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PERSONAL EXPOSURE TO MICROBIAL AEROSOLS

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ACADEMIC DISSERTATION

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ABSTRACT

At present very little is known about total exposures to bioaerosols. Short-time stationary samples correlate poorly with the health effects and probably represent, at best, only surrogates of the true exposure, because during a single day, an individual will be exposed to different concentrations of bioaerosols in several environments. It is necessary to understand better this phenomenon if we are to pinpoint determinants and find associations between exposure and health effects.

A random sample of 81 teachers was selected from 823 teachers working in two municipalities in Eastern Finland for the wintertime measurement period (1998 - 1999). Bioaerosol and other particles were collected on filters using personal sampling and microenvironmental measurements in homes and at work. Particle mass, black smoke, viable and total microorganisms were analysed from each filter. The 24-hour sampling period was repeated twice. Questionnaires of home and workplace characteristics and events during the sampling period were filled in after the measurements. The homes and working places were also inspected for visible signs of moisture by a civil engineer.

The personal particle mass (57 $\mu g/m^3$), total fungi (12200 spores/m^3) and viable fungi (33 cfu/m^3) mean concentrations were higher than home (17 $\mu g/m^3$, 10800 spores/m^3 and 30 cfu/m^3) or workplace (34 $\mu g/m^3$, 12000 spores/m^3 and 19 cfu/m^3) concentrations. Personal (1.10×10^{-5} 1/m) and work (1.12×10^{-5} 1/m) BS concentrations were higher than the home (0.67×10^{-5} 1/m) concentration. Total and viable bacteria concentrations in workplace (145000 cells/m^3, 1090 cfu/m^3) were higher than the personal exposure (86400 cells/m^3, 715 cfu/m^3) or home concentrations (60600 cells/m^3, 338 cfu/m^3). Personal exposure mass concentrations were only moderately correlated with home or work concentrations ($r=0.3$, $p<0.001$) while black smoke concentration of personal and home/work filters showed better correlations ($r=0.6$-0.7, $p<0.001$). There was no correlation between personal and home/work fungal concentrations.

Those samples with higher viable fungal concentrations also had a higher diversity of fungi than the samples with lower concentrations. The concentration ratio of viable fungi and bacteria counts to total counts of fungi and bacteria was close to 1:100. The estimated mass concentrations of total fungi and bacteria were less than 1% of the total particle mass concentration. Variation in the concentration of *Penicillium* explained between 25 up to 95 % of the variations of viable fungal concentration in personal exposure, home and workplace environment. There was an association between personal exposure and home concentration of viable fungi and between personal exposure and home and work concentrations of viable bacteria. However, the results also indicate that observation of a certain fungus in a main microenvironment does not necessarily coincide with findings from personal exposure samples.

There were several determinants (such as behaviour, traffic or building factors) contributing to personal exposure and increasing home and workplace concentrations of particles and microbes. There were common determinants for both personal exposure and microenvironments such as teaching subjects, condensation on window, ventilation and traffic, which point to the presence of potential pollutant sources in these microenvironments.

Personal exposure measurements of bioaerosols in indoor environments are feasible ways to assess the real exposure to bioaerosols. In addition to personal bioaerosol exposure, stationary sampling in main microenvironments is often needed to determine the extent to which the microenvironment influences the measurements of total exposure and to design cost effective strategies to reduce exposure and related health effects.

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Kuopio, October 2004 Mika Toivola

ABBREVIATIONS

AODC	Acridine orange direct counts
BS	Black smoke
CFU	Colony forming unit
DG18	Dichloran 18% glycerol agar
d_{pa}	Aerodynamic diameter (µm)
DNA	Deoxyribonucleic acid
EC	Elemental carbon
GM	Geometric mean
IgG	Immunoglobulin G
IL	Interleukin
MEA	2% malt extract agar
MEM	Microenvironment monitor
NO	Nitric oxide
ODTS	Organic dust toxic syndrome
PEM	Personal monitor
PM	Particular matter in air
PM_1	Particulate matter in air with a 50% cut-off aerodynamic diameter of 1 µm
$PM_{2.5}$	Particulate matter in air with a 50% cut-off aerodynamic diameter of 2.5 µm
PM_{10}	Particulate matter in air with a 50% cut-off aerodynamic diameter of 10 µm
PVC	Polyvinyl chloride
QA	Quality assurance
SOP	Standard operation procedure
TNFα	Tumor necrosis factor alpha
TYG	Tryptone yeast glucose agar

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four original articles, which are referred to in the text by the Roman numerals.

I Toivola M., Alm S., Reponen T., Kolari, S., Nevalainen A. 2002. Personal exposures and microenvironmental concentrations of particles and bioaerosols. Journal of Environmental Monitoring 4:166-174.

II Toivola M., Nevalainen A., Alm S. Personal exposure to particles and microbes in relation to microenvironmental concentrations. Indoor Air (in press).

III Toivola M., Alm S., Nevalainen A. 2004. Viable fungi and bacteria in personal exposure samples in relation to microenvironments. Journal of Environmental Monitoring 6:113-120.

IV Toivola M., Alm S., Roponen M., Hirvonen M.-R., Nevalainen A. Determinants of personal exposure to particles and microbial aerosols. (Manuscript).

CONTENTS

1 INTRODUCTION

Exposure to particle material in air has been claimed to evoke many adverse health effects. However, the concept of exposure is a complex one. Particles include organic, inorganic and biological material and they may originate from various sources such as soil, vegetation, combustion processes, traffic and industrial sources. Particles from animals, plants, microorganisms and soil derived material are usually called bioaerosols. How, where and when an individual becomes exposed to particles and bioaerosols are basic issues if one wishes to understand the causal connections between exposure and subsequent health effects.

Many adverse health effects associated with airborne particles have been reported in many contexts; for example the link between exposure to outdoor air particles and increased mortality and ischemic heart disease (Dockery and Pope 1994, Schwartz et al. 1996). Long-term exposure to combustion-related fine particulate air pollution is an important environmental risk factor for cardiopulmonary and lung cancer mortality (Pope et al. 2002). Several health disorders and respiratory diseases, *e.g.* allergic alveolitis, asthma and organic dust toxic syndrome (ODTS) have been associated with exposure to organic dust in work environments, such as farms, remedial work and sawmills (Lacey and Crook 1988, Lacey and Dutkiewicz 1994) where airborne microbe concentrations are usually high. Respiratory health effects like wheeze, cough and asthma have been associated with building-related moisture damage in residential, school and office environments (Waegemakers et al. 1989, Dales et al. 1991, Dekker et al. 1991, Brunekreef et al. 1992, Spengler et al. 1994, Peat et al. 1998, Bornehag et al. 2001) where airborne microbial concentrations are usually low, only 0.01-10% of those found in dusty work environments. Also non-respiratory symptoms have been reported in moisture damaged buildings (Husman 1996). Although many types of particulate pollutants have been linked with various health effects, there is still insufficient knowledge about the causative agents involved.

One reason preventing a deeper understanding of the role of fungal and bacterial particles as causative agents in non-infectious symptoms is the insufficient knowledge about the personal exposure to these particles as well as its temporal and spatial variations. The exposure to bioaerosols has usually been assessed by stationary sampling. However, bioaerosol concentrations vary in time and space, and an individual will be moving around from one microenvironment to another, spending various periods of time in each of them. There is very limited information available on the personal exposure to microbes collected with sampling from an individual's

breathing zone or with portable monitors which would better mimic the actual exposure of an individual than can be obtained with stationary sampling.

2 REVIEW OF THE LITERATURE

2.1 Exposure assessment

Exposure to a pollutant is defined as an event during which a person comes into contact with the pollutant of interest (Ott 1982, Ott 1995, Nieuwenhuijsen 2003). The contact may take place between the substance in an environmental medium (such as air, water, soil, food) and the surface of the human body such as skin or respiratory tract. The chain of events starts from the source of a pollutant and ends as the dose causing a health effect as illustrated in Fig. 1. According to this definition, exposure has units of concentration and time. After uptake of a substance into the body, it is referred to as the dose (Fig. 1). Dose and dose-response are outcomes of an exposure, because there is no dose without exposure (Ott 1982).

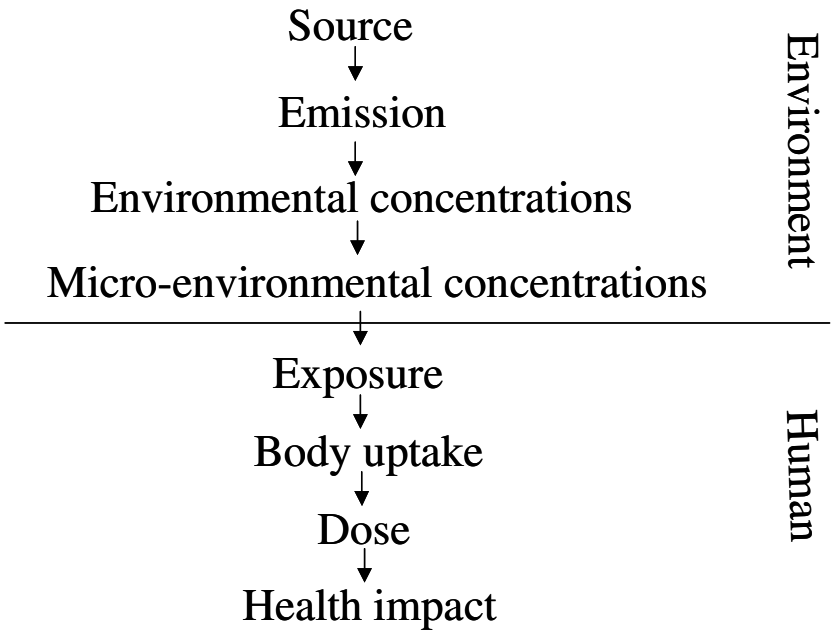

Fig. 1. Exposure model to air pollutants (Adopted from Nieuwenhuijsen 2003).

Quantification of exposure, *i.e.* exposure assessment has been described as one of the main components of environmental health risk assessment in conjunction with hazard identification and dose-response assessment (NRC 1983).

There are three general types of measurements aiming at exposure assessment; direct (personal), indirect (microenvironmental and questionnaires) and biological monitoring (Lioy 1990). Microenvironments are well-defined surroundings *e.g.* home, office, automobile that can be treated as homogenous or well characterized with respect to the concentration of some pollutant or other agent (Duan 1982, EPA 1992). Personal exposure measurements of airborne particles have been conducted in several studies (Wallace 1996, Janssen et al. 1998, Koistinen et al. 1999), while microbial exposure assessments have been based generally on microenvironmental samples (Pasanen 2001, Meklin et al. 2002). Personal exposure to microbes has been assessed mainly in work environments, *e.g.* during remediation of moldy structures (Rautiala et al. 1998). Microbial exposure has also been assessed by combining microenvironmental measurements with biological monitoring and by determination of microbe specific IgG antibodies in exposed individuals (Erkinjuntti-Pekkanen et al. 1999, Hyvärinen et al. 2003, Patovirta et al. 2003). However, the correlation between IgG antibodies and microbial findings in environmental samples has not been especially good (Hyvärinen et al. 2003). This may be largely due to methodological problems both with antibody measurements and with microbial measurements based only on culturing. One important target for microbial exposure assessment is an indoor environment known or suspected to have moisture and mould damage. Air samples, dust, surface and material samples are recommended to be used for environmental monitoring (Pasanen 2001). There is no single method to determine comprehensively microbial growth and microbial exposure. The spatial and temporal variation in the numbers of airborne microbes is large (Hunter et al. 1988, Pasanen et al. 1992, Hyvärinen et al. 2001a) and thus to avoid the problems arising from these variations, house dust samples have been used to assess fungal exposures because of the ease of sampling and their representativeness for long-term exposure (Dillon et al. 1996, Flannigan 1997, Verhoeff and Burge 1997). However, little is known about the inhalation exposure to possible causative agents of microbes that may be responsible for the large variety of health effects observed in the epidemiological studies.

2.2 Bioaerosols and their sources

According to Baron and Willeke (2001) an aerosol is an assembly of liquid or solid particles suspended in a gaseous medium like air long enough to be observed and measured. Aerosols include bioaerosols, which are defined as particles that are living or originate from living organisms (ACGIH 1999). Bioaerosols contain a heterogeneous mixture of particles from plant, animal and microbial origin (Dillon et al. 1996). The particle size distribution of bioaerosols is wide: from small viruses (20-300 nm) up to bacterial cells (0.5-30 µm), fungal spores (1.5-30 µm) and some of the larger pollen grains (over 0.1 mm) (Reponen et al. 2001). In this work, fungi and bacteria are considered as the main types of bioaerosols. In indoor environments, bioaerosols may include also particles from house dust mites, cockroaches and other insects, and skin scales from humans and pets (Flannigan 2001).

The main source of indoor air fungi is usually outdoor air (Burge 1990, Levetin et al. 1995). Fungal spores are always present in the outdoor air, with natural soil and dead plant material being the main sources. Season has an impact on the numbers of fungal propagules present in the indoor air (Ren et al. 2001). During summer and autumn one is more likely to detect higher fungal concentrations than during spring and winter. In the Nordic countries, the snow cover on the ground reduces the concentrations in wintertime thus the outdoor air has virtually no influence on the indoor air mycoflora during the winter (Reponen 1992b).

Human activities like handling of firewood and foodstuffs, cleaning and other household activities may temporarily increase fungal concentration in the indoor air (Hunter et al. 1988, Lehtonen et al. 1993). Also the presence of pets and bedding materials used by pets may increase fungi in indoor air (Lehtonen et al. 1993, DeKoster and Thorne 1995, Ren et al. 2001). Fungal spores can be carried indoors on clothes from highly contaminated environments such as cow barns (Pasanen et al. 1989, Burge 1990). In some cases, such as firewood, it may be a question of a true source of bioaerosol particles, and in other cases such as cleaning, the increase in airborne concentration may be a result of resuspension of previously settled particles.

Moisture and mould problems in buildings have been reported to increase the indoor air concentration of fungi (Pasanen et al. 1992, Hyvärinen et al. 1993, Miller et al. 2000, Meklin et al. 2003). However, water damage or observation of mould growth have not necessary been related to the measured viable fungi in indoor air (Strachan et al. 1990, Ren et al. 2001). Pasanen et al. (1992)

have reported that moisture problems in the urban environment do not necessarily increase total viable spore concentration, but have an impact on the composition of fungal flora. Temporal and spatial variations in the concentration of fungi have been detected by short time sampling methods (Ross et al. 2000, Hyvärinen et al. 2001a, Ren et al. 2001, Herbarth et al. 2003).

Location, frame type and age of the building have been found to influence the concentration of indoor fungi. Fungal concentrations are lower in the urban/suburban residences than in the rural residences (Pasanen et al. 1992). The building materials used in the building frame have an effect on the indoor air fungal levels, since higher airborne fungal concentrations have been detected in wooden school buildings than in concrete school buildings (Rand et al. 1999, Ellinger et al. 2000, Meklin et al. 2003). Furthermore, microbes accumulate over time in a building and the age of a residential building has been shown to gradually increase the concentration of airborne fungi (Pasanen et al. 1992). However, Meklin et al. (2003) did not find any significant association between concentrations of airborne fungi and the age of the school buildings which clearly differ from residences in activities, size and in furnishings.

2.3 Particles and black smoke

The US Environmental Protection Agency (EPA) has defined four terms for categorizing particles of different sizes: ultrafine $d_{pa} \leq 0.1$ µm, fine 0.1 µm< $d_{pa} \leq 2.5$ µm, coarse 2.5 µm < $d_{pa} \leq 10$ µm and supercoarse $d_{pa} > 10$ µm (EPA 2002). In Fig. 2, the sizes of the various particles are illustrated. The term PM_{10}, called "coarse particles" includes particles having an aerodynamic diameter of less than or equal to 10 micrometers, and $PM_{2.5}$, called "fine particles", includes particles having an aerodynamic diameter of less than or equal to 2.5 micrometers. The shape and density of the particle affect its aerodynamic diameter and thus the aerodynamic diameter characterizes the filtration, respiratory deposition and separation of the particle (Hinds 1999).

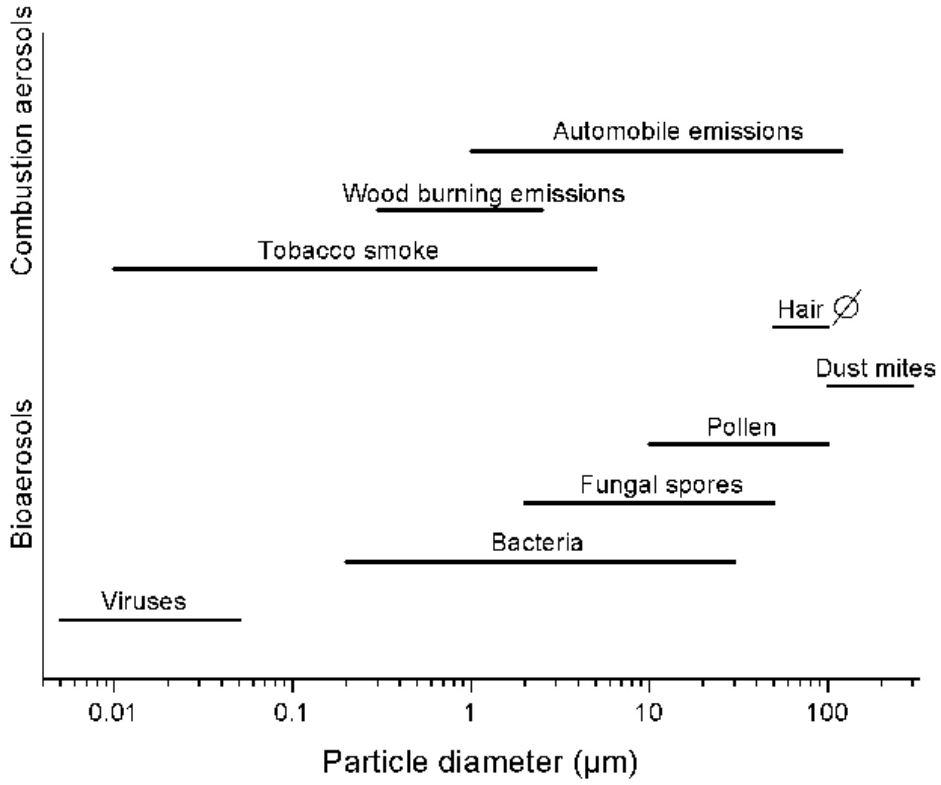

Fig. 2. Sizes of some indoor particles (Adapted from Owen et al. 1992).

Instead of using the terms “fine” and “coarse” particles, the terms “inhalable”, “thoracic” and “respiratory” particles are used in occupational hygiene. The 50% cut-off diameter for the thoracic fraction is 10 µm and for the respirable fraction it is 4 µm (CEN 1993). The inhalable fraction of total airborne mass fraction consists of particles that are inhaled through the nose and mouth. Particles greater than approximately 50 µm in diameter can enter the nose and mouth, and particles >10 µm are deposited on the ventilation pathway surfaces above the trachea. Fine particles gain entry to the alveolar region of the lungs (Rodes and Wiener 2001).

The fine particles measured in the Helsinki area consist mainly of sulphate (21%), nitrate (12%), ammonium (9%) and other material (43%). Coarse particles mainly originate from crustal matter (59%) (Pakkanen et al. 2001). The black smoke (BS) mainly consists of carbon or soot particles generated in combustion processes, such as energy production and car engines (Gray and Cass 1998, Kinney et al. 2000) and it is a marker component for local traffic (Vallius et al. 2000, Pakkanen et al. 2000).

2.4 Sources and determinants of particle exposure

In indoor air, cigarette smoking has been reported to be the most important source of fine and coarse particles (Wallace 1996). The estimated particle concentration increases in homes with smokers are between 25 and 45 μg/m^3. Cooking has been identified as the second most important particle source (Wallace 1996, Özkaynak et al. 1996). Also particle matter from the outdoor air may penetrate into indoor air (Long et al. 2001). Furthermore, there are other significant sources of particles that are not so well known. In several studies, it has been reported that personal exposure to particles is higher than the indoor or outdoor concentrations of particles measured with stationary samplers (Wallace 1996). This finding has been explained partly by the so-called "personal cloud" (Rodes et al. 1991, Wallace 1996, Janssen et al. 2000). During the day, when individuals are active, the particle increase can be as high as 50%. It has been suggested that the personal cloud mainly consists of coarse particles which can more easily resuspend than fine particles (Thatcher and Layton 1995, Wallace 1996, Luoma and Battermann 2001).

Instead, in outdoor air, the major sources of fine particles are fossil fuel combustion, vegetation burning and processing of metals (Holman 1999). Traffic is an important source of both fine particles and coarse particles in urban areas due to the road dust lifted by the traffic and wind (Pakkanen et al. 2001). While fossil fuel combustion is a major source of black smoke (BS) emission (Blakemore et al. 2001), traffic has been shown to be the most important local black carbon source (Pakkanen et al. 2000, Vallius et al. 2000). In many European cities, over 90 % of elemental carbon (EC) originates from traffic sources (Hamilton and Mansfield 1991). In particular, diesel engines are known to emit EC (Watson 1994, Kerminen et al. 1997). It has also been shown that sources for fine particles tend to be regional in nature, but fine particles are also capable of travelling long distances (Chow et al. 1994). The coarse particles are mainly fugitive dust from industry, agriculture, construction and demolition and fly ash from fossil fuel combustion (Holman 1999).

2.5 Sampling methods of particles and microbial aerosols

The physical principles utilized in the sample collection techniques for microbes and other particles are similar. The methods used in airborne microbe sampling are mainly based on filtration, impaction or liquid impingement (Willeke and Macher 1999, Reponen et al. 2001). Filters, impactors, virtual impactors and cyclones are widely used in the sampling of particles (Lee and

Mukund 2001, Marple et al. 2001). Cascade impactors were developed in the 1940s, and they are still commonly used in microbiological measurements in home and work environments (Nevalainen et al. 1992, Reponen et al. 1994, Seuri et al. 2000, Wu et al. 2000a). Inertial classification of particles in impactor samplers is achieved by turning the gas flow and capturing the particles with sufficient inertia to cross gas streamlines and to escape the flow impinging on a plate (*e.g.* growth media) or to penetrate into the collection probe (Marple et al. 2001). The Andersen 1, 2 - or six-stage impactors are widely used impactor samplers, which collect microbes directly onto growth medium (Andersen 1958). The impinger and filter samples need to be cultivated separately on growth media (Palmgren et al. 1986, Kenny et al. 1999, Lin et al. 1999, Lin et al. 2000). The collection of the microbes directly onto the cultivation plate provides more a gentle method if one wishes to count viable microbes than collection on the filter. During the sampling, the desiccation effect due to airflow may be decrease the viability of microbes (Wang et al. 2001).

Microbial sampling with filtration has been widely used, especially in stationary and personal monitoring in heavily contaminated work environments (Palmgren et al. 1986, Karlson and Malmberg 1989, Blomquist 1994, Heldal et al. 1996, Rautiala et al 1998, Rautiala et al. 2003). Filtration has also been used in other particle monitoring devices in indoor and outdoor air (Fischer et al. 2000, Janssen et al. 2000, Götschi et al. 2002). Filter samplers usually contain a body, which includes a filter holder and an inlet section, which can control the aspiration flow rate on sampling and size distribution of collected particles. In exposure measurements in the work environments, several types of filter samplers have been used. The most commonly used filter samplers have traditionally been 37 mm plastic filter cassette with a closed face and an open face, the IOM personal inhalable sampler, the seven hole personal sampler, the GSP personal sampler, the PAS-6 personal sampler, the PERSPEC personal sampler and the CIP10-I personal sampler (Vincent 1995, Kenny et al. 1997). IOM and GSP samplers were reported to have the best accuracy and precision of those samplers (Kenny et al. 1997). Furthermore, a button sampler has been designed in the University of Cincinnati. This sampler is based on the aerodynamic properties of a bluff body which allows smooth flow over its surface in a fast moving wind (Kalatoor et al. 1995, Aizenberg et al. 1998). This means that the sampling efficiency of the button sampler follows well the inhalability convention even in windy conditions (Aizenberg et al. 2000).

In Table 1, examples of personal samplers and sampling studies are listed. Although personal dust exposure has commonly been measured in work environments (Ogden et al. 1993, Tsai et al. 1996, Nieuwenhuijsen et al. 1999, Nielsen et al. 2000, Harper et al. 2002, Harper et al. 2004) the

applications of microbial personal sampling have been rather limited (Milton et al. 1996, Rautiala et al. 1996, Kenny et al. 1997, Alwis et al. 1999, Nieuwenhuijsen et al. 1999, Mitakakis et al. 2000, Nielsen et al. 2000). In addition, personal exposure samples have been mainly collected by samplers, which have no exact cut size. In the large exposure studies PTEAM and EXPOLIS, total personal exposure to particles $PM_{2.5}$, PM_{10} has been assessed for 12 or 48 hours measurement periods (Clayton et al. 1993, Özkaynak et al. 1996, Koistinen et al. 1999, Oglesby et al. 2000). The measurement period in those studies included exposure in the home and in workplaces as well as in all other environments where the study subjects spent their time.

Table 1. Examples of samplers used in studies focusing on personal exposure.

Sampler	Exposure environment	Pollutant	Study
37 mm filter holder	Remediation work Nickel alloy production Wood working Fiberglass insulation manufacturing	Total and viable microbes Nickel Viable microbes Endotoxins	Rautiala et al. 1996 Tsai et al. 1996 Alwis et al. 1999 Milton et al. 1996
25 mm filter holder, closed face	Biowaste collection	Endotoxin, dust, total and viable microbes	Nielsen et al. 2000
$PM_{2.5}$ cyclone (GK2.05) with 37 mm filter holder	Total exposure Total exposure Total exposure	$PM_{2.5}$ $PM_{2.5}$, elements $PM_{2.5}$, absorption coefficient (BS)	Koistinen et al. 1999 Oglesby et al. 2000 EXPOLIS study Janssen et al. 2000
37 mm filter holder with inlet-nozzle section and impactor plate	Total exposure	PM_{10}, elements PM_{10}, elements	Clayton et al. 1993 Özkaynak et al. 1996 PTEAM
Small inertial impactor	Total exposure	$PM_{2.5}$, PM_{10}, Mn	Pellizari et al. 1999
IOM (Institute of Occupational Medicine) personal inhalable sampler	Nickel alloy production Cotton manufacture Agriculture	Nickel Cotton dust Dust, endotoxin, crystalline silica	Tsai et al. 1996 Ogden et al. 1993 Nieuwenhuijsen et al. 1999
Button sampler	Wood-products industries Wood-products industries House cleaning	Wood dust Wood dust Total dust	Harper et al. 2004 Harper et al. 2002 Hauck et al. 1997
PAS (Personal air sampler), with filter held within IOM sampling head	Total exposure	Pollen and mould spores	Mitakakis et al. 2000

2.6 Analysis methods for microbes and other airborne particles

Although the sample collection techniques used for microbes and other airborne particles possess some similarities, there are considerable differences in the analysing methods used. Microbial analyses are mainly based on cultivation or staining of microbes before calculating or identification, whereas particles are analysed by direct counting from air or by gravimetrical techniques.

After the sampling, microbial concentrations can be determined using cultivation, microscopy, molecular biological, immunochemical or biochemical methods (Larsson and Saraf 1997, Nugent et al. 1997, Pasanen et al. 1997, Tuomi et al. 1998, Douwes et al. 1999, Keller et al. 1999, Samson 1999, Saraf 1999). Traditional cultivation methods are often based on direct impaction on a growth media (Verhoeff et al. 1992, DeKoster and Thorne 1995, Miller et al. 2000). After incubation for one week, fungal colonies can be identified by their morphological appearance to the genus or species level.

Only some of the airborne microbes are viable or able to grow on the culture media (Burge and Otten 1999, Näsman et al. 1999). The total concentration of microorganisms, including viable and non-viable microorganisms can be counted from samples collected on a filter with microscopical methods. The sample can also be collected on a slide or tape. Microorganisms can be detected by staining with a fluorescent stain (*e.g.* acridine orange) and counting by epifluorescence microscopy. Various other microscopic methods are available, from scanning electron microscopy to the image analysers as presented by Morris (1995).

The most common aerosol particle property measured is the mass. Several samplers classify particles according to their size, *e.g.* $PM_{2.5}$ or PM_{10}. Other possible analyses for collected particles include particulate carbon, elemental, anion and cation analysis as well as microscopic or electron beam analysis (Chow 1995, Solomon et al. 2001). The black smoke (BS) method is intended for the measurement of a BS index and is based on the measurement of reflectance (ISO 1993). Total carbon is classified into elemental carbon or black carbon, organic carbon and carbonate carbon. Their analysis methods are based mainly on thermal or optical techniques (Solomon et al. 2001).

2.7 Ambient concentrations of microbial aerosols and particles

2.7.1 Microbes in outdoor air

Fungal concentrations in outdoor air vary widely according to location, altitude, season, climate and time of day (Madelin and Madelin 1995). Examples of reported levels of airborne fungi and bacteria in indoor and outdoor environments and in personal samples are shown in Table 2. Seasonal variation in bioaerosols has been observed to be remarkable. In air sampling, carried out in Connecticut, the mean concentrations of culturable fungal propagules in outdoor air were highest in the summertime, 1200 cfu/m^3 whereas the mean wintertime concentration was 505 cfu/m^3 (Ren et

al. 1999). In fall and spring, mean concentrations were 607 and 830 cfu/m^3. Similar results were reported by Kuo and Li (1994) in the subtropical climate of Taiwan. Reponen et al. (1992) have reported levels of outdoor air in the Northern climate of Finland. In wintertime, when snow cover eliminates outdoor sources, fungal geometric mean (GM) concentration was only 20 cfu/m^3, while the summertime mean was 950 cfu/m^3. In summary, in outdoor air, typical summertime mean concentrations are around 10^3 cfu/m^3, while in winter conditions the concentrations are much lower, of the order of magnitude of 10^1–10^2 cfu/m^3. Fall and spring means are between these extremes, *i.e.* 10^2 cfu/m^3.

2.7.2 Microbes in indoor air

Fungal concentrations of indoor air also vary with location, season, climate and time of day (Verhoeff et al. 1992, Li and Kuo 1993, Levetin et al. 1995, Hyvärinen et al. 2001a, Medrela-Kuder 2003) because outdoor air is the main source of the airborne fungi found in indoor air (Flannigan et al. 1991, Levetin et al. 1995). The climatic conditions also have a major impact on indoor airborne fungal concentrations. In warm climates, the concentration levels of viable fungi in dwellings and school buildings have ranged from 10^2 to 10^4 cfu/m^3 and total fungi from 10^2 to 10^4 spores/m^3 (Levetin et al. 1995, Su et al. 2001a, Su et al. 2001b) (Table 2). In cold subartic climates, such as in Scandinavia, concentration levels in dwellings and schools usually remain at 10^2 cfu/m^3 during the warm seasons but during the wintertime, when there is snow cover on the ground, levels as low as 10^1 cfu/m^3 have been reported (Reponen et al. 1992, Dotterud et al. 1995, Smedje et al. 1997, Bartlett et al. 1999, Hyvärinen et al. 2001a, Meklin et al. 2003). Higher airborne fungal concentrations have been reported in moisture and mould problem buildings than in reference buildings, although the concentrations levels usually have remained lower than in dusty work environments (Macher et al. 1991, Hyvärinen et al. 1993, Górny et al. 1999, Miller et al. 2000, Pessi et al. 2002).

In dwellings in rural environments, higher fungi concentrations have been reported (from 10^2 to 10^3 cfu/m^3) than in urban dwellings (Pasanen 1992). In dusty work environments, such as agricultural environments, sawmills and handling waste or woodchips, much higher concentrations of fungi have been reported. Concentrations of viable fungi have ranged from 10^3 to 10^7 cfu/m^3 with total fungi from 10^4 to 10^7 spore/m^3 (Kotimaa et al. 1987, Eduard et al. 1990, Hanhela et al. 1995, Lappalainen et al. 1996, Kullman et al. 1998, Mandryk et al. 2000, Pillai et al. 2002) (Table 2). During the demolition work of building structures, viable fungal concentrations have increased up

to 10^4 cfu/m^3 and total concentrations of fungi up to 10^6 spore/m^3 (Rautiala et al. 1996, Rautiala et al. 2004).

The fungal flora present in indoor air reflects largely the fungal profile present in outdoor air (Reponen et al. 1992, Li and Kendrick 1996, Wu et al. 2000b). The most commonly found fungal genera in indoor air are *Penicillium* together with *Cladosporium*, *Aspergillus* or yeasts (Dungy et al. 1986, Hunter et al. 1988, Pasanen 1992, Pasanen et al. 1992, Beguin and Nolard 1994, Kuo and Li 1994, Rand 1999, Burge et al. 2000, Cvetnic and Pepeljnjak 2001, Hyvärinen et al. 2001a, Meklin et al. 2003). These fungal genera and groups are common, irrespective of the climate or continent, for example, *Cladosporium* seems to be the most dominating genus in outdoor air around the world. In moisture and mold problem buildings, composition of fungal flora may change (Pasanen et al. 1992, Hyvärinen et al. 1993, DeKoster and Thorne 1995). This is due to the microbial growth on moist building materials. In addition to the most commonly observed microbial genera, *Penicillium,* yeasts, *Cladosporium* and *Aspergillus spp.* also less frequent genera are detected on moldy materials, such as *Acremonium, Aspergillus versicolor, Aureobasidium, Stachybotrys* and Sphaeropsidales (Hyvärinen et al. 2002). Indeed, certain fungi that are not part of the normal flora of indoor environments have been listed as indicator organisms of moisture and mould damage. The international workshop “Health Implications of Fungi in Indoor Environments” in Baarn, the Netherlands 1992 produced a list of fungi containing many indicator organisms, *i.e., Aspergillus fumigatus, Aspergillus versicolor, Exophiala, Fusarium, Phialophora, Stachybotrys, Trichoderma* (Samson 1994).

Table 2. Examples of reported levels of airborne fungi and bacteria in indoor and outdoor environments. The wintertime and summertime concentrations are shown together, separated by "/", as are the fall and spring concentrations.

Environment	Mean (GM) concentration	Season	Study
Outdoor,	**Viable fungi (cfu/m^3)**		
Finland	20/950	w/s	Reponen et al. 1992
Taiwan	54/1230	w/s	Kuo and Li 1994
	374/379	f/sp	
Connecticut	505/1200 *	w/s	Ren et al. 1999
	607/830*	f/sp	
Cracow, lecture hall	106/1211*	w/s	Medrela-Kuder 2003
	557/508*	f/sp	
Indoor,	**Viable fungi (cfu/m^3)**		
Amsterdam, damp house	645	f	Verhoeff et al. 1992
Taiwan, homes	140-3200*	May-Jun	Li and Kuo 1993
Sweden, dwellings	300	w	Björnsson et al. 1995
USA, schools	56-624*	w	Levetin et al. 1995
	494-2100*	f, s	
Handling of grain	53000	f-w	Lappalainen et al. 1996
Demolition work	23000		Rautiala et al. 1996
Finland, reference dwellings	58/160	w/f	Hyvärinen et al. 1993
Taiwan, schools	9672/4381	w/s	Su et al. 2001a
Taiwan, homes	20552/6798*	w/s	Su et al. 2001b
	11083/11096*	f/sp	
Sweden, schools	200	w	Smedje and Nordbäck 2001
Cracow, lecture hall	353/939*	w/s	Medrela-Kuder 2003
	690/501*	f/sp	
Finland, schools	37	w	Meklin et al. 2003
	Total fungi (spores/m^3)		
Sawmill	$9x10^6$*		Eduard et al. 1990
Sweden, dwellings	288-1287*	w	Björnsson et al. 1995
USA, schools	1541-9999*	w	Levetin et al. 1995
	$2.1x10^6$	f/s	
Handling of grain	$1.3x10^6$	as	Lappalainen et al. 1996
Demolition work	17600		Rautiala et al. 1996
Wisconsin, barns	19000	as	Kulman et al. 1998
Sweden, schools	17600	w	Smedje and Nordbäck 2001
	Viable bacteria (cfu/m^3)		
Sweden, dwellings	400	w	Björnsson et al. 1995
Demolition work	14000		Rautiala et al. 1996
Finland, dwellings	~400/~600	w/f	Hyvärinen et al. 2001b
Sweden, schools	360	w	Smedje and Nordbäck 2001
Finland, schools	646	w	Meklin et al. 2003
	Total bacteria (cells/m^3)		
Sawmill	$7.2x10^5$*		Eduard et al. 1990
Sweden, dwellings	52000	w	Björnsson et al. 1995
Sweden, schools	22800	w	Smedje and Nordbäck 2001

season: w=winter, s=summer, f=fall, sp=spring and as=all season

* arithmetic mean

2.7.3 Particles in outdoor air

The seasonal and temporal variations are also typical to ambient concentrations of respirable particles. Seasonal concentrations of coarse particles in ambient air can be affected by soil dust (Muir and Laxen 1995, Harrison et al. 1997, Vallius et al. 2000). Examples of mass concentrations in indoor and outdoor air are shown in Table 3. In a residential area of Helsinki, the concentration in winter period of PM_1 was 6 $\mu g/m^3$, $PM_{2.5}$ 9 $\mu g/m^3$, PM_{10} 13 $\mu g/m^3$ and black smoke (BS) $1.5x10^{-5}$ 1/m but in two Central European cities, Alkmaar and Erfurt, $PM_{2.5}$ and BS concentrations were much higher (Vallius 2000, Ruuskanen et al. 2001). As reported in the PEACE study of 14 European research centers, the median concentration of PM_{10} ranged from 11 $\mu g/m^3$ in Scandinavian rural sites to 92 $\mu g/m^3$ in Athens, Greece, adjacent to a busy street (Hoek et al. 1997). In the EXPOLIS study, the $PM_{2.5}$ outdoor concentration was 37 $\mu g/m^3$ in Athens whereas $PM_{2.5}$ concentrations were lower in Basel, Helsinki and Prague (Götschi et al. 2002). Outdoor BS levels in EXPOLIS study were also highest in Athens, $3.3x10^{-5}$ 1/m.

In Kuopio city situated in eastern Finland previously reported concentrations of PM_{10} in wintertime were 24.5 $\mu g/m^3$ in an urban part, and 20.7 $\mu g/m^3$ in a suburban part (Reponen et al. 1996). The sources of PM_{10} were also identified. The main sources of PM_{10} in Kuopio area were soil and street dust (48%), traffic exhausts (14%), heavy fuel oil burning (12%), wood burning (11%) and unidentified sources (15%) (Hosiokangas et al. 1999). In Birmingham, vehicles were the major contributors to both $PM_{2.5}$ and PM_{10} (Harrison et al. 1997).

2.7.4 Particles in indoor air

The concentrations of particles in indoor environments have attracted much less research interest than the outdoor concentrations. However, there are some reports from different cities around the world. In residences of Riverside, California, mean indoor air concentration of $PM_{2.5}$ was 37 $\mu g/m^3$ and for PM_{10}, 95 $\mu g/m^3$ (Özkaynak et al. 1993, Özkaynak et al. 1996). In Amsterdam, PM_{10} indoor air concentrations varied from 22 $\mu g/m^3$ in homes in low traffic areas to 37 $\mu g/m^3$ in high traffic areas (Fischer et al. 2000). The outdoor air was the major source of both fine ($PM_{2.5}$) and coarse particles (PM_{10}) indoors, accounting for 76% and 66%, respectively (EPA 1996). Smoking and cooking were the two other important indoor $PM_{2.5}$ and PM_{10} sources, accounting for 4-5% of the total (EPA 1996, Morawska et al. 2003). In European cities, Amsterdam, Athens, Basel and Prague $PM_{2.5}$ indoor air concentrations were between 15 $\mu g/m^3$ to 36 $\mu g/m^3$ and in Helsinki, 10 $\mu g/m^3$

(Fischer et al. 2000, Janssen et al. 2000, Götschi et al. 2002). Black smoke (BS) levels in indoor air in those European cities ranged from $0.78x10^{-5}$ 1/m to $2.9x10^{-5}$ 1/m (Fischer et al. 2000, Janssen et al. 2000, Götschi et al. 2002) (Table 3).

Work environments with high microbial concentrations are often also dusty work environments, such as agricultural environments, sawmills and facilities handling of waste or woodchips. In such places, total dust concentrations from 0.01 to 16 mg/m^3 have been reported (Lappalainen et al. 1996, Kullman et al. 1998, Mandryk et al. 2000, Melbostad and Eduard 2001) (Table 3). These levels are as much as three orders of magnitude higher than the particle mass concentrations present in indoor air of urban dwellings, even sites adjacent to roads with heavy traffic.

Table 3. Examples of mass concentrations of particles in indoor and outdoor air. The concentrations in different seasons is separated by "/"

Environment	**Mean concentration (µg/m^3)**				**Season**	**Study**
Outdoor,	**$PM_{2.5}$**	**PM_{10}**	**BS**	**Total dust**		
Riverside, CA	52	97				Özkaynak et al. 1993
Bristol		26	6			Muir and Laxen 1995
Kuopio, suburban, urban site		18, 23	9, 13		w	Reponen et al. 1996
Birmingham, centre		22/25			w/s	Harrison et al. 1997
Kuopio		19			w	Hosiokangas et al. 1999
Toronto	15	24			Jun-Aug	Pellizari et al. 1999
Amsterdam, high traffic homes	25	43	3.0[a], 2.8[a2]		Jan-Mar	Fisher et al. 2000
Helsinki	9.4/9.9	13/22	15/13[a,b]		w/sp	Vallius et al. 2000
Amsterdam, Helsinki	21, 12		1.8, 2.1		w/sp	Janssen et al. 2000
Alkmaar, Erfurth, Helsinki	27, 42, 9.4		1.8, 4.0, 1.4[b]			Ruuskanen et al. 2001
Athens, Basel, Helsinki,Prague	37, 19, 11, 27		3.3, 1.4, 1.0, 3.0 [c]		w and s	Götschi et al. 2002
Indoor,						
Riverside, CA	37	98				Özkaynak et al. 1993
Handling of grain				6.2mg/m^3	f-w	Lappalainen et al. 1996
Wisconsin, barns				0.7mg/m^3 [#]	as	Kulman et al. 1998
Toronto	21	30			Jun-Aug	Pellizari et al. 1999
Amsterdam, high traffic homes	27	37	2.1[a], 2.2[a2]		Jan-Mar	Fisher et al. 2000
Amsterdam, Helsinki	14.9, 10.2		1.8, 1.6[c]		w/sp	Janssen et al. 2000
Helsinki, non-ETS exposed	8.2				as	Koistinen et al. 2001
Athens, Basel, Helsinki,Prague	36,21,10,34		2.9, 1.4, 0.8, 2.7[c]		w and s	Götschi et al. 2002
Brisbane	11.2				f/w	Morawska et al. 2003

season: w=winter, s=summer, f=fall, sp=spring and all season [#] geometric mean
[a] $PM_{2.5}$ sample, [a2] PM_{10} sample [b] (1/m)x10^6, [c] (1/m)x10^5

2.8 Personal exposure to microbial aerosols and particles

2.8.1 Microbes

Personal microbial exposure measurements have been done mainly in dusty work environments. Task-specific exposure levels have varied from 10^4 to 10^7 spore or bacteria/m^3 in agricultural environments (Melbostad and Eduard 2001) (Table 4). During the remediation work, the fungi concentration level in personal samples varied from 10^4 to 10^5 cfu/m^3 (Rautiala et al. 1998). In sawmill workers, personal exposure varied from 10^3 to 10^4 cfu/m^3 (Mandryk et al. 2000). Personal exposure levels to microbes have seldom been studied in home environments. In an Australian study in home environments using one hour exposure measurements, personal exposure median level of *Alternaria* spores was 33 spores/m^3 (Mitakakis et al. 2000) and of *Cladosporium* spores, 8 spores/m^3.

2.8.2 Particles

Personal exposure to $PM_{2.5}$ and PM_{10} has been reported in several studies (Clayton et al. 1993, Wallace 1996, Janssen et al. 1998, Clayton et al. 1999, Ebelt et al. 2000, Evans et al. 2000). Three large-scale studies that quantified personal exposure to PM under normal conditions have been reported in the literature, EPA's Particle Total Exposure Assessment Methodology (PTEAM) study (Clayton et al. 1993); the Toronto, Ontario study (Pellizzari et al. 1999); and the Air Pollution Exposure Distribution within Adult Urban Populations in Europe (EXPOLIS) exposure study (Jantunen et al. 1999); (EPA 2001). In the PTEAM study, daytime mean personal PM_{10} concentrations were 150 µg/m^3 and the overnight personal PM_{10} concentration was 77 µg/m^3 (Clayton et al. 1993) (Table 4). Daytime personal concentrations were over 50% higher than indoor or outdoor concentrations (95 µg/m^3) and overnight personal concentrations were similar to the indoor (63 µg/m^3) and outdoor (86 µg/m^3) levels. In the Toronto area, personal PM_{10} concentrations were also much higher (68 µg/m^3) than indoor (30 µg/m^3) or outdoor (24 µg/m^3) PM_{10} concentrations. The corresponding $PM_{2.5}$ concentrations were; personal 28 µg/m^3, indoor 21 µg/m^3 and outdoor 15 µg/m^3 (Pellizzari et al. 1999). Resuspension of particles caused by personal activities (the so called personal cloud) mainly affected coarse particle concentrations (Janssen et al. 2000). However, much of difference between the personal, indoor and outdoor PM_{10}, $PM_{2.5}$ levels can be attributed to tobacco smoking. In the EXPOLIS study, personal $PM_{2.5}$ exposure in the Helsinki area was lower (19 µg/m^3) than in Toronto (28 µg/m^3) (Koistinen et al. 2001). Inorganic

secondary particles, primary combustion and soil were the dominant source types for personal exposure $PM_{2.5}$ concentration (Koistinen et al. 2003). From the personal $PM_{2.5}$ exposure, residential indoor $PM_{2.5}$ concentrations explained 76% of personal leisure time exposure variation and workplace concentrations explained 66% of the workday exposure variation (Kousa et al. 2002).

In dusty work environments, Melbostad and Eduard (2001) have reported not only microbial exposure, but also the task-specific exposure level to total dust in agricultural environments, *e.g.*, threshing, animal tending, manuring and handling of grain or hay and these varied from 0.4 to 5 mg/m^3. Personal dust exposure at sawmills was around the same level as found in agricultural environments or even higher (from 0.83 to 12 mg/m^3) (Mandryk et al. 2000) (Table 4).

Table 4. Examples of reported levels of airborne fungi and bacteria and particles in personal exposure samples. The wintertime and summertime concentrations are shown together, separated by "/", as are the fall and spring concentrations.

Environment	**Mean (GM) concentration**				**Season**	**Study**
Microbes,	**Total fungi (spores/m^3)**					
Norwegian farmers, handling of grain	$1.3x10^6$				as	Melbostad and Eduard 2001
Finland, remediation work	$3x10^6$-$8x10^6$ (range)					Rautiala et al. 2002
	Viable fungi (cfu/m^3)					
Australia, sawmills	34000					Mandryk et al. 2000
Finland, remediation work	$9.6x10^4$-$1.7x10^5$ (range)					Rautiala et al. 1998
Norwegian farmers, handling of grain	**Total bacteria (cells/m^3)** $5.5x10^6$				as	Melbostad and Eduard 2001
Environment	**Mean concentration (µg/m^3)**				**Season**	**Study**
Particles,	**$PM_{2.5}$**	**PM_{10}**	**BS**	**Total dust**		
Riverside, CA, daytime		150			f	Clayton et al. 1993
Riverside, CA		144				Özkaynak et al. 1993
Toronto	28	68			Jun-Aug	Pellizari et al. 1999
Australia, sawmills				1.6mg/m^3 (#		Mandryk et al. 2000
Amsterdam, Helsinki	15.3, 10.0		1.7, 1.5(c		w/sp	Janssen et al. 2000
Norwegian farmers, handling of grain				19mg/m^3 (#	as	Melbostad and Eduard 2001
Helsinki, non-ETS exposed	9.9				as	Koistinen et al. 2001

season: w=winter, s=summer, f=fall, sp=spring and as=all season, # geometric mean
c $(1/m)x10^5$

3 AIMS OF THE STUDY

The principal aim of this study was to explore the personal exposure of individuals to airborne microbes and particles during wintertime. The detailed objectives of this study were:

1. To develop an approach and protocols for investigation of bioaerosol exposure (I).
2. To compare the personal exposure of teachers to microbes and particles with concentrations in microenvironmental measurements at home and in work, and with a time weighed microenvironmental model (I, II).
3. To determine the associations between airborne microbes and particles in personal and microenvironmental samples (II).
4. To investigate the qualitative composition of airborne microbes in personal and in microenvironmental samples (II, III).
5. To identify the determinants of bioaerosol and particle exposure in teachers (IV).

4 MATERIALS AND METHODS

4.1 Study design

A short background questionnaire (15 questions) was sent out in October 1998 to all elementary schoolteachers in two municipals. The questionnaire contained questions about their health and the indoor environment in their home and workplace. From these teachers, a random sample of 81 individuals was chosen for wintertime measurement period (November 98 - March 99) when the snow cover eliminates outdoor airborne microbes.

The study design was an application of the design developed in the EXPOLIS study for particle exposure (Jantunen et al. 1999). A 24-hour sample for bioaerosol and other particles was collected using personal sampling and microenvironmental sampling in homes, and an 8-hour sample was collected in the workplaces. The button personal sampler (SKC, Eighty Four, PA, USA) (Kalatoor et al. 1995) that has a low wind sensitivity and low intersample variability was utilized. The sampling procedure was repeated two times for each individual.

The mass concentration of collected particles was determined gravimetrically and the blackness of the filter was assessed by using a reflectometric method. Viable microorganisms collected on the filter were cultured and the total number of microorganisms was counted with an epifluorescence microscope (Fig. 3).

At the end of each sampling period, a questionnaire was filled in concerning the events of the previous 24 hours possibly affecting the exposure. After the measurements, an extensive background questionnaire was filled in. This covered details of health symptoms as well as home and workplace characterization. A technical investigation was conducted by a civil engineer for signs of moisture or mould damage in both homes and workplaces according to a checklist developed in previous studies (Nevalainen et al. 1998).

The study was conducted during the same time period as several other studies concerning microbial problems and their health effects in schools (Taskinen 2001, Immonen 2002, Meklin 2002).

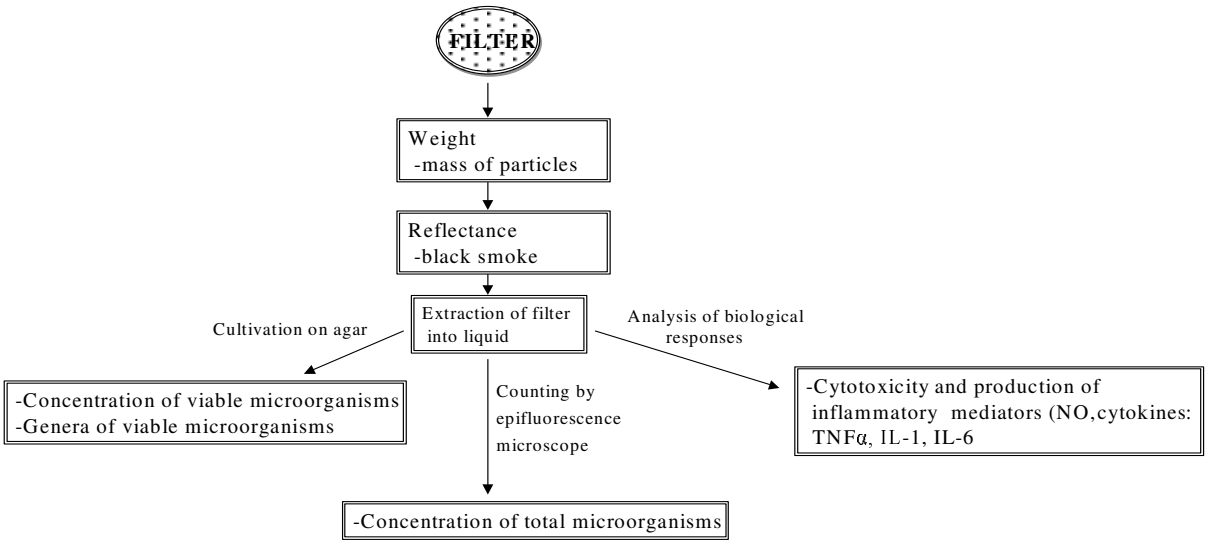

Fig. 3. Processing of the filter sample. (Cytotoxicity and inflammatory mediators are not reported in this thesis).

4.2 Study location and target population

The study population were elementary school teachers from Kuopio and Siilinjärvi area in eastern Finland. During the wintertime measurement periods, the average temperature was -7°C, lakes were frozen and the land was under snow cover.

At the beginning of the study there were 823 elementary school teachers working in these areas. The background questionnaire was sent in October 1998 to all teachers in these two municipals. A total of 562 (68%) of the teachers responded by filling and returning the short questionnaire. Of the respondents, 88 % wanted to participate in the exposure study, 2 % would not be living in the area any longer during the measurement periods and 10 % refused to participate in the exposure study. From the voluntary teachers, a random sample of 81 individuals was chosen for the measurements. According to the short background questionnaire analyse the persons selected in the exposure study were fairly representative of the larger 562 respondents teachers population (I, Table 1).

4.3 Monitoring methods

4.3.1 Personal exposure monitoring (PEM)

The personal exposure monitor (PEM) was an aluminium briefcase including a modified pump (AFC 400, BGI Inc., Waltham, MA, USA), a button sampler (SKC Inc., Eighty Four, PA, USA) (Fig. 4) and noise absorption material (total weight 3.5 kg) (Fig. 5). The samples were collected with a flow rate of 4 l/min on a 25 mm PVC filter (0.8 µm pore size, Millipore, Bedford, MA, USA). PVC filters were chosen because of their high collection efficiency, low toxicity and low pressure-drop. The sampling period was repeated two times for each individual.

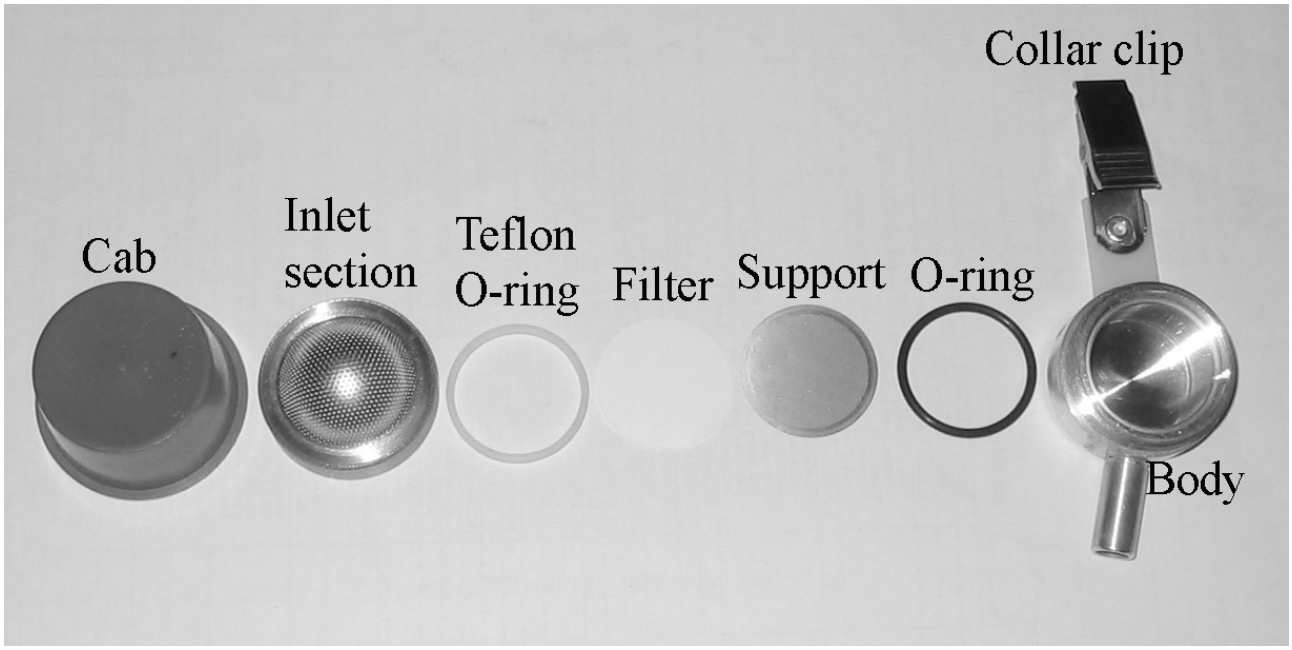

Fig. 4. Button sampler.

Fig. 5. The personal exposure monitor (PEM), with the button sampler.

4.3.2 Microenvironmental monitoring (MEM)

The microenvironmental monitor (MEM) consisted of the pump (PQ100, BGI Inc., Waltham, MA, USA) and button sampler with the PVC filter (Fig. 6). The flow rate was preset to 4 l/min and the pump was programmed to start and stop automatically to take the 24 hour sample from the home environment and an 8 hour sample from the school environment (I).

Fig. 6. The microenvironmental monitor (MEM).

Personal sample volumes were normalized to 760 mmHg air pressure at 20 ºC temperature. PEM pumps had volumetric flow control and the MEM pumps had mass flow control and thus PEM flows were normalized with conditions in which the MEMs were calibrated. Temperature data were collected by Vaisala humidity & temperature indicator (HM31 Vaisala, Finland) and air pressure data of the laboratory used in calibration by a mercury manometer.

4.3.3 Filter weighing

The mass concentration of collected particles was analysed gravimetrically. A microbalance (Mettler MT5 by Mettler-Toledo AG, Greinfensee, Switzerland) with a reading precision of 1 µg was used for weighing the PVC filters. The filters were equilibrated to weighing room conditions for a minimum of 2 hours according to NIOSH manual (1994) before the weighing session and deionised with a Po-210 deioniser (Staticmaster 1269 by Cahn Inc, USA) before weighing. Temperature, relative humidity and air pressure were recorded during the weighing session. In the weighing, two consecutive weighings of the same filters had to agree within 1 µg to be accepted (Koistinen et al. 1999). The same weighing room and the standardized weighing procedure were used throughout the study.

4.3.4 Black smoke analysis

The term black smoke (BS) is used here as a term to indicate the absorption coefficient of filters. Black smoke is assumed to indicate particles like soot from burning process. The light absorption coefficients of the PVC filters were measured with a black smoke method according to the ISO protocol (ISO 1993) by a smokestain reflectometer (M43D, Diffusion Systems Limited, London U.K.). The absorption coefficient was measured on five points on filter surface in a dark chamber.

4.4 Microbial analysis

4.4.1 Extraction of filter

On the same day, after the weighing and the reflectance measurements, the particles were extracted from the filters with 5 ml dilution buffer (distilled water with 42.5 mg l^{-1} KH_2PO_4, 250 mg l^{-1} $MgSO_4$, 8 mg l^{-1} NaOH, and 0.02% Tween 80) using an ultrasonic bath (15 min) and a shaker (15 min). After the extraction, the suspension was divided into two, for microbiological analyses (2.1

ml) and toxicological analyses (2.9 ml) (toxicological analyses are not reported in this thesis) (Fig. 3).

4.4.2 Viable microbes

The concentrations of viable fungi and bacteria and genera of the viable fungi were determined by the cultivation method. Diluted suspension (1:10 and 1:100) was plated on two fungal growth media, 2% malt-extract agar (MEA) and dichloran glycerol 18 agar (DG18), and on a bacterial medium, tryptone-yeast-glucose agar (TYG). Fungi were incubated for seven days at 25 ºC, and bacteria for up to 14 days at 20 ºC. The total number of viable bacteria colonies was counted after five days and actinomycete colonies after 14 days of incubation. The detection of actinomycete colonies was based on their typical dry appearance. Fungi were identified morphologically on a genus level.

4.4.3 Total microbes

The total concentration of the collected microbes on the filters was determined by the acridine orange direct counting method (AODC) (Hobbie et al. 1977, Palmgren et al. 1986). The samples were filtered and stained with 0.01% (w/v) acridine orange. The stained microbes were counted with an epifluorescence microscope (Olympus BH-2, Olympus Optical Co., Tokyo, Japan). Forty randomly chosen fields were counted by using 1000 x magnification. Fungal spores and bacteria were distinguished roughly from each other by their size (for bacteria, less than 1.5 µm).

4.4.4 Time weighed microenvironmental model

A time weighed microenvironmental model was used to assess average personal exposure to pollutants according to equation 1 (I).

$$TWC_{hw} = C_{home} \times t_{home} / (t_{home} + t_{work}) + C_{work} \times t_{work} / (t_{home} + t_{work}), \quad (1)$$

where TWC_{hw} is pollutant concentration of home and workplace measurements with time weighing; C is the concentration of the pollutant; t is the time spent at home and in work.

4.5 Questionnaires (I-V)

Three different questionnaires were used in this study. The first background questionnaire contained 15 questions about the health status of the individuals and the indoor environment in the home and workplace. Immediately after each measurement period, the subjects participating in the exposure study (n=81) filled in a 24 hours time activity and exposure questionnaire to collate all the events of the previous 24 hours possibly affecting the exposure. Details of health symptoms as well as home and workplace characterisation were screened by an extensive background questionnaire conducted at the end of the second measurement of the exposure study (n=81). The representativeness of the subsample was analysed by comparing the characteristics of this group with those of the whole population of respondents (n=562).

4.6 Quality assurance (QA)

Representativeness of the exposure study sub-sample (n=81) was analysed. Detailed standard operating procedures (SOP) and working instructions were created for analysis and sampling. The sampling monitors and analytical methods were tested during a short pilot study. Flow rate before the sampling had to be within 2.5% and after sampling within 10% of the 4 l/min flow rate. Comparability of samples was assessed by duplicate samples (5% of all samples). Contamination of samples was assessed by blank samples (10% of all samples).

4.7 Data analysis

Statistical analyses were conducted with SPSS for Windows, version 9.0. - 12.0. All concentration values under the detection limit were replaced with a half of the detection limit in the database. The concentrations of airborne microbes were not normally distributed (I-IV).

Statistical analyses utilized both nonparametric and parametric tests to test the representativeness of the 81 teachers participating (I), differences between the personal, home and workplace environments (I, III) and the difference between field duplicates (I). The proportion of the pollutant types explaining the variation of other pollutants (II, III), the associations between personal exposure and the time weighted model and between different microbes (III), microbial differences between urban and rural areas (III) and differences and agreement between MEA and DG18 media

(III) were tested. Statistical differences inside the determinant groups and the proportions of the classified determinant types explaining the variation of pollutants concentrations and toxicity of collected particles were analysed with one way analysis of variance test (IV). The proportions of the determinants explaining the variation of pollutants concentrations and toxicity of particle material were analysed with multiway analysis of variance test custom model with main effects (IV). Details of statistical analyses are described in each original publication (I-IV).

5 RESULTS

5.1 Quality assurance (QA) (I)

There were no statistically significant differences in the characteristics of the exposure study sub-sample of individuals (n=81) and all of the respondents of the short screening questionnaire (n=562) (I Table 1). The field blank filters showed a systematic increase in concentrations of particle mass and total bacteria during the field measurements in all microenvironments (I Table 2). The average field blank mass increase was subtracted from all of the particle mass results. A small increase in total fungi, viable fungi and viable bacteria was also detected in some field blank filters from the home and work sampling sites. This increase was not subtracted from the results.

The calculated detection limits for mass concentrations were 2.9 $\mu g/m^3$ in personal samples, 16.9 $\mu g/m^3$ in home samples and 20.2 $\mu g/m^3$ in workplace samples. The detection limits for total fungi and bacteria concentrations were 4051 spores/m^3 (for bacteria, cells/m^3) in personal and in home samples, and 12152 spores/m^3 in workplace samples. For viable fungi and bacteria concentrations, the detection limits were 4 cfu/m^3 in personal and home samples, and 12 cfu/m^3 in workplace samples. The detection limits in workplace measurement samples were higher than in home or personal samples due to the shorter sampling time (8 hours versus 24 hours). There were variations noted between the duplicates (CV%= 9.9-78.7) although the differences were not statistically significant (p=0.158-1). Details of QA results are shown in publication I.

5.2 Personal exposure and microenvironmental concentrations

The personal particle mass (57 $\mu g/m^3$), total fungi (12200 spores/m^3) and viable fungi (33 cfu/m^3) mean concentrations were higher than home (17 $\mu g/m^3$, 10800 spores/m^3 and 30 cfu/m^3) or workplace (34 $\mu g/m^3$, 12000 spores/m^3 and 19 cfu/m^3) concentrations (I Fig. 1 and 2, II Table 1). Personal (1.10×10^{-5} 1/m) and work (1.12×10^{-5} 1/m) BS concentrations were higher than the home (0.67×10^{-5} 1/m) concentration. Total and viable bacteria concentrations in workplace (145000 cells/m^3, 1090 cfu/m^3) were higher than the personal exposure (86400 cells/m^3, 715 cfu/m^3) or home concentrations (60600 cells/m^3, 338 cfu/m^3). However, there were statistically significant associations between personal BS and viable bacteria exposures and both home and work BS concentrations (II). Furthermore, there were associations between personal exposure and the home

concentration of viable fungi and between personal exposure and home and work concentration of viable bacteria, whereas poorer associations were detected with work concentrations and particle mass, total bacteria with home or work concentrations (II, III). Black smoke (BS) concentrations explained best the variation of particle mass concentrations in personal exposures and in home concentrations while in highly populated workplaces, viable bacteria concentrations explained the largest variation within the particle mass concentrations (II Table 4).

5.3 Viable fungal genera in personal exposure and in microenvironments

The geometric mean (GM) of total concentrations of viable fungi varied between 3-12 cfu/m^3 in the different environments (I, II). Fungal concentrations and the diversity of personal exposure and home samples were higher in the rural (GM= 15 and 15 cfu/m^3) environment than in the urban environment (GM= 11 and 5 cfu/m^3) (III). The concentrations of viable fungi and bacteria were about 1 % of the total fungi and bacteria concentrations in the wintertime measurements (I). Those samples with higher fungal concentrations had also higher diversity of fungi than samples with lower concentrations. Total number of fungal genera was 39 for personal, 34 for home and 23 for work samples. The most common fungi in personal exposure and home samples were *Penicillium spp.* and in the workplace samples, the most common group was the yeasts. The geometric mean concentration was 0.6-3.7 cfu/m^3 for *Penicillium* and mainly under 1 cfu/m^3 for other fungi. The *Penicillium* concentration explained as much as 95 % of the variation of personal exposures, 80 % of home and 25 % of workplace concentration variation of total viable fungi (III).

5.4 Correlations (II, III)

Personal exposures to viable fungi correlated with home concentrations ($r=0.6$, $p<0.001$). With respect to viable bacteria, personal exposure correlated with both home ($r=0.7$, $p<0.001$) and work ($r=0.5$, $p<0.001$) concentrations. Similarly, personal black smoke (BS) exposures correlated with both home ($r=0.6$, $p<0.001$) and work ($r=0.6$, $p<0.001$) BS concentrations (II). In contrast, personal exposure to particle mass did not correlate well with home or workplace concentrations.

When correlations between various pollutants were analysed, the black smoke concentrations in personal exposure and at home correlated with personal exposure to particle mass ($r=0.4$, $p<0.001$) and with home ($r=0.4$, $p<0.001$) concentrations of particle mass. In workplaces, viable bacteria concentrations correlated ($r=0.6$, $p<0.001$) best with particle mass concentrations, but no other

significant correlations were identified (II). The estimated mass concentrations of total microbial aerosols (viable and non-viable) fungi and bacteria were less than 1% of the particle mass concentration in wintertime measurements as assessed by mean fungal spore size 2.6 µm (II).

The time weighted microenvironmental models of BS and viable bacteria correlated moderately well (r=0.7) with personal exposures (II, III). The correlations with other pollutants were poorer (r=0.2-0.6) and the model underestimated personal exposures of particle mass, viable fungi, total fungi and total bacteria (II, III).

5.5 Determinants (IV)

5.5.1 Determinants of personal exposure

The determinants of the personal exposure varied according to the pollutant being measured. The most important determinants of personal exposure are shown in Table 5. Personal exposure to particles was elevated by teaching of practical subjects, time spent in car (>35 min) and by having a spouse, especially a spouse with a blue-collar professional status. The black smoke personal exposure was elevated by the time spent in a car, but also by having a low (<=325) number of pupils in school and living in a rented apartment. Personal exposure to total fungi concentrations was elevated by teaching of practical subjects, and by having a low number of pupils in the school. Personal exposure to viable fungi was increased by dogs, contact with potential microbe sources, living in a family house and condensation inside of the inner glass window at home. Total bacterial concentrations of personal exposure were elevated by teaching of practical subjects, low traffic density near to the home and having a low number of pupils in school. Personal exposure to viable bacteria was higher in men than women and in younger persons (27-40 and 40-48 years) compared to older persons (over 48 years). Furthermore, having a spouse, especially a blue-collar spouse, as well as moisture damage and visible mould growth at home elevated personal exposure to viable bacteria. (IV Table 2, Table 5)

5.5.2 Determinants of home concentrations

The major descriptors of particle concentrations in the home were heavy traffic near to the home, having a spouse or spouse with a blue-collar professional status and the number of persons living in the house. Traffic also elevated black smoke concentration at home, as did burning of candles and

living in an apartment. In the home, the total concentration of fungi was increased by cats and potential microbial sources. In the home, several determinants of viable fungi could be defined. Home location in a rural area, animals at home, income of family over 50000€, fireplaces, presence of extractor hood in the kitchen, potential microbe sources and condensation inside of the inner glass window elevated viable fungi concentration in the home. Owning cats, having a spouse with a blue-collar professional status and the presence of visible mould growth elevated total bacteria concentrations in the home. Cats, having a spouse and a spouse with a blue-collar professional status, building year (built after 1980 compared to built 1960-1979), ventilation systems without cleaning and moisture damage in home increased viable bacteria concentrations in the home. (IV Table 4, Table 5)

5.5.3 Determinants of workplace concentrations

In the classrooms, teaching of practical subjects and gravitational ventilation increased particle mass concentration. The age of the school building (building year <1960) increased the black smoke concentration in school. In the workplace, no significant determinant for total fungi could be detected. In the school, gravitational ventilation elevated viable fungi concentration. Both total bacteria and viable bacteria concentrations were higher in primary schools than in upper level schools. (IV Table 6, Table 5)

Table 5. The main determinants of personal exposure and occurrence of those determinants at home or in the workplace.

Pollutant	Determinant of personal exposure	Same determinant at home (H) or workplace (W)
Particle mass	Teaching of practical subjects	W
	Work status of spouse	H
	Time spent in car	na
Black smoke	Number of pupils in school	-
	Time spent in car	na
	Owner of dwelling	-
Total fungi	Teaching of practical subjects	-
	Number of pupils in school	-
Total bacteria	Traffic near to home	-
	Teaching of practical subjects	W
	Number of pupils in school	-
	Visible mould growth	H
Viable fungi	Dogs	-
	Contact with potential microbe sources	H
	Condensation inside of the inner glass window	H
Viable bacteria	Gender	-
	Age	-
	Work status of spouse	H
	Moisture damage	H
	Visible mould growth	-

na=not analysed from home or workplace, - = not the same determinant at home or workplace

6 DISCUSSION

6.1 Monitoring methods of particles and microbial aerosols

Personal exposure to bioaerosols has usually been assessed by indirect stationary measurement methods (ACGIH 1999, Pasanen 2001). Exposure has been estimated in some particular microenvironment, although bioaerosols, such as fungi and bacteria, are present everywhere in our normal environments and thus, exposure occurs wherever a person is moving or spending his/her time. The measurements have usually involved sampling over a relatively short time although it is well known that the concentrations vary in time and space in each environment (Hyvärinen et al. 2001a). Thus, one must question how well such short time measurements in a single microenvironment reflect an individual's actual exposure to the pollutant in question. This study was initiated to fill this gap in our knowledge for the personal exposures to microbial aerosols. Personal exposure to particle matter (PM) had been previously studied by personal monitoring in a number of studies (Wallace 1996, Janssen et al. 1998, Koistinen et al. 1999). Microbial aerosols are part of the airborne particle matter, although little attention has been paid to them in studies focusing on PM exposure. In this study, the approaches and methods used in PM exposure studies were applied to study personal exposure to microbial aerosols.

Both airborne particles in general and more specifically, bioaerosols have been intensively studied, but usually in separate studies and by different researchers. However, people are simultaneously exposed to many specific pollutants in their normal life. Until now, the health effects of individual pollutants as well as their interactions are still poorly understood. The sampling method used here provided access to several pollutants, with simultaneous collection of both microbes and other particles in the mixture by using collection methods which were not exclusive for either particle matter or microbes. The personal aerosol samplers used in exposure studies are mainly based on filter collection with inlets like impactors or cyclones with specific cut points, such as 2.5 μm or 10 μm (Lioy et al. 1990, Thomas et al. 1993, Koistinen et al. 1999, Janssen et al. 2000). In dusty work environments, personal exposure studies of microbes have usually been conducted during a workday with 37 mm filter cassettes, sampling the whole size range of airborne particles. However, the 37 mm filter cassette is no more accepted for workplace monitoring, but according to SFS-EN 481, the samplers used at workplaces should follow the ACGIH/CEN/ISO inhalable convention. Size-selective personal samplers have been developed for this purpose and become commercially available (Kalatoor et al. 1995, Kenny et al. 1999, Agranovski et al. 2002). The button sampler used

in this study has been designed to follow the inhalable convention curve. It also has lower intersample variability and higher uniformity of particle deposition than the 37 mm filter cassettes (Hauck et al. 1997, Agranovski et al. 2002).

The filter sampling used in this study, may have an effect on the culturability of microbes, because of desiccation of the cells on the filter during the collection (Wang et al. 2001). Therefore, the total concentrations of microbes on the filters were also determined by direct counting with a microscope. The direct counting method used here has the disadvantage of having a relatively high detection limit, which compromises its use in indoor environments where there are relatively low microbial concentrations. The proportion of viable fungi of the total concentration of airborne microbes varies. In our study, the proportion of viable fungi and bacteria of their total numbers was approximately 1%. In a previous study, the proportion of the airborne culturable fungi in comparison with the total spore concentration varied between 0.2 and 7.4% before the dismantling of building structures and between 0.2 and 37% during the dismantling (Rautiala et al. 1996). In some situations, the corresponding ratio of viable fungi and bacteria can be even less than 1% of total counts (I, Rautiala et al. 1996), which is also commonly observed for environmental bacteria in water and soil (Atlas and Bartha 1993, Szewzyk et al. 2000). Collection and analysing the samples for airborne microbes is a major research challenge. Given the complexity and diversity of microbial bioaerosols, the present methods still need further refinement to resolve the methodological problems of microbial exposure assessment.

6.2 Concentrations of particles and microbial aerosols

Personal exposures to particles have mainly been studied and measured as exposures to $PM_{2.5}$ or PM_{10}. The levels of particle mass observed in this study were 17-57 $\mu g/m^3$, rather similar to the levels reported for $PM_{2.5}$ and PM_{10} in large field studies, PTEAM and EXPOLIS (Pellizzari et al. 1999, Koistinen et al. 2001). Also in the Toronto, Ontario study rather similar levels of the inhalable fraction of particles have been reported (Clayton et al. 1993). Similarly to our results, the personal $PM_{2.5}$ and PM_{10} exposures in these studies have also been higher than in stationary samples. This is evidence of the phenomenon of the "personal cloud" which contributes to the personal exposure compared to stationary sampling. When persons are active, the particle increase can be even 50% and it has been suggested that the personal cloud mainly consists of coarse particles (Thatcher and Layton 1995, Wallace 1996). However, the proportion of personal cloud from total personal exposure to particles was not detected in this study.

Reflectance of filters is expressed as light absorption (1/m). The results were not transformed into $\mu g/m^3$ since the transformation used to calculate mass concentration is filter specific and no transformation is available for the filter used in the present study. For this reason, it is not possible to make any direct comparison of black smoke concentrations between different studies. However, the black smoke concentrations of personal exposure, home and workplace in this study are at the same level as those for outdoor air in Kuopio area (Penttinen et al. 2000) and in Amsterdam (Janssen et al. 2000) or in the indoor air in Helsinki and Basel (Götschi et al. 2002). In another study, much higher indoor and outdoor concentrations were reported from Amsterdam (Fischer et al. 2000) but these measurements were made at a site with a high traffic intensity. In Athens and Prague, BS levels were more than twice as high as those found in this study (Götschi et al. 2002).

Total fungi concentrations $10^4 – 10^6$ spores/m^3 measured by the filter method have previously been reported from dusty work environments, such as farms or during the house repair work (Lappalainen et al. 1996, Rautiala et al. 1996). In this study, total fungi concentrations were 100 times lower than the concentrations reported from dusty work environments. However, total fungal concentrations similar to this study have been detected in Finnish schools and offices in our previous study (Toivola et al. 1999). On the other hand, higher total fungal concentrations were found both from Swedish homes (Björnson et al. 1995) and in Swedish schools (Smedje and Norbäck 2001) than in this study. Part of the differences may be due to methodological aspects, since much shorter sampling times (1-5 h) were used than in our study (8-24 h). The measurements were also made during the active hours which probably show the peak levels of airborne fungi as indicated in chapter 2.2.

As could be expected, total bacteria concentrations measured by the filter method in dusty work environments, such as woodchip handling, agriculture and sawmills (Eduard et al. 1990) have been much higher compared to the findings of this study. However, Björnson et al. (1995) reported about two times higher total bacteria concentrations in Swedish homes compared to the bacteria concentrations in this study. Instead, the bacteria concentrations in Swedish schools were only a third of those measured in this study (Smedje and Norbäck 2001). The differences in homes may partly be due to the different sampling approach as has been discussed with the total fungal counts above. However, this does not explain the difference observed in the schools, but the differences may be due to differences in the efficiency of ventilation in schools.

In this study, viable fungal concentrations were much lower than those reported from dusty work environments (Lappalainen et al. 1996, Rautiala et al. 1996), but also slightly lower than those reported in normal Finnish homes and schools when measured with a six-stage impactor (Hyvärinen et al. 2001a, Meklin et al. 2002). This may be partly caused by desiccation during the sampling but also by the long sampling periods that also covered quiet hours in the nighttime.

In this study *Penicillium* was the most common fungal genus, as has been reported previously in several studies (Pasanen 1992, Hyvärinen et al. 1993, Kuo and Li 1994). In this study, the *Penicillium* concentration explained between 25-95% of the variation of viable fungi concentration of home, of personal exposures and of workplace concentrations. Observation of a fungus at home or in the workplace does not always coincide with fungal findings of personal exposure samples. In samples where there were higher fungal concentrations, also more fungal genera were detected than in samples with lower concentrations. This suggests that some fungal sources were also present in the environments studied.

The concentrations of viable bacteria found in schools were comparable to the concentrations found in normal residences or schools (Liu et al. 2000, Hyvärinen et al. 2001b). Viable bacteria concentrations were from 1-10% of the concentrations reported by Rautiala et al. (1996) from a dusty work environment.

6.3 Personal exposure in relation to microenvironmental concentrations

The personal exposure mean concentrations of particle mass and viable fungi were higher than those in home and workplace samples (I, II). Also in the EXPOLIS study, Koistinen et al. (2001) reported higher personal $PM_{2.5}$ exposure concentrations than those in stationary samples. In some previous studies, high personal concentrations of total dust and workplace concentrations have been reported in dusty work environments as well as higher personal sample concentrations than stationary workplace sample concentrations (Kulman et al. 1998, Mandryk et al. 2000, Melbostad and Eduard 2001). A major factor contributing to higher personal exposures compared to stationary sampling is the resuspension of particles caused by personal activity, the so-called personal cloud (Janssen et al. 2000). This applies mainly to coarse particles and therefore, to the mass concentrations. The personal exposure levels to black smoke (BS) were at the same level as workplace concentrations but significantly higher than home levels. On the other hand, Janssen et al. (2000) reported slightly higher BS level at home than in personal exposure for elderly people in

Amsterdam and Helsinki. Differences between this study and study in Amsterdam and Helsinki may be mainly due to differences of behaviour *i.e.*, activities, time spent in workplace and home between teachers and elderly people. The total concentrations of fungi in work environments were higher than those measured at home or with personal exposure monitoring. This finding differs from that of viable fungi. It may be that non-viable fungi are typically part of the settled dust in a school building where the activity of school children causes elevated levels in the air during the workday. The concentrations of viable fungi in the home environments were lower than those detected in personal exposures but higher than in work environments. This may be explained by the normal sources of fungi detected in homes, such as cooking, cleaning and having pets (Lehtonen et al. 1993). Both the total bacteria and viable bacteria concentrations were highest in the work environments and lowest in homes. Schools are highly populated indoor environments, and since humans are a major source of airborne bacteria, the high concentrations of bacteria may be a result of crowdedness.

The personal exposure concentrations of black smoke correlated with home and work BS concentrations better than did the concentrations of particle mass in those environments. Similar results have also been reported in previous studies among elderly people (Janssen et al. 2000). However, correlations between personal particle mass exposures and both home and workplace concentrations were weaker in this study than those reported for PM_{10} (Monn et al. 1997, Janssen et al. 1998), which may due to the fact that also particles larger than PM_{10} were collected in this study. The BS explained best the variation in the particle mass concentrations in personal exposures and home concentrations. However, the BS is an indicator of fine (<2.5 μm) and ultra fine soot particles derived from combustion processes (Vallius et al. 2000) and the button sampler collects also larger particles.

In the samples of the present study, which were collected in wintertime, total mass of fungi and bacteria accounted for less than 1% of the total particle mass. In an office building measured in the autumn the proportion of viable fungi and bacteria number concentration was <1% (Luoma and Batterman 2001). However, this means higher total microbial proportion than in this study, because concentrations of total airborne fungi and bacteria are 100-fold those of viable fungi and bacteria. This is a feasible conclusion since the airborne loads of fungi and bacteria are much higher in summer and autumn than in winter (Reponen et al. 1992). There is not much data on the proportions of microbial aerosols of the total particle matter, but in numerical terms, 37% of all outdoor air

particles over 0.2 μm appeared to be of biological origin in Germany in the summertime (Matthias-Maser and Jaenicke 1994).

The time weighted microenvironmental model was a useful method to assess personal exposure for BS and viable bacteria concentrations (II, III). However, the model underestimated personal exposures of particle mass, viable fungi, total fungi and total bacteria concentrations. This indicates that there were other relevant sources in addition to home and workplace for these pollutants.

6.4 Determinants of particles and microbial aerosols

Teaching of practical subjects, time spent in a car, having a spouse and having a spouse with a blue-collar occupational status were the major determinants for personal exposure to particle matter. There were slight correlations between personal particle exposures and particle mass concentrations at home, where also having a spouse and a spouse with a lower occupational status were determinants for particle mass concentration. It has been previously reported that $PM_{2.5}$ exposure is higher for persons with lower occupational status than higher occupational status (Rotko et al. 2000). The reason why there is an increase in the personal exposure associated more with the marital and socioeconomical status than the number of occupants, remains to be studied in the future. The results of this study also indicate that the number of inhabitants at home may increase particle concentrations. The number of people may increase the activities inside the building and this may increase the resuspension of particles and the generation of new particles (Wallace 1996).

There was also a slight correlation between personal particle exposure and particle mass concentration in the workplace. The common determinant of personal exposure and workplace concentration was teaching practical subjects, *i.e.* woodwork, textile work, arts and crafts and cooking. These are all particle-creating activities which explains both the increase in the particle concentrations in the work environment and its contribution to the personal exposure. Time spent in a car increased personal particle exposure as did the presence of traffic near to the home. Traffic also increased the particle concentration at home. Traffic density has been reported to be a major determinant for particle exposure of individuals in large cities (Janssen et al. 1998, Pakkanen et al. 2001). One interesting aspect in our study was that the effect of traffic could be seen even in areas of relatively low traffic intensity. The towns included in this study have 20000-88000 inhabitants and the traffic densities are ~20000 vehicles/day at the maximum. Thus the traffic is a major descriptor of particle exposure in both cities and less densely populated areas. Smoking has been the

strongest determinant of fine particles for personal exposure and indoor concentration (Wallace 1996, Janssen et al. 1998). In our study, there were only a few smokers and the effect of smoking was thus not detected.

Time spent in a car was the most important determinant for personal exposure to black smoke (BS). Although personal exposure to BS correlated with home and workplace BS concentrations, there was no common determinant for these concentrations. However, the presence of traffic near to the home was the main determinant of home BS concentration. Traffic and combustion processes are the main sources for BS and indoor BS predominantly originates from the outdoor air. BS outdoor levels explained 62% of the indoor variations in Helsinki (Götschi et al. 2002). Similarly to the particle results, it is interesting to note that traffic acts as a descriptor of BS concentrations even in areas of relatively low traffic density. Other determinants for personal BS exposure were the number of pupils in school and ownership of dwelling. Tenants of the rented dwellings may be exposed more to BS than homeowners because rented dwellings are often located in urban areas close to heavy traffic.

Personal exposure to total fungi was mainly related to the school environment. The main determinants for total fungi exposure were teaching of practical subjects and a lower number of pupils in school. However, none of those determinants was important for the workplace concentrations and there was no correlation between personal and workplace total fungi concentration. There may be some other, so far unexplained, determinants for personal total fungi exposure. In general, the most important fungal spore source is the outdoor air (Garrett et al. 1998), but in this study, conducted in wintertime, the contribution of outdoor spores was probably minimal (Reponen et al. 1992).

Fungal exposure was also assessed in terms of concentrations of viable fungi. Condensation inside of the interior window glass was the main determinant of viable fungi concentrations both in personal exposure and the home environment. Condensation in interior window glass is a surrogate of high humidity or insufficient ventilation instead of being a real determinant itself. Thus, observations of condensation suggest a source of recently released fungal spores that are detected as viable counts rather than as total numbers of spores. This is an important observation since in the dry indoor air conditions typical of a cold wintery climate, condensation is an unusual and undesired phenomenon. Nevertheless, the results show that it contributes to the fungal exposure experienced by the occupants. Dogs also increased personal viable fungal exposure. Pets have been

reported to increase fungal concentration in homes (Lehtonen et al. 1993, DeKoster and Thorne 1995, Ren et al. 2001). Fungal concentrations and the diversity of personal exposure and exposure at home were higher in rural areas than in urban areas. This has also been shown earlier by Pasanen et al. (1992) in a study comparing rural homes with urban homes. The personal exposure to viable fungi correlated with home concentrations, whereas the correlation with workplace concentrations was poorer and there was no common determinant. In the qualitative analysis of the samples, it was noted that the fungal findings were not always the same in the individual's personal samples and in the microenvironmental samples in the places he/she had visited. Therefore, it can be concluded that the detection of a fungus in a certain microenvironment does not always coincide with findings from personal exposure samples.

Teaching of practical subjects also increased personal exposure to total bacteria. Teaching these subjects means handling of wood, textiles, craft material and foodstuffs. However, the levels observed in this study were only 0.1-1% of the levels measured in agricultural work (Melbostad and Eduard 2001). There were slight associations between personal total bacteria exposure and home and workplace concentrations. Humans are considered to be important sources for indoor air bacteria (Nevalainen et al. 1991, DeKoster and Thorne 1995). Interestingly, observations of visible mould growth increased exposure to total bacteria and concentration at home, but not exposure or concentration of fungi. Meklin et al. (2002) did not find clear differences in the microbial flora between moisture damage and reference school buildings. This raises the question of how important fungal exposures are in comparison to bacteria in situations of indoor mould growth in school buildings.

With respect to viable bacteria, the essential determinants of personal exposure were gender, age, having a spouse or work status of the spouse, moisture damage and visible mould growth. Men and young persons were exposed to more bacteria than women and older people. This may be due to gender- related physiological differences or activities. It is known that humans are important bacterial sources and their emissions depend on gender and physical activity (Noble et al. 1976, Nevalainen et al. 1991, DeKoster and Thorne 1995). Similarly to the concentrations of total bacteria, moisture damage and visible mould growth were determinants of exposure to viable bacteria. This supports the suggestion that the role of bacteria exposures should be further studied in connection with indoor moisture and microbial problems.

7 CONCLUSIONS

An approach and quality assurance protocols for simultaneous studies of personal exposure to microbial aerosols and particles were developed and tested with random sample of teachers. It can be assumed that the developed protocols are feasible also in other non-industrial living environments with low microbial concentrations. The use of duplicates and blanks is recommended to control for possible contamination and to guarantee the accuracy of the methods used.

The personal exposures to particle mass, black smoke and fungi were higher than the concentrations in the two main microenvironments, the home and the workplace. This emphasizes the importance of the personal cloud and the activities of individuals. It may also point to other sources and important microenvironments for exposure to these pollutants. Although the time spent in these other microenvironments is short, sporadic high concentrations encountered there may have an influence on the total personal exposure. In contrast, bacterial concentrations in workplaces were higher than those in personal samples. The workplaces were busy, heavily populated school buildings which apparently contribute to the exposure to airborne bacteria. For certain pollutants, such as black smoke the time weighted model using information detected in many microenvironments is useful method to assess the personal exposure.

The associations of personal exposure to microenvironmental concentrations of airborne microbes and particles were only moderate. However, correlations between personal exposure and home concentrations of viable fungi and bacteria indicate that the home dominates the personal exposure to microbial aerosols compared to work environment. With respect to black smoke, both home and workplace concentrations correlated with personal exposure, while the correlations concerning particle mass concentrations were poor. This is in line with earlier studies and is explained by different sources and behaviour of the two types of airborne particles.

The concentration of viable microbes in the indoor air was only about 1% of the total concentrations of microbes and less than 0.01% of the total particle mass. Both the concentration of viable fungi and the diversity of fungal genera were higher in personal samples than in home and work samples. This may point to exposure environments for fungi other than home and workplace. On the other hand, environmental samples also contain fungal genera that were not detected in personal samples, thus, fungal findings in a microenvironment do not always coincide with the findings from personal exposure.

There were several determinants for personal exposure, home concentration and workplace concentration of pollutants, but none of them was dominant. There were common determinants for both personal exposure and microenvironmental concentrations, which suggests presence of pollutant sources in these microenvironments. Therefore, source-related information concerning *e.g.* building characteristics is valuable in exposure assessment studies.

Personal exposure needs to be measured to assess the real exposure to bioaerosols. Methodological aspects are critical in the assessment of microbial exposures. For example, a long sampling time with filters may influence the viability of fungi and bacteria, thus, other methods not based on culturing such as direct microscopy, DNA techniques and immunochemical or chemical marker analyses, may provide useful alternatives. However, stationary sampling in the main microenvironments is also important to elucidate their importance as microbial sources and to design cost effective strategies to reduce exposure and related health impacts.

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