UMEÅ UNIVERSITY MEDICAL DISSERTATIONS

New Series No.1052 – ISSN – 0346 – 6612 – ISBN 91 – 7264 –148 – 7

From the Department of Public Health and Clinical Medicine,
Occupational Medicine, Umeå, Sweden

Volatile organic compounds from microorganisms

-identification and health effects

Anna-Sara Claeson

Umeå 2006

Arbetslivsinstitutet
National Institute for Working Life

UMEÅ UNIVERSITET

ISSN 0346 – 6612 – 1003
ISBN 91 – 7264 – 148 – 7

Printed by Arbetslivsinstitutet, Bo Sellman, Umeå, 2006

”What we get from this adventure is just sheer joy.
And joy is, after all, the end of life.
We do not live to eat and make money.
We eat and make money to be able to enjoy life.
That is what life means and what life is for.”

-George Leigh Mallory

Abstract

Damp building materials are subjected to degradation processes due to moisture and also microbial growth, with both of these giving rise to emissions of volatile organic compounds (VOCs) that may contribute to indoor air health problems. The overall aim of this thesis was to investigate emissions of reactive and non-reactive VOCs from damp building materials and from the microorganisms growing on them, and also to investigate the possible health impact of these compounds.

Three studies were carried out in order to study emissions of VOCs. The first investigated emissions from a mixture of five fungi (*Aspergillus versicolor*, *Fusarium culmorum*, *Penicillium chrysogenum*, *Ulocladium botrytis* and *Wallemia sebi*) and the second emissions from the bacterium *Streptomyces albidoflavus*. In both studies the microorganisms were cultivated on three different building materials (pine wood, particle board and gypsum board) and one synthetic media, MEA and TGEA respectively. The bacterium was also cultivated on sand. Air samples from the cultures were collected on six different adsorbents and chemosorbents to sample a wide range of compounds such as VOCs, aldehydes, amines and light-weight organic acids. The samples were analyzed with gas chromatography, high-pressure liquid chromatography and ion chromatography. Mass spectrometry was used for identification of the compounds. Alcohols and ketones were the predominant compound groups identified. The bacterial culture growing on TGEA emitted ammonia, methylamine, diethylamine and ethylamine. The third study dealt with secondary emissions collected from buildings with moisture and mould problems. Samples were taken when the materials were dry and also after they had been wet for a week. Most alcohols and ketones could be identified from the wet materials. Trimethylamine and triethylamine, were identified from sand contaminated by *Bacillus*. One study looked at the development of a method for analysis of primary and secondary amines with LC-MS/MS. A three-step process was developed, with the first step screening the samples for NIT derivatives with selected reaction monitoring, SRM. In the second step a precursor ion scan gave the $[M+H]^+$ ion, and the last step involved fragmentation with a product ion scan. It was possible to separate and identify all the investigated amines, which showed that the method was both specific and selective and therefore well suited for the analysis of amines in complex environments. The last study comprised two exposure studies. In study 1 each participant took part in two exposure conditions, one with air from mouldy building materials and one with blank air for a 60 minute period. In study 2 each participant was exposed four times (for a period of 10 min) at random to air from mouldy building materials and blank air, with and without nose-clip. The participants rated air quality and symptoms before, during and after each exposure. Exposure to moderate VOC levels resulted in reports of perceived poor air quality, but no such results were received when exposing the participants to low VOC levels.

Summary in Swedish

Nedbrytning av byggnadsmaterial till följd av fukt och mikrobiell växt ger upphov till emissioner av flyktiga organiska ämnen. Dessa kan vara en orsak till hälsoproblem relaterade till inomhusluft. Det övergripande syftet med denna avhandling var att identifiera reaktiva och icke-reaktiva flyktiga organiska ämnen emitterade från fuktigt byggnadsmaterial med växt av mikroorganismer och dessutom att undersöka möjliga hälsoeffekter av dessa.

Tre studier genomfördes för att studera emissioner av flyktiga organiska ämnen. I den första undersöktes emissioner från en blandning av fem olika mögelsvampar (*Aspergillus versicolor*, *Fusarium culmorum*, *Penicillium chrysogenum*, *Ulocladium botrytis* och *Wallemia sebi*). I den andra undersöktes emissioner från en bakterie, *Streptomyces albidoflavus*. I båda studierna odlades mikroorganismerna på tre olika byggnadsmaterial (furu, spånskiva och gips) och ett syntetiskt medium, MEA respektive TGEA. Bakterierna odlades även på sand. Luftprover från odlingarna togs på sex olika adsorbenter och kemosorbenter för att provta flyktiga organiska ämnen, aldehyder, aminer och lågmolekylära organiska syror. Proverna analyserades med gaskromatografi, vätskekromatografi och jonkromatografi. Masspektrometri användes för att identifiera föreningarna. Alkoholer och ketoner var de vanligast förekommande ämnesgrupperna som identifierades. Vid växt på TGEA emitterade *S. albidoflavus* ammoniak, metylamin, dietylamin och etylamin. Den tredje studien rörde sekundära emissioner från byggnadsmaterial hämtade från byggnader med fukt- och mögelproblem. Prover togs då materialen var torra och även efter att ha stått fuktigt i en vecka. I denna studie identifierades också mest alkoholer och ketoner från de blöta materialen. Trimetylamin och trietylamin emitterades från sand som var kontaminerad med *Bacillus*. I en studie utvecklades en metod för analys av primära och sekundära aminer med LC-MS/MS. En metod i tre steg utvecklades, i det första steget analyserades proverna med avseende på derivat av NIT-aminer med SRM (Selected Reaction Monitoring). I det andra gav ett föräldrajonscan $[M+H]^+$-jonen. I det sista steget gav ett produktscan fragment som kunde användas för identifiering. Det var möjligt att separera och identifiera alla de i studien undersökta aminerna vilket visade att metoden var både specifik och selektiv och därför väl lämpad för analys av aminer i komplexa miljöer. Den sista studien bestod av två exponeringsstudier. I studie 1 deltog varje försöksperson i två försök (á 60 minuter vardera), ett med låga halter av luft från mögligt byggnadsmaterial och ett med ren luft. Studie 2 bestod av fyra 10 minuters exponeringar, slumpvis för medelhöga halter av luft från mögligt byggnadsmaterial och ren luft och dessutom med och utan näsklämma. Försökspersonerna bedömde luftkvaliteten och symptom före, under och efter exponering. Vid exponering för medelhöga nivåer av flyktiga organiska ämnen emitterade från mögel och fuktigt byggnadsmaterial rapporterade försökspersonerna signifikant sämre luftkvalitet. Exponering för låga halter av dessa ämnen gav inga sådana reaktioner.

Original papers

This thesis is based on the following papers:

1. Anna-Sara Claeson, Jan-Olof Levin, Göran Blomquist and Anna-Lena Sunesson
Volatile metabolites from microorganisms grown on humid building materials and synthetic media.
Journal of Environmental Monitoring, 2002, 4, 667-672

2. Anna-Sara Claeson and Anna-Lena Sunesson
Identification using versatile sampling and analytical methods of volatile compounds from *Streptomyces albidoflavus* grown on four humid building materials and one synthetic medium.
Indoor Air, 2005, 15 (suppl.9), 1-8

3. Anna-Sara Claeson, Maria Sandström and Anna-Lena Sunesson
Volatile organic compounds (VOCs) emitted from building materials affected by microorganisms.
Manuscript submitted

4. Anna-Sara Claeson, Anders Östin and Anna-Lena Sunesson
Development of a LC-MS/MS method for the analysis of volatile primary and secondary amines as NIT (naphthylisothiocyanate) derivatives.
Analytical and Bioanalytical Chemistry, 2004, 378, 932-939

5. Anna-Sara Claeson, Steven Nordin and Anna-Lena Sunesson
Effects on perceived air quality and symptoms of exposure to microbially produced metabolites and compounds emitted from damp building materials.
Manuscript submitted

This thesis was financed by grant from the Centre for Environmental Research (CMF)

CONTENTS

Abbreviations

ANOVA	Analysis of variance
APCI	Atmospheric pressure chemical ionization
BUT(s)	Tear film break-up time (self-reported)
CBS	Centraalbureau voor schimmelcultures
CSS	Chemical sensitivity scale
DG-18	Dichloran glycerol agar
DNPH	Dinitrophenylhydrazine
ESI	Electrospray ionization
eV	Electron volt
FID	Flame ionization detection
GC	Gas chromatography
HPLC	High performance liquid chromatography
MANOVA	Multivariate analysis of variance
MDF	Medium density board
MEA	Malt extract agar
MS	Mass spectrometry
MVOC	Microbial volatile organic compound
NaOH	Natrium hydroxide
NIOSH	National Institute for Occupational Safety and Health
NIT	Naphthylisothiocyanate
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbons
PCA	Principal component analysis
PLS	Partial least squares
PVC	Poly vinyl chloride
Q1,Q2,Q3	Quadrupole 1, 2, 3
RH	Relative humidity
SBS	Sick building syndrome
SIM	Single ion monitoring
SRM	Selected reaction monitoring
TGEA	Tryptone glucose extract agar
TVOC	Total volatile organic compounds
UPSC	Uppsala University culture collection of fungi
VOC	Volatile organic compound
WHO	World Health Organization
WP	Wood preservatives

Aims of the study

The general hypothesis of this thesis is that water damage and growth of microorganisms on building materials generate emissions of both reactive and non-reactive volatile organic compounds and that these emissions are able to cause health problems similar to those found in sick buildings, such as irritation of the eyes, nose and throat, as well as to give rise to complaints about indoor air quality.

This main hypothesis generated the following specific objectives:

-to identify volatile organic compounds produced by a mixture of moulds and also of one strain of bacterium which grows on building materials and laboratory media.

-to identify reactive compounds such as amines, aldehydes and organic acids from a mixture of moulds and also of one strain of bacterium growing on building materials and laboratory media.

-to develop a specific and sensitive method for screening unknown samples for trace amounts of primary and secondary amines and to be able to evaluate the structures of those amines.

-to identify volatile organic compounds from materials affected by microbial growth taken from water damaged buildings.

-to assess perceived air quality, health effects and cognitive performance of low to moderate levels of volatile organic compounds emitted from damp building materials and a mixture of moulds growing on those materials.

Introduction

People spend most of their time indoors, and as older and naturally ventilated buildings have been replaced by more energy efficient buildings, the number of people experiencing negative health effects from their indoor environment has been increasing since the 1970s (93). Non-specific problems found in buildings are often called “sick building syndrome” (SBS) or “building related health problems”. The symptoms have been defined by the World Health Organization (WHO) and include mucous membrane irritation (eye, nose and throat irritation), neurotoxic effects (headaches, fatigue and irritability), asthma and asthma-like symptoms (chest tightness and wheezing) and skin symptoms (dryness and irritation) (143). A typical feature of SBS is that the symptoms disappear when the person leaves the building (114), although more recent studies indicate that some symptoms such as nasal hyper reactivity may be chronic (31).

No single environmental factor or group of factors has been established as the cause for SBS although there are many suggestions. These factors are, for example, air contaminants such as volatile organic compounds (VOCs), bacteria and fungi, dust, dampness and poor ventilation. Personal factors such as female gender, stress and job satisfaction have also been suggested (19, 93). Most probably the health problems related to indoor air are of multifactorial origin consisting of a number of factors acting together (93). This thesis has focused on VOCs and mould and their involvement in the non-specific health problems found in sick buildings.

Today there is general agreement on a relation between dampness in buildings and health effects such as respiratory symptoms, coughing, wheezing, asthma and also general symptoms such as tiredness and headache (14, 15, 83). The causative agents have not yet been discovered, although organic chemicals, mites and microbial agents have been suggested. Damp or wet building materials are subjected to degradation processes with the emission of chemical compounds as a result, and the water content of the materials also supports microbial growth. Fungal growth has been considered as one of the most likely causes of health problems in buildings, but reported indoor air spore levels have shown to correlate poorly with reported symptoms (15, 54, 85). The microbial growth is often hidden behind carpets or ceilings which can be one explanation for the lack of relation between measured spore levels in indoor air and health symptoms. However, microorganisms emit volatile organic compounds known as MVOCs (microbial volatile organic compounds) during growth and the MVOCs are able to permeate through building structures, in so doing adding to the total mixture of VOCs to which those humans staying indoors are exposed. Nevertheless, since no consistent relationship between health problems and MVOCs has been found the interference of MVOCs in the SBS complex of problem is still questionable.

Microorganisms

Microorganisms are present everywhere in both indoor and outdoor environments. The group includes bacteria, protozoa, algae, viruses and fungi. This thesis focuses on fungi and bacteria commonly found in an indoor environment.

Bacteria and fungi are heterotrophic, which means that they utilize organic molecules as sources of both carbon and energy, such as simple sugars or amino acids. In indoor environments moulds are able to grow rapidly on almost any surface because their general temperature, nutrient and pH requirements are usually fulfilled, therefore the primary limiting factor is the availability of moisture. Different species prefer different growth conditions which means that there are always some fungi or bacteria able to grow in any humid indoor conditions. Species such as *Penicillium*, *Eurotium* and *Aspergillus* begin to grow when the relative humidity (RH) exceeds 75-80% and are called primary colonizers. Secondary colonizers (e.g. *Cladosporium*) appear at a RH of 80-90% and tertiary colonizers at RH above 90%. Examples of tertiary colonizers are species of *Fusarium*, *Stachybotrys* and also actinomycetes (4, 41, 90). A number of fungal species are commonly found indoors and *Penicillium* and *Aspergillus* are two of the most abundant genuses. Examples of other moulds also commonly found are *Alternaria*, *Cladosporium*, *Mucor* and *Ulocladium* (107).

Mould growth on different materials is usually accompanied by bacterial growth although bacteria are studied far less than fungi. The mesophilic actinomycete *Streptomyces* is commonly found in the indoor air of buildings affected by microbial growth (84, 112). This bacterium belongs to the ascomycetes which constitute a group of bacteria growing in the form of branching, filamentous cells that either form spores or reproduce by fragmentation of hyphae. This method of growing and reproducing resembles that of fungi. Actinomycetes are important for the degeneration of many materials including rubber, plastics, and other materials that are difficult to break down (69). In some Finnish studies the species *Streptomyces* has been identified in up to 70% of the investigated mouldy buildings and has therefore been proposed to be an indicator of water-damaged buildings (55, 84, 112).

Fungi and bacteria are capable of producing a wide variety of biochemical compounds. These products are volatile compounds formed via primary and secondary metabolism (e.g. MVOCs) and more complex substances (e.g. toxins) that are usually not volatile. Primary metabolism is shared by most living systems and is required for producing compounds essential to the organism such as materials for growth, development and reproduction.

Secondary metabolism has a lower priority and the process starts after active growth has ceased, although the distinction between primary and secondary

metabolism is not absolute (6, 119). Secondary metabolites have diverse chemical structures and are usually distinct products of particular groups of organisms and sometimes even strains (119). The function of secondary metabolites in the organism is not clear, but the process seems to have many different purposes owing to their remarkable variety and many different chemical structures. Most of these metabolites are excreted into their surroundings by the organism, and it has therefore been proposed that they could be waste material or a means of detoxification of the organism. The best known secondary metabolites are antibiotics, toxins and dyes (6). MVOCs can also constitute an important regulatory factor in determining the interrelationship between organisms in microbial ecosystems (53).

Microorganisms and human health

The health effects of fungi and bacteria may be caused by the cells themselves, bacterial endotoxins, bacterial exotoxins, fungal mycotoxins, fungal cell-wall components or microbially produced volatile organic compounds (MVOCs). The ways in which fungi and bacteria may affect human health can be categorized into three groups: allergic reactions, infections and toxic responses (44, 104). In addition, exposure to MVOCs is believed to be responsible for a number of non-specific symptoms such as eye, nose and throat irritation and fatigue which are often found in connection with building related health problems (97, 104).

Volatile organic compounds

There are several definitions of VOCs because volatility depends on many different parametrics such as boiling point, vapour pressure, molecular weight, and size. WHO has classified a number of indoor air pollutants where the volatility of the compounds depends on the boiling point. According to this a compound is volatile up to a boiling point of 300 ºC (142). Researchers investigating indoor air quality usually consider all organic vapour-phase compounds measured by their sampling and analysis methods to be VOCs.

VOCs in indoor air

Over 350 VOCs have been identified in indoor air, and it has been shown that indoor concentrations of many pollutants are often higher than those typically found outdoors (57). Building products are usually the major contributors to the pollution of indoor air, but VOCs are generated from a wide variety of other sources including furniture, solvents, human activities, dampness, microorganisms, and infiltration of outdoor air etc (57).

Because of the many sources (and the use of different sampling and analysis methods) indoor concentrations of VOCs vary considerably, but "normal" (both problem and non-problem buildings) TVOC (total count of VOC) concentrations are often less than 0.5 mg/m^3 , while the concentration of single compounds rarely exceeds 50 µg/m^3, and is most often even below 5 µg/m^3 (17). Although many studies have tried to find associations between high levels of TVOC and health problems, none has yet been identified (7, 82, 114). TVOC concentrations are generally greater in new buildings, and the highest emission rates from new building construction occur during the first six months and decay within a year (17). These are called primary emissions and consist mainly of non-bound VOCs from accelerators, additives, antioxidants, monomers, plasticizers, solvents and unreacted raw material (63). Factors including moisture, alkali, high temperature, UV-light, maintenance etc. affect materials and may result in secondary emissions because of their decomposition, hydrolysis and oxidation. This also contributes to indoor air pollution, but at a much lower emission rate. However, secondary emissions may increase over time and may also last for long periods or even continue throughout the life of the building product (63, 138, 140). Former studies investigating emissions from materials have focused on primary emissions and have resulted in extended use of low-emitting materials, but today secondary emissions are regarded as being more relevant to health. This depends on the time aspect of emission decay and also the fact that primary emissions consist of stable volatile organic compounds such as for example toluene, decane, limonene and dichloromethane (18, 57, 140). The secondary emissions appear to be more reactive, such as aldehydes, fatty acids and alcohols (138). A list of VOCs (from both primary and secondary emissions) emitted from building materials frequently found in indoor air is presented in Table 1. A more complete list can be found in Brown *et al* (1999) (17).

Table 1. Examples of emissions from common building materials.

Compound Group	Source	Examples of common compounds
Hydrocarbons	Carpet, paint, plastics, PVC*, glue, WP*, sealant	**Decane, dodecane, toluene, styrene, ethylbenzene, hexane, trimethylbenzenes**
Terpenes	Wood, linoleum, glue, particle board, MDF*	**α-Pinene, limonene**
Aldehydes	Wood, linoleum, paint, carpet, particle board, plywood, fibreboards, PVC*, MDF*	**Formaldehyde, nonanal, decanal, hexanal**
Alcohols	Linoleum, paint, plastics, PVC*, glue, floor varnish	**2-Ethyl-1-hexanol, 2-butoxyethoxyethanol, phenol, 1,2-propandiol, butoxyethanol, 1-butanol**
Ketones	Linoleum, paint, plastics, glue	**Acetone, butanone,**
Ethers and esters	**Paint, plastics, glue, PVC*,**	**Urethane, ethyl acetate, glycolether, glycolether ester**

*PVC=Polyvinyl chloride floor covering, WP=Wood Preservatives, MDF=medium density board (5, 17, 57, 63, 130, 138, 147)

VOCs from microbial sources

Microorganisms are able to produce a number of different MVOCs including alcohols, esters, hydrocarbons, terpenes, ketones, sulfur containing compounds and carboxylic acids (Table 2).

Table 2. Examples of compounds and compound groups emitted by microorganisms (Modified from Wilkins *et al* (2000) (134)).

Compound Group	Subgroups	Examples of common compounds
Hydrocarbons	Alkanes, alkenes, dienes, trienes	Octane, 1-octene
Terpenes	Hemi- (C_5 hydrocarbons, alcohols, ketones) Mono- (C_{10} hydrocarbons, alcohols, ethers, ketones) Sesqui- (C_{15}, C_{11}, C_{12} hydrocarbons, alcohols, ketones) Di- (C_{20} hydrocarbons)	Isoprene, limonene, geosmin
Alcohols	Saturated, unsaturated, branched	1-Octen-3-ol, 2-methyl-2-propanol
Carboxylic acids and esters	Saturated, unsaturated, branched, diols, ketols	Acetic acid, ethyl acetate
Ketones	Methyl(2-)ketones (saturated, branched) Ethyl(3-)ketones (saturated, unsaturated) Cyclic-	2-Butanone, 3-methyl-2-pentanone, 2-hexanone, 3-hexanone, cyclopentanone
Sulfur derivatives	Thiols, mono, di, trisulfides, S-methyl thioesters, thioethers	Dimethyldisulfide
Aromatic compounds	Hydrocarbons, alcohols, ethers, ketones, phenols	Styrene
Nitrogen containing heterocyclics	Alkoxypyrazines, indoles, pyrroles, alkylfurans, γ- and δ-lactones	3-Methylfuran

(11, 13, 37, 39, 40, 67, 107, 108, 134).

It is generally unclear if the compounds found in relation to microbial growth really are metabolic products or if microbial growth and/or moisture promote

emission of compounds from a substrate (97). However, some metabolic pathways have been identified for a number of common MVOCs and are described below. Hydrocarbons are often found in relation to fungal growth and are thought to be produced via oxidative breakdown of fatty acids (133). 1-Octen-3-ol serves as a precursor in the formation of 1,3-octadiene and styrene is derived from aromatic amino acids or from decarboxylation and oxygenation of monoterpenes. Oxygenated aromatic compounds are also formed in this way (133). Terpenes and terpene derivatives are produced via the mevalonic acid pathway and production of higher levels of terpenes is triggered by lack of nutrients (9).

Alcohols such as 1-octen-3-ol, 3-methyl-1-butanol and 2-methyl-1-propanol have been found to be emitted in the greatest quantities when moulds grow on media containing carbohydrates (9). Other commonly found alcohols are 1-pentanol, 2-heptanol, 2-nonanol, 1-hexanol, 2-methyl-1-butanol, 2-ethyl-1-hexanol and 3-octanol. Eight-carbon alcohols and ketones are produced by fatty acid degradation from linoleic acid and linolenic acid (9, 134). Many alcohols are also formed via the Erlich pathway by decarboxylation and reduction of amino acids (8). For example 2-methyl-1-propanol is formed from valine and 3-methyl-1-butanol from leucine (8). C2-C4 alcohols and ketones (ethanol and acetone) are products of fundamental biochemical processes such as glycolysis and the Krebs cycle for nearly all organisms (132). The precursors for methylketones, such as 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone are fatty acids (9, 34, 61).

Esters are formed from acids and alcohols (60, 72). Ethyl acetate is commonly found to be emitted from microbial growth on different materials. Acetates have been found in relation to the growth of *Penicillium* (71) and seem to be involved in the production of Acetyl CoA in the Krebs cycle for use in the production of citric acid (8). Sulfur-containing compounds are produced from degradation of amino acids containing sulfur such as methionine and cysteine (4).

There are a large number of metabolic pathways, but only a few have been described here together with common products. Many other metabolites and metabolic pathways exist, but a detailed description of these was not the main objective of this thesis.

Metabolites from bacteria

Actinomycetes and especially the genus *Streptomyces* are well-known producers of secondary metabolites, for example, antibiotics. Despite this, few studies on the production of volatile organic compounds by these organisms have been carried out, and identification of the strains used is often not provided (4, 100).

Streptomyces are known for their capacity to produce compounds with strong earthy-like smells and low odour thresholds, such as geosmin (trans-1,10-dimethyl-trans-9-decalol) and 2-methylisoborneol (48, 109). Sulfur-containing compounds such as sulfides and sulfur esters were produced in large amounts by *Streptomyces albidoflavus* during growth on TGEA (tryptone glucose extract agar) (109). Alcohols, esters, ketones and terpenes have also been identified as metabolites produced by different species of *Streptomyces* and many of these compounds have also been identified in relation to fungal growth (100, 109, 132).

Bacterial cultures have been shown to produce patterns of metabolites that differ from those of fungal cultures. Actinomycetes grown on agar-based media produce more branched ketones than do fungal cultures (e.g. 3-methyl-2-butanone, 4-methyl-2-pentanone), and cyclopentanone has been proposed to be a unique compound emitted from bacteria (132). The common fungal metabolites consisting of eight-carbon compounds (e.g. 3-octanone, 1-octen-3-ol, 3-octanol, 1-octene, 1,3-octadiene) are absent in cultures of *Streptomyces* (62, 100, 132). Sesquiterpenes seem to be more common products of cultures consisting of bacteria than of those consisting of mould, and bacteria also seem to produce a wider variety of these compounds (62, 101, 109).

Factors affecting emission of MVOCs

The species and the substrate composition are the most important factors for the production of MVOCs (109). Furthermore, moisture and temperature influence the emission of MVOCs, and a prolonged growth phase due to a lower temperature may influence the production of certain compounds and extend the time for maximum production (109). Other environmental factors such as pH of the substrate, light and levels of CO_2 or O_2 probably also influence the MVOC pattern. The substrate composition has a great influence on both qualitative and quantitative production of volatile metabolites. In general, nutrient-rich media such as laboratory substrates promote both larger quantities and other types of metabolites than do nutrient-poor media such as building materials (134). The emissions of VOCs change with the growth phase. These changes are influenced by the changes in the substrate as microorganisms grow and successively use different nutrients (9, 94).

MVOCs emitted from microbial growth on building materials

There are numerous studies concerning metabolite production resulting from microorganisms growing on laboratory media (10, 94, 100, 107). The information from such studies may have limited value other than for the prediction of potential types of MVOCs because the building materials often contain lower amounts and

other types of available nutrients. The emissions from *Streptomyces albidoflavus* grown on TGEA consisted of up to 80% of sulfur-containing compounds, whereas when *S. albidoflavus* was grown on gypsum board no sulfur compounds were emitted at all (109). It is therefore of great importance to study metabolite production resulting from cultures growing on different building materials in order to provide an indication of which metabolites may be expected from buildings affected by microbial growth. However, studies using the same building materials may not give the same result because potential nutrients available in building materials vary greatly, for example, the nutrient content in wood. Contamination of building materials by soil or dust may also add enough nutrients to support growth and emission of MVOCs (9). Reported MVOCs detected from building materials contaminated with known species of fungi or bacteria are shown in Appendix I.

Why measure MVOCs?

Analysis of VOCs produced by microorganisms has been used as an indicator of fungal growth in stored cereals and food when other signs of microbial contamination could not be detected (13, 99). The use of MVOCs to identify hidden microbial growth has also been used in buildings. A group of selected MVOCs, such as 3-methyl-1-butanol, 1-octen-3-ol, 2-heptanone and 3-methylfuran among others, is considered to be only of microbial origin and these have therefore been used as marker compounds (33, 105, 129, 130). However, there is currently no consistent evidence that levels of MVOCs are higher in buildings with microbial growth than in those without.

Most of the MVOCs are found in very low concentrations and also to have other sources in indoor environments, which makes them unsuitable as marker compounds (43, 98) Emissions from *Aspergillus* and *Stachybotrys* grown on gypsum board were investigated in order to find unique MVOCs (substances that have no other sources than fungi and bacteria) (42). 3-Methy-1-butanol, 2-methyl-1-propanol, terpeniol and 2-heptanone were suggested to be unique in the study involving *Aspergillus,* while in the study involving *Stachybotrys* only one compound was suggested to be unique, 1-butanol, which has many other sources in other contexts. Before MVOC analysis can be used as a reliable indicator of mould growth in buildings, if at all possible, it is necessary to identify a larger number of metabolites and in particular more specific ones from cultures grown on materials commonly found in buildings today.

MVOCs have also been seen as helpful in the identification and classification of closely related microorganisms or even different microbial species and strains on the basis of their MVOC profile (40, 133). Media for the production of characteristic MVOC patterns have been developed, and a comparison of the MVOC pattern of three species investigated has shown that it may be possible to

identify the mould species growing on building material (134). Larsen and Frisvad (1999) investigated the MVOC pattern of 47 different taxa of *Penicillium* and discovered that each of these produced a pattern of MVOC unique enough to be used for classification on the species level (70, 71). In another study cultivation undertaken on five different building materials did not show patterns unique enough for differentiation between genuses (98).

Reactive compounds

Indoor air consists of a mixture of hundreds of different compounds, and reactions occur both in gas phase and on surfaces. This creates new compounds whereby the air may contain a new composition of compounds with qualities other than those in the original mixture. Some of the formed compounds are more stable than their precursors, but some react further to produce yet more different compounds. Most studies investigating indoor air have focused on compounds that can be sampled on commonly used adsorbents which favour identification of non-polar compounds such as alcohols (91, 139). Most likely the chemical mixture of indoor air also includes compounds that are difficult to sample and analyse using traditional techniques (139).

Reactions among commonly occurring indoor pollutants have a great impact on the composition of the compounds found in indoor air (126, 128). Reactions likely to occur and generate compounds capable of affecting health are those that take place between ozone and unsaturated compounds (e.g. terpenes). Terpenes are found everywhere in indoor air, and the most important sources for ozone besides outdoor-indoor transport are photocopiers, laser printers and electrostatic precipitators. This reaction produces hydroxyl radicals able to react with both saturated and unsaturated organic compounds in order to produce aldehydes and ketones, carboxylic acids and other radicals; some of these products may be more irritating than their precursors (23, 125). Other important reactions taking place in indoor air are those between NO_x and ozone which produce the nitrate radical. This radical reacts fast with certain unsaturated organic compounds and polycyclic hydrocarbons (PAH) (126), producing, for example, carbonyl nitrates such as 1-nitroxy-2-propanone (23).

There are certain conditions which promote indoor reactive chemistry. For example, oxidations increase with rising levels of ozone and terpenes in indoor air. Hydrolysis takes on greater importance in environments containing high levels of moisture. Low ventilation rates promote reactions between compounds in the gas phase, and dirty surfaces will provide more surface reactions. High indoor temperatures increase both the reaction rates for most reactions and the emission rate (126).

In this thesis I have focused on a few reactive compound groups capable of being emitted in relation to microbial growth rather than on the reactive chemistry itself. A compound can be either chemically reactive such as alkenes capable of reacting with, for example, ozone or NO_2 or it can be biologically reactive. A biologically reactive compound is a compound which forms chemical bonds to receptor sites in the mucous membranes, for example, formaldehyde and acrolein (1, 141). In this thesis a reactive compound is defined as biologically reactive, and focus is on three such compound groups: amines, organic acids and aldehydes, as described below.

Amines

Low concentrations of reactive metabolites, such as amines, have been suggested as one possible explanation for indoor air health problems such as sick building syndrome (SBS) (91, 139). Low molecular, volatile amines are often used in the manufacturing of industrial chemicals such as rubbers, plastics and other polymers, dyestuffs and corrosion inhibitors (58, 146). Amines are also formed and emitted as by-products in the metabolism of microorganisms, plants and animals (58, 94, 144). In metabolism, volatile amines are produced from the decarboxylation of neutral amino acids. But decarboxylation is not the only biosynthetic route; an amination of carbonyl compounds can also take place with the formation of ethylamine, diethylamine and trimethylamine. Trimethylamine has also been found to be produced by bacteria from choline (144).

Many amines have a very unpleasant smell; the detection thresholds range from 1 mg/m^3 for ammonia to 20 $\mu g/m^3$ for propylamine (74). They are also very potent irritants to skin, eyes, mucous membranes and the respiratory tract (45). Some amines are even regarded as toxic or, as dimethylamine, capable of reacting with NO_x and OH radicals to form carcinogenic nitrosamines (26, 146). Nitrosamines can also be formed through chemical reactions with nitrite or nitrate. Through this reaction primary amines form short-lived species which react to form mainly alcohols. Secondary amines form stable N-nitrosamines and tertiary amines seem to produce a range of labile N-nitrosoproducts (58). The toxicological potential of the amines and their occurrence in many diverse environments makes it important to monitor the concentrations both in ambient, workplace and indoor air.

Organic acids

Fatty acids are both substrates and products in the metabolism of MVOCs and it has been shown that octanoic acid can be produced from linoleic and linolenic acid. Acids such as 2-methylpropanoic acid, butanoic acid, 2-methylbutanoic acid, pentanoic acid and hexanoic acid can be formed through lipolysis of triglycerids or amino acids (9). However, from a health perspective potentially more irritating VOCs are also more interesting such as carboxylic acids of low molecular weight,

for instance, formic acid, acetic acid, propionic acid and butyric acid. These low molecular weight organic acids are widely used in commercial organic synthesis, as additives in the food industry and in the manufacturing of plastics and rubbers (49), and they are known to be hazardous to the skin and to cause eye irritation. Some of these are also known to be products of microbial metabolism. Acetic acid, for example, is the most abundant fatty acid excreted by yeasts (8). Isobutyric acid and isovaleric acid have been identified from *Brochothrix thermosphacta* when growing on a medium containing glucose, ribose or glycerol, and *Shewanella putrefarans* has been found to produce formic acid at extremely high levels (115). In some organisms organic acids have been found to be produced by bacteria and to have an antifungal effect (77).

Aldehydes

Aldehydes are of great concern because of their impact on health. For example, formaldehyde is well known for its irritative effect and is classified as carcinogenic (79, 148). Other saturated and unsaturated aldehydes are also suspected to be irritative to the eyes and mucous membranes and they are also highly odorous; such examples are acrolein, glutaraldehyde, acetaldehyde and furfural.

Carbonyls such as aldehydes are present everywhere in indoor and outdoor environments. The primary sources are exhaust gases from motor vehicles and industry. In indoor air the primary sources come from gases from building and furnishing materials and emissions from certain consumer products. Aldehydes are also formed through chemical reactions with ozone (79, 127, 149).

Aldehydes have been found to be produced by microorganisms. Acetaldehyde is formed through oxidative carboxylation of acetolactate, a by-product of the synthesis of leucine in yeasts (8). Unsaturated fatty acids may be transformed to volatile aldehydes such as hexanal, heptanal and nonanal, and the precursors of 2-decenal, 2-undecenal and 2-heptenal are linoleic- and linolenic acid (65). In some studies investigating the emission of VOCs during microbial growth the concentration of aldehydes decreased as though the microorganisms had consumed the aldehydes (67, 111).

Chemestesis and olfaction

Airborne chemicals are detected by two separate chemosensory systems in humans. In one the odours are detected by the olfactory receptors in the olfactory mucosa in the upper back portion of the nasal cavity and are mediated by the olfactory nerve. The other constitutes the trigeminal system which is there to sense irritation, a system also referred to as chemestesis. Chemestetic sensations are detected by nociceptors in the ocular, nasal and oral mucosae and mediated by the trigeminal

nerve. These two systems, the olfactory and chemestetic, interact to enable us to experience the chemicals in our environment.

There are several differences between the systems. Perceived irritation has a longer reaction time and may persist for a longer time than perceived odour (24, 141). Perceived irritation is more resistant to sensory adaptation (3, 92). This was seen in a study where people were exposed to low concentrations of a mixture of 22 different compounds that normally occur in indoor air. During the first 30 minutes of exposure there was an acute effect that showed no signs of adaptation (81). In another study an increase in irritation over time was observed (52).

The detection threshold has also been shown to be lower for odours than for chemestetic sensations (24). The low levels found in indoor air will therefore most probably be detected in the first instance by olfaction, after which an increase in concentration to a certain level will start to affect the trigeminal nerve endings (25). Generally, studies investigating odour and sensory potency thresholds have shown that for many compound groups such as alcohols, ketones, carboxylic acids, aldehydes and acetates the sensory potency increased with the lengthening of the carbon chain (20, 22). However, mixtures of VOCs below the irritation level have been shown to have complete additivity concerning the irritative effect and the interactions may even be hyperadditive (21, 23). The odour and irritation thresholds for a small number of MVOCs are listed in Table 3.

Table 3. Odour and irritation thresholds for some commonly reported MVOCs.

(M)VOC	Odour thresholds (mg/m^3)	Irritation thresholds (mg/m^3)
1-Octen-3-ol	0.005-100[1]	-
2-Methyl-1-propanol	0.003[2] 0.36-225[3]	**300[3]**
3-Methyl-1-butanol	36-126[3]	**360[3]**
3-Octanone	31[3]	**260[3]**
Geosmin	**0.01-0.36[1]** **0.007[2]**	-

[1] (102)
[2] (59)
[3] (95)

Airborne chemicals are believed to activate the receptors in the trigeminal system by either physical adsorption or chemical reaction (1, 29). Non-reactive chemicals such as saturated alkanes, alkylbenzenes, alcohols, ketones and ethers interact via

physical adsorption including non-covalent bonds such as electrostatic interactions, hydrogen bonds, van der waals attractions and hydrophobic interactions. The sensory irritation potency of these compounds increases with heightened lipofilicity (1). Reactive compounds such as amines, aldehydes, ozone as well as unsaturated alcohols and ketones react with the receptor by acid/base reactions, hydrolysis, redox reactions and condensation reactions, which can partly explain their potency (1, 139). The different irritation thresholds may in some way reflect the differences in chemical reactivity among compounds, which in turn highlights the importance of chemical reactivity for an understanding of health problems in relation to exposure to VOCs.

VOCs and health

A number of health problems have been identified in relation to buildings and SBS. In this thesis the definition of health effects is based on previous studies regarding exposure to VOCs and SBS problems. Such health effects were either perceived poor air quality parameters (e.g. stuffy air, smell) or self-reported sensory measures (e.g. irritation of the eyes, nose, throat and skin, headaches, tiredness etc).

Dampness increases the risk of developing health problems in buildings (14, 15, 83). Various types of exposures are related to building dampness such as house dust mites, moulds and bacteria. Building dampness also increases emissions of VOCs which are due to degradation or microbial activity. The hypothesis that VOCs have an impact on health problems in indoor air is supported by a number of studies that have reported negative health effects in relation to low ventilation rates (16, 47, 123).

Many of the symptoms described in relation to SBS have also been reported in studies regarding exposure to VOCs. In a study by Mølhave *et al* (1986) (81) subjects were exposed to 22 compounds known to be common indoor pollutants. The health effects were evaluated using a questionnaire, and significant effects of exposure were found for questions relating to general air quality, odour, ability to concentrate and/or mucous membrane irritation. The effect was acute and showed no sign of adaptation (81). A significant increase in eye and throat irritation and headaches was also found in a study by Hudnell *et al* (1992) using the same mixture. In this study as well no adaptation was seen regarding irritative effects; it was only seen for the olfactory. This was interpreted in terms of both the trigeminal and olfactory systems being activated by the mixture (52). In yet another study the same mixture of VOCs was used. Both a questionnaire and objective measurements was used, such as test of lung function and biomarkers of airway inflammation. Significant effects were found on lower, upper and non-respiratory symptoms, but the objective measurements did not support the findings (89). One problem with these studies is that the concentration used during exposure is far from relevant for

indoor air levels and is therefore difficult to transfer to a real situation. The compounds found in indoor air are often at $\mu g/m^3$ levels or even below. Therefore there existed for some time the hypothesis that the sum of all VOCs could be an indicator for poor indoor quality, but today it is generally agreed that the concept of TVOC does not have any biological relevance.

There is little evidence to support a relationship between non-reactive VOCs at relevant indoor air concentrations and health effects. In a study by Wargocki *et al* (1999) (124) exposure to low levels of a mixture of VOCs emitted from an old carpet showed effects on performance and perceived air quality. It is probably the existence of different kinds of compounds and the way in which these interact, rather than their total concentration, which are important for health. Even if complete additivity of irritative effects is assumed, it is difficult to account for health problems arising from compounds known to be part of the indoor air mixture today (21, 139).

In search for other answers to indoor air health problems, more recent exposure studies have evaluated the effect of exposure to products resulting from the reaction between ozone and different unsaturated hydrocarbons. Usually limonene or pinene but also isoprene has been used. The results from these studies are not conclusive. A mouse bioassay showed significant sensory irritation from exposure to the products resulting from the reaction between ozone and isoprene (131). Controlled short-term exposures to a mixture of ozone and limonene showed that these products have a negative effect on perceived air quality (113). In yet another study, 130 women were exposed to ozone, a mixture of 23 VOCs ("Mølhave mixture" + limonene) and stress. The exposure time was 3 hours. In this study no significant main or interaction effects were seen on subjective or objective health effects from exposure to the VOC mixture and ozone. Regardless of exposure conditions the subjects reported a significantly greater number of severe symptoms of anxiety during the conditions of stress (38). Exposure to a mixture of VOCs and ozone using objective measurements to evaluate the resulting nasal effects found no significant differences between exposure conditions either (73).

The products generated from the reactions between ozone and the unsaturated hydrocarbons identified so far are probably not the only answer to the health problems found in indoor air. There could be other strong airway irritants present that are formed and not analyzed that also help account for the symptoms (131).

MVOCs and health

There are no studies as yet able to prove any relationship between health effects and MVOCs at levels occurring in buildings. A few epidemiological studies have suggested a relation between exposure to low levels of MVOCs and non-specific

health symptoms. However, the higher prevalence of symptoms in relation to MVOC exposure was not always statistically significant (27, 33). In a study of allergic symptoms among children, mouldy odour along skirting boards was found to be associated with rhinitis and eczema, although the measured MVOC did not provide evidence of this relationship (47). Exposure studies to unrealistically high concentrations of 3-methylfuran, 1-octen-3-ol and 2-ethyl-1-hexanol found minor irritation effects and changes in nasal lavage biomarkers (121, 122). A mouse bioassay was also undertaken measuring the effects of a mixture of three MVOCs: 1-octen-3-ol, 3-octanol and 3-octanone. 1-Octen-3-ol became 7.3 times more irritative in mixture form than on its own. The authors concluded that the synergistic effect seen among these three compounds was still not sufficient to have an impact on human health at realistic concentrations (66). In another study VOCs from moulds were found to affect the mucociliary functions of respiratory mucosa of guinea pigs. However, it is difficult to extrapolate results from bioassays to observe the effects on building occupants (56).

Cultivation of microorganisms

Six different microorganisms frequently found in indoor environments were cultivated in order to measure metabolite production. In **Papers 1** and **5** a mixture of five mould species was used: *Aspergillus versicolor* (UPSC 2027), *Fusarium culmorum* (UPSC 1981), *Penicillium chrysogenum* (UPSC 2020), *Ulocladium botrytis* (UPSC 3539) and *Wallemia sebi* (UPSC 2502). The isolates were obtained from the Uppsala University culture collection of fungi (UPSC). In **Paper 1** the mixture of fungi was cultivated on gypsum board, pine wood and particle board and also on malt extract agar (MEA), a medium which favours the growth of these microorganisms (*Wallemia sebi* was grown on dichloran glycerol agar, DG-18). The cultivation was carried out in 2 l culture flasks made of glass (Fig. 1). Filtered, humidified air was constantly passed through the flasks (30 ± 2 ml/min) to keep the material saturated with moisture during the whole cultivation period. 100 ml of autoclaved and demineralised water was poured together with the spore suspensions into each flask. For each media, two cultivation flasks and one blank were prepared. The cultures on the building materials were maintained for 63 days and those on the synthetic media for 23 days. In **Paper 5** 1 ml of the fungal mixture was inoculated on small pieces of building materials (study 1: 165 and 175 pieces of pinewood and particle board respectively; study 2: 150 pieces of each material).

In **Paper 2** (and **4**) two strains of the bacterium *Streptomyces albidoflavus* (CBS 431.51 and CBS 416.34) were inoculated on gypsum board, pine wood, particle board, sand and tryptone glucose extract agar (TGEA), a medium which favours the growth of actinomycetes. Both strains were obtained from the Centraalbureau voor Schimmelcultures (CBS) at the Institute of the Royal Academy of Arts and Sciences in the Netherlands. In **Paper 2** the cultivation was done in the same way as in **Paper 1** except for the fact that the spore suspension now consisted of the bacterium *S. albidoflavus*. The cultivation period was also different; in the first study the cultures were maintained for 25 days on the building materials and for 23 days on the synthetic media (involving the strain CBS 431.51), and in the second study (CBS 416.34) the cultures on gypsum board and sand were maintained for 126 days and on pinewood and particle board for 98 days.

In **Paper 3** secondary emissions due to degradation were measured by collecting material from 20 different buildings with moisture problems. Seven different materials were represented: carpet, concrete, gypsum board, insulation, plastic, sand and wood. In order to determine the microorganisms growing on the different materials the samples were microscopically investigated and inoculated. Air samples were collected twice from all the building materials, in the first instance when these materials had been placed in the culture flasks and were “dry”, that is, in their original condition on arrival at the laboratory. The materials were then

soaked in autoclaved water and left to stand for one week in order to restart the active growth of microorganisms, and samples were then taken again.

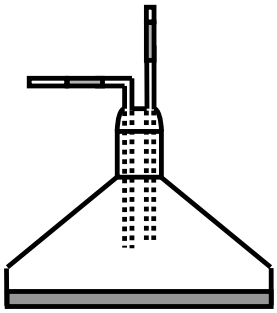


Figure 1. The culture flask used in **Papers 1, 2** and **3**.

Methods for sampling and analysis of VOCs

The VOCs measured depend primarily on the sampling and analytical technique used, and in order to sample a wide range of compounds different methods have to be used. All methods have both advantages and disadvantages and are more suitable for certain compounds or compound groups. The following sampling techniques and analytical methods were used in this thesis.

Sampling of volatile compounds

Eight different sampling methods were used in **Papers 1-5** in order to sample VOCs, amines, aldehydes and carboxylic acids. Tenax TA is a commonly used adsorbent for sampling of indoor air VOCs, partly because it has a low background and is thermally stable, and partly because of its ability to sample a wide range of compounds. A sampling range of C7-C26 is recommended, although it is used down to C4. Tenax TA is a very hydrophobic, porous polymer (2,6-diphenyl-p-phenyloxide) and is not suitable for sampling of highly volatile compounds due to its low specific surface area (30 m^2/g) (30). Carbopack B, also used in **Paper 1**, is another non-polar commonly used adsorbent. It consists of graphitized carbon black and has a larger specific surface area (100 m^2/g) (80).

It is not possible to use the same adsorbent for all types of reactive compounds; often a chemosorbent must be used. This is a sorbent which is coated with a reagent. The reagent reacts with the compound(s) of interest to form a derivative. The established derivative is stable and can be desorbed and analyzed as usual. Low molecular weight aldehydes are preferably sampled with DNPH (2,4-dinitrophenylhydrazine) impregnated adsorbents. DNPH reacts with the carbonyl group on either an aldehyde or a ketone rapidly and quantitatively form stable hydrazones through a condensation reaction (75).

Amines are difficult both to sample and analyse because of their high volatility and polarity, basic character and high solubility in water. The method used for sampling of amines in **Papers 1, 2, 3** and **4** offers a sensitive and selective method for the sampling of amines in the gas phase. Primary and secondary amines react rapidly and quantitatively with NIT (naphthylisothiocyanate) impregnated XAD-2 tubes to form stable thiourea derivatives (78). These derivatives are desorbed and analyzed by HPLC-UV or HPLC-MS (high performance liquid chromatography with either ultraviolet or mass detection). Sampling of tertiary amines requires other methods. For methyl- and ethyl-substituted aliphatic amines sampling on activated charcoal is recommended (2). Activated carbons are thermally stable and have a chemical heterogenous surface that adsorbs compounds through non-specific and specific interactions such as hydrogen-bridges. These adsorbents cannot be desorbed by thermal desorption (30). An alternative method used for

mostly cyclic tertiary amines or amines with long carbon chains is another type of porous polymer, XAD-2 (2).

The most common method of determining aliphatic carboxylic acids in the air involves trapping in liquid such as water or aqueous solutions of NaOH (117). Sampling can also be carried out with a solid sorbent, silica gel, which was used in **Paper 1**. Sampling on silica gel is a method described by NIOSH and OSHA, and silica is good for the sampling of very polar compounds (86).

Gas chromatography

Gas chromatography is used for the separation of mixtures of volatile compounds whereby the sample is vaporized and carried by an inert carrier gas (often He). The gas moves the sampled compounds through a column where the compounds are separated with respect to size, polarity and other qualities decided by the type of column. Transfer of the sampled compounds to the gas chromatographic column was made either by thermal desorption or by solvent extraction followed by splitless injection.

In thermal desorption the compounds collected on the adsorbent tubes are desorbed by heating the tube in a stream of carrier gas. The gas transfers the compounds to the gas chromatograph. Before entering the column the sample is reconcentrated in a cold trap in order to avoid broad tailing peaks. In solvent extraction the sample is extracted from the adsorbent with an appropriate solvent and a small part of the sample is thereafter injected on to the GC-column. There are several potential drawbacks in using solvent extraction such as the possible introduction of volatile impurities, the masking effect of the solvent peak and the loss of very volatile compounds during concentration of the elute. The main advantage of thermal desorption is that the whole sample is available for analysis. This means that it is a very sensitive method, and because there is no need for solvent extraction no solvent peak appears in the chromatogram. This is in contrast to the solvent-based techniques where the sample is diluted during extraction and only a small part of the sample is injected on to the GC, and also the solvent peak can cover compounds.

After separation in the GC-column, each component produces a separate peak in the detector output. The detectors connected to the gas chromatograph used in this thesis were either a mass detector or a flame ionization detector (FID).

High performance liquid chromatography

Liquid chromatography (LC) is an analytical chromatographic technique that is useful for separating mixtures of less volatile or polar compounds dissolved in a

solvent. A column coated with a stationary phase is used and the mobile phase is pumped through the column with a high-pressure pump. The most common detector for liquid chromatography is the UV-detector. It utilises light in the ultraviolet area, and when a component in the sample passes the detector parts of the radiation are absorbed by the sample.

Ion chromatography

Ion chromatography is a form of high-pressure liquid chromatography used for analysis of aqueous samples containing common anions, such as fluoride, chloride, nitrite, nitrate, and sulfate, and common cations, such as lithium, sodium, ammonium, and potassium, using conductivity detectors. It is also commonly used for biochemical species such as amino acids and proteins.

Mass spectrometry

Mass spectrometry is a powerful technique used to identify and measure a wide variety of biological and chemical compounds. The mass spectrometer converts the sampled compounds into gaseous ions, and the most common ionization process for gas phase analysis involves bombardment of the molecule with electrons, electron impact ionization (EI). The molecule is given enough energy to eject one of its electrons and become positively charged. The bombardment of electrons also results in fragmentation of the molecule; this gives a number of ions with different mass-to-charge (*m/z*) ratios. The fragmentation of each molecule is unique and is used as a chemical fingerprint to characterize the analyte.

The mass spectrometric analyses following gas chromatographic separation in this thesis have all been carried out by means of electron impact ionization with an electron energy of 70 eV. The mass spectrometer was operated in full-scan between *m/z* 35-300 in order to identify unknown compounds in **Papers 1, 2, 3** and **5**.

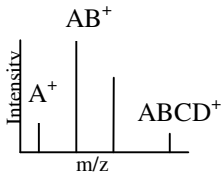
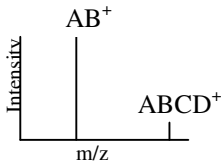
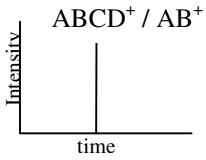
The coupling of liquid chromatography (LC) and mass spectrometry (MS) has resulted in important advances, especially in biomedical and biochemical research. The interfaces predominantly used for the formation of ions are electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). Both interfaces serve for the transfer of the LC eluent into the gas phase and the ionization of the analytes, and are considered soft-ionization techniques, producing protonated or deprotonated molecules. In electrospray the sample is introduced through the ion spray probe and nebulized by a jet of gas, and then sprayed through a high-voltage sprayer, creating a mist of small highly charged droplets. The ions in the droplets evaporate from the surface through “ion evaporation”. The APCI interface utilizes heat and a stream of gas to vaporize the solvent and a corona

discharge to ionize compounds in the gas phase at atmospheric pressure. In **Paper 2** and **4** ESI was used together with a triple quadrupole mass spectrometer.

There are a number of modes of operation in which different pieces of information can be obtained. In full scan all ions between given intervals of *m/z* values are detected, which is necessary for the identification of unknown compounds. Where there is a need to detect only one or a few compounds analysis is carried out with selected ion monitoring (SIM). In this mode only a few characteristic ions are detected which help increase sensitivity. A triple quadrupole mass spectrometer offers further possibilities, and besides the two modes of operation mentioned above it can also carry out a daughter ion scan, selected reaction monitoring (SRM) and a precursor ion scan. In a daughter ion (or product ion) scan the first quadrupole is held constant so that at any given time only one specific *m/z* value can pass. The molecule then passes a collision cell (the second quadrupole), collides with a collision gas and finally fragmentizes. The third quadrupole is set to scan during a given interval of *m/z* values. This results in a spectrum from a chosen molecule and is most acceptable for structure elucidations.

SRM involves studying a selected molecular ion in the first quadrupole, and to verify the result a specific fragment is recorded in the third quadrupole. In a precursor ion scan the second mass analyzer (Q3) is fixed to the fragment mass of interest and the first mass analyzer (Q1) is scanned over a range. The resulting mass spectrum will display the masses of all the compounds which produced the specified fragment mass (Table 4).

Table 4. Fragmentation pattern for daughter ion scan, precursor ion scan and selected reaction monitoring in MS-MS analysis (Q = Quadrupole).

ABCD $\xrightarrow{e^-}$	**Q1**	**Collision cell (Q2)**	**Q3**
Daughter (product) ion scan	Static (parent mass selection) $ABCD^+$	Fragmentation (All masses pass)	
Precursor (parent) ion scan	Scanning $AB^+\rightarrow$		
Selected reaction monitoring	Static (parent mass selection) $ABCD^+$		

Comparing UV and MS detection

The advantages of using MS detection compared to UV detection have been shown in many areas, such as analysis of aldehydes (46, 64, 150) and isocyanates (32). Samples from complex environments may contain unknown or unexpected compounds, and LC with UV detection gives little or no qualitative information on the analytes, information necessary for the identification of unknown compounds. Using a conventional LC together with MS/MS detection will result in approximately the same level of sensitivity as micro LC with UV detection, but chromatographic methods in conjunction with UV detection do not have sufficient specificity or selectivity for screening trace levels of unknown compounds such as amines. Changing the detection system to a mass spectrometer enhances the specificity of the method. Using a mass detector also improves selectivity because separation is possible in both the mass analyzer and the LC-system. This decreases the risk of not detecting compounds due to coelution.

Multivariate methods

Nowadays it is often very easy to collect large amounts of data. In the past few variables were investigated, but today a large number of variables are often included in the analysis as it is no longer time-consuming to collect data. This makes new demands on evaluation methods; large data sets require the use of multivariate projection methods. By translation of a multivariate data table into a series of projections with the aid of principal component analysis (PCA) and partial least squares (PLS) a data set can be summarized and visualized. The projection method is also used to find quantitative relationships among variables.

Principal component analysis (PCA), the basis for multivariate data analysis, is a projection method used to obtain an overview of systematic variations in a data matrix (35). The projection helps to observe grouping of objects, time trends and outliers, and uncovers the relationships between observations and variables. A matrix of data with N rows (observations) and K columns (variables) is used, in which the variables give the number of dimensions and the observations are plotted on to the K-dimensional space (K=number of variables). A principal component is calculated which best approximates the data in the least square sense. Conceptually, this component can be understood as a line, and each observation is projected on to this line. This gives the coordinates, scores, of each observation along this component (line). The plotting of the scores of two principal components is known as a score plot and similar observations will end up close to each other. Information about which variables are responsible for the patterns seen among the observations is given by the loadings. The loadings are given by the angle between the principal component and the variable, and a value close to zero means that the variable has little effect on the component. Variables containing similar information are grouped together.

PLS (partial least squares) is a regression extension of PCA used to combine the information in two blocks of variables, X and Y, to each other (51, 135-137). It derives its usefulness from its ability to analyze data with many noisy, collinear and even incomplete variables in both X and Y, and precision improves with the increasing number of relevant X-variables. This method differs from PCA in that each observation corresponds to two values: one in X-space and one in Y-space. The PLS models are interpreted in the same way as the PCA, with score plots and the PLS counterpart for loading plots, weight plots. A multivariate approach was used in **Paper 3** in order to investigate patterns of genus specific emissions from a number of microbially infested building materials.

Exposure studies

Paper 5 includes two exposure studies; in Study 1 27 healthy students were exposed to low levels of (M)VOCs and in Study 2 the participants (24 students) were exposed to moderate levels of (M)VOCs.

In order to expose the subjects to the whole mixture of VOCs emitted from microorganisms and building materials, small pieces of pinewood and particle board were inoculated with a mixture of moulds (see "Cultivation of microorganisms"). Pieces of the building materials were placed in petri dishes and saturated with moisture, and these petri dishes were then placed in a box made of sheet metal (1.5 x 1.0 x 0.6 m^3). In Study 1 the box was coupled to an exposure chamber (Fig. 2), and air (5 l/min) was blown through the box via a spore filter into the exposure chamber. In Study 2 the air was taken directly from the box containing the pieces of mouldy building materials, and led via a pump through a spore filter into a modified fresh-air hood originally designed for respiratory protection of exposure to the face. The air flow through the box was approximately 20 l/min. The air from the metal sheet box was continuously monitored by sampling on Tenax TA, which was taken both directly from the box and from inside the exposure chamber (only in Study 1). The levels of TVOC and MVOC showed only minor fluctuations during the two exposure periods.

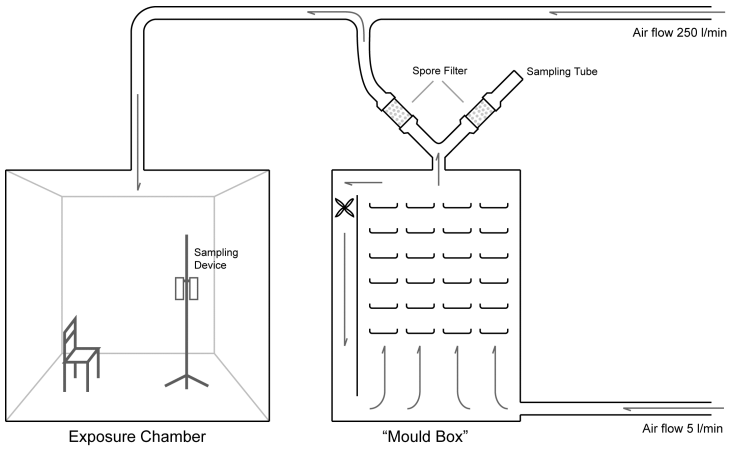


Figure 2. The experimental set-up used in study 1, **Paper 5**. In study 2 the same "mould box" was connected to a fresh air hood instead of the exposure chamber.

Rated air quality and symptoms
The air in the box and the fresh air hood was evaluated by means of a questionnaire from which ratings on the perceived air quality and symptoms were carried out. The choice of symptoms was based on previous studies regarding exposure to VOCs and SBS problems (81, 143). The questions were as follows: "How would you describe the air quality in this room at this very moment, regarding stuffy air, dry air, smell and unpleasant smell?" and "To what extent do you at this very moment experience the following symptoms: smarting pain/irritation in the eyes, stinging/irritation of the skin, redness of skin, dryness/irritation of the nose, nasal congestion, nasal discharge, dryness of the mouth, dryness of the throat/hoarseness, coughing, tiredness, heaviness/pressure on the head/headache, nausea, problems with attention, and feeling bad-tempered?" A Borg's CR-10 scale was used for the rating (12). This is a verbally anchored ratio scale suitable for measuring intensities of most kinds of sensory perception and experience. It has descriptive adjectives that correspond to specific numbers on the scale (nothing at all, 0; extremely weak, 0.5; very weak, 1; weak, 2; moderate, 3; strong, 5; very strong, 7; extremely strong, 10), with "absolute maximum" located outside of the number scale in order to avoid ceiling effects.

Eye irritation was also measured using the self-reported tear-film break-up time method (BUT(s)) (145). BUT(s) was measured before and after each session.

Procedure
Before the first exposure session the subjects participating in both Studies 1 and 2 filled in a questionnaire called the Chemical Sensitivity Scale (CSS). This is a tool for identifying self-reported affective reactions to and behavioural disruptions caused by odorous/pungent substances (88). In Study 1 each participant took part in two exposure conditions, one with air from mouldy building materials and one with blank air lasting a period of 60 min. The participant rated air quality and symptoms four times in each session: before exposure, after 30 and 60 minutes of exposure, and 15 min after exposure. A cognitive task was conducted after 45 min of exposure. The cognitive task consisted of the Digit Symbol Test (76) and the Pattern Comparison Test (96) in order to assess attention and processing speed.

In Study 2 each participant was exposed to four different exposure conditions in random order: (1) air from mouldy building materials without nose-clip, (2) blank air without nose-clip, (3) air from mouldy building materials with nose-clip and (4) blank air with nose-clip. The exposure was performed on four separate days and lasted for 10 min. The same aspects of air quality and symptoms as in Study 1 were rated in this study. The ratings were given before exposure, after 10 min of exposure, and 60 min after exposure.

Results

Identification of MVOCs

Paper 1. Volatile metabolites from microorganisms grown on humid building materials and synthetic media

83 compounds were identified to be emitted from the building materials and MEA, most of them having been sampled on Tenax TA (Appendix II). Dimethyl disulfide was the only compound produced on all media. 1-Octen-3-ol, 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-octanone were found on all materials except particle board.

The amount of metabolites were found to vary with nutrient availability, that is, the highest emissions were produced from cultures on MEA and the lowest from cultures on gypsum board and particle board. The metabolites produced by the fungal mixture grown on pine wood consisted almost entirely of ketones and alcohols. A greater variety of compounds including ketones and alcohols was identified from fungal growth on particle board, gypsum board and MEA. On particle board a number of different hydrocarbons such as isoprene, heptane and octane was identified together with compounds such as 1,3,5-trioxepane and dimethoxymethane.

The emissions from microorganisms growing on gypsum board consisted mainly of very low concentrations of terpenes, such as pinene, camphene and 3-carene, although alcohols were also produced. The number of compounds emitted from microbial growth on gypsum board was less than that from the other investigated materials. Microbial growth on the synthetic medium MEA, on the other hand, resulted in high concentrations of a wide variety of compounds.

No aldehydes or carboxylic acids could be identified as metabolites in this study; instead the concentration of aldehydes was found to decrease during fungal growth. No amines could be identified with the alternative methods used, although some compounds containing nitrogen could be identified such as pyridine, 2-methylpyridine, nitromethane, ammonia and hexanenitrile during growth on particle board.

Paper 2. Identification using versatile sampling and analytical methods of volatile compounds from *Streptomyces albidoflavus* grown on four humid building materials and one synthetic medium

Metabolites were identified from cultures growing on gypsum board, pine wood, particle board and TGEA (Appendix II). No metabolites could be identified from the growth of *Streptomyces* on sand. The most common compound groups emitted were ketones and alcohols, although terpenes including several different

sesquiterpenes were also emitted when grown on pine wood, gypsum board and TGEA.

Only one compound, 1-butanol, was found to be emitted from all materials and none of the common eight-carbon fungal metabolites (3-octanone, 1-octen-3-ol and 3-octanol) was found. Other interesting compounds found to be emitted from bacterial growth on particle board and gypsum board were 3-methylfuran and 2-pentylfuran.

Few compounds were detected with the alternative methods. Thus, aldehydes, tertiary amines or carboxylic acids were not detected from any of the materials. Methylamine, ethylamine, diethylamine were identified from bacterial growth on TGEA. Another compound, probably a secondary amine, was also present but could not be identified.

Paper 3. Volatile organic compounds (VOCs) emitted from building materials affected by microorganisms

Emissions of VOCs were measured from wet and dry building material collected from buildings with mould problems. The emissions not only increased after wetting but also changed noticeably in composition. In general the dry materials emitted hydrocarbons and aldehydes and the wet materials emitted ketones and alcohols. No primary or secondary amines were identified, and the measuring of lower aldehydes resulted in low concentrations of formaldehyde and acetaldehyde, emitted in equal amounts during wet and dry conditions. A number of furanes such as 3-methylfuran, 2-ethylfuran, 2,5-dimethylfuran and tetrahydrofuran were identified when sampling with Carbopack X.

Carpet, for example, emitted substantially more ketones such as 2-heptanone, 2-hexanone, 2-octanone and 2-pentanone when wet. Higher aldehydes (hexanal, heptanal and octanal) were found to be emitted in larger quantities from the dry materials. Wet concrete also emitted methylated ketones such as 3-methyl-2-pentanone and 4-methyl-2-hexanone. The most abundant compound emitted from the wet concrete was 2-ethyl-1-hexanol. Emissions from pieces of gypsum board differed considerably from each other, although the general trend could be seen in these materials as well: emissions of aldehydes and hydrocarbons from the dry materials and alcohols and ketones from the wet materials. From the wet pieces both propanoic and hexanoic acid were identified. Insulation was the only material which showed a pattern differing from that of the other materials; the highest concentrations were emitted from the dry materials, and the emission pattern did not change much between dry and wet conditions. Emissions from plastic, on the other hand, changed dramatically between conditions. Emissions from the wet pieces consisted almost entirely of acids (acetic, butanoic, hexanoic, pentanoic and propanoic acid) and these compounds could not be found at all during the dry condition. A total change in the emission pattern was also observed in sand.

Almost no emissions could be found from the dry material, but from the wet material both alcohols and ketones were identified as well as trimethylamine and triethylamine. Hydrocarbons were identified from dry wood and terpenes from wet wood.

The microorganisms growing on the materials were also identified, and 12 different strains were found, including *Aspergillus*, *Penicillium*, *Cladosporium* and also *Bacillus* and *Streptomyces*. A PLS model was calculated to investigate patterns of genus specific emissions from the materials. The model included all VOC emissions from all wet and dry materials and showed that the emissions were specific for the kind (mould or mould and bacteria) of microorganism growing. The model including the dry material generated three components (R2Y=0.939, Q2=0.6) and the model with the wet materials five principal components (R2Y=0.986 and Q2=0.731). A score plot shows the separation between the two groups dependent on the VOCs emitted from the materials (see **Paper 3**, Figure 1).

Amine analysis

Paper 4. Development of a LC-MS/MS method for the analysis of volatile primary and secondary amines as NIT (naphthylisothiocyanate) derivatives

A three-step process for screening and identification of volatile amines was developed. For the first step characteristic fragments were detected for the protonated naphthylisothiocyanate $[NIT+H]^+$. The samples were screened with selected reaction monitoring (SRM) for the ion pairs *m/z* 144/127 and 186/128; fragments shown to be characteristic for the primary and secondary NIT derivatives respectively. These fragments provided information on the amine content of a sample and indicated whether the amines were primary or secondary. The second step in the process of identifying amines involved a precursor ion scan which gave information on the $[M+H]^+$ ion, and then in the third step a product ion scan of the identified precursor ion gave the fragments necessary for identification.

A structure elucidation scheme was constructed from the product ion spectra of 18 NIT-amine derivatives involving both primary and secondary amines, saturated and unsaturated, aromatic amines and branched amines (n-, iso-, sec-, and tert-).

To evaluate the method, two environmental applications were tested. Air samples were taken from a bacterial culture and a tyre repair shop. Ammonia and six different amines, methylamine, ethylamine, dimethylamine, cyclohexylamine, aniline and tert-butylamine, were found and identified from these two environments. The limit of detection was determined for four amines: proylamine-NIT 0.12 ng/µL, butylamine-NIT and aniline 0.16 ng/µL and dibutylamine 0.25 ng/µL.

Exposure studies

Paper 5. Effects on perceived air quality and symptoms of exposure to microbially produced metabolites and compounds emitted from humid building materials

Study 1

The concentrations of MVOCs identified in the chamber during exposure were fully comparable with the levels found in indoor air, even though the TVOC concentrations were slightly higher than normal due to a high background manifested in the blank air condition.

No significant differences were found between the mould and clean air conditions for perceived air quality and symptoms (MANOVA), BUT(s) or the cognitive tasks (ANOVA) (Table 5). The effect of gender was included in the MANOVA, and a significant main effect was found at 30 and 60 min of exposure but not 15 min after exposure. Post-hoc, two-way ANOVAs yielded no significant main effects of gender for any air quality or symptom variable. A significant main effect of gender was also seen on the digit symbol test, but there were no significant interaction effects between exposure and gender.

Linear regression analysis was used to investigate the correlation between CSS and the sum of the ratings of air quality and symptoms, but no such correlation was identified (at 30 min of exposure: $p=0.41$, $R^2=0.03$; 60 min of exposure: $p=0.16$, $R^2=0.08$; or after exposure: $p=0.28$, $R^2=0.05$).

Table 5. Results from two-way MANOVAs of ratings of air quality and symptoms, self-reported tear-film break-up time (BUT(s) and performance on cognitive tasks (main effect of gender is given within brackets).

		Degrees of freedom	F	P
Rating of perceived air quality and symptoms.	**30 min exp:**	18,8 (18,8)	1.64 (3.28)	0.24 (0.05)
	60 min exp:	18,6 (18,6)	2.00 (3.95)	0.20 (0.05)
	After exp:	18,7 (18,7)	1.51 (0.72)	0.30 (0.73)
BUT(s)		1,25 (1,25)	0.56 (0.81)	0.46 (0.38)
Digit Symbol		1,25 (1,25)	1.34 (5.42)	0.26 (0.05)
Pattern comparison		1,25 (1,25)	0.01 (0.21)	0.97 0.65

Study 2

The concentrations of MVOC were about 10-100 times higher and the levels of TVOC about 100 times higher than in Study 1. A three-way MANOVA including the variables exposure, nose-clip and gender showed no significant main effects but did show a significant interaction between exposure and nose-clip [$F(13,7)=1.20$, $p=0.41$]. This interaction suggests that regardless of exposure condition the participants reported more symptoms when not using the nose-clip, which prompted separate analyses of the nose-clip conditions. Therefore, two separate MANOVAs ("with nose-clip" and "without nose-clip") were carried out, which showed a significant effect of mould exposure when not using the nose-clip (Table 6).

Table 6. Results from two-way MANOVAs of ratings of air quality and symptoms, self-reported tear-film break-up time (BUT(s)).

		With nose-clip			Without nose-clip		
		DF	**F**	**P**	**DF**	**F**	**P**
Rating of perceived air quality and symptoms.	**10 min exp:**	13,8	1.16	0.43	18,5	5.46	0.035
	After exp:	13,7	0.81	0.65	18,5	1.33	0.41
BUT(s)		1,20	0.95	0.34	1,22	0.12	0.73

Post-hoc, one-way ANOVA showed significant differences among ratings concerning perceived air quality, such as "stuffy air" [$F(1,22)=28.25$, $p=0.00$], "smell" ($F(1,22)=35.98$, $p= 0.00$) and "unpleasant smell" [$F(1,22)=30.85$, $p=0.00$]. No significant effects were seen on the other variables ($\alpha=0.003$, Bonferroni correction), although the ANOVA showed strong tendencies of more "skin irritation" [$F(1,22)=4.73$, $p=0.04$], "redness of skin" [$F(1,22)=3.09$, $p=0.09$], "nasal irritation" [$F(1,22=3.46$, $p=0.07$] and "tiredness" [$F(1,22)=3.57$, $p=0.07$] in the mould condition compared to the blank condition.

Regression analysis between ratings of air quality and symptoms and CSS score did not show a significant correlation (without nose-clip: $p=0.11$, $R^2=0.11$; with nose-clip: $p=0.37$, $R^2=0.04$).

Discussion

Identification of MVOCs/VOCs

The growth of microorganisms generates volatile metabolites (MVOCs), but the lack of knowledge about metabolic routes makes it generally unclear whether all compounds found in relation to microbial growth really are a metabolic product or whether microbial growth and/or moisture promote(s) emission of compounds from a substrate. Examples of this are benzene, methyl benzene and xylene which were identified as metabolites in a study by Ezeonu *et al* (1994) (36). These compounds do not have a natural metabolic route and it is therefore questionable whether these compounds really are of microbial origin (91, 134). Microbial growth seems to be able to alter the emissions from the substrate it grows on. Aldehydes have in earlier studies, as well as in **Paper 1**, been shown to decrease during growth of microorganisms in laboratory experiments (67, 111). However, the main objective of this study was not to identify "true" MVOCs but rather to identify VOCs that could possibly result in contamination in indoor air, regardless of whether the compounds were emitted due to microbial growth, moisture or both.

The general hypothesis of this thesis was that fungal and bacterial growth on building materials gives rise to VOCs of both a reactive and a non-reactive nature. A number of different methods were used in order to sample VOCs, amines, aldehydes and carboxylic acids, and a large number of compounds were identified but few compounds were found with the alternative methods used. The most common compound groups found were ketones and alcohols, involving typical MVOCs such as 2-methyl-1-propanol, 1-octen-3-ol, 3-mehyl-1-butanol, 2-heptanone, 2-octanone and 2-nonanone (5, 33, 39, 67, 108, 130, 134). The emissions varied greatly between different growth media, and only one compound was identified as being emitted from all media: in **Paper 1** dimethyldisulfide and in **Paper 2** 1-butanol. The difficulty in using single MVOCs as specific tracer compounds is therefore confirmed (65, 107). The use of a group of compounds to identify mould growth in houses has been proposed, but given the variety of compounds identified in **Papers 1** and **2** an unrealistically large group of compounds would be needed. Still, only a few of the compounds are specific; most of the identified compounds also have other natural sources for being present in indoor air which makes them inappropriate as tracer compounds. Identifying MVOCs from materials taken from buildings with mould problems also shows that the MVOCs identified so far are not enough for the identification of dried-up water damaged material (**Paper 3**).

A number of earlier studies have been concerned with metabolite production from microorganisms growing on different laboratory media such as MEA and TGEA. Knowledge of the impact of growth media on emissions makes relevant an investigation into whether these compounds can also be found where

microorganisms grow on building materials. In **Papers 1** and **2** some compounds previously identified from laboratory media were also found to be emitted from microbial growth on building materials, for example 3-methylfuran, pyridine, 2-methyl-pyridine and nitromethane. Different growth media also have an impact on how fast compounds are produced/emitted. Nutrient rich media, high temperature and long cultivation periods seem to promote the production of potentially more irritative and odorous metabolites (109). For example, 3-methylfuran and 2-pentylfuran were identified from *Streptomyces* growing on particle and gypsum board. On gypsum board these compounds were emitted after 110 days of cultivation. The time aspect might explain the absence of metabolites where *Streptomyces* grew on sand as reported in **Paper 2**. The impact of material on emissions could also be seen in **Paper 3**. Typical MVOCs were more commonly emitted from materials that seem to have few nutrients to support microbial growth, such as sand, concrete and carpet. **Paper 3** identified differences not only between materials but also between emissions from the same kind of materials. Probably no two water damaged sites or pieces of material are identical in microbial composition, for which reason all emissions produced from the materials differ as well. Identifying VOCs from materials can therefore only be seen as examples of what can be emitted under existing circumstances.

Many of the compounds found to be emitted from *Streptomyces* growing on building materials have also been identified as metabolites from fungal cultures. Significant differences were also identified; for example none of the common eight-carbon fungal metabolites were identified from the cultures consisting of bacteria. This has also been found in earlier studies (100, 132). In **Paper 2**, *S. albidoflavus* was cultivated on different media, whereas methylamine, ethylamine and dimethylamine were identified during cultivation on TGEA. Amines (trimethylamine and triethylamine) were also identified in relation to bacterial growth in **Paper 3**. The knowledge that under the right conditions *Streptomyces* are able to produce potentially irritating compounds would make interesting further study of emissions from bacteria and also the monitoring of bacteria in an indoor environment. The amines are not only irritating but also can react with other compounds in indoor air such as NO_x or OH radicals to form carcinogenic nitrosamines (26, 146). The number and variety of sesquiterpenes identified from materials inoculated with bacteria was greater than those identified from materials inoculated with the mould mixture (**Paper 1** and **2**), and the same pattern has emerged in earlier studies (62, 100, 109). These compounds are interesting not only because some of these have a very characteristic mouldy smell (e.g. geosmin) but also because unsaturated organic compounds such as terpenoids and sesquiterpenes have been identified to react with ozone or NO_x at significant rates, producing compounds that may be harmful to humans (126). A mouse bioassay was able to find evidence for airway irritation from mixtures of isoprene and ozone as well as from a mixture of isoprene, ozone and NO_2. Perceived air quality was also found to be poorer when exposing people to a mixture of ozone and limonene (113). Both

isoprene and limonene together with other monoterpenes are commonly found in relation to microbial growth (**Paper 1, 2** and **3**) (68). Exposure studies have so far failed to find a connection between the products of reaction and health effects (38, 73). The absence of significant health effects could be due to the short exposure times. In **Paper 3** a number of interesting compounds was found on materials with bacterial growth, for example, amines as mentioned earlier and tetrahydrofuran. These compounds were also partly responsible for the separation between the groups in the multivariate PLS model. All these results indicate that further study should focus on bacteria and their impact on an indoor environment. This has also been suggested by a number of Finnish studies (50, 55, 84, 112)

There is evidence of a relation between indoor dampness and health effects. The complete change in emission patterns when building materials are wet compared to when dry may be one explanation (**Paper 3**). Secondary emissions are suspected to pose greater health problems than are primary emissions because they are more reactive and are emitted over a longer period of time. Secondary emissions due to moisture and possibly also microbial growth were identified in **Paper 3**. For example, 2-ethyl-1-hexanol was identified from all the wet samples. This compound is a known irritant to the upper airways, and has also been linked to an increased frequency of asthma (87, 116, 118). Furanes (3-methylfuran, 2-ethylfuran, 2,5-dimethylfuran and tetrahydrofuran) were also identified in relation to dampness in **Paper 3**. 3-Methylfuran has a characteristic fungal smell and has also been suggested to contribute to building-related illness (122).

Most of the compounds identified in **Papers 1, 2** and **3** were collected on Tenax TA and identified with GC-MS, and few of these if any can individually account for the irritation complaints found in indoor air at the measured concentration levels. However, several MVOCs identified have a low odour threshold, are known irritants, and might together be capable of giving rise to health complaints. A possible relation between a group of VOCs and health was found in a study using multivariate methods (110). Differences in VOC content in the air in problem and non-problem buildings were identified, and these differences could only be seen to be the whole group of compounds acting together. Synergism among low levels of several compounds has been proposed as one possible explanation for indoor air health problems. However, studies investigating the effect of mixtures of MVOCs and VOCs have concluded that the levels of compounds identified are still too low to be likely to cause health effects (21, 66). Instead the health effects may come from compounds that we cannot or at least have not measured. Hägerhed-Engman *et al* (2006) (47) found a relation between smell along the skirting board together with a low ventilation rate and an increased risk of certain health effects, although no specific compounds could be detected. This result shows that the human sense of smell is far more sensitive than chemical sampling and analytical techniques. Another indirect proof that immeasurable chemicals derived from indoor air chemistry may be partly responsible for sensory effects was presented by Sundell

et al (1993) (106). They found a lower TVOC level in the room air than in the supply air, and found these so-called lost TVOC to be inversely proportional to the symptoms found in relation to sick building syndrome. In more recent studies low ventilation has been seen to correlate strongly with discomfort and health problems in buildings. Volatile organic compounds can be ventilated, unlike for example personal factors proposed to be involved in SBS. This provides further indirect proof that the VOCs in indoor air play an important role (123).

Amine analysis method

Amines are known irritants and are also known to react to form other potentially more irritating compounds. Amines have been found in diverse environments and also in relation to microbial growth (**Paper 2**) (58, 94, 144). In general, methods more sensitive and effective in sampling and detecting reactive compounds are needed in indoor air research (91, 139). In **Paper 4**, a method for screening and identifying primary and secondary amines in the air was developed. The method proved to be at least as sensitive as other methods using HPLC-UV, with the advantages of mass spectrometric detection such as structural information about compounds and the opportunity to separate compounds in the mass analyzer. The specificity and selectivity of this method increase the chances of discovering amines in samples collected in complex environments. The mass spectrometer also makes it easier to identify unknown or unexpected compounds in a given sample.

The detection limit was well below the concentrations that the human chemical senses can detect, and also well below the threshold limit values available for some of the compounds. The sense of smell can for example detect propylamine at concentrations down to 20 $\mu g/m^3$, whereas the analytical system was able to detect approximately 0.6 $\mu g/m^3$ (74). There is no occupational threshold limit value set for this compound.

With the developed analysis method given above we were able to detect amines from bacterial growth on TGEA; the same samples were also analyzed with HPLC-UV but none of the amines were identified. This shows the importance of a specific and selective method in order to identify unknown compounds at trace levels in a complex matrix.

Exposure studies

There are still very few studies linking VOCs at relevant concentrations to health effects; most studies only use a few selected compounds at rather high levels of concentration when evaluating health effects, whereas indoor air exposure contains a mixture of compounds always at low concentrations. In **Paper 5** the entire mixture of VOCs emitted from both building materials and the moulds growing on those materials was used in two exposure studies to investigate its possible effects on perceived air quality and health similar to the effects found in sick buildings. Short-term (10 min) exposure to the mixture of VOCs at concentrations slightly

higher than that found in indoor air did not show any health effects, although it significantly affected the subjective reports concerning perceived air quality. These reactions may serve as chemical warnings and may be the first signs of health hazards. No such results were found when exposing people to low levels of VOCs for 60 min.

During exposure to moderate levels of VOCs the subjects reported not only more nasal irritation but also more skin symptoms, and the effects were more pronounced without the nose-clip. One explanation for this could be that activation of the chemosomatosensory part of the trigeminal nerve by stimulation of nociceptors in the nasal cavity may also cause increased sensitivity to chemosensory stimulation to other areas that are mediated by the trigeminal nerve. Another explanation, proposed by Dalton (2003) (28) and Hudnell *et al* (1992) (52), is that olfactory stimulation may lower the level of sensory irritation.

The results indicated that there is a relationship between VOC exposure and certain symptoms, although this finding was not statistically significant. The absence of clear-cut effects on symptoms may have a number of explanations. Too short exposure time is one of them (103). Irritation symptoms have been shown to increase during a working day (141), and in a controlled study of exposure to mixtures of VOCs the irritating effect has been found to increase during the first 30 min of exposure (81). Temporal issues may also be of relevance regarding effects on cognition. Wargocki *et al* (1999) (124) have concluded that time-consuming tasks requiring vigilance are needed if they are to have any effect on cognition, which may explain the non-significant effects on attention and processing speed in **Paper 5**. Other explanations for the absence of symptoms during exposure to low levels of VOCs are that the concentrations may be too low, or that the background (blank-air condition) was too high and could have masked the possible effects from exposure. Another possibility is that critical VOCs may not have been present in the mixture or that the VOCs could also be adsorbed on particles. MVOCs have been found to adsorb on respirable particles of house dust, and it has been suggested that these particles contribute to the health effects caused by indoor air (120). In **Paper 5** the particles were not measured, but if present most of them were probably removed by the spore filter situated at the inlet of the exposure chamber. The use of healthy subjects for exposure has been discussed in other studies (38, 122, 141) and there may be a need to expose sensitized subjects in order to detect any effects when using such short exposure times and low concentrations. It is generally agreed that the problem with SBS is probably a multifactorial one, and therefore no powerful effects can be expected when testing only one factor.

Conclusions

- A large number of volatile organic compounds are emitted during microbial growth on building materials, and ketones and alcohols are the most common compound groups. Production is dependent on both species and medium and it is therefore important to identify more metabolites using a variety of relevant materials as a growth medium.

- The alternative methods used in this study for sampling more volatile compounds (Carbopack B and X), aldehydes (DNPH), amines (NIT) and carboxylic acids (silica) provided little or no additional information. This may be due to these compounds either not being produced or else being produced at concentrations below the detection limit.

- *Streptomyces albidoflavus* under the right conditions are capable of producing reactive compounds such as amines.

- Many MVOCs/VOCs are potential sensory irritants and may individually or in combination exceed irritation thresholds. Many of the emitted compounds can also react to produce other more irritative compounds.

- A specific and selective method for screening samples for unknown amines was developed. This proved to be a general method well suited for screening and identifying low concentrations of both primary and secondary amines as well as ammonia.

- Dampness is a strong influence on emissions from both building materials and the microorganisms growing on them. The emission pattern changes when materials are wet, and this change as a result of secondary emissions might be one of the factors responsible for the origin of sick building syndrome.

- VOCs emitted from damp building materials and the moulds growing on them resulted in an increased number of reports of perceived poor air quality during brief exposure to moderate conditions. Stimulation of chemical warning systems (the nasal chemosensory part of the trigeminal system and the olfactory system) may possibly enhance skin symptoms.

- MVOCs cannot be used as a reliable method for finding mould growth either in buildings or on dried-up water damaged materials.

Future perspectives

Since bacteria have been identified as a probable risk factor for negative health effects in indoor air, there is a need to study further metabolite production from the growth of indoor bacteria such as *Streptomyces*. It would also be of value to study emissions from both bacteria and mould growing on other building materials. The mixture of bacteria and mould may be capable of generating other compounds.

Development of better methods for the sampling and analysis of reactive compounds such as low-molecular acids is also needed. Different methods should be used to study reaction products from emissions generated by mould growth together with ozone, for example, sesquiterpenes.

A better understanding of the cause of SBS could be acquired by exposing already sensitized people to low levels of emissions from mould or bacteria growing on building materials.

Acknowledgements

Jag vill tacka alla som på olika sätt har hjälpt mig genom åren, TACK!

-Anna-Lena Sunesson – för att du alltid har tid, är positiv och klok och dessutom en god vän. En bättre handledare kan man inte önska sig!

-Steven Nordin, Janne Levin, Bengt Järvholm och Göran Blomquist – för åsikter och hjälp där det behövts.

-Margit Sundgren, Annika Hagenbjörk-Gustavsson och Margaret Rhen – för hjälp i labbet och god vänskap.

-Roger Lindahl, S-O Westermark, Anders Jakobsson och Bertil Nordström – för hjälp med tekniska detaljer och försöksuppställningar.

-Peter von Zweigbergk– min kära rumskamrat, arbetet skulle inte ha varit hälften så kul utan dig!

-Jessica Körning-Ljungberg – för många skratt och för att du är så bra på att fylla på energi och göra mig peppad att fortsätta skriva.

-Metha Karlsson – för att du hjälper mig hålla reda på praktiska saker (tänk att man kan fylla i blanketter på så många olika fel sätt).

-Ninni Broms Dahlgren – för all litteratur du hjälpt mig att hitta.

-Leif Nilsson och Jessica Fahlén för hjälp med statistiken.

-Jörgen Nätterlund för hjälp med figuren.

-Johannes van den Berg- för hjälp vid slutspurten.

-Alla försökspersoner som ställt upp i mina försök.

-Alla övriga arbetskamrater på ALI för att ni gör det roligare att komma till jobbet!

-Åsa S – min ”tvillingsyster”, vi är lika på väldigt olika sätt. Tack för att du är en god vän som alltid tror på mig. Du gör mig ”starkare, snabbare och modigare”!

ACKNOWLEDGEMENTS

-Hanna, Jenny och Christina – mina cykel-, styrketränings-, skid- och klätterkompisar, vad skulle jag göra utan våra tränings- och fika stunder (som tenderar mer och mer att bara bli fikastunder…)!

-Familjen (Mamma Britt och pappa Johan och bröderna Petter och Olle) - för att ni finns, synd att ni inte finns lite närmare…

-Jonas – för att du är min bästa vän i alla lägen och för att du gör mig så glad!

-Jakob och Viktor för att ni hjälper mig hålla reda på vad som är viktigt i livet.

-I finally also want to thank the Centre for Environmental Research (CMF) for generous funding of this thesis.

Kram på er alla ☺

References

1. **Alarie, Y., M. Schaper, G. D. Nielsen, and M. H. Abraham**. 1998. Structure-activity relationships of volatile organic chemicals as sensory irritants. Archives of Toxicology **72**:125-140
2. **Andersson, B. and K. Andersson**. 1989. Determination of tertiary amines in air. Applied and Industrial Hygiene **4**:175-179
3. **Arts, J. H. E., C. de Heer, and R. A. Woutersen**. 2006. Local effects in the respiratory tract: relevance of subjectively measured irritation for setting occupational exposure limits. International Archives of Occupational and Environmental health **79**:283-298
4. **Batterman, S. A.** 1995. Sampling and analysis of biological organic compounds. In: Bioaerosols Indoor Air research series ed: H.A, Burge, Boca Raton, Lewis publishers 249-268
5. **Baumann, M., L. Lorentz, S. Batterman, and G. O. Zhang**. 2000. Aldehyde emissions from particleboard and medium density fiberboard products. Forest Products Journal **50**:75-82
6. **Bentley, B. and J. W. Bennett**. 1988. Biosynthesis of secondary metabolites. In Berry, D. R. (ed) Physiology of Industrial Fungi. Oxford. Blackwell Scientific Publishers. 161-183
7. **Berglund, B. and I. Johansson**. 1996. Health effects of volatile organic compounds in indoor air. Archives of the Center for Sensory Research **3**:
8. **Berry, D. R.** 1988. Products of primary metabolic pathways. In Berry, D. R. (ed) Physiology of Industrial Fungi. Oxford. Blackwell Scientific Publications. 130-160
9. **Bjurman, J.** 1999. Release of MVOCs from microorganisms. In Organic Indoor Air Pollutants - Occurrence, Measurement, Evaluation. Ed. T. Salthammer 259-273
10. **Bjurman, J. and J. Kristensson**. 1992. Production of volatile metabolites by the soft rot fungus *Chaetomium globosum* on building materials and defined media. Microbios **72**:47-54
11. **Bjurman, J., E. Nordstrand, and J. Kristensson**. 1997. Growth-phase-related production of potential volatile organic tracer compounds by moulds on wood. Indoor Air **7**:2-7
12. **Borg, G. and E. Borg**. 2001. A new generation of scaling methods: Level-anchored ratio scaling. Psychologia **28**:15-45
13. **Börjesson, T., U. Stöllman, and J. Schnürer**. 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on cereal grains. Applied and Environmental Microbiology **58**:2599-2605
14. **Bornehag, C. G., G. Blomquist, F. Gyntelberg, B. Järvholm, P. Malmberg, L. Nordvall, A. Nielsen, G. Pershagen, and J. Sundell**. 2001. Dampness in buildings and health. Indoor Air **11**:72-86
15. **Bornehag, C. G., J. Sundell, S. Bonini, A. Custovic, P. Malmberg, S. Skerfving, T. Sigsgaard, and A. Verhoeff**. 2004. Dampness in buildings as a risk factor for health effects, EUROEXPO: a multidisciplinary review of the literature (1998-2000) on dampness and mite exposure in buildings and health effects. Indoor Air **14**:243-257
16. **Bornehag, C. G., J. Sundell, L. Hägerhed-Engman, T. Sigsggard, S. Janson, and**

N. Aberg. 2005. "Dampness" at home and its association with airway, nose and skin symptoms among 10,851 preschool children in Sweden: a cross-sectional study. Indoor Air **15**:48-55

17. **Brown, S. K.** 1999. Occurrence of Volatile Organic Compounds in Indoor Air. In Organic Indoor Air Pollutants - Occurrence, Measurement, Evaluation. Ed. T. Salthammer 171-184
18. **Brown, S. K., M. R. Sim, M. J. Abramson, and C. N. Gray**. 1994. Concentrations of volatile organic compounds in indoor air - a review. Indoor Air **4**:123-134
19. **Burge, P. S.** 2004. Sick building syndrome. Occupational and Environmental Medicine **61**:185-190
20. **Cometto-Muniz, J. E. and W. S. Cain**. 1994. Perception of odor and nasal pungency from homologus series of volatile organic compounds. Indoor Air 140-145
21. **Cometto-Muniz, J. E. and W. S. Cain**. 1997. Agonistic sensory effects of airborne chemicals in mixtures: odor, nasal pungency and eye irritation. Perception and Psychophysics **59**:665-674
22. **Cometto-Muniz, J. E., W. S. Cain, and M. H. Abraham**. 1998. Nasal pungency and odor of homologous aldehydes and carboxylic acids. Experimental Brain Research **118**:180-188
23. **Cometto-Muniz, J. E., W. S. Cain, and M. H. Abraham**. 2004. Chemosensory additivity in trigeminal chemoreception as reflected by detection of mixtures. Experimental Brain Research **158**:196-206
24. **Cometto-Muniz, J. E., Cain W S, and Abraham M H**. 2004. Detection of single and mixed VOCs by smell and by sensory irritation. Indoor Air **14**:108-117
25. **Cometto-Muniz, J. E., W. S. Cain, and M. H. Abraham**. 2005. Determinants for nasal trigeminal detection of volatile organic compounds. Chemical Senses **30**:627-642
26. **Dabek-Zlotorzynska, E. and W. Maruszak**. 1998. Determination of dimethylamine and other low-molecular-mass amines using capillary electrophoresis with laser-induced fluorescence detection. Journal of Chromatography B **714**:77-85
27. **Daisey, J. M., W. J. Angell, and M. G. Apte**. 2003. Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. Indoor Air **13**:53-64
28. **Dalton, P.** 2003. Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicology Letters **140-141**:239-248
29. **Damgård Nielsen G**. 1991. Mechanisms of activation of the sensory irritant receptor by airborne chemicals. Critical Reviews in Toxicology (CRC) **21**:183-208
30. **Dettmer, K. and W. Engewald**. 2002. Adsorbent materials commonly used in air analysis for adsorptive enrichment and thermal desorption of volatile organic compounds. Analytical and Bioanalytical Chemistry **373**:490-500
31. **Edvardsson, B., B. Stenberg, J. Bergdahl, N. Eriksson, G. Lindén, and L. Widman**. 2005. The medical and social prognosis of sick buildings syndrome - a follow-up study focusing on female patients. Indoor Air '05, The Proceedings of the 10th International Conference on Indoor Air Quality and Climate 3706-3710
32. **Ekman, J.** 2002. Air sampling method comparisons and LC-MS/MS analysis of derivatised isocyanates. **Licentiate Thesis** Umeå University, Sweden
33. **Elke, K., J. Begerow, H. Opperman, U. Krämer, E. Jermann, and L. Duneman**. 1999. Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID-a new feasible approach for the

detection of an exposure to indoor mould fungi? Journal of Environmental Monitoring **1**:445-452

34. **Engel, K.-H., J. Heidlas, W. Albrecht, and R. Tressl**. 1989. Biosynthesis of chiral flavor and aroma compounds in plant and microorganisms. ACS Symposium series. **380**:8-22
35. **Eriksson, L., E. Johansson, N. Kettaneh-Wold, and S. Wold**. 2001. Multi- and megavariate data analysis-principles and applications. Umetrics AB
36. **Ezeonu, I. M., D. L. Price, R. B. Simmons, S. A. Crow, and D. G. Ahearn**. 1994. Fungal production of volatiles during growth on fiberglass. Applied and Environmental Microbiology **60**:4172-4173
37. **Fiedler, K., E. Schütz, and S. Geh**. 2001. Detection of microbial volatile organic compounds (MVOCs) produced by moulds on various materials. International Journal of Hygiene and Environmental Health **204**:111-121
38. **Fiedler, N., R. Laumbach, K. Kelly-McNeil, P. Lioy, Z. H. Fan, J. Zhang, J. Ottonweller, P. Ohman-Strickland, and H. Kipen**. 2005. Health effects of a mixture of indoor air volatile organics, their ozone oxidation products and stress. Environmental Health Perspectives **113**:1542-1548
39. **Fischer, G., T. Müller, R. Schwalbe, R. Ostrowski, and W. Dott**. 2000. Exposure to airborne fungi, MVOC and mycotoxins in biowaste-handling facilities. International Journal of Hygiene and Environmental Health **203**:97-104
40. **Fischer, G., R. Schwalbe, M. Möller, and R. Ostrowski**. 1999. Species-specific production of microbial volatile organic compounds (MVOC) by airborne fungi from a compost facility. Chemosphere **39**:795-810
41. **Flannigan, B.** 1992. Indoor microbiological pollutants - sources, species, characterization and evaluation. In Chemical, Microbiological, Health and Comfort Aspects of Indoor Air Quality - State of the Art in SBS. Ed. H. Knöppel and P. Wolkoff. Kluwer Academic Publishers, Amsterdam. 73-98
42. **Gao, P., F. Korley, J. Martin, and B. T. Chen**. 2002. Determination of unique microbial volatile organic compounds produced by five *Aspergillus* species commonly found in problem buildings. American Industrial Hygiene Association Journal **63**:135-140
43. **Gao, P. and J. Martin**. 2002. Volatile metabolites produced by three strains of S*tachybotrys chartarum* cultivated on rice and gypsum board. Applied Occupational and Environmental Hygiene **17**:430-436
44. **Górny, R. L., T. Reponen, K. Willeke, D. Schmechel, E. Robine, M. Boissier, and S. A. Grinshpun**. 2002. Fungal fragments as indoor air biocontaminants. Applied and Environmental Microbiology **68**:3522-3531
45. **Greim, H., D. Buty, H. J. Klimisch, M. Oeben-Negele, and K. Ziegler-Skylakakis**. 1998. Toxicity of aliphatic amines: structure - activity relationship. Chemosphere **36**:271-195
46. **Grosjean, E., P. G. Green, and D. Grosjean**. 1999. Liquid chromatography analysis of carbonyl (2,4-dinitrophenyl)hydrazones with detection by diode array ultraviolet spectroscopy and atmospheric pressure negative chemical ionization mass spectrometry. Analytical Chemistry **71**:1851-1861
47. **Hägerhed-Engman, L.** 2006. Indoor environmental factors and its associations with asthma and allergy among Swedish pre-school children **Ph. D Thesis**:Lund University, Sweden
48. **Harris, N. D., C. Karahadian, and R. C. Lindsay**. 1986. Musty aroma compounds

produced by selected molds and actinomycetes on agar and whole wheat bread. Journal of Food Protection **49**:964-970

49. **Hekmat, M. and R. G. Smith**. 1991. Determination of low molecular weight organic acids collected on silica gel air sampling tubes by using ion-exclusion chromatography. American Industrial Hygiene Association Journal **52**:332-335
50. **Hirvonen, M. R., K. Huttunen, and M. Roponen**. 2005. Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. Indoor Air **15**:65-70
51. **Höskuldsson, A.** 1996. Prediction methods in science and technology. Thor Publishing, Copenhagen, Denmark
52. **Hudnell, K. H., D. A. Otto, D. E. House, and L. Mølhave**. 1992. Exposure of humans to a volatile organic mixture. II. Sensory. Archives of Environmental Health **47**:31-38
53. **Humphris, S. N., A. Bruce, E. Buultjens, and R. E. Wheatly**. 2002. The effects of volatile microbial secondary metabolites on protein synthesis in *Serpula lacrymans*. FEMS Microbiology Letters **210**:215-219
54. **Husman, T.** 1996. Health effects of indoor-air microorganisms. Scandinavian Journal of Work and Environmental Health **22**:5-13
55. **Hyvärinen, A., T. Meklin, A. Vepsäläinen, and A. Nevalainen**. 2002. Fungi and actinobacteria in moisture-damaged building materials - concentrations and diversity International Biodeteroration & Biodegradation **49**:27-37
56. **Joki, S., V. Saano, T. Reponen, and A. Nevalainen**. 1993. Effect of indoor microbial metabolites on ciliary function in respiratory airways. Proceedings of Indoor Air '93 **1**:259-263
57. **Jones, A. P.** 1999. Indoor air quality and health. Atmospheric Environment **33**:4535-4564
58. **Kataoka, H.** 1996. Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis. Journal of Chromatography A **733**:19-34
59. **Keller, R., K. Senkpiel, and H. Ohgke**. 1999. Use of MVOC measurements and odor perception as indicator of mould in indoor areas. In: Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control. Ed. E. Johanning. 533-537
60. **Kempler, G. M.** 1983. Production of flavour compounds by microorganisms. Advances in Applied Microbiology **29**:29-51
61. **Kinderlerer J. L. and B. Kellard**. 1984. Ketonic rancidity in coconut due to xerophilic fungi. Phytochemistry **23**:2847-2849
62. **Kiviranta, H., A. Toumainen, M. Reiman, S. Laitinen, J. Liesivouri, and A. Nevalainen**. 1998. Qualitative identification of volatile metabolites from two fungi and three bacteria species cultivated on two media. Central European Journal of Public Health **6**:296-299
63. **Knudsen, H. N., U. D. Kjaer, P. A. Nielsen, and P. Wolkoff**. 1999. Sensory and chemical characterization of VOC emissions from building products: impact of concentration and air velocity. Atmospheric Environment **33**:1217-1230
64. **Kölliker, S. and M. Oehme**. 1998. Structure elucidation of 2,4-dinitrophenylhydrazone derivatives of carbonyl compounds in ambient air by HPLC/MS and multiple MS/MS using atmospheric chemical ionization in the negative ion mode. Analytical Chemistry **70**:1979-1985

65. **Korpi, A.** 2001. Fungal volatile metabolites and biological responses to fungal exposure **Ph. D Thesis**: University of Kuopio, Finland
66. **Korpi, A., J. Kasanen, Y. Alarie, V.-M. Kosma, and A.-L. Pasanen**. 1999. Sensory irritating potency of some microbial volatile organic compounds (MVOCs) and a mixture of five MVOCs. Archives of Environmental Health **54**:347-352
67. **Korpi, A., A. L. Pasanen, and P. Pasanen**. 1998. Volatile compounds originating from mixed microbial cultures on building materials under various humidity conditions. Applied and Environmental Microbiology **64**:2914-2919
68. **Kuzma, J., M. Nemecek-Marshall, W. H. Pollock, and R. Fall**. 1995. Bacteria produce the volatile hydrocarbon isoprene. Current Microbiology **30**:97-103
69. **Lacey, J.** 1988. Actinomycetes as biodeteriogens and pollutants of the environment. Actinomycetes in Biotechnology. Ed. M. Goodfellow, S.T. Williams, M. Mordarski. Academic Press. London 359-432
70. **Larsen, O. T. and J. C. Frisvad**. 1999. Characterization of volatile metabolites from 47 *Penicillium* taxa. Mycological Research **99**:1153-1166
71. **Larsen, T. and J. Frisvad**. 1995. Chemosystematics of P*enicillium* based on profiles of volatile metabolites. Mycological Research **99**:1167-1174
72. **Latrasse, A., C. Alabouvette, and J. Sarris**. 1988. Novel aromas of fungal origin. Symbiosis **2**:235-245
73. **Laumbach, R. J., N. Fiedler, C. R. Gardner, D. L. Laskin, Z. H. Fan, J. Zhang, C. J. Weshler, P. J. Lioy, R. B. Devlin, P. Ohman-Strickland, K. Kelly-McNeil, and H. M. Kipen**. 2005. Nasal effects of a mixture of volatile organic compounds and their ozone oxidation products. Journal of Occupational and Environmental Medicine **47**:1182-1189
74. **Lestremau, F., Desauziers V, and J. L. Fanlo**. 2001. Formation of artefacts during air analysis of volatile amines by solid phase micro extraction. The Analyst **126**:1969-1973
75. **Levin, J. O., R. Lindahl, and K. Andersson**. 1988. High-performance liquid-chromatographic determination of formaldehyde in air in the PPB to PPM range using diffusive sampling and hydrazone formation. Environmental Technology Letters **9**:1423-1430
76. **Lezak, M.** 1995. Neurophysiological assessment, 3rd edition. Oxford University Press, New York
77. **Lind, H., H. Jonsson, and J. Schnürer**. 2005. Antifungal effect of dairy propionibacteria - contribution of organic acids. International Journal of Food Microbiology **98**:157-165
78. **Lindahl, R., J. O. Levin, and K. Andersson**. 1993. Determination of volatile amines in air by diffusive sampling, thiourea formation and high-performance liquid chromatography. Journal of Chromatography **643**:35-41
79. **Liu, W., L. Zhang, B. J. Turpin, C. P. Weisel, M. T. Morandi, T. H. Stock, S. Colome, and L. R. Korn**. 2006. Estimating contributions of indoor and outdoor sources to indoor carbonyl concentrations in three urban areas of the United States. Atmospheric Environment **40**:2202-2214
80. **Matisova, E. and S. Skrbakova**. 1995. Carbon sorbents and their utilization for the preconcentration of organic pollutants in environmental samples. Journal of Chromatography A **707:145**-179
81. **Mølhave, B., B. Bach, and O. F. Pedersen**. 1986. Human reactions to low concentrations of volatile organic compounds. Environment International **12**:167-

175

82. **Mølhave, L., G. Clausen, B. Berglund, J. De Ceaurriz, A. Kettrup, T. Lindvall, M. Maroni, A. C. Pickering, U. Risse, H. Rothweiler, B. Seifert, and M. Younes**. 1997. Total volatile organic compounds (TVOC) in indoor air quality investigations. Indoor Air **7**:225-240
83. **National Academy of Sciences**. 2004. Damp indoor spaces and health. Board on health promotion and disease prevention, Institute of medicine. The National Academies Press, Washington, D.C.
84. **Nevalainen, A.** 1991. The indoor air quality in Finnish homes with mould problems. Environment International **17**:299-302
85. **Nevalainen, A. and M. Seuri**. 2005. Of microbes and men. Indoor Air **15**:58-64
86. **NIOSH-method 2011.** 1994. Formic acid. Manual of analytical methods (NMAM) P. M & Cassinelli, M.E (Eds.) (4th ed.). Cincinnati, OH, USA: National Institute for Occupational Safety and Health.
87. **Norbäck, D., G. Wieslander, K. Nordström, and R. Wålinder**. 2000. Asthma symptoms in relation to measured building dampness in upper concrete floor construction, and 2-ethyl-1-hexanol in indoor air. International Journal of Tuberculosis and Lung Disease **4**:1016-1025
88. **Nordin, S., E. Millqvist, O. Löwhagen, and M. Bende**. 2003. The chemical sensitivity scale: Psychometric properties and comparison with the noise sensitivity scale. Journal of Environmental Psychology **23**:357-365
89. **Pappas, G. P., R. J. Herbert, W. Hendersson, J. Koenig, B. Stover, and S. Barnhart**. 2000. The respiratory effects of volatile organic compounds. International Journal of Occupational and Environmental Health **6**:1-8
90. **Pasanen, A.-L., T. Juutinen, M. J. Jantunen, and P. Kalliokoski**. 1992. Occurrence and moisture requirements of microbial growth in building materials. International Biodeterioration and Biodegradation **30**:273-283
91. **Pasanen, A. L., A. Korpi, J. P. Kasanen, and P. Pasanen**. 1998. Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. Environment International **24**:703-712
92. **Prah, J. D., M. W. Case, and G. M. Goldstein**. 1998. Equivalence of sensory responses to single and mixed volatile organic compounds at equimolar concentrations. Environmental Health Perspectives **106**:739-744
93. **Redlich, C. A., J. Sparer, and M. R. Cullen**. 1997. Sick-building syndrome. The Lancet **349**:1013-1016
94. **Rivers, J. C., J. D. Pleil, and J. W. Wiener**. 1992. Detection and characterization of volatile organic compounds produced by indoor air bacteria. Journal of Exposure Analysis and Environmental Epidemiology **suppl.1**:177-188
95. **Ruth, J. H.** 1986. Odor thresholds and irritation levels of several chemical substances: a review. American Industrial Hygiene Association Journal **47**:142-151
96. **Salthouse, T. A. and R. L. Babcock**. 1991. Decomposing adult age differences in working memory. Developmental Psychology **27**:763-776
97. **Samson, R. A.** 1985. Occurrence of moulds in modern living and working environments. European Journal of Epidemiology **1**:54-61
98. **Schleibinger, H., D. Laussmann, C. Brattig, M. Mangler, D. Eis, and H. Ruden**. 2005. Emission patterns and emission rates of MVOC and the possibility for predicting hidden mold damage? Indoor Air **15**:98-104
99. **Schnürer, J., J. Olsson, and T. Börjesson**. 1999. Fungal volatiles as indicators of

food and feeds spoilage. Fungal Genetics and Biology **27**:209-217

100. **Schöller, C., H. Gürtler, R. Pedersen, S. Molin, and K. Wilkins**. 2002. Volatile metabolites from actinomycetes. Journal of Agricultural and Food Chemistry **50**:2615-2621
101. **Schöller, C., S. Molin, and K. Wilkins**. 1997. Volatile metabolites from gram-negative bacteria. Chemosphere **35**:1487-1495
102. **Siegmund, B. and B. Pollinger-Zierler**. 2006. Odor thresholds of microbially induced off-flavour compounds in apple juice. Journal of Agricultural Food and Chemistry **54**:5984-5989
103. **Smeets, M. and P. Dalton**. 2005. Evaluating the human response to chemicals: odor, irritation and non-sensory factors. Environmental Toxicology and Pharmacology **19**:581-588
104. **Stetzenbach, L. D.** 1998. Microorganisms and indoor air quality. Clinical Microbiology Newsletter **20**:157-161
105. **Ström, G., J. West, B. Wessèn, and U. Palmgren**. 1994. Quantitative analysis of microbial volatiles in damp Swedish houses. In Health Implications of Fungi in Indoor Environments. Eds R.A Samson, B Flannigan, M.E. Flannigan, A.P. Verhoeff, O.C.G. Adan, E.S. Hoekstra. Elsevier Amsterdam. 291-305
106. **Sundell, J., B. Andersson, K. Andersson, and T. Lindvall**. 1993. Volatile organic compounds in ventilating air in buildings at different sampling points in the buildings and their relationship with the prevalence of occupant symptoms. Indoor Air **3**:82-93
107. **Sunesson, A. L.** 1995. Volatile metabolites from microorganisms in indoor environments - sampling, analysis and identification. **Ph. D Thesis**:Umeå University, Sweden
108. **Sunesson, A. L., C. A. Nilsson, B. Andersson, and G. Blomquist**. 1996. Volatile metabolites produced by two fungal species cultivated on building materials. Annals of Occupational Hygiene **40**:397-410
109. **Sunesson, A. L., C. A. Nilsson, R. Carlson, G. Blomquist, and B. Andersson**. 1997. Influence of temperature, oxygen and carbon dioxide levels on the production of volatile metabolites from *Streptomyces albidoflavus* cultivated on gypsum board and tryptone glucose extract agar. Annals of Occupational Hygiene **41**:393-413
110. **Sunesson, A. L., I. Rosèn, B. Stenberg, and M. Sjöström**. 2006. Multivariate evaluation of VOCs in buildings where people with non-specific building-related symptoms perceive health problems and in buildings where they do not. Indoor Air **16**:383-391
111. **Sunesson, A. L., W. H. J. Vaes, C. A. Nilsson, G. Blomquist, B. Andersson, and R. Carlson**. 1995. Identification of volatile metabolites from five fungal species cultivated on two media. Applied and Environmental Microbiology **61**:2911-2918
112. **Suutari, M., E. Rönkä, U. Lignell, H. Rintala, and A. Nevalainen**. 2002. Characterisation of *Streptomyces spp*. isolated from water-damaged buildings. FEMS Microbiology Ecology **39**:77-84
113. **Tamas, G., C. J. Weschler, Toftum J, and P. O. Fanger**. 2006. Influence of ozone-limonene reactions on perceived air quality. Indoor Air **16**:168-178
114. **Ten Brinke, J., S. Selvin, A. Hodgson, W. Fisk, M. Mendell, C. Koshland, and J. Daisey**. 1998. Development of new volatile organic compound (VOC) exposure metrics and their relationship to "sick building syndrome" symptoms. Indoor Air 140-152

115. **Tsigarida, E., I. S. Boziaris, and G.-J. E. Nychas**. 2003. Bacterial synergism or antagonism in a gel cassette system. Applied and Environmental Microbiology **69**:7204-7209
116. **Tuomainen, A., M. Seuri, and A. Sieppi**. 2004. Indoor air quality and health problems associated with damp floor coverings. International Archives of Occupational and Environmental Health **77**:222-226
117. **Uchiyama, S., E. Matsushima, S. Aoyagi, and M. Ando**. 2004. Simultaneous determination of C_1 - C_4 carboxylic acids and aldehydes using 2,4-Dinitrophenylhydrazine-impregnated silica gel and high-performance liquid chromatography. Analytical Chemistry **76**:5849-5854
118. **van Thriel, C., A. Seeber, E. Keisswetter, M. Blaszkewicz, K. Golka, and G. A. Wiesmüller**. 2003. Physiological and psychological approaches to chemosensory effects of solvents. Toxicology Letters **140-141**:261-271
119. **Vining, L. C.** 1990. Functions of secondary metabolites. Annual Reviews of Microbiology **44**:395-427
120. **Wady, L. and L. Larsson**. 2005. Determination of microbial volatile organic compounds adsorbed on house dust particles and gypsum board using SPME/GC-MS. Indoor Air **15**:27-32
121. **Wålinder, R., L. Ernstgård, E. Gullstrand, D. Norbäck, P. Venge, and G. Wieslander**. 1999. Acute effects of experimental exposure to four volatile compounds associated with water-damaged buildings and microbial growth. Proceedings of Indoor Air 606-611
122. **Wålinder, R., L. Ernstgård, G. Johanson, D. Norbäck, P. Venge, and G. Wieslander**. 2005. Acute effects of a fungal volatile organic compound. Environmental Medicine **113**:1775-1778
123. **Wargocki, P., J. Sundell, W. Bischof, G. Brundrett, P. O. Fanger, F. Gyntelberg, S. O. Hanssen, P. Harrison, A. Pickering, O. Seppänen, and P. Wouters**. 2002. Ventilation and health in non-industrial indoor environments: report from a European multidisciplinary scientific consensus meeting (EUROVEN). Indoor Air **12**:113-128
124. **Wargocki, P., D. P. Wyon, Y. K. Baik, G. Clausen, and P. O. Fanger**. 1999. Perceived air quality, sick building syndrome (SBS) symptoms and productivity in an office with two different pollution loads. Indoor Air **9**:165-179
125. **Weschler, C. J.** 2001. Reactions among indoor pollutants. The Scientific World **1**:443-457
126. **Weschler, C. J.** 2004. Chemical reactions among indoor pollutants: what we've learned in the new millennium. Indoor Air **14**:184-194
127. **Weschler, C. J. and H. C. Shields**. 1997. Potential reactions among indoor pollutants. Atmospheric Environment **31**:3487-3495
128. **Weschler, C. J., J. R. Wells, D. Poppendieck, H. Hubbard, and T. A. Pearce**. 2006. Workgroup report: Indoor chemistry and health. Environmental Health Perspectives **114**:442-446
129. **Wessén, B. and K. O. Schoeps**. 1996. Microbial Volatile Organic Compounds-What substances can be found in sick buildings? Analyst **121**:1203-1205
130. **Wessén, B., G. Ström, U. Palmgren, K. O. Schoeps, and M. Nilsson**. 2001. Analysis of microbial volatile organic compounds. In: Microorganisms in home and indoor work environments. Diversity, health impacts, investigation and control. Eds. B Flannigan, R.A Samson, Amsterdam, Elsevier 267-274

131. **Wilkins, C. K., P. A. Clausen, P. Wolkoff, T. S. Larsen, M. Hammer, K. Larsen, V. Hansen, and G. D. Nielsen**. 2001. Formation of strong airway irritants in mixtures of isoprene/ozone and isoprene/ozone/nitrogen dioxide. Environmental Health Perspectives **109**:937-941
132. **Wilkins, K.** 1996. Volatile metabolites from actinomycetes. Chemosphere **32**:1427-1434
133. **Wilkins, K. and K. Larsen**. 1995. Variation of volatile organic compound patterns of mold species from damp buildings. Chemosphere **31**:3225-3236
134. **Wilkins, K., K. Larsen, and M. Simkus**. 2000. Volatile metabolites from mold growth on building materials and synthetic media. Chemosphere **41**:437-446
135. **Wold, S., C. Albano, W. J. Dunn, U. Edlund, K. Esbensen, P. Geladi, S. Hellberg, E. Johansson, W. Lindberg, and M. Sjöström**. 1984. Multivariate data analysis in chemistry. In: Chemometrics: Mathematics and Statistics in Chemistry, (Ed. B R Kowalski) **D. Riedel Publishing Company, Dordrecht, Holland**:
136. **Wold, S. and M. Josefson**. 2000. Multivariate calibration of analytical data. Encyclopedia of Analytical Chemistry 1-27
137. **Wold, S., M. Sjöström, and L. Eriksson**. 1999. PLS in chemistry. In: The Encyclopedia of Computational Chemistry (Eds: Schleyer, P v R; Allinger, N L; Clark, T; Gasteiger, J; Kollman, P A; Schaefer III, H F; Schreiner, P R) John Wiley & Sons, Chichester:2006-2020
138. **Wolkoff, P.** 1999. How to measure and evaluate volatile organic compound emissions from building products. A perspective. The Science of the Total Environment **227**:197-213
139. **Wolkoff, P., P. A. Clausen, B. Jensen, G. D. Nielsen, and C. K. Wilkins**. 1997. Are we measuring the relevant indoor pollutants? Indoor Air **7**:92-106
140. **Wolkoff, P. and G. D. Nielsen** 2001. Organic compounds in indoor air - their relevance for perceived indoor air quality? Atmospheric Environment **35**:4407-4417
141. **Wolkoff, P., C. K. Wilkins, P. A. Clausen, and G. D. Nielsen**. 2005. Organic compounds in office environments - sensory irritation, odor, measurements and the role of reactive chemistry. Indoor Air 1-13
142. **World Health Organization.** 1989. Indoor air quality: organic pollutants. EURO reports and studies No.111 Copenhagen
143. **World Health Organization (WHO)**. 1986. Indoor air quality and research. Euro Reports and Studies 103
144. **Wright, J. L. C. and L. C. Vining**. 1976. Secondary metabolites derived from non-aromatic amino acids. Filamentous Fungi **2**:475-502
145. **Wyon, N. M. and D. P. Wyon**. 1987. Measurement of acute response to draught in the eye. Acta Ophtalmologica **65**:385-392
146. **You, J., W. Zhang, Q. Zhang, L. Zhang, C. Yan, and Y. Zhang**. 2002. Development of a precolumn derivatization method for the determination of free amines in wastewater by high-performance liquid chromatography via fluorescent detection with 9-(2-hydroxyethyl)acridone. Analytical Chemistry **74**:261-269
147. **Yu, C. and D. Crump**. 1998. A review of the emission of VOCs from polymeric materials used in buildings. Building and Environment **33**:357-374
148. **Zhang, J., Q. He, and P. J. Lioy**. 1994. Characteristics of aldehydes: concentrations, sources, and exposures for indoor and outdoor residential microenvironments. Environmental Science & Technology **26**:146-152
149. **Zhang, J., W. E. Wilson, and P. Lioy**. 1994. Indoor air chemistry: formation of

organic acids and aldehydes. Environmental Science & Technology **28**:1975-1982

150. **Zurek, G. and U. Karst**. 2000. 2,4-Dinitro-3,5,6-trideuterophenylhydrazones for the quantitation of aldehydes and ketones in air samples by liquid chromatography-mass spectrometry. Journal of Chromatography A **869**:251-259

APPENDIX I

MVOCs identified from building materials with known microbial species. The numbers (1-7) represent different studies listed below. 1=(108) W=pine wood, G=Gypsum board+mineral wool, 2=(36) F=Fiberglas, 3=(134) G=Gypsum board, C=Cardboard mat, 4=(11) W=Pine wood, 5=(67) C1= Gypsum board covered with wallpaper and plastic film, C2=Chipboard and glass wool, C3=Ceramic tile attached to aggregate block, 6=(37) CW=Conifer wood, B=Beech wood, 7=(109) G=gypsum board.

	1	***2***	***3***	***4***	***5***	***6***	***7***
COMPOUNDS							
Hydrocarbons							
1-Heptene			C				
1-(1,1-Dimethylethyl)-4-ethylbenzene	G						
1,3-Octadiene			C			CB,B	
1,3,6-Octadiene isomers						CB	
1,3,5-Heptatriene						CB,B	
2,6-Dimethyl-2,4,6-octatriene						B	
3-Methyl-1-heptene						CB	
Benzene		F					
Dimethylhexadiene			C				
Octadiene isomer			C				
Pentane		F					
Methyl benzene		F					
Xylene		F				CB	
Alcohols							
1-Butanol			G				
1-Hexanol	W				C1,2,3		
1-Octen-3-ol	G		G,C	W	C1	CB,B	
1-Octanol							
1-Pentanol			C		C2		
2-Butanol			C				
2-Ethyl-1-hexanol	G	F				B	G
2-Methyl-1-butanol	G,W						
2-Methyl-1-propanol	G,W		G,C				G
2-Heptanol			G				
2-Hexanol			G				
2-Nonanol			G				
2-Octanol			G				
2-Propanol			C				
2,6-Dimethylphenol						B	
3-Methyl-1-butanol	G,W			W	C1,2		
3-Methyl-2-butanol					C1		

APPENDIX I

3-Pentanol			C				
6-Methyl-heptanol						B	
Ethanol		F					
Trimethylhexanol						B	
Ketones							
2-Butanone	G,W				C3	CB,B	
2-Heptanone	W		G	W		CB,B	
2-Hexanone	W		G,C		C3	CB,B	
2-Nonanone			G				
2-Octanone			G			CB,B	
2-Pentanone	W		G		C3	CB,B	
3-Methyl-2-butanone							G
3-Methyl-2-pentanone	W				C3		G
3-Octanone	G		G		C2	B	
3-Pentanone			C				
4-Heptanone						B	
Acetone	W				C1	CB,B	
Acetophenone						B	
Ethers							
1-Methoxy-3-methylbutane	G,W						
1,3-Dimethoxy benzene						CB	
2,3,5-Trimethylfuran	G						
2,5-Dimethylfuran	W						
3-Methylfuran	G		C				
3-Methylanisole	G					CB	
4-Allylanisole				W			
4-Ethylanisole						B	
Anisole			G,C				
Dimethylanisole						CB	
m-Methylanisole			G				
o-Methylanisole			G				
Esters							
2-Methylpropionate			G				
Ethyl acetate							
Methyl acetate	W						
Methyl benzoate			C				
Propyl acetate	W						
Sulfur compounds							
Dimethyl disulfide	G		C				
Dimethyl trisulfide	G						

APPENDIX I

Terpenes and terpene derivatives							
1-Methoxy-4-(1-methylethyl)-benzene	G						
α-Pinene			C				
β-Pinene			C				
β-Myrcene						CB	
β-Farnesene						B	
m-Cresol						CB	
Geosmin	G						G
Isoprene			C			B	
Limonene		F			C1	CB	
Monoterpene ($C_{10}H_{16}$)	G						
Monoterpenol ($C_{10}H_{16}O$)	G						
Sesquiterpene ($C_{15}H_{24}$)	G					CB,B	G
Sesquiterpenol ($C_{15}H_{24}O$)	G						
Terpenoid compound ($C_{13}H_{20}$)	G						
Terpenoid compound ($C_{13}H_{20}$)	G						
Terpinen-4-ol						CB	
Others							
Arsenous acid		F					
Cyclotrisiloxane		F					
Methylpyrazine						CB,B	
Pyridine						CB	

APPENDIX II

Microbially produced compounds from cultures grown on building materials and MEA or TGEA in Paper 1 and 2 (X=mould mixture, S=Streptomyces, Q=Qualifier (MS library fit) in %, R=Reference compound). Numbers within brackets=retention time.

	Pinewood	***Particle board***	***Gypsum board***	***MEA/ TGEA***	***Q/R***
COMPOUNDS					
Hydrocarbons					
1-Hexene	X	X			94
1-Methyl-4-1,4-cyclohexadiene	S				96
1-Methyl-4-(1-methylethyl)-benzene			X, S		97
1-Octene		X			93
1,2,4-Triethyl-benzene				S	89
1,2,4,4-Tetramethylcyclopentene		X			91
2-Methyl-1,3-butadiene (Isoprene)		X		S	R
2,4-Dimethyl-heptane				X	83
2,5 Dimethyl-2,4-hexadiene				X	80
3-Ethylidene-1-methyl-cyclopentene				X	91
4-Methyl-octane				X	81
Ethylbenzene				X	R
Decahydro-2-methyl-naphtalene			S		97
Decahydro-naphtalene			S		97
Dodecane			S		95
Hexane		X			90
Heptane		X			R
Isopropenyltoluene			S		95
Nonane		X			R
Octane		X			94
Styrene	X				R
Tridecane			S		97
Undecane			S		95
Alcohols					
1-Butanol	S	S	S	S	90
1-Heptanol	X				90
1-Hexanol	X,S	X	S		R
1-Octen-3-ol	X		X	X	R
1-Pentanol			X,S		R
1-Penten-3-ol	S				R
1-Propanol			S		83
2-Ethyl-1-hexanol	S				R
2-Heptanol	X,S				90
2-(2-Propenyl)-phenol				S	90

2,3-Dimethyl-2-butanol	S				83
2,4,5-Trimethyl-phenol			S		80
2-Methyl-1-butanol	X		X,S	X,S	R
2-Methyl-1-propanol	X,S		X,S	X,S	R
2-Methyl-2-propanol	S				83
2-Methyl-3-buten-2-ol	X	X			91
3-Methyl-1-butanol	X,S		X,S	X,S	R
3-Methyl-2-butanol	X,S				83
3-Methyl-2-buten-1-ol	X			S	90
3-Methyl-3-buten-1-ol				X,S	R
3-Octanol				X	R
4-Methyl-1-hexanol	X				83
4-Methyl-1-3-cyclohexen-1-ol			X		96
Benzyl alcohol				S	97
Benzenemethanol			S		93
Ethanol	S	S			R
Isopropylalcohol		S			86
Phenylethylalcohol				S	95
Ketones					
2-Butanone	X,S	X	S	X,S	R
2-Dodecanone			S		86
2-Heptanone	X	X	S		R
2-Hexanone	X,S	X			R
2-Methyl-5-1-cyclohexanone	X				96
2-Nonanone	X,S				97
2-Octanone	X		X,S	X	93
2-Pentanone	X,S	X			R
2-Methyl-5-1-cyclohexanone	S				96
2,4-Pentanedione				X	86
2,6,6-Trimethyl-bicyclo-heptan-3-one		X			90
3-Cyclohepten-1-one				X	91
3-Hexanone			S		80
3-Hydroxy-2-butanone			S		86
3-Methyl-2-butanone			S	S	90
3-Methyl-2-pentanone	X		S	X,S	R
3-Octanone	X			X	R
4-Methyl-2-hexanone	X,S			S	91
5-Methyl-2-heptanone				S	91
5-Methyl-2-hexanone				S	91
6-Methyl-2-heptanone				S	91
Acetone			X	X	R
Cyclopentanone	X			X,S	R
Pulgone			X		91

APPENDIX II

Sulfur compounds					
2,4-Dithiapentene				S	90
Dimethyl disulfide	X	X	X,S	X,S	R
Dimethyl sulphide				X,S	80
Dimethyl trisulfide				S	96
Diphenylsulfone				X	96
Methanethiol				S	90
Methyl-methanothiosulfonate				S	96
Methyl-(methylthio)disulfide				S	96
S-Methyl-3-methylbutanethioate				S	80
S-Methyl-butanethioate				S	80
S-Methyl-ethanethioate				S	91
Ethers					
1-Ethyl-4-methoxy-benzene				X	91
1-Methoxy-3-methyl-benzene				X	95
2-Pentylfuran		S	S	X	91
2,5-Dimethylfuran	X				R
3-Methylfuran	X	X	S	X	R
Esters					
Acetic acid, pentyl ester	X				86
Ethyl acetate	S			S	90
Ethyl isobutyrate				S	94
Ethyl phenylacetate				S	87
Ethyl propionate				S	80
Methyl acetate			X		R
Propanoic acid ester (Cas:74367-33-2)			X		64
Propanoic acid ester (Cas:74367-34-3)			X		90
n-Propyl acetate	X,S				R
Nitrogen compounds					
2-Furancarbonitrile				S	95
2-Methylpyridine		X			R
Ammonia		X		S	R
Diethylamine				S	R
Ethylamine				S	R
Hexanenitrile		X			R
Methylamine				S	R
Nitromethane		X			R
Pyridine		X			R
Terpenes					
1,3,3-Trimethyl-tricycloheptane			X		91

2-Carene	S			X	98
3-Carene			X,S		R
3-Thujen-2-ol			X		78
Borneol			X,S		R
Camphene			X		R
(+)-Epi-bicyclosesquiphellandrene				X	90
S(-)-Limonene			X	X	R
α-Pinene			X,S		R
D-Verbenone			X,S		93
Sesquiterpene ($C_{15}H_{24}$) (22.78)	S				99
Sesquiterpene ($C_{15}H_{24}$) (23.75)				S	90
Sesquiterpene ($C_{15}H_{24}$) (23.81)				S	90
Sesquiterpene ($C_{15}H_{24}$) (23.95)			S	S	91
Sesquiterpene ($C_{15}H_{24}$) (24.26)	S		S	S	90
Sesquiterpene ($C_{15}H_{24}$) (24.62)	S		S	S	80
Sesquiterpene ($C_{15}H_{24}$) (24.74)				S	80
Sesquiterpene ($C_{15}H_{24}$) (25.04)	S			S	86
Sesquiterpene ($C_{15}H_{24}$) (25.14)				S	93
Sesquiterpene derivative ($C_{15}H_{22}$) (24.44)				S	96
Sesquiterpene derivative ($C_{12}H_{20}$) (20.64)				S	90
Trans-1,10-dimethyl-trans-9-decalol (Geosmin) (23.64)				S	R
Acids					
2-Ethyl-hexanoic acid				X	91
Acetic acid				X	R
Hexanoic acid			X	X	83
Octanoic Acid				X	90
Others					
1,3,5-Trioxepane		X			83
2-Butyl-2-octenal			X		96
2-Ethyl-hexanal			S		90
2-Methyl-propionic acid	S				90
2,3,5,6-Tetramethyl-p-benzoquinone				X	81
Dimethoxymethane		X			R