they collect practically all the fungal spores (> 2 µm) and also the bacterial spores (1µm) with reasonable efficiency.

It should be noted that impactor collection directly on culture medium may be a harsh treatment for biological particles that are vulnerable to physical stress. For example, the viability of airborne bacteria may be compromised during the collection phase (Stewart et al., 1995). Therefore, inertial impaction on culture medium is most suitable method to collect particles that are relatively resistant to such stresses. Microbial spores are usually more tolerant than vegetative cells (Cox and Wathes, 1995).

#### **4.5.4.2 Impingers**

Impingers are another application of impactor techniques. In these samplers, collection is based on liquid impingement of aerosol particles into a collection liquid, that is, the air to be sampled is bubbled through the sampling liquid and the particles are washed into the liquid, while the air is removed from the sampler (Lin et al., 1999). The main advantage is their flexibility in terms of analytical techniques that may be used to analyse the samples and effective concentration range. The portions of the collecting liquid can be concurrently examined by various techniques, which is

particularly beneficial for routine analysis of bioaerosols with unknown microbial contents. Since liquid samples can be either concentrated (by filtration) or serially diluted to facilitate the analysis, the impingers can be used over a wide range of bioaerosol concentration.

The bubbling of the liquid may re-aerosolise collected particles and to facilitate the evaporation of the liquid, thus reducing the collection efficiency. Additives to the collection liquid such as antifoam, peptone water, physiological saline, phosphate-buffered saline, or others may be useful to prevent foaming and loss of collection liquid and minimize injury to the cells.

A more recent application of impinger technique is the BioSampler centrifugal impinger (Willeke et al., 1998; Lin et al., 2000). By using a mineral oil, the evaporation problems often encountered in traditional impinger can be avoided.

To address some of the limitations of commercially available impingers (large pressure drops, liquid evaporation, etc.) a new sampler has been recently developed by Agranovski et al (2002). Although this sampler cannot be classified strictly as an impinger, the bioaerosols are still sampled into a liquid. In this method, a porous medium is submerged in a liquid layer and the air contaminated with particles, is blown through it. The gas carrier is split into a multitude of very small bubbles. The particulates are scavenged by these bubbles, and, thus effectively removed into the liquid. The advantage of this method is that there is very little stress on the sampled microorganisms due to the very small pressure drops. This leads to recovery rates for very sensitive microorganisms such as *P. fluorescence* of over 60% after 8-h sampling. The small pressure drops (portable pumps) make this method preferable as a personell sampler (Agranovski 2007).

#### **4.5.4.3 Filter sampling**

Samples of airborne particles can also be collected on filters. The collection is facilitated by a pump which may be set at different volumes of flow, e.g., 2 l/min or 10 l/min. Collection of airborne particles on filters is based on several physical mechanisms, and the collection efficiency is good especially for particle sizes larger than the diameter of the pores (Willeke and Macher, 1999). Filter sampling may not be the ideal method for sampling viable microbes, since the desiccation effects caused by air passing the filter at the sampling phase may compromise the viability of the collected cells. Instead, filter collection is suitable for sampling of airborne endotoxin and microbial components (Pasanen, 2001). Among filter types that are suitable to sampling of airborne microbial components are membrane filters, e.g., polycarbonate, cellulose ester or Teflon filters, and glass fibre filters. Although no generally accepted



protocols exist for choice of filter type for various purposes, glass fibre and teflon filters may yield highest recoveries of airborne endotoxin (Douwes et al, 1995).

Filters can be used as personal samplers or stationary sampling, and a special inlet can be used to improve the uniformity of particle deposition (Aizenberg et al., 2000). A filter sample can be used for a number of different analyses. For example, biological activity of the collected sample, such as inflammatory potential *in vitro* can be determined from filter samples (Reponen et al. 2003). Filters may be used for sampling of mycotoxins if using high volumes of air (10-100 m<sup>3</sup>), and can subsequently be analysed chemically or by determining the cytotoxicity of the collected material in an *in vitro* test (Johanning et al., 2002; Pasanen, 2001).

#### **4.5.4.4 Passive samplers**

The passive samplers that are based on gravitational sedimentation of particles, can be used to identify the microbial species present in the ambient air. They are not suitable for the quantification of biological particles, as they do not give information on the volume of air being sampled. These methods are strongly affected by both the size of particles, due to the differences in sedimentation rate and air movements. However, passive sampling may provide a means to sample airborne organisms without compromising their viability. Passive samplers, often used for sampling of volatile compounds from air, has been recently developed for purposes of airborne particle sampling (Wagner and Leith 2001a, 2001b). Their suitability for bioaerosol particle sampling is yet to be shown.

#### **4.5.5.5 Comparison of samplers**

Samplers for bioaerosol collection have been widely evaluated and a number of comparison studies for devices have been reported (Nakhla and Cumings, 1981; Gillespie et al., 1981; Lundholm, 1982; Placencia et al., 1982; Smid et al., 1989; Nevalainen et al. 1992; Jensen et al., 1992; Macher, 1993; Cage et al., 1996; Pahl et al., 1997; Griffiths and Stewart, 1999; Bellin and Schillinger, 2001). No sampler is ideal for all the purposes of bioaerosol sampling, since the characteristics of the agents to be collected as well as the aims of the sampling vary.

When enumeration of viable microorganisms is a focus of sampling, the impingers and impactors with a sufficiently low cut off size (1 µm) are preferable samplers to ensure the effective collection of small spores and bacterial cells. In comparison studies, they have been shown to recover biggest numbers of culturable microorganisms. However, there are differences in how the characteristics of different

samplers affect the colony formation of the collected particles. While the impactors collect the particles directly on agar surface, the liquid impingement may break agglomerates of microbial cells resulting in a separate colony from each individual cell. Thus, in case of side-by-side comparison of these samplers, the liquid impinger should give higher numbers of CFU's. Another general rule should help in selecting the appropriate sampler: culture-plate impactors are limited to environments where airborne levels of viable microorganisms are expected to be below than 10,000 CFU/m<sup>3</sup>. In environments with high microbial concentrations, liquid impingers or filters are preferable samplers. Analyses based on cultivation of microorganisms give information about the microbial species present, but they underestimate the total number of organisms in the air.

In practical situations, the investigator has to make a choice for the optimal sampler for sampling purposes. As mentioned earlier, the causal relationships between biological agents and health effects are poorly known, and thus the agent causing the possibly observed health effect is not necessarily known. However, the measured agent may still be a good surrogate of the exposure, or it may be an indicator of the contamination of the environment. The measurements of the airborne contaminants are relevant and helpful in the assessment of the problem and its remediation. The prerequisite is that the sampling has been adequately performed with a well designed sampling strategy and according to a good quality assurance system.

#### **4.5.4.6 Real time sampling methods**

With the increasing threat of bioterrorism and the spread of infectious diseases such as SARS, the real-time detection and identification of such harmful microorganisms became an urgent civilian and military requirement. However, measurement and detection of biological aerosols in real time has been a difficult task. Several techniques have been developed in recent years that enable real to near real time analysis of bioaerosols. Among these techniques, bioaerosol mass spectrometry, real-time quantitative polymerase chain reaction (real-time qPCR), and ultraviolet laser-induced fluorescence (UV-LIF) based systems are the most promising ones. A recent review by Chen and Li (2007) gives more details of these techniques. As the only commercially available instruments are the UV-LIF based systems, they are the ones discussed in this chapter.

Live organisms (bacteria and fungi) consist of numerous intracellular biological molecules associated with energy yielding reactions. The fluorescent characters of these endogenous molecules at specific excitation and emission wavelengths make them very attractive probes for biological detection and characterization. The ideal

target fluorophore should have an intrinsic fluorescence spectrum that distinguish it from other materials and have sufficient fluorescence intensity to produce a strong signal. One of the most promising candidate molecules are the reduced nicotiamide adenine dinucleotides [NADH and NAD(P)H]. A commercially available instrument, the Ultraviolet Aerodynamic Particle Sizer (UV-APS) manufactured by TSI, designed to differentiate between biological and non biological aerosols (Hariston et al., 1997; Brosseau et al., 2000; TSI-Incorporated, 2000), has been developed utilising the fluorescences properties of these molecules. The instrument uses a commercially available laser source at 348 nm for excitation and targeting NADH, NAD(P)H and riboflavin which have characteristic broad emission bands peaked at 450 and 560 nm (Agranovski et al., 2005). It has been extensively characterised both for laboratory generated bacterial aerosols (Agranovski et al 2004) as well as aerosols of fungal origin (Kanaani et al 2007). Besides laboratory evaluations it was also characterised in field conditions (Agranovski et al, 2004) and has been shown to be a promising technique for early identification and enumeration of particles carrying biologically active material. It can be used in environments with very high concentrations, such as animal housing facilities ( $\sim 10^6$  microorganisms/m<sup>3</sup>), to background measurements if coupled with a concentrator. The main advantage of this technique besides being real time is that it gives the size distribution of the airborne viable particles. The main disadvantage, besides a relatively high cost, is that it is not immune to interference from other fluorescent particles that do not have a biological origin. The limitations of the instrument are highlighted in more detail below:

- (1) While the UVAPS has demonstrated the ability to sort fungi (*Penicillium* and *Aspergillus niger*) by fluorescence percentage and size, when cultures were exposed to the same controlled growth conditions, it was unable to sort ambient air samples in the field due to the presence of non-biological aerosols, which may also fluoresce at the same wavelength (355nm) as spores, complicating the interpretation of the resulting fluorescence signals (Kanaani et al., 2007a);
- (2) Many non-biological aerosols, such as the particles produced from the agar washing, peptone water, broth media, canola oil and talcum powder, also produced relatively strong fluorescent signals (Agranovski et al., 2003; Kanaani et al. 2007b), which suggests that the UVAPS is not selective for the specific microbial fluorescent molecules it was designed for; and
- (3) While the ability of the UVAPS was capable of measuring fungal spores, it was limited to measuring only individual bacterial spores (Agranovski et al., 2003; Kanaani et al. 2007b).

## **4.6 Analyses of biological agents**

There are a number of techniques that can be used for enumeration or quantification of the collected bioaerosol or other biological material. The most commonly used technique to analyze fungi or bacteria is based on cultivation of the collected microbes into visible colonies that can be counted with naked eye and microscopic inspection.

However, only a small fraction of environmental microbes can be cultured in a laboratory (Atlas and Bartha 1993). The indoor agents that are the focus of this document, are usually non-infectious microbes, and therefore, the viability of the organism is not necessarily the prerequisite for its effects; non-viable microbial material may have similar allergenic or immunotoxic properties as the viable organisms (Hirvonen et al. 1997). In order to assess the whole range of both viable and non-viable microbial material, methods that are not dependent on the viability of the organisms are also needed. Such techniques applied to analyses of biological agents are direct counting of the total number of biological particles by microscope, DNA based analyses, such as PCR, determination of specific chemical markers of the microbial biomass, immunochemical methods to identify agents with antigen properties, such as cat, dog or house dust mite allergen, or bioassays to determine substances with toxic or inflammatory properties, used for example for determination of endotoxin, and chemical analyses of microbial metabolites (ACGIH 1999, Stetzenbach 2002).

Further, real-time quantitative polymerase chain reaction (QPCR) methods have been developed for microbial detection. QPCR is based on amplification of specific DNA-regions, monitoring of the amplification continuously using fluorescent dyes, and quantification of the target based on standards (Heid et al., 1996). Since the detection is based on DNA, it is not dependent on the cultivability of the microbes. Methods have been developed for detection of indoor molds and bacteria (Haugland et al., 1999 and 2004, Rintala and Nevalainen, 2006) and used for determination of microbes in indoor samples, e.g. house dust (Meklin et al., 2004, Vesper et al., 2005) and air samples (Zeng et al., 2004). The main advantages of real-time PCR are its quantitative property and high specificity. It is also rapid and high throughput applications can be developed. Also, it can be applied to almost all kinds of sample material. The QPCR technique is not yet used in routine analysis of indoor samples; however it is a promising tool for exposure assessment.

### **4.6.1 Cultivation methods for microbes**

Enumeration of fungi and bacteria on growth media, often solidified by agar, is the most commonly used method to quantify the microorganisms present in the sample.

As presented above, the cultivation method only works if the organism is viable and will germinate and grow on the media used. Thus, the investigator should be aware that without specific resuscitation techniques, only 1-10% of airborne microbes can be cultured on the commonly used media. Long time air-sampling, causing desiccating effects, or other harsh treatment may further decline the survival of the collected microorganisms. Enumerating the viable microbes provides, however, a way to characterize the microbial load of an indoor environment; it can be used to describe differences between the outdoor air and indoor air; between buildings or rooms, and the cultural methods are irreplaceable in identification of a location of microbial growth or other source of microbial contamination.

The major benefit of the cultivation method is the information about the species present, and the possibility to isolate individual microbial strains for further studies. Most indoor fungi can be identified morphologically by microscopic inspection, while identification of bacterial colonies usually needs a series of steps including staining, biochemical tests and DNA sequencing (Krieg, 2001).

#### **4.6.1.1 Cultivation methods for microfungi**

Only a limited portion of all the fungi in the indoor environment will grow on a given culture medium, and therefore, the conclusion of the genera present is always biased by the media selected. The composition of the growth medium is selective, and the more common or faster growing species may outgrow the less frequent but possibly important genera and species. Hence, using more than one medium is necessary if a comprehensive picture of the mycoflora is desired. For example, viable *Stachybotrys* spores can only be detected on a medium with high water activity ( $a_w > 0.95$ ), such as malt extract agar (MEA), corn meal agar (CMA), vegetable juice media (V8), and cellulose agar (Andersen and Nissen, 2000; Jarvis 1989; ASTM 1994).

Fungal colonies on a cultivation medium can be counted with naked eye, after 4-8 days of incubation at 25°C. For fungal colonies, identification to genus level and some times sub-genera levels can be made in parallel with the counting, since the identification of fungi is based on their colonial morphology or macro morphology (size, texture and colour of colony), and micro morphology observed in a microscope (mycelium, conidia and the conidiophores) (Samson et al., 2000). However, species-level identification is a demanding task only to be done by experienced mycological laboratories, and it usually demands sub-cultivation of pure cultures to 2-4 media which differentiates the species in that particular genus (Samson et al., 2000). Even then, the identification of certain species and sub-species within the genera *Penicillium*, *Aspergillus*, *Trichoderma* and *Stachybotrys*, requires biochemical

analyses for precise identification (Samson et al., 2000; Frisvad, 1989; Thrane et al., 2001; Lübeck et al., 2000; Andersen et al., 2002) .

Currently the use of dichloran-glycerol agar (DG18) combined with malt extract agar MEA or V8 (with antibiotics) is recommended for indoor fungi, but in cases where especially xerophilic fungi, as *Wallemia* and *Eurotium* are of interest, a lower  $a_w$  medium such as Czapek yeast agar (CYA) with 40% sucrose (Samson et al., 2000) is recommended. Other commonly used fungal media are cellulose agar (CA), potato dextrose agar (PDA), corn meal agar (CMA), Rose bengal agar (RBA) and Saboroud dextrose agar (SDA) (Cruz, 2002).

Determination of the fungal diversity is important in determining if a building is having mould or moisture related problems. For an experienced investigator, the presence of different species and their relative distribution may be more important criteria in assessing the microbiological indoor air quality than the number concentrations. The microfungi of indoor environments have been reviewed by e.g., Wanner (1993) and Verhoeff et al. (1996a), and examples of fungal types typically found in moisture-damaged indoor environments have been presented in Samson et al. (1994) and in Gravesen et al. (1994). For concentrations of fungi reported from different countries, see Chapter 2.

#### **4.6.1.2 Cultivation methods for bacteria**

Commonly used growth media for indoor bacteria are tryptic soy agar (TSA), nutrient agar, and tryptone yeast glucose (TYG) agar, or R<sub>2</sub>A medium (ACGIH 1999, Nevalainen 1989, Hyvärinen et al. 1991). Bacterial counts in indoor environments correlate strongly with the number of people present, and many bacteria in indoor air derive from humans, representing the normal flora of human skin (Nevalainen, 1989). High numbers of bacteria in indoor air thus reflect an insufficient ventilation efficiency. The diversity of indoor bacteria has not been well characterized, but in a study made in a cold climate, the majority of the bacteria found on TYG medium were gram-positive cocci, followed by coryneforms and gram negative bacteria (Nevalainen 1989).

Identification of bacterial species requires first of all a pure culture. Nowadays, a polyphasic approach for species identification is recommended, starting with morphological techniques such as colony and cell morphology and gram staining. Physiological tests, such as utilization of different substrates and antibiotic sensitivity, and biochemical characteristics, e.g., cell wall composition are determined, followed by genetic techniques such as 16S rRNA gene sequencing, DNA fingerprinting etc. (Krieg, 2001). The use of reference strains and standardized methods is essential.

While the fungal flora of an indoor sample can usually be assessed with a single morphological analysis, bacterial identification of an environmental sample is a far more tedious task. Bacterial identification is usually done in order to find the presence of certain genus or species, and the description of the whole bacterial flora is seldom relevant due to the large number of bacterial colonies in the sample, their diversity and the need of expertise resources in the identification. Instead, the endotoxin activity which reflects the amount of gram-negative species can be determined (see 4.6.2.3), or the total amount of bacterial biomass determined as muramic acid, a chemical marker of all bacteria (Szponar and Larsson, 2001).

However, a large group of environmental bacteria, actinomycetes, can be distinguished from the unspecific bacterial plates by their characteristic appearance. They form dryish, powdery or leathery colonies, as opposed to common bacterial colonies that are moist and smooth. It may be relevant to count the number of actinomycetes separately from the total number of viable bacteria. In some cases, the presence of actinomycetes (mostly of the genus *Streptomyces*) may indicate microbial growth in the building (Nevalainen et al., 1991, Samson et al. 1994), although in agricultural environments, they are common and part of the normal bacterial flora (Pasanen et al., 1989).

If determination of specific bacteria are wanted, e.g., mycobacteria (Katila et al., 1989) or specific pathogens, an appropriate growth media combined with defined incubation temperature and time is needed. For *Legionella pneumophila* bacteria which may have their source and transportation mechanism within a building and its ventilation system, a detection and identification scheme is presented below.

#### **4.6.1.3 Legionella**

Samples for determining the presence of *Legionella pneumophila* are taken from water tanks, water pipes, cooling tower water reservoirs or other water reservoirs into sterile bottles, kept in cold (<10°C) during the transportation, and analyzed within 24 hours of sampling. Detection of legionella bacteria from water samples is usually performed by media culturing method. This can be done according to a standard method (ISO 11731) using buffered charcoal yeast extract agar with  $\alpha$ -ketoglutarate (BCYE $\alpha$ ) and BCYE $\alpha$  supplemented with glycine, vancomycin, polymixin and cycloheximide (GVPC) (ASTM 1999). To detect minor concentrations of legionellae, water samples are concentrated by filtration or centrifugation. Acid wash (pH 2.2, 4 min) and heat pretreatment (50 °C for 30 min) are used as decontamination methods. Also a portion of unconcentrated water is directly inoculated on GVPC medium. The inoculated plates are incubated at 36 °C $\pm$ 1 °C for 10 days. Suspected legionella

colonies are identified with growth tests, and for species and subspecies level identification using, for example, the immunofluorescence methods or the agglutination tests. PCR-techniques for legionella are mainly used for verification of the source of infection by comparing the strains isolated from infected patients and those from water samples. However, most of the legionella infections are diagnosed by detection of legionella antigens in the urine samples (Kohler et al. 1981).

#### ***4.6.2 Non-cultivation methods for detection of microorganisms***

As mentioned in the previous section, the methods based on cultivation do not detect dead or damaged spores, hyphal fragments and other non-cultivable biological particles. However, these may have equally toxic or inflammatory properties as viable spores (Hirvonen et al., 1997). With non-culture methods, also non-viable particles may be detected. They may also be faster methods than those based on culture. Non-culture methods are based on microscopical counting, determination of chemical markers of microbial biomass; enzyme activity, immunochemical techniques, DNA based analyses or bioassays.

##### **4.6.2.1 Direct microscopy**

Direct microscopy of a bulk sample is a useful tool in mycological analysis, which can either be performed under a dissection microscope or, more commonly, from tape slides stained with lactofusin (Samson et al., 2000) which are then inspected under a light microscope. The spores can be counted for a semiquantitative assessment, and in cases where conidiophore structures can be seen, also identified to genus level. The presence of fungal growth is seen as high numbers of spores and presence of mycelial structures. Quantitative analyses of e.g. dust samples by microscopic counting requires the use of specific techniques, e.g. two-phase technique to diminish disturbance of background matrix (Pasanen et al. 1997).

Direct microscopic studies need a person who is familiar with the microscopic appearance of microbial spores and structures, mites, insects or other biological agents in question. For reviews of direct microscopic methods applicable to indoor environments, see Wanner et al. (1993;1996b); Health Canada (1995) and Pasanen (2001).

##### **4.6.2.2 DNA based methods**

DNA based methods are independent on the culturability of the microbes, since they detect the genetic material of the organism, called the DNA (deoxyribonucleic acid).



Detection of bacterial and fungal DNA with polymerase chain reaction (PCR) is a promising and specific technique, which is already widely used for example in clinical microbiology and food hygiene (Tang and Persing, 1999). Applications for environmental purposes are still under development, and mostly qualitative or semi quantitative (Bartlett et al., 1997; Borneman and Hartin, 2000; Buttner et al., 2001; Rintala et al., 2001; Stetzenbach, 2002), although quantitative methods have also been published (Haugland et al., 1999; Cruz-Perez et al., 2001; Roe et al., 2001). With environmental samples, problems are caused by PCR inhibiting substances and sometimes by ineffectiveness of the amplification of the wanted DNA (Wilson, 1997; von Wintzengerode et al., 1997).

Polymerase chain reaction (PCR) is used to amplify a specific DNA fragment from the sample million-fold, so that it can be visualized or measured in one way or another, which makes it possible to detect very low amounts of indoor contaminants. The specificity of the method is dependent of the primers, which are short DNA fragments specifying the start and the end of the amplified DNA, and therefore, the primer design should be done very carefully. Specificity problems with microfungi are caused by the incompatibility of the fungal taxonomy based on phenotypical criteria with the DNA sequence information. Attention should also be paid to the selection of the DNA isolation method. Fungi and bacterial spores have a much more robust cell wall structure than the vegetative bacteria, and thus are more difficult to break for the release of DNA (Haugland et al., 1999, Kuske et al., 1998). In addition, sufficient purification of the DNA to remove the PCR-inhibitors has to be performed (Miller 2001, Haugland et al., 2002).

#### **4.6.2.3 Endotoxin determination**

Endotoxin (LPS, lipopolysaccharide) is a structural component of gram negative bacteria, and its amount reflects the amount of gram negative bacteria in the environment in question. Endotoxin in the environment is usually measured by using the *Limulus* amoebocyte lysate (LAL) assay. The LAL assay is commercially available and inexpensive. It contains a mixture of proenzymes that are activated by endotoxin (through an endotoxin specific factor C) in a cascading set of reactions (Iwanaga, 1993). Modern versions of this assay are based on the activation of the clotting enzyme present in mixed lysates of the *Limulus polyphemus* (horseshoe crab). Several LAL-based assays have been developed including turbidimetric and chromogenic methods (Iwanaga, 1978; Teller et al., 1979). The turbidimetric method measures an increase in light scattering in proportion to endotoxin concentration, whereas the chromogenic methods measure an increase in chromophore release from synthetic substrates with increasing endotoxin concentrations. Both techniques are most frequently used in the kinetic mode, where the rate of increase in turbidity

(turbidimetric method) or optical density (chromogenic method) is measured over time. The LAL assay is very sensitive and has a broad measurement range (0.01 - 50 Endotoxin Units (EU)/ml  $\approx$  1 pg/ml - 5 ng/ml). Endotoxin concentrations are expressed in Endotoxin Units (EU) rather than in weight units. Each lot of lysate is standardized according to the standard endotoxin (usually *E. coli* endotoxin) and varies from lot to lot.

#### **4.6.2.4 $\beta$ -glucans**

$\beta$ -glucans are glucose polymers present in the cells of filamentous fungi, yeasts and some bacteria. They are used as a marker of fungal biomass. There are currently two methods to measure environmental  $\beta(1,3)$ -glucans. One is a glucan-specific *Limulus* amoebocyte lysate (LAL) assay. The assay is based on the same principles as the LAL assay described for endotoxin measurements (see above). However, rather than activating factor C glucans activate factor G leading to a series of enzymatic reactions resulting in a colour reaction (chromogenic LAL assay) that is proportional to the amount of glucan present in the sample. A turbidimetric variant of this assay is also available. The glucan specific LAL assay is commercially available. An immunoassay for  $\beta(1-3)$ -glucans has also been developed (Douwes et al., 1996). In this assay,  $\beta(1-3)$ -glucans in the test sample inhibit the binding of affinity-purified rabbit anti-glucan antibodies to the glucans coating the microtiter plate. Quantitation is achieved by labeling the rabbit antibodies with an enzyme-linked anti-rabbit antibody.

#### **4.6.2.5 Ergosterol**

Ergosterol is the principal sterol present in membranes of hyphae and spores of filamentous fungi and thus provides a chemical marker for fungal biomass (Flannigan 1997). Ergosterol determinations may detect quantities down to 500 pg of ergosterol, corresponding to 200 fungal spores (Young, 1995; Dales et al., 1999). Ergosterol determination can be used for quantification of fungal biomass on building materials (Szponar and Larsson, 2000; Pasanen et al., 1999; Nielsen and Madsen, 2000) and used as a marker of fungal exposure in air or dust samples (Axelsson et al., 1995; Saraf and Larsson, 1996; Dales et al., 1998).

#### **4.6.2.6 Chemical markers of bacterial biomass**

Analogically to ergosterol as a chemical marker for fungal biomass, there are also markers that reflect the biomass of bacteria, covering both viable and non-viable bacterial material. Muramic acid is a major component of the glycan backbone of the

cell wall peptidoglycan. Since muramic acid has not been found elsewhere in nature, including fungi, it can be used as a unique marker for peptidoglycans. The method is useful for even trace levels of microorganisms in chemically complex matrices, such as house dust (Szponar and Larsson, 2001, Saraf 1999). Similarly, 3-hydroxy fatty acids can be used as markers of endotoxin (Saraf et al., 1997; Szponar and Larsson, 2001).

#### **4.6.2.7 Methods based on enzymatic activity**

Microorganisms contain a number of enzymes that can be detected in very low quantities, by a synthetic substrate similar to the real substrate the enzyme is cleaving in its habitat.

Currently two methods are found in the literature:

- Fluorescein diacetate hydrolytic activity (by determining fluorescein) will both detect the presence of bacteria and/or moulds in wood samples (Thelander et al., 1993; Bjurman, 1993)
- Determination of the activity of  $\beta$ -N-acetylglucosaminidase, an enzyme in the chitinase pathway (Miller et al., 1998). The test is commercially available as the Mycometer Test<sup>TM</sup> in several countries.

#### **4.6.3 Methods to determine microbial products**

In addition to microbial biomass, cells and spores and the structural components of microbial cells described above, also microbial products may be of interest in indoor studies. Among such products are microbial volatile organic compounds (MVOC), which are volatile end products of microbial metabolism, mycotoxins that are toxic secondary metabolites of many fungi, and extracellular polysaccharides (EPS) produced by fungi.

#### **4.6.4 MVOC analyses**

In buildings with water damage and microbial growth, characteristic smells of mold, cellar or earth are often observed. These odorous substances are volatile end products of microbial metabolism, MVOC (microbial volatile organic compounds), and thus their presence mainly indicates the active phase of microbial contamination. Their detection from air has been considered a possibility to locate the indoor sources of odour and hidden microbial growth behind the interior surfaces without opening the structures. It is assumed that MVOC enter indoors e.g. through water vapour barriers more easily than spores, as they can be transported by diffusion. The MVOC compounds often reported in the field settings include, e.g., 3-methylfuran, 2-methyl-

1-propanol, 3-methyl-2-butanol, 2-pentanol, 1-octen-3-ol, 2-methylisoborneol and geosmin. The concentrations of individual compounds vary from a few ng/m<sup>3</sup> to 1 mg/m<sup>3</sup>. These compounds have been found in the indoor air of buildings both with and without dampness and microbial problems, as well as in outdoor air (Korpi, 2001). Because of the overlapping of the concentrations in different environments, it is difficult to recognise a microbial problem in a building on the basis of the MVOC measurements alone, or to establish any reference values for MVOC.

The methods to sample and analyze MVOC presented in the literature vary, comparative data on different methods is scantily available, and currently there is no consensus or general recommendation for these methods.

MVOC can be collected from the ambient air with either active or passive sorbent sampling using e.g., activated charcoal, graphitized carbon blacks, silica gels and polymers (Batterman 1995, Sunesson 1995). MVOC are usually analysed with a high resolution gas chromatography (GC) equipped with a mass selective detector (GC-MS) or a flame ionisation detector (GC-FID), and identified according to their mass spectra (Batterman 1995, Sunesson 1995, Korpi et al. 1998). The sample preparation depends on the sorbent used: e.g., for Tenax polymers, the sample is led from the adsorbent to the GC column by a thermal desorption cold trap injector (Sunesson 1995), whereas for charcoal sorbents (such as Anasorb), desorption with solvent (e.g. methylene chloride) is required before leading into the GC (Ström et al. 1994). The type of column and chromatographic conditions vary between the studies depending on the MVOC selection. Carbonyl compounds (aldehydes and ketones) collected in DNPH-silica Sep-Pak cartridges are analysed by high-pressure liquid chromatography (HPLC) after elution with acetonitrile (Korpi et al. 1998, Schleibinger and Ruden 1999).

#### **4.6.4.1 Mycotoxins**

Many mycotoxins, such as macrocyclic trichothecenes and sterigmatocystin have been detected in indoor environments (Dietrich et al., 1999; Engelhart et al., 2002). They have also been found in contaminated building materials (Tuomi et al., 2000, Nielsen et al., 1999). Mycotoxin analysis is a difficult task requiring specialised analytical chemists with knowledge of fungal metabolites as well as highly specialised equipment. Moreover, there is still limited experience concerning toxin analyses from building materials compared to, e.g., feed and food analyses, and the previously developed methods do not necessarily apply to these materials.

Air samples for mycotoxins can be collected on polycarbonate membrane filters (0.2 µm of pore size) at air flow rates of 10-20 l/min. Sampling from a moldy material can

be done by swabbing or by vacuuming on a 0.3-0.45µm of pore size filter. The sample is then extracted by a polar solvent to avoid the interference of the many polymers and pigments present in building materials (Pasanen et al. 1993, Nielsen, 2002).

Gas chromatographic methods in combination with mass spectrometry and/or fluorescence are needed for the analyses. Also HPLC, TLC, FTIR techniques and immunochemical assays have been developed for mycotoxin analyses (Tuomi et al 1998, Tuomi et al 2000, Dietrich et al 1999, Ostrowski 1999). Currently, analyses of mycotoxins in the indoor environment should only be performed with a research purpose or in cases of extreme exposure, since the methodology has not been developed into routine use.

#### **4.6.4.2 Extracellular polysaccharides (EPS)**

Extracellular polysaccharides (EPS) are stable carbohydrates secreted or shed during fungal growth, with an antigenic specificity usually at the genus level. However, EPS from *Aspergillus* and *Penicillium* spp. are cross reactive. Although at present there is no evidence for a pathogenic role of EPS in allergic or inflammatory reactions to fungal components, EPS may be a quantitative marker for fungal biomass in house dust. Antibodies have been developed against certain extracellular polysaccharides from these organisms (β-D-galactofuranosyl residues from mycelial galactomannans) (Kamphuis et al., 1991; Notermans and Soentoro, 1986), which have then been used to develop a sandwich ELISA to detect EPS from *Aspergillus* and *Penicillium* in house dust samples (Douwes et al., 1998). Both molds appear indoors and are particularly important in buildings where water damage is evident. Genus-specific EPS assays may provide important qualitative information allowing differentiation between indoor and outdoor fungal genera. EPS assays are not yet commercially available and there is only limited experience with this method in field studies.

#### **4.6.5 Determination of allergens**

Allergens may be determined in indoor air samples collected on a filter through which indoor air is sucked for a given period of time at a specified flow. However, levels are generally extremely low and often only represent a “snap shot” measurement. Therefore, this is seldom done. Most often, settled house dust is collected from dust reservoirs such as living and bedroom floors, and mattresses. Dust samples are subsequently extracted in water or buffer at room temperature, with or without additives such as e.g. Tween-20.

Antibody-based immunoassays, particularly enzyme-linked immunosorbent assays (ELISA) are widely used for the measurement of aeroallergens and allergens in settled dust in buildings. These assays use antibodies with specificity for the target allergen and an enzymatic reaction with a substrate is used in detection. In radio-immunoassays (RIA), radio-labelling is used for detection.

To date, the house dust mite allergens, *Der p* I, *Der f* I, and *Der p/f* II have been most widely investigated and the methods have been well described (Platts-Mills and Chapman, 1987; Platts-Mills and de Weck, 1989; Price et al., 1990). Methods for assessment of exposure to allergens from animals (Schou et al., 1991; Swanson et al., 1985) and cockroaches (Pollart et al., 1991), have also been published. Methods to measure fungal allergens are not widely available mainly due to the fact that fungal allergen production in nature is highly variable and dependent on many factors such as substrate, temperature etc. Due to this variability it is very difficult to develop specific antibody based immunoassays that detect the relevant fungal allergens in a specific environment.

#### **4.6.6 Bioassays**

As the causative agents for the indoor moisture-associated health problems are not yet well known, the exposure assessment for biological contaminants of the indoor environment may be difficult to focus to any single agent. Air concentrations of microbial toxins and many biological agents with inflammatory properties may be very low, and their concentrations may possibly vary greatly in time and space, similarly to the concentrations of airborne spores (Hyvärinen et al. 2001). Bioassays are interesting alternatives to provide information about the biological properties of the agents. The most commonly used bioassay is the *Limulus* test for endotoxin (see 4.6.2.3). New applications of bioassays for indoor environmental samples or microbial isolates from indoor environments are cytotoxicity tests such as MTT test which detects cytotoxicity in mammalian cells *in vitro* induced e.g. by microbes or their metabolites (Hirvonen 1997a,b; Johanning et al., 1998; Hirvonen et al 2001; Hanelt et al., 1994; Gareis et al. 1999). In addition, *in vitro* production of inflammatory mediators such as cytokines, nitric oxide (NO) and reactive oxygen species (ROS), induced by microbes or their products can be measured (Ruotsalainen et al.1998, Hirvonen et al., 1998; Huttunen et al., 2003; Roponen et al., 2002, Jussila et al., 2002). Such responses in mammalian cells tell about the potential of the exposing agent to initiate inflammatory responses in the cell, and possibly in the organism (Jussila, 2003). Effects of mycotoxins have been described by assays based on detection of protein or DNA synthesis inhibition (Abado-Becognee et al., 1998; Tajima et al., 2002). An assay using a more specific mode of action, as the protein

translation inhibition which is highly selective against trichothecenes (Yike et al., 1999), has also been published.

Proinflammatory markers can also be measured from the humans as a response for exposure to biological agents. However, proinflammatory markers are elevated in all kinds of inflammation processes, and they cannot be considered biomarkers in a strict sense, since the prerequisite for a biomarker is high specificity. Although actual biomarkers for biological agents are not yet available, elevated levels of proinflammatory markers in a nasal lavage sample have been linked with exposure and symptoms in a moisture damaged indoor environment (Hirvonen et al., 1999; Wålander et al., 2001).

#### **4.7 General principles for interpretation of results**

As a general rule, biological agents are present everywhere and a certain background concentration can usually be found in any normal environment with a method sensitive enough. Background concentrations may vary according to the agent, season and location. For example, fungal spores are always present in outdoor air, from where they also are transported indoors, but the outdoor air concentration may have a remarkable seasonal variation, for example, with minimal concentrations in winter of a cold climate and maximum counts in late summer and fall (Reponen et al., 1992). In warm climates, seasonal variation has less importance. The indoor mycoflora usually resembles that of outdoor air. If indoor flora clearly differs from that outdoors, it can be interpreted as an indication of an indoor source of microbes. Besides climate, factors that affect the background concentrations are also normal activities of occupants, such as walking, cleaning and cooking (Lehtonen et al., 1993, Buttner and Stetzenbach, 1993) and the building characteristics, such as age, ventilation and even the frame material of the building (Meklin et al., 2002).

The sampling strategy and interpretation of results should be based on a rationale that can be applied to different situations. Whatever samples are taken, it is essential to plan in advance, how the results of the samples from different locations will be interpreted. Since the sources and source strengths as well as the aerosolization behaviour of biological agents vary by location and conditions, it is difficult to give absolute numbers of microbes or biological contaminants that would indicate indoor contamination similarly in all the conditions, in different climates and in different buildings. Therefore, if the goal of the measurements is to identify an amplification site of the biological agent, which acts as a source of contamination, it may be simply a question of finding differences in the concentrations or in the microbial composition between different locations.

The distributions of microbial concentrations are usually near log-normal distributions. That means that the concentration differences are rather expressed as orders of magnitude, e.g., 10, 100 or 1000 CFU/m<sup>3</sup> than in exact absolute numbers. For example, 100 CFU/m<sup>3</sup> would be considered practically equal to 110 CFU/m<sup>3</sup> and 1000 CFU/m<sup>3</sup> would be nearly similar to a concentration 1100 CFU/m<sup>3</sup>. As there are usually no general databases nor routine monitoring data available for indoor biological agents, the responsibility of adequate interpretation of sampling results is on the investigator. In order to facilitate relevant comparison of the results and make the maximum benefit of them, each investigator should develop a database on “normal” samples that express the background levels of the microbes or biological agents and their concentration distributions in the given conditions.

#### **4.8 Quality assurance and quality control (QA/QC)**

Quality assurance (QA) is an essential part of all sampling and analysis of biological agents. The quality assurance plan should cover the field sampling, sample transportation, laboratory analyses and the report that gives accurate documentation on the measurements and the results. In all microbial sampling, a number of field blanks should be included. The blank samples are treated in similar way than the actual samples but their purpose is to confirm that the sampling materials or procedures did not contaminate the samples. Part of the quality assurance of the sampling is also to use a reasonable number of duplicate samples. Since the cost of the sampling and analyses are often the limiting factor in the sampling plan, the number of samples and number of duplicates must be decided case to case. As a general rule, one single sample of anything rarely allows a proper assessment of the quality of the indoor environment. Two samples (e.g., one pair of duplicates) may give information on the very microenvironment where the sample was taken. The concentration in air varies in time and space, opposite to bulk samples that represent a more integrated sample. Therefore, the number of air samples needed for reasonable conclusions is usually more than that of bulk samples. As a rule of thumb, a minimum of 6-10 air samples per building and 6-10 air samples in the outdoor air or in an appropriate control location, are needed for proper conclusions (ACGIH 1999).

Quality assurance is an integrated system of activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure a product or service meets defined standards of quality within a stated level of confidence. QA programmes are required for the following reasons:

- To improve the quality of investigations
- To generate reliable, reproducible results
- To establish inter-laboratory comparability in laboratory testing



- To establish the credibility of the laboratory
- Motivating the staff for further improvement
- To ensure the production of legally defensible data of known and documented quality.

The quality of investigation and laboratory results depends not solely upon the laboratory undertaking this analysis. However, there are many pre-analytical and post-analytical factors, which influence the quality of the end result to a significant extent. Some of the important factors influencing quality are listed here:

### **Personnel**

The quality of investigation and laboratory results is directly proportional to the training, commitment and motivation of the technical staff. The laboratory shall be under the overall direction of a qualified person. The laboratory staff must be knowledgeable in the field of environmental microbiology and mycology. All laboratory personnel must be qualified by education and experience to produce reliable analytical results. It is important that laboratory employees receive proper and regular training in analytical methods, laboratory procedures and safety education.

### **Facilities and equipment**

A laboratory must have adequate space, ventilation, facilities and equipment for the services provided, as well as appropriate occupational health safety procedures to protect the employees, according to national standards. Good housekeeping and cleanliness of the laboratory are necessary to ensure the integrity of samples and analytical procedure. Good quality equipment is essential to generate quality results.

### **Analytical Methods**

Each laboratory should have Standard Operating Procedure Manuals (SOPMs) for the collection, transport, storage, processing, analysis and retention of samples as well as for recording of results, reporting of results, procedure of quality control, maintenance of equipment, housekeeping and related practices. SOPMs should be periodically reviewed and revised and strictly followed in the laboratories.

### **Internal Quality Control (IQC)**

IQC denotes a set of routine procedures undertaken by the staff for continuously and concurrently assessing laboratory work so that quality results are produced by the laboratory. This is the backbone of a good quality assurance programme (QA). The IQC must include the following steps:

- routine checking and calibrating of equipment and instruments to ensure adequate performance

- routine checking of culture media, water, and analytical reagents for appropriate sterility, microbial growth, and analytical reactions
- data quality assurance, quality assurance records, and documentation of deficiencies and corrective actions
- housekeeping, cleanliness, and appropriate waste treatment and disposal practices
- maintenance of a reference cultures collection

### **External Quality Assessment (EQA)**

External Quality Assessment (EQA) is a system of objectively assessing the laboratory performance by an outside agency. This assessment is retrospective and periodic but is aimed at improving the IQC. Laboratory accreditation is a formal recognition of an organisation's technical competency to perform specific tests, types of tests, or calibrations.

### **External Quality Control (EQC)**

Continued participation in the proficiency analytical testing program. In USA, the American Industrial Hygiene Association initiated an Environmental Microbiology Proficiency Analytical Testing program (EMPAT) in 1996. In Germany, the state health agency Baden-Württemberg (Landesgesundheitsamt Stuttgart) initiated EQC-program in 2001.

### **Post-analytical factors**

Transcription errors, incomplete reports, and improper interpretation can adversely influence the laboratory results and results of investigation. Evaluation and interpretation of sampling data should be performed by an experienced health professional.

#### ***4.8.1 Collaboration between laboratory and field-investigators***

In most cases, the person who is taking the samples in the field, i.e., from indoor air or from different parts of the building surfaces, materials or structures does not do the laboratory analyses, but is sending the sample to a laboratory that is providing such analytical services. It is important to establish a good communicative relationship with the laboratory to ensure the flawless transportation, storage and handling of the sample, and relevant documentation of the results. As part of the quality assurance system, the investigator should confirm about the quality assurance system of the laboratory, and agree about the procedure how samples can be sent for analysis. The investigator also needs to develop a plan for data interpretation prior to sample collection. Details that should be clarified and documented into written protocols are e.g.:

- How many samples can be sent at one time?
- How should the samples be packed?
- How much of material sample does the laboratory need for the analysis in question?
- Are there limitations in transportation schedules, e.g. concerning weekdays or the delay between sampling and the analysis?

The samples should be clearly marked or numbered, but to ensure a blinded analysis, the details of the sample should not be given at this point. At the interpretation phase of the results, the numbers or list of organisms found may not be informative enough to make a conclusion about the findings. At this point, the field investigator may discuss the results with the laboratory experts, to make best available conclusion about what the findings may indicate.

Investigations of an indoor air problem of biological origin may either follow the appearance of symptoms compatible with exposure to biological agents, or the detection of potentially problematical biological material in a building environment. Such investigations require expertise and experience in the areas of environmental microbiology and mycology, allergology, public and occupational health, industrial hygiene, chemistry and building dynamics. Problems in the indoor air quality are often remarkably complex and multifactorial, and the investigations may be difficult. To facilitate this demanding work, the application of quality assurance principles and practices presented above is highly recommended.

## **4.9 Conclusions and recommendations**

From issues concerning the assessment of biological agents, the following general conclusions can be drawn:

- There are a numerous methods available for qualitative and quantitative assessment of biological agents in indoor environments, but no method has been validated well enough to have gained a status of a standard method
- The validation of exposure assessment methods is at present compromised by the fact that the causal relationships between individual biological agents in indoor environments and health effects are not established
- Assessment of biological agents is a complex task including specific demands for both sampling and analytical phases.
- Present rapid development in biomedical research will hopefully provide new alternatives for assessment of biological agents in indoor environments

For future actions, research and development work, it is recommended that

- Research efforts are focused on effective application of new techniques in molecular biology into environmental microbiology and analyses needed for indoor air research and building investigations
- In all studies on biological agents in indoor environments, a well developed quality control system should be used to ensure a high quality of the data
- Databases should be developed, e.g., on national basis, on the distributions of concentrations of biological agents to facilitate the assessment of the biological indoor air quality

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## 5. Biological Agents and Air Pollution Interactions

### 5.1 Biological agents and non-biological particles in the air

Both outdoor and indoor air contain mixtures of biological agents as well as non-biological particles including tobacco smoke, cooking generated particles, motor vehicle exhaust particles or dust and organic and inorganic gases (HC, CO, SO<sub>2</sub>, NO<sub>2</sub>, O<sub>3</sub>, etc) (Pei-Chih et al., 2000; Hargreaves et al., 2003; Portnoy et al., 2004; Jo and Seo, 2005) (Sessa et al., 2002; Górný, R. and Dutkiewicz, 2002; Law et al., 2001; Spengler et al., 2001; Sheldon et al., 1993; Gauvin et al., 2002).

To date little work has been done to investigate interactions and associations between particles of biological and non-biological origin, instead, physical, chemical and biological properties of particles have most often been studied in isolation (Samet J.M. 1995; Fantuzzi et al., 1996; Zhang et al., 1994; Sessa et al., 2002). Most atmospheric research to date has focused on characterisation of isolated airborne pollutant(s) to provide for example, information on their sources and sinks or concentration levels and trends in concentrations, without, however considering the whole air pollutants mixture (Wallace et al., 1987; Brown et al., 1994; Saglani and McKenzie, 2002, Bholah and Subratty, 2002.). However, the various pollutants coexist and have similar variation patterns in both indoor and outdoor air, as illustrated in the Table 2.10. It is therefore likely that interactions may occur between biological and non-biological pollutants that could affect their behaviour in the air and ultimately the effect they have on health. It has already been shown that the physical and chemical properties of non-bioaerosol particles do affect their dynamics and behaviour in the air (Cox and Wathes, 1995).

**Table 2.10.** Average indoor and outdoor pollutants concentrations measured in 14 residential houses, Brisbane, between May and August 1999 (adopted from Morawska et al. 2001).

	OUTDOOR		INDOOR	
	Average	Range	Average	Range
Supermicrometer Particle (particle m <sup>-3</sup> )	(1.87±1.38) x 10 <sup>6</sup>	(0.45 ~ 3.96) x10 <sup>6</sup>	(1.74±1.27) x 10 <sup>6</sup>	(0.51 ~ 3.86) x 10 <sup>6</sup>
Submicrometer particle (particle m <sup>-3</sup> )	(2.47±1.41) x 10 <sup>9</sup>	(0.57 ~ 4.87) x 10 <sup>9</sup>	(2.10±1.21) x 10 <sup>9</sup>	(0.53 ~ 4.00) x 10 <sup>9</sup>
PM <sub>2.5</sub> (µg.m <sup>-3</sup> )	11.1±4.4	4.7 ~ 18.4	10.8±3.8	4.4 ~ 15.3
Fungi (CFU/m <sup>3</sup> )	1133±759	463 ~ 3125	810±389	400 ~ 1675
Bacteria(CFU/m <sup>3</sup> )	376±383	75 ~ 1625	425±249	81 ~ 850
TVOCs* (µg.m <sup>-3</sup> )	39.2±37.5	5.9 ~ 136.7	87.5±61.5	27.2 ~ 229.5

\*TVOCs – total volatile organic compounds

Among other characteristics, the size of the particle will determine how long it may remain airborne and how far it will travel, as well as the site of deposition in the respiratory tract. While the largest particles ( $>10\mu\text{m}$ ; inhalable fraction) are deposited in the upper airway (nose, pharynx), thoracic particles ( $<10\mu\text{m}$ ) can penetrate the respiratory tract beyond the larynx, respirable particles ( $<4\mu\text{m}$ ) can penetrate to the unciliated airways of the lung (alveolar region) (Mark, 1998). Unattached or attached allergen particles that are in the ultra fine ( $<0.1\mu\text{m}$ ) or submicrometer size range, respectively, can penetrate to the deepest parts of the respiratory tract. Fungal allergens are proteins of a nanometer size range occurring on the surface of the fungal spore. It was shown for example that the deposition of fungal spores in the lung and their effect on human health depends on their composition (genera and species), concentration, and size (Horner et al., 1995, Reponen et al., 1996).

Thus, examining associations between biological and non-biological particles present in the air is the first step towards understanding the similarities and differences in their dynamics as well to explain the interactions occurring between them.

## **5.2 Indoor and outdoor air**

### **5.2.1 Indoor Air Pollutants**

The sources of indoor air pollution may include: (1) combustion of oil, gas, kerosene, coal, wood, and tobacco products; (2) building materials and furnishings, asbestos-containing insulation, wet or damp carpet, and cabinetry or furniture made of certain pressed wood products; (3) products for household cleaning and maintenance, personal care, or hobbies; (4) central heating and cooling systems and humidification devices; (5) and outdoor air pollution. The relative importance of any single source depends on how much of a given pollutant it emits and how hazardous those emissions are. Some sources, such as building materials and furnishings, release pollutants continuously. Other sources related to activities carried out in the home, such as smoking or the use of cleaning products, release pollutants intermittently. It has to be pointed out, that without obvious indoor sources, outdoor air is the major source of indoor air contamination.

The common indoor air pollutants originated from the specific to the indoor environment sources include combustion products (e.g., particulates, carbon monoxide, carbon dioxide, nitrogen dioxide, sulfur dioxide, hydrocarbons), volatile organic compounds (formaldehyde, fragrance products, pesticides, solvents, cleaning agents), heavy metals (airborne lead, mercury vapor), radon, and biological pollutants (bioaerosols, gases and vapors produced by living organisms).



### **5.2.2 Outdoor Air Pollutants**

Natural outdoor air pollutants include oxides of sulphur and nitrogen from volcanoes, oceans, biological decay, lightning strikes and forest fires, VOCs and pollen from plants, grasses and trees, and particulate matter from dust storms. Typical outdoor air pollutants from man-made activities include nitrogen oxides, carbon monoxide, sulphur dioxide, hydrocarbons and particulate matter. All these pollutants are primary pollutants. Common sources of these primary pollutants include power stations, industrial plants, and road transport (carbon monoxide, particulate matter and nitrogen oxides). Ozone is a secondary pollutant, formed in the air as a result of chemical reactions. Outdoor pollutants penetrate indoors and their contribution to indoor concentration depends on the building ventilation system, as well as the pollutant specific dynamics (e.g. see Chapter 3.3).

### **5.2.3 Airborne Particulate Matter**

Most biological agents are in particle form and it is important to consider them in the context of airborne particulate matter, in general. Airborne particulate matter is a mixture of solid particles and liquid droplets suspended in gaseous medium, which vary in concentration, nature and size distribution. The particles can be, for example, combustion products, mechanical dust or bioaerosols (Sessa et al., 2002; Law et al., 2001; Spengler et al., 2001; Sheldon et al., 1993; Gauvin et al., 2002), and can act as carriers of adsorbed chemicals, bio-contaminants or condensed gases. Fine particles are defined as those with an aerodynamic diameter equal to or below 2.5  $\mu\text{m}$ , coarse particles above 2.5  $\mu\text{m}$ , while ultra fine particles are those below 0.1  $\mu\text{m}$ . In the terminology of the standards used in many countries,  $\text{PM}_{10}$  and  $\text{PM}_{2.5}$  fractions are mass concentrations of airborne particulate matter with aerodynamic diameter smaller than 10  $\mu\text{m}$  and 2.5  $\mu\text{m}$  respectively. In terms of number, the vast majority of airborne particles are in the ultra fine range (Morawska et al., 1999a, Morawska et al., 1999b, Morawska et al., 2007). Non-biological particles that are airborne are generated by a multiplicity of sources. Ultrafine particles are related mainly to combustion, gas to particle conversion or nucleation processes. They typically contain a mixture of components including soot, acid condensates, sulfates and nitrates, as well as trace metals and other toxins. Larger particles are generated from mechanical processes including grinding, breaking and wear of material and dust re-suspension.

### **5.3 Behaviour of biological agents in the air**

Biological particles are unique, in terms of their biological component, nevertheless they can be considered in terms of their physical characteristics such as size, mass, shape, hygroscopic properties, etc. The two critical questions to answer are:

1. Whether the behaviour of biological particles in the air is governed by their physical characteristics, and thus does not differ from the behaviour of non-biological particles of similar physical characteristics
2. What role non-biological particles play in terms of acting as carriers of biological particles?

The second question is of critical importance for the transport of submicrometer biological particles, such as viruses or allergens.

#### ***5.3.1 Release of Biological Agents in the Air***

Biological agents may be released into the air as single unattached microbial cells (viruses, bacteria, fungi), as the clumps composed of a number of microorganisms, or may be attached (as intact cells or cell fragments) to the other suspended particles. Both fine and coarse atmospheric particles were found to contain materials of biological origin, including microorganisms, pollen, pollen allergens, debris of spores and bacteria, epithelial cells, or insects, (Matthias-Maser, 1998). In regards to the complex particles, whether individual biological agent will be aerosolized as a counterpart of chemically complex particle or will be attached to non-biological particles as a result of their interactions in the environment largely depends on their properties (e.g., size), sources, and the methods of dissemination. For example, humans and animals are the primary sources of bacteria and viruses in the indoor air. Coughing, sneezing, and talking may result in release of either single or aggregated microorganisms. If not inhaled, they will settle on the surfaces (e.g., carpet), which may preserve their infectivity, but later may be disturbed due to human activities and subsequently re-released into the air. However, the re-aerosolised cells may be this time attached to the dust particles and thus differ in size from the originally released, will affect the site of the deposition in the respiratory tract, and thus bioresponse. There is a possibility that for example secondary dispersal of fungal allergens on the surface of smaller particles or spore fragments occurs as a result of an attachment process (Ormstad et al, 1998).

The fragmentation of fungal spores, to produce smaller and submicrometer fungal particles, has been reported in many studies. Górny et al. (2002) reported that fungal fragments aerosolized along with spores from contaminated agar and that fragments

number release is much higher than fungal spores. Kildesø et al. (2003) found particle fragments of a size less than the spore size for *A. versicolor* and *T. harzianum* at 3m/s, and fungal fragments were detected by an Ultraviolet Aerodynamic Particle Sizer (UVAPS), when a continuous air flow contacted the surface of the *Penicillium* or *Aspergillus niger* mycelia at speed of 5.5, 7.1, 8.5 and 10.2 m/s (Kanaani et al., 2007a).

### **5.3.2 Dynamics of Biological Agents in the Air**

It is expected that the dynamics of particles in the air is governed mainly by particle physical characteristics of which size is one of the most important (Cox and Wathes, 1995). A recent study by Kanaani et al. (2007b) has investigated the effect of ventilation rates on fungal spore deposition rates in a chamber of 20.4 m<sup>3</sup>. The study reported that the deposition rates of fungal spores (*Aspergillus niger* and *Penicillium* species) and other aerosols (canola oil and talcum powder) were similar, especially at a very low air exchange rate (0.009 h<sup>-1</sup>) and they all showed deposition rates that were a function of particle size. Therefore biological and non-biological particles of similar sizes would display the same dynamics in the air and thus respond similarly to the forces acting on, and processes affecting them. The type of physico-chemical processes that could be of importance include: dispersion, interaction with other types of particles, transport, removal from the air by deposition on surfaces, penetration to, and deposition in the respiratory tract or penetration through ventilation and filtration systems (Cox and Wathes, 1995).

### **5.3.3 Concentrations**

When considering the behaviour and ultimately the fate of biological particles in the air it is important to keep in mind that the concentration of these particles is usually significantly lower than the concentration of particles that are not of biological origin ((submicron, fine and coarse particles), in both the indoor and outdoor environment (Torres et al., 2002).

Many studies have investigated the concentrations of bioaerosols in the indoor and outdoor environment. A study by Lee et al. (2006) reported that the concentration of airborne fungi in six Cincinnati homes is typically between 0-1362 CFU/m<sup>3</sup>, while the concentrations of airborne fungi in environmental samples measured outdoors and indoors (in 14 residential suburban houses in Brisbane) was reported to be  $1133 \pm 759$  CFU/m<sup>3</sup> and  $810 \pm 389$  CFU/m<sup>3</sup>, respectively (Hargreaves et al., 2003). The bacterial and fungal concentrations in the indoor and outdoor air at 42 bars, 41 Internet cafes, 20 homes and 44 classrooms at 11 elementary schools in Daegu, Korea, measured under uncontrolled environmental conditions during both summer and winter, were

found to be between  $10\text{-}10^3$  CFU  $\text{m}^{-3}$  (Jo and Seo, 2005). Pastuszka et al. (2000) found that the typical level of bacteria indoors is about  $10^3$  CFU  $\text{m}^{-3}$  in homes and  $10^2$  CFU  $\text{m}^{-3}$  in offices. The fungal concentration in winter ranged from  $10\text{-}10^2$  CFU  $\text{m}^{-3}$  in healthy homes and  $10\text{-}10^3$  CFU  $\text{m}^{-3}$  in homes with mold problems, while in summer it reached up to  $10^3$  CFU  $\text{m}^{-3}$  in healthy homes and  $10^3\text{-}10^4$  CFU  $\text{m}^{-3}$  in moldy buildings.

In terms of non-biological aerosols, Morawska et al. (1998) found that the average concentration of submicrometer particulate concentration in centre of the subtropical city of Brisbane, Australia, was  $7.4 \times 10^9$  particles/ $\text{m}^3$ . Long-term measurements of particle number size distributions (submicrometer particles) were also conducted in a moderately polluted site in the city of Leipzig, Germany, and the mean number concentration for non-biological aerosols was found to be  $1.7 \times 10^{10}$  particles/ $\text{m}^3$  (Wehner and Wiedensohler, 2003). Thus, the concentration of biological aerosols is  $10^6$  times or six orders of magnitude lower than the concentration of non-biological aerosols.

#### **5.3.4 Interactions**

Whilst the fact of interaction between anthropogenic pollutants and biological materials is broadly recognised, the mechanisms of such interactions are not well understood. There could be various interactions occurring between the two types of particles, of which the most likely would be coagulation that results from particle collisions. The process is strongly dependent on particle concentration and also on particle size and is governed by the rate of diffusion of particles towards each other. The process is faster when a small particle of high diffusion coefficient diffuses to a larger particle with a large surface (Willeke and Baron, 1993). For example in a mixture of fungal spores and combustion aerosols (motor vehicle emissions) present in the air, fungal spores are much bigger than the combustion aerosols that are predominantly in the lower submicrometer range, and therefore fungal spores can carry combustion aerosols attached to their surface. On the other hand, fungal allergens, which are in the submicrometer range are very likely to coagulate with the combustion aerosols since they are of similar sizes and the concentration of the spore allergens is usually significantly lower than the concentration of the combustion aerosols (by an order of magnitude or more). Thus it is recommended to study associations of fungal allergens with submicrometer particles, rather than associations with fungal spores, as fungal allergens are also in the submicrometer size range.

Non-biological particles may serve as carriers of fungal allergen molecules into the lung independently of the whole fungal spore. In the case of non-viable combustion particles such as tobacco smoke or cooking-generated particles ( $<1 \mu\text{m}$ ), such an interaction would have serious implications, as allergen molecules could conceivably

be carried deeper into the lung than a fungal spore would be expected to penetrate. At the very least, the aerodynamic behaviour of a coupled particle within the lungs may be expected to be quite different from that of two non-associated particles. Therefore, it is of critical importance to determine whether associations occur between non-biological and biological aerosol particles, and if so what the nature of interactions causing the associations might be.

The majority of studies on this issue were concerned with aeroallergens, from which pollen allergen have received the most attention. There are two major consequences of influence of air pollutants on pollen allergens: modification of allergens and the formation of allergen-loaded fine particles, either by inducing a higher production of allergens with pollen grains still located in their anthers or through interactions in the atmosphere (Masuch et al., 1997). A comprehensive review on potential interaction between air pollutants and aeroallergens can be found in Emberlin (1995) and Monn and Koren (1999).

### **5.3.5 Allergenic and Toxic Properties**

A wide range of microorganisms such as bacteria, moulds, actinomycetes, amoebae and nematodes has been identified as sources of offending allergens and toxins (Carrer et al., 2001). It has to be noted, that unlike infectious agents, microorganisms may not have to be viable and intact to retain allergenic and toxic properties. While the majority of studies on bioaerosols are concerned with the intact microorganisms, their microbial toxins and allergens may be also aerosolized with the cell debris attached to the airborne particles and, consequently, be associated with the particulates of sizes outside the typical for the intact organisms size range. This fact should be taken into account when designing a particular study and developing the sampling and analytical strategy. For example, in studies concerned with allergic or toxic potential of airborne microorganisms, in addition to the characteristics (e.g., concentration and composition) of total microbial population, it is also important to investigate allergen-containing fragments using appropriate sampling methods and immunochemical methods of analysis. While no relevant study was published in regards to the microbial toxins or allergens, the phenomenon of interaction between aeroallergens and non-bacterial particulates was often observed for pollen allergens. Although pollen grains have sizes greater than 10  $\mu\text{m}$  and may, therefore, be associated only with particles larger than 10  $\mu\text{m}$ , pollen allergens were found in fine (Solomon et al., 1983; Rantio-Lehtimäki et al., 1994; Spiekma, 1990; Spiekma et al., 1995; Schäppi et al., 1996) and ultra-fine particle such as diesel soot particles (Knox et al., 1997; Ormstad et al., 1998; Glikson et al., 1995). Consequently, due to their large size, intact pollen grains are deposited in the upper respiratory tract (nose, pharynx) and cause allergic responses such as hay fever (Wilson et al., 1973), whereas

the allergen-loaded fine particles are penetrated into lower airways causing allergic asthma (Suphioglu et al., 1992).

### **5.3.5 Lung Deposition**

It is important to understand the dynamics of biological agents and interactions with other airborne pollutants, as it is the dynamics that will determine for example whether the particles will be deposited in the lung, while the interactions will determine what additional components they will carry or whether they will be carried by different pollutants.

A study by Geiser et al. (2000) demonstrated that *Calvatia excipuliformis* puffball spores (3µm diameter), inhaled by Golden hamsters, were distributed over a large surface area of the lungs, where group A hamsters inhaled the aerosol by spontaneous breathing and group B hamsters inhaled the aerosol by continuous negative-pressure ventilation. The results showed that 67.2% and 89.8% of the spores that were deposited within the alveoli, for group A and B, respectively. The intrapulmonary conducting airways retained 32.3% in group A and 10.0% in group B, while <0.5% was deposited on the extrapulmonary mainstream bronchi and trachea.

### **5.3.6 Summary**

In summary, while there are different sources responsible for generation of biological and non-biological particles, the reasons for the associations could be twofold. On one hand biological and non-biological particles could behave in the air in a similar way, as reported by Kanaani et al. (2007), and thus respond similarly to the forces acting on, and processes affecting them. On the other hand, there could be interactions occurring between the two types of particles, of which the most likely would be coagulation that results from particle collisions or when small particles are attracted to and carried by larger particles, as reported by Houba et al. (1996). This implies that the behaviour of biological particles in the air cannot be investigated in isolation but consideration has to be given to the characteristics of non-biological particles (concentration, size distribution), as the presence of the later could have an important impact on the biological particles.

## 5.4 Case studies

There have been only a very limited number of studies reported in the literature that investigated interactions between biological and non-biological particles.

- One study on this topic showed that cat and pollen allergen, (birch pollen, Bet v 1) is absorbed by diesel exhaust emission particles Ormstad et al. (1998). To date, the investigations of this nature for fungal allergens are also very limited.  *$\alpha$ -amylase* allergens, which are derived from *Aspergillus niger*, were the most frequently reported as a cause of allergy for bakers working in both small and large bakeries (Dockery et al., 1993; Houba et al., 1996). The presence of these  *$\alpha$ -amylase* allergens were tested using Sandwich enzyme immunoassay with affinity-purified polyclonal rabbit IgG antibodies and the  *$\alpha$ -amylase* allergen was found in all stages of the cascade impactor (in some bakeries), predominantly carried by particles larger than 5  $\mu\text{m}$  (for all the tested bakeries) (Houba et al., 1996). In another study of outdoor air components, the cytoplasmic content of spores and pollen was often found attached to vehicle exhaust particles (Glikson et al., 1995).
- The objectives of a study reported by Hargreaves et al. (2002), was to examine associations occurring primarily in indoor air of residential houses between the concentration levels of submicrometer and supermicrometer particles and biological particles including fungi, bacteria, dust mite allergen (Derp1), cat allergen (Feld1) and cockroach allergen (Blag1). Findings of this study in relation to the associations between biological and non-biological particles or transport mechanisms of biological particles can be summarised as follows.

Dust mite allergen (Derp1) and cat allergen (Feld1) were found in all 14 houses investigated, while cockroach allergen (Blag1) was found only in 4 houses. For sensitised individuals, concentrations of 10  $\mu\text{g/g}$  Derp1 are considered a risk factor for severe symptoms, while concentrations of 2  $\mu\text{g/g}$  considered as a risk for sensitisation (Platts-Mills and De Weck, 1989). In this study 93% of houses had concentrations of Derp1 greater than 10  $\mu\text{g/g}$ . Those residents with health problems were found to have higher Feld1 concentration in their living rooms and it is thus likely that this allergen contributes to their respiratory health problems. In this study Feld1 was not only found in houses with cats but also in those houses with other pets and no pets, although at lower concentrations which may suggests that Feld1 is transported in some manner between the houses. In a study of allergens in school dust conducted by (Munir et al., 1993), pet allergens were found to be present in the hair and clothes of pet owners, thereby becoming carriers of the allergens, transporting them to other places. Blag1 on the other hand, was found in the living room and kitchen of 4 houses, however, it was undetectable in the bed samples in

those same houses. While only very small concentrations were detected in these four sampled areas, lack of the allergen in other areas in the houses may indicate that Blag1 is transported in a different way to Feld1.

Weak but statistically significant inverse relationships were found between airborne fungal concentrations and supermicrometer particle concentrations in the outdoor and living room air under minimum ventilation conditions. The relationship can be attributed to the fact that both fungi and supermicrometer particles occur in the same size range and are counted in terms of number, and that the meteorological conditions at which supermicrometer particle concentrations are elevated are different to those required for the release of total fungi. A similar negative correlation was found between bioaerosol counts and PM<sub>10</sub> concentrations in the outdoor air study conducted by Glikson and co-workers in 1995.

No statistically significant associations between the fungal spore concentrations and submicrometer particle concentrations were discovered under the experimental conditions of the study. While fungal spores are much bigger than non-viable particles in the submicrometer range, it was considered that fungal allergens, which are in the submicrometer range, could be present in the air in direct proportion to the numbers of culturable fungal spores. Thus, a relationship between particles and allergens could be inferred from an observed relationship between particles and fungal spores. Lack of a relationship could mean that either the assumption of a direct relation between the fungal spore and the allergens is not correct or may be attributed to the experimental design. In case there is no direct relation between the fungal spore and the allergen concentrations, relation between the fungal spore and submicrometer particles would not be expected, due to the differences in size. This means that in any future studies direct measurements of the allergens should be conducted for investigations of associations with non-viable submicrometer particles.

The study showed that there is a certain aspect of similarity in behaviour between fungal spore and submicrometer aerosols. It was shown that similarly to what was reported in the literature about the dispersion of particles from motor vehicle emissions, fungal concentrations decrease markedly at distances greater than 150 m from the major source, the park. With the park acting as a primary source of total fungi and particularly *Cladosporium*, a dispersion effect was observed with airborne fungal concentration decreasing with the distance from the park. A similar trend was shown by Hitchins et al. (2000) in relation to the concentration of submicrometer particles originating from vehicle emissions from a major road.



Under minimum ventilation conditions both fungal and submicrometer particle concentrations were lower than under normal ventilation conditions. In these respects fungi and submicrometer particles behaved in a similar manner. However, a difference noted was that while fungal indoor to outdoor ratios were below one, the ratios for submicrometer particle concentrations were closer to one. Therefore, it is possible that different experimental conditions may uncover interactions occurring between fungi and submicrometer particles.

## **5.5 Effect of atmospheric gases on bioaerosol composition.**

Interaction of biological agents with gaseous contaminants can result in altered biological properties of viable aerosols that will determine the human bioresponse, if inhaled. Ozone, olefins, nitrogen oxides, sulfur oxides are major gaseous pollutants affecting viability and thus infectivity of pathogenic airborne microorganisms. The interactions of bioaerosols with these air contaminants were studied most comprehensively and have been reviewed by Strange and Cox (1976), Donaldson (1978), Spendlove and Fannin (1982), and Cox (1987). Other pollutants studied include CO, C<sub>2</sub>H<sub>2</sub>, C<sub>2</sub>H<sub>4</sub>, C<sub>2</sub>H<sub>8</sub>, HF, HCl, HCOH (de Mik and de Groot, 1973; Cox et al., 1973). All of these compounds interact both individually and synergistically with intrinsic factors within specific organisms (Berendt et al., 1972). The effect of any one compound on viability/ infectivity can be different for different agents. The allergenic properties, however, have been known to be retained even when microbes are no longer viable. Moreover, gases such as O<sub>3</sub> and NO<sub>2</sub> may increase the allergen content in pollen by induction of stress-related proteins (Masuch et al., 1997; Behrendt et al., 1997). Behrendt et al. (1997), who investigated interactions between pollen and air pollutants in a floating chamber under controlled conditions, have found that, in contrast to NO<sub>2</sub> and O<sub>3</sub>, exposure to SO<sub>2</sub> may result in a reduction of allergen release. They have also concluded that air pollutants may modulate the bioavailability of grass pollen allergens.

While very little research has been done in this field, considerable explorations have been made by the food technology industry regarding the effects of various atmospheric gases on the viability of microorganisms. The development of Modified Atmosphere Packaging (MAP) and Controlled Atmosphere Storage (CAS) have required experimentation with various levels of atmospheric gases, in order to observe inhibitory effects on food spoilage organisms. Since many of these organisms are also found commonly in bioaerosols, correlations may be made with confidence.

Of the three major gases (oxygen, nitrogen and carbon dioxide) used in MAP, CO<sub>2</sub> is the only one that has significant and direct anti-microbial activity. Increased levels of CO<sub>2</sub> appear to increase the lag phase and also the generation time during the

logarithmic phase of the organisms involved (Phillips, 1996). This inhibition by CO<sub>2</sub> is both temperature and concentration dependent. Several attempts have been made to explain this anti-microbial effect and these hypotheses have been summarized by Farber (1991), as follows:

- Alteration of cell membrane function including effects on nutrient uptake and absorption;
- Direct inhibition of enzymes or decreases in the rate of enzyme reactions;
- Penetration of microbial membranes leading to intracellular pH changes;
- Direct changes to the physico-chemical properties of proteins.

The inhibitory influence of CO<sub>2</sub> has differential outcomes for microorganisms (FDA/CFSCAN, 2001), however the most pronounced effect is found to be on aerobic bacteria, which are inhibited by moderate to high levels of CO<sub>2</sub>. While the result of MAP on yeasts is negligible, moulds, as aerobic microorganisms, exhibit similar growth inhibition by CO<sub>2</sub> to the aerobic bacteria. This may be caused by CO<sub>2</sub> concentrations as low as 10% (Molin, 2000), but is not necessarily fungicidal (Littlefield et al., 1996).

The other major atmospheric gases are less effective for inhibition of microbial growth, although increased nitrogen levels will result in retardation of the growth of aerobic organisms, and very high levels of oxygen can inhibit the growth of both anaerobic and aerobic microorganisms, since the optimal O<sub>2</sub> level for growth (21% for aerobes, 0-2% for anaerobes) is surpassed (FDA/CFSCAN, 2001). This treatment is referred to as “oxygen shock” and while very effective, requires levels of 70-100% oxygen (Day, 1996), which is neither safe nor practicable in a large-scale operation. More recently, super-atmospheric O<sub>2</sub> levels have been used to control microbial levels, but were only effective if O<sub>2</sub> levels approaching 100kPa were used, or if lower pressures (40kPa O<sub>2</sub>) were employed in combination with 15kPa CO<sub>2</sub> (Kader & Ben-Yehoshua, 2000; Wszelaki & Mitcham, 2000). Most of the work with modified oxygen levels, however, has been done using low levels of O<sub>2</sub> to inhibit anaerobic organisms. While having some success at levels of 1-5% O<sub>2</sub>, these results do not invite comparison with the bioaerosol situation, in which most of the organisms are expected to be aerobic, or at least oxygen tolerant, rather than anaerobic.

Modified atmospheres have been trialled in attempts to control bacterial (Gram positive and Gram negative, with and without endospores), fungal and viral multiplications, with varying degrees of success. The most effective use of these manipulations was in control of those microorganisms with an aerobic metabolism, such as bacteria and fungi. While it is probably not feasible to consider use of such methods to manipulate bioaerosols on a large scale, there is a strong possibility of adapting CAS technology for air remediation purposes.

## **5.6 Synergistic effects of biological agents and other airborne pollutants**

There has been very limited work done to investigate whether there are any synergistic effects of biological agents and other airborne pollutants. The handful of studies published on some aspects of this topic are summarised below.

One study concluded that pollen allergens interact with other sources of fine particles in polluted air, which could cause the allergens to become concentrated in polluted air and to trigger asthma attacks (Knox et al., 1997).

It has been shown that the total protein content and major allergen release differs greatly between freshly collected pollen from rural meadows and pollen from areas near high-traffic roads. The pollutants seem to make the surface of the pollen exine more fragile and also to trigger a mucosal reaction making the mucosa more exposed to pollen allergens. The pollutants also act as allergen adjuvants, causing a greater production of IgE and worsening of allergic symptoms (Peltre, 1998). Besides being the source of allergens, pollen grains might also have important implications on early events as initiators of allergy as the pollen grain secretes significant amounts of eicosanoids namely prostaglandin E2 and leukotriene B4 (Behrendt et al., 1999).

As for the interaction between biological and non-biological agents, airborne particles such as diesel exhaust particles have an adjuvant activity for IgE antibody and cytokine production (Takafuji et al. 1987; Fujimaki et al. 1995). Intranasal instillation of ragweed allergens with diesel exhaust particles to allergic patient significantly increased allergen-specific IgE production and enhanced Th2 – type cytokine mRNA expression compared to those of allergic patients instilled with ragweed alone (Diaz-Sanchez et al. 1997).

There was a strong suggestion made for the synergism between different indoor environmental exposures, as it was shown that the combination of exposure to environmental tobacco smoke, damp housing and high level of Fel d 1 ( $>8 \mu\text{g/g}$ ) resulted in a very high risk of sensitisation to cats (odds ratio of 42) (Lindfors et al., 1999). In a study from New Zealand, there was a modest association between asthma and the presence of cat in home both in early life and currently (7-9 years), compared to owning a cat in just one of these times or not owning a cat at all (Wickens et al., 2000). In a recent study of Biswas et al. (1999) a significant association between acute respiratory infections in children and parental smoking and use of solid fuels in household stoves was observed.

## **5.7 Summary of gaps in knowledge and recommendations**

Despite the critical role biological aerosol particles play in atmospheric systems, there are only hypotheses and speculations as to the mechanisms governing their behaviour in the air, and at the interface between air and the living or non-living environment.

The type of physico-chemical processes that are of importance include: dispersion, interaction with non-viable aerosols, transport, removal from the air, penetration through ventilation and filtration systems, or penetration to, and deposition in the respiratory tract. There is number of reasons for this very limited knowledge in this area, and the main ones of them can be summarized as follow:

- Significant experimental complexity of simultaneous characterisation of biological and physical nature of biological aerosol particles. In terms of the experimental design, the difficulty in measuring concentrations of biological and non-biological particles relate to different response time of instruments and differences in their flow rates which often results in a time difference between these two types of measurements. Both biological and non-biological particle concentrations change with time, and thus any time difference introduced between the two measurements, may affect the outcome when associations are investigated. Additionally, since the rate of change of these two concentrations may not be the same, more reliable results could be achieved if time series of biological and non-biological concentration could be compared.
- While, however, measurements can easily be conducted to obtain for example time series of particle concentrations, due to practical and financial limitations, the number of fungal samples that can be taken for analyses is limited (often to one sample per specific condition). Future experimental designs should target solutions that would enable measurements of these concentrations to take place as close in time as possible, and to attempt to measure time series of both biological and non-biological particles. The availability of the real time Ultraviolet Aerodynamic Particle Sizer instrument (UV APS), has contributed to many recent investigations on bioaerosols. However, the UVAPS needs to be developed further, in order to provide more reliable real time investigations of bioaerosols.
- Methods to accurately assess the concentration of biological aerosol particles in the submicrometer size do not exist (UVAPS can not detect lower than 0.5  $\mu\text{m}$ ). Classical methods used to sample these particles have never been characterised for their physical properties (sampling efficiency) in the submicrometer range. Therefore, much developmental work is needed before accurate and reliable information on the behaviour of airborne biological aerosol particles can be generated and such data applied to design of mathematical models and strategies to control and prevent spread through air.
- There is a lack of fundamental experimental data on the size distribution and concentration of biological particles in the size range between 0.5 and 20  $\mu\text{m}$ , and their relative content in the total number of particles in the atmosphere.

Contributions to biological aerosol particles from different indoor and outdoor sources are also unknown.

- There is only limited understanding of the mechanisms with which biological particles interact with the human respiratory tract, with some basic questions in relation to their mechanisms of interaction still to be answered. For example, the large size of pollen particles results in only very small probability of their penetration through the nasal area to the deeper parts of the respiratory tract, yet clear links have been established between the presence of pollen particles in the air and allergic reactions. There are only hypotheses available as to the mechanisms of this process. Another significant question is whether non-biological particles play a role as carriers of biological particles, as reported in a limited number of studies, thus affecting the resident time in the air, or transport mechanisms and delivery to the human respiratory tract.

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## **6. Aspects for the health care professionals with respect to health effects potentially due to biological agents in the indoor environment**

### **6.1 Symptoms, diagnosis, treatment and causal analysis**

Results of epidemiological investigations into the health effects of biological agents in the indoor environment usually either establish an association between exposure and response in a sample of individuals or establish that no association exists or are inconclusive. In all cases the results only apply for a group and do not establish or exclude an association for an individual. Patients typically present with a variety of symptoms, and the challenge for both the physician and the patient is to

- To have the symptoms cured
- To find out the causes for recurrent or persistent symptoms
  - Internal causes
  - External causes such as specific environmental exposures
  - Individual susceptibility

If external causes are to be supposed (i.e. after internal causes have been investigated and eliminated as potentially responsible), it is necessary to find out if the observed health effect is associated with a specific environmental exposure. In such cases, physicians may need to probe for additional information about where symptoms occur and what seems to trigger or exacerbate them. Patients often observe these associations before health care workers. For diseases that are rarer, physicians are more likely to think about where and when a potential exposure could have occurred. There are many possible environmental factors that could be contributing to the problem, not all of them building-related.

The physician's workup of a patient concerned about building-related exposures should begin with a careful, thorough history of non-specific (headaches, fatigue, dry skin) and specific symptoms (e.g., wheeze and cough, or sinusitis).

If the patient has no idea about potential environmental causes, the physician should:

Ask about the outdoor environment

- Ask about the indoor environment
- Ask about the occupational environment
- Ask about temporal and spatial association between environment and symptoms

- Suggest potential environmental causes and raise the patient's awareness to them
- Refer to the complexity of causal associations and to the potential multi-causality of symptoms
- Recommend professional assessment of environmental conditions and exposure measurements (who is to pay?)
- Recommend to reduce exposure if it is obvious or avoidance of exposure by evacuating the worker to other places or rooms

If the patient has already some suggestion of a potential association, the physician should

- Refer to the complexity of causal associations and to the potential multi-causality of symptoms
- Recommend professional assessment of environmental conditions (who is to pay?)
- Recommend to reduce exposure if it is obvious

Symptoms are often related to the route of exposure, which in most cases is inhalation, and to a lesser extent, skin contact. However, in the case of allergic reactions, ingestion of allergens in the nutrition cannot be ignored. In assessing the potential of environmental causation, an important consideration is whether symptoms have a temporal variation (see above). For example, do symptoms change over the course of a day, are there changes between the work days, the weekend, vacations or seasonal variations? Although patients may attribute health effects to a particular exposure, the clinician should always be alert and raise awareness for other possible contributing factors, including personal medical history (e.g. allergy history), co-existing physical and psychological illness, other potential environmental or occupational exposures, and family medical history.

Potential building related exposures and associated health effects are presented in Table 6.1. This table also specifies effects, markers of effect and markers of exposure, and diagnostic criteria to be applied for establishing a diagnosis.

Table 6.1 Building-related illnesses related to exposure to indoor biological agents

Organ System	Exposure	Internal Markers of Exposure/Effect	Clinical Effect	Diagnostic criteria	Comment
Upper Airways Nose, sinuses and Throat	Fungi, allergens, irritants	IgE, IgG, (IgA), IgM, other immunologic and inflammatory markers	Rhinitis, sinusitis, laryngitis, nose bleeding	History, exam, laboratories, imaging	Consider cost regional priorities & availabilities
Mouth, throat	Microbial irritants, allergens	Isolation of fungi / yeast on mucous membranes / gingiva	Glossodynia, Gingivitis, swollen mucous membranes	History, exam, biopsy	Exclude Sjögren's syndrome and related diseases
Lower Airway and Lung	Fungi, allergens, mites, bacterial and fungal by-products	IgE, IgG, (?IgA), IgG, other immunologic and inflammatory markers	Bronchitis, asthma, bronchiolitis, RADs, ABPA, allergic alveolitis (HP)  toxic alveolitis/ and pneumonitis	History, exam, laboratories, imaging, lung function tests, provocation tests  PEF-follow-up	Consider cost regional priorities & availabilities
Combined Upper and Lower Airways	Legionella, fungi, yeasts	Bronchoalveolar lavage, biopsy	Legionnaire's disease  Aspergillosis  Fungal rhino-sinusitis	History, exam, laboratories, imaging, culture,	Consider cost regional priorities & availabilities
Skin/Mucous Membrane	Microbial irritants, allergens	IgE, cell-mediated response	Urticaria, dermatitis (allergic vs. irritant, toxic), conjunctivitis	History, exam, laboratory, provocative skin testing, biopsy	Inspection of patient important  Photographs
Joints, muscles	Microbes	Immune complexes, IgG complement 3 & 4	Tender, swollen joints	X-ray, ultrascan	Normal differential diagnostics needed
Other Organs Central nervous system, immune system, liver, kidney, endocrine system	Fungi, bacteria, organic dusts, microbial by-products	?organ-specific markers  (Ochratoxin A elevation in serum)	Overlapping diagnoses, differential diagnoses (exclusion of unrelated conditions)	History, exam, ?laboratory or functional testing,  Biopsy	Testing should focus on clinical effects  Consider cost regional priorities & availabilities
Eyes, conjunctiva	Microbes, irritants, allergens	IgE, cell-mediated response	Allergic / toxic conjunctivitis	History, exam, provocation test, cell swab, biopsy	Differential diagnostics: Sjögren's syndr, other collagenosis, dry eye

## **6.2 Conclusions and recommendations**

The challenge is to correctly identify and reduce the exposure, and this requires a multi-disciplinary intervention involving the experienced and knowledgeable (with respect to potential environmental causes) physician, the patient, the family, health care professionals, environmental specialists, and in case of building-related symptoms and illnesses building owners or managers. Even when a specific causal agent is not necessarily be established, improvement of building environmental conditions or moving away from the building often results in significant clinical improvement of building-related diseases.

## **6.3 Future research needs**

There is a need for improvements in the physician's tools for diagnosis, treatment and prevention of building-related illnesses in individuals and groups due to microbial agents. Specific gaps exist in:

- (1) Awareness of the physician and of the patient of potential environmental causes
- (2) Understanding of the relationship between ambient concentration of biological agents and their uptake;
- (3) Markers of exposure and early biological markers of disease, particularly for agents that act through non-immunologic mechanisms;
- (4) Understanding of immune mediated and non-immune mediated health effects, including irritation and toxic effects; the basic pathophysiology and clinical effects of biological agents, including uptake, susceptibility factors, clinical outcomes, diagnostic tests, and treatment modalities.
- (5) Understanding of interactions between biological agents, indoor and outdoor environmental exposures, other environmental condition and personal health history
- (6) Clinical effects in different populations, including susceptible groups or individuals with immune compromise or other disorders, and the cumulative or long-term effects in humans.

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