EUROPEAN COLLABORATIVE ACTION

INDOOR AIR QUALITY & ITS IMPACT ON MAN

Environment and Quality of Life

Report No. 12

Biological Particles in Indoor Environments

Commission of the European Communities
Directorate General for Science, Research and Development
Joint Research Centre - Environment Institute

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Biological Particles in Indoor Environments

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1. INTRODUCTION

This report is concerned with the strategy and methodology for investigating four major categories of biological particle in the indoor air of private houses, non-industrial workplaces and public buildings (excluding hospitals). These particles are mites and their faeces; dander from pets and other furred animals; fungi, including moulds and yeasts; and bacteria, including actinomycetes.

The document does not deal with pollen or insect-derived particles, although both may affect respiratory health. Because it largely originates from plants outdoors, the concentration of pollen is generally much lower in indoor air than in outdoor air. Respiratory problems are primarily associated with exposure to the higher concentrations of pollen outdoors. Airborne particles emanating from cockroaches (particularly *Blatella germanica* and *Periplaneta americana*) may be of significance in the health of individuals in socioeconomically disadvantaged groups, and particles from the cat flea or the common clothes moth may also affect health (Mathews 1989). Nevertheless, the number of cases involving these insect-derived particles is small in relation to cases involving the four categories dealt with later in this report.

Evidence indicates that some viral illnesses such as the common cold and measles may be transmitted via indoor air, e.g. measles in schools (Riley *et al.* 1978), and rates of viral infection in buildings with heating, ventilating and air conditioning (HVAC) systems with recirculating air may be higher than in naturally ventilated buildings (Brundage *et al.* 1988). However, it is generally held that person-to-person transmission is the principal cause of outbreaks of most viral diseases, e.g. mumps. Therefore, although they are of great epidemiological significance, such viruses and other pathogenic organisms from human and animal sources are excluded from further consideration in this report.

There have been various reports linking the four categories of biological particle in houses, non-industrial workplaces and public buildings with one or more of the following allergic manifestations among the occupants:

(1) Rhinitis, with "hay fever" symptoms such as nasal congestion, runny nose, sneezing, conjunctivitis and lacrymation;

(2) Asthma, with symptoms which include wheeze, tightness of the chest and shortness of breath;

(3) Humidifier fever, with symptoms including fever, chills, muscle ache and malaise, but no obvious respiratory effects;

(4) Extrinsic allergic alveolitis (hypersensitivity pneumonitis), with acute pneumonia-like bouts of fever, cough, tightness of the chest and lung infiltration, or chronic development of cough, shortness of breath and infiltration of lungs; and

(5) Atopic allergic dermatitis.

Biological particles have also been implicated in sick building syndrome (SBS) and organic dust toxic syndrome (ODTS). SBS is associated primarily with offices and other non-industrial premises (Report No. 4), but no quantitative relationship between particulates and health effects in these environments has yet been established. The acute systemic reaction to inhalation of massive amounts of dust associated with mouldy plant products, known as ODTS, occurs among workers handling agricultural materials (Sorenson 1989) and municipal waste (Sigsgaard *et al.* 1990).

Although each of the categories of biological particle has been implicated in one or more of these allergic diseases, allergens produced by house dust mites and in the dander of furred domestic animals are generally seen to be the most important cause of disease episodes in atopic individuals. For example, in a U.K. study some 80% of asthmatic children were allergic to the house dust mite (Price *et al.* 1990), and a Swedish study showed that 57% of asthmatic children were allergic to at least one type of furred animal (Kjellman and Pettersson 1983). Various investigations have shown that numbers of house dust mites are higher in damp houses (e.g. Burr *et al.* 1980), so that the incidence of mite-induced asthma is likely to be higher in damp housing. The major mammalian sources of allergens are the dander (skin scales) of cats and dogs, but dried urine and saliva of these animals are also potential sources of allergens (Knysak 1989). Among atopic children, there appears to be a greater incidence of allergy to cats than to dogs. Mice, rats and a number of other rodents which are popular as pets may also contribute to problems of allergy, e.g. hamsters, desert-rats (gerbils) and guinea pigs (Knysak 1989).

Although most investigations have shown that far fewer respiratory patients with suspected allergy react to moulds than to house dust mites and animal dander (e.g.

Beaumont *et al.* 1985), mould allergy among atopic children is frequent and may be severe. Recent epidemiological studies have also observed a strong association between reported dampness and mould in houses and respiratory symptoms in children (Strachan and Elton 1986; Martin *et al.* 1987; Strachan 1988; Brunekreef *et al.* 1989; Platt *et al.* 1989; Dales *et al.* 1991b) and adults (Dales *et al.* 1991a) occupying the houses. Strachan (1988) and Strachan *et al.* (1990) have, however, cautioned against reporting bias among participants in surveys and highlighted the need for further valid and objective data. Nevertheless, it has been reported that the effect of moulds or dampness may be comparable in magnitude to the effect of passive smoking on pulmonary function (Strachan 1988; Brunekreef *et al.* 1989). Correlation has been noted between reported rhinitis in children and winter indoor levels of yeast and the moulds *Cladosporium* and *Epicoccum* and between reported childhood wheeze (including physician-confirmed asthma) and indoor levels of *Aspergillus* spp. in winter and *Alternaria* in summer (Su *et al.* 1990, 1991), but it has also been suggested that a non-allergic mechanism may account for the increased respiratory symptoms among adults and children in damp houses (Dales *et al.* 1991a,b).

Attention must therefore be given to other biological factors which could possibly affect health. Firstly, there is the inhalation of airborne toxigenic organisms. The cell-wall lipopolysaccharide fractions of Gram-negative bacteria are endotoxins, and the symptoms provoked by respiratory challenge with these endotoxins, or the whole cells, are very similar to those found in typical acute episodes of humidifier fever (Rylander 1986). The spores of toxigenic moulds may contain very high concentrations of mycotoxins, and the dissemination of spores of one of these moulds, *Stachybotrys atra*, which had colonised ducts, insulation and structural materials, has been reported to be the cause of chronic health problems in members of one household (Croft *et al.* 1986). Other toxigenic moulds which may be prevalent indoors include *Aspergillus versicolor*, *Penicillium* spp., such as *P. brevicompactum* and *P. viridicatum*, and *Phoma* spp., or more rarely *Paecilomyces variotii* and *Trichoderma viride*. Mycotoxins must certainly be viewed as potentially hazardous factors present in airborne particles in homes and non-industrial environments, but considerably more research effort will need to be expended before their real impact on human health can be satisfactorily assessed.

A second factor which may be involved is the complex mixture of fungal volatiles, including 1-octen-3-ol, 2-octen-1-ol and 1,10-dimethyl-9-decalol (geosmin), which account for mouldy odours in damp houses. According to Samson (1985), some individuals do not react to these, some become nauseous and others may be quite ill. Tobin *et al.* (1987) have indicated that acute respiratory responses may range from a feeling of stuffiness to wheeze. Low toxicity to experimental animals has been demonstrated for 1-octen-3-ol and a range of other volatiles reported to be produced by moulds (Sorenson 1989). At present, however, there is insufficient information upon which to base an objective assessment of the impact of microbial volatiles on health. Although we regard the detection and measurement of such volatiles as being important, we consider that they, like mycotoxins, are outside the scope of the present document.

The fungi and bacteria implicated in allergic diseases generally live as saprotrophs (Flannigan *et al.* 1991), being able to lead an independent existence under damp conditions by utilizing for growth and multiplication the organic matter in wall coverings, soil (e.g. plant pots), dust, humidifiers, air ducts and soft furnishings, such as upholstered furniture and carpets. However, some of these organisms which can lead a saprotrophic existence in buildings are also opportunistic pathogens of man. For example, in exceptional cases the mould *Aspergillus fumigatus* may invade the lung tissue of debilitated individuals and from there become systemic, especially in the case of immunocompromised patients. Members of the bacterial genus *Legionella*, which can proliferate in water supplies and humidifiers, include *L. pneumophila*, the infectious agent of the acute respiratory disease, Legionnaires' Disease and the non-pneumonic disease Pontiac Fever. Outbreaks of this disease have been associated with hotels, hospitals and other large public buildings in particular.

Since there are numerous confounders, it is impossible to gauge the scale of health problems which are directly attributable to biopollutants in non-industrial workplaces, schools and other public buildings. It is also not possible to deduce the full extent of the problem in homes, although evidence of links between damp home conditions, mite numbers, mould growth, and respiratory health has accumulated. Damp housing is a serious problem in the Netherlands (Verhoeff *et al.* 1990), where around 15% of houses may be affected to some extent by dampness, and in the United Kingdom it has been estimated that around 20% of all households experience some mould growth or damage to decorations due to dampness (Environment Committee 1991). Mould growth in any

building is in itself undesirable and controllable, but in addition it is an indicator that conditions of relative humidity may also be favourable for yeasts, bacteria and mites, which (especially in the case of mites) can likewise affect respiratory health. The presence of mould growth in houses may also affect the occupants psychologically. Whilst the economic effects of ill-health resulting from these biopollutants cannot be quantified, one additional economic burden associated with mould and bacterial growth in buildings is the cost of remedial treatment which will prevent growth, or of more frequent redecoration where growth is not controlled.

This document is prepared at a time when the traditional approach to assessment of microbiological pollutants in indoor air is increasingly under question. It recognises that, in assessing the possible role of bioaerosols on health, (1) spatial and temporal fluctuations in numbers of airborne micro-organisms (Hunter *et al.* 1988) have to be taken into account; (2) not just culturable spores and cells, but the total numbers of allergenic (or toxigenic) particles as well as the antigenic components, i.e. the macromolecular organic dust components (MOD) of biological origin (Gravesen *et al.* 1990), are important; and (3) that both qualitative and quantitative comparison between outdoor and indoor air is necessary to indicate hidden sources of moulds, yeasts and bacteria indoors. Bearing in mind that there is unlikely ever to be universal agreement on either strategy or methodology, it therefore sets out for consideration strategies and methodologies for sampling four main categories of bioaerosol (mites, dander from furred pets, fungi and bacteria) which take into account recent research and thought.
A working group of WHO also dealt with biological contaminants in indoor air (WHO 1990b). This working group concluded that generally methods for collecting environmental samples of biological contaminants have not been standardised and that also most laboratory procedures for analysis have not yet been standardised. In the present document, sampling and analytical procedures for bioparticles will be discussed more in detail.

For each of the four categories of bioparticle dealt with in the main part of the document, the occurrence and health effects are outlined and specific problems in houses and workplaces/public buildings noted. Strategies are described for sampling the air and/or dust in naturally ventilated buildings and for large buildings with central and local HVAC systems. Specific methods for collection and analysis are detailed. Because of its particular importance, *Legionella* is given special attention in Chapter 6. The

interpretation of data is discussed and, whilst it may not be possible to give reference values, representative observed values from reported investigations are appended. Conclusions on strategies and methodology are presented in the final part of the document.

It should be stressed that at present there are no sampling or analytical methods available which provide accurate data for reliable assessment of the health risk arising from exposure to biological particles. Hence, the methods of collection and analysis presented in this report only provide information about the presence of biological particles in indoor environments. Furthermore, general sampling strategies for biological particles in indoor environments cannot be given, because the sampling strategy is highly dependent on the aim of an investigation.

2. HOUSE DUST MITES

2.1. Health effects

House dust allergy was recognised as early as the 1920s when dust extracts were found to give relevant positive reactions in skin tests on asthmatics. More than 20 years ago, the role of mites of the family Pyroglyphidae as the most important source of house dust allergens, was established. The importance of house dust mites in human health lies in their allergenic properties. The most important mite species in this context are *Dermatophagoides pteronyssinus*, *D. farinae*, *D. microceras* and *Euroglyphus maynei* (Mosbech 1985). However, a variety of non-pyroglyphid (storage) mites in the genera *Acarus*, *Lepidoglyphus*, *Tyrophagus* and *Glycyphagus*, can live in ordinary house dust as well, especially in damp houses (Mosbech 1985; Revsbech and Dueholm 1990).

In the last decade, considerable progress has been made in the identification, purification and characterization of allergens produced by *Dermatophagoides* spp. Their major allergens (Der p I, Der p II, Der f I, Der f II and Der m I and Der m II) are well characterised and purified (Lind 1981; Platts-Mills and Chapman 1987). Der p I, Der f I and Der m I have been shown to be faecal allergens, and very high concentrations of Der p I are present in mite feaces. Faecal pellets of the mites are about 25 µm in size, but can break into smaller particles which can be inhaled (Platts-Mills and Chapman 1987).
Allergy to mite allergens is fairly common in the atopic population. The reported prevalences among asthmatics varies from 45-85% (Platts-Mills and de Weck 1988). Sensitivity to mites is therefore a severe risk factor for asthma. For individuals with manifest allergy to house dust mite, exposure to 500 mites/g dust corresponding to 10 µg Der p I/g dust, is considered to be a level that provokes acute asthma attacks (Platts-Mills and de Weck 1988).

2.2. Occurrence

The natural food source of house dust mites appears to be skin scales, primarily from man, and/or fungi growing on skin scales. However, many other food sources may be used (Platts-Mills and de Weck 1988). Laboratory studies have shown that house dust mites

require particular conditions of temperature and humidity in order to grow. For *D. pteronyssinus* the optimum conditions are 25 °C and a relative humidity of 70-80%. The humidity seems to be the most critical factor in the survival and development of a house dust mite population (Mosbech 1985).

House dust mites have been identified in most parts of the world and mite-allergen levels in house dust have traditionally been assessed by counting isolated mite bodies under the light microscope. The earliest studies on mite counts reported seasonal and geographic variation in the numbers of mites in house dust attributable to differences in humidity. For example, in temperate climates, the lowest numbers are found in winter and the highest numbers in summer. In tropical countries, high numbers of mites can generally be found throughout the year. At high altitudes, the numbers of mites are lower. Environmental conditions also influence the species distribution. *D. pteronyssinus* usually dominates in continiously humid conditions, whereas *D. farinae* tends to predominate in areas where there is a prolonged period (more than 3 months) of dry weather. In most cases, it is unusual for other mite species to account for more than 10% of the mite population (Platts-Mills and Chapman 1987).

The highest numbers of mites in houses are usually found in mattresses, bedding, pillows, children's stuffed toys, upholstered furniture, and carpeting. Overall, the number of mites may vary from 10 to more than 1000 mites/g house dust (Pollart *et al.* 1988).

The amounts of mite allergen differ, as expected, between different locations, seasons and different geographic areas. However, mite allergen levels in house dust do not correlate well with mite numbers in house dust. For example, in temperate climates levels of Der p I in dust may stay high until January, even though living mite numbers fall in September. It is important to realize that dead or degraded mite bodies still have their allergenic properties (Platts-Mills and Chapman 1987; Pollart *et al.* 1988). As is the case for the mite numbers, there is a wide range in the levels of house dust mite allergens in house dust (<10 ng to >300 000 ng/g dust).

2.3. Available sampling methods

Air sampling

Several techniques have been described for volumetric sampling of airborne particles using cascade impactors or high volume samplers in combination with membrane filters. These techniques have the advantage that they sample airborne allergens and so may be more representative of exposure than assays of settled dust. However, a practical disadvantage of airborne sampling is that long sampling periods (2 - 24 h) are required, whereas exposure to short periods of high mite allergen concentrations may clinically be (more) important (Platts-Mills and de Weck 1988).

Mites themselves are not seen in air samples. Furthermore, in undisturbed rooms amounts of airborne mite allergens are small and therefore difficult to detect, even after prolonged sampling. Most of the allergens are bound to faecal pellets, which only become airborne as a result of disturbance, and very little allergen is associated with particles that will remain airborne for more than a few minutes (Pollart *et al.* 1988). In addition, no reliable information is available at present which will support adoption of a standard method for air sampling of house dust mite allergens. According to an International Workshop held in 1987 (Platts-Mills and de Weck 1988, see below) airborne sampling has not been shown to be better than dust sampling for primary measurement of mite infestation. Therefore, air sampling of house dust was not recommended. Since asthma is triggered by inhaled antigen, the measurement of airborne levels is more likely to be relevant in assessing the exposure, as indicated by Price *et al.* (1990). Therefore, research to compare the usefulness of air and dust sampling is recommended. If airborne sampling is presently undertaken, dust samples should also be obtained (Sporik *et al.* 1990).

Dust sampling

An International Workshop held in 1987 under the auspices of the International Association of Allergology and Immunology, UCB Institute of Allergy, the American Academy of Allergy and Immunology and WHO proposed the following procedure for sampling dust to measure house dust mites and their allergens (Platts-Mills and de Weck 1988). Some additions are made to this.

Vacuum cleaners used for settled dust sampling can be equipped with a special attachment

(e.g. the ALK (Allergological Laboratory, Denmark) allergen mouthpiece) to collect dust on a paper filter (38 cm^2, pore size 6 μm), or the dust can be collected into a disposable paper bag within the vacuum cleaner. The first method is less time-consuming, because cleaning of the sampling device with alcohol between two samplings is easier or even unnecessary if each sample is obtained with a new sampling device. If possible, the suction rate of the vacuum cleaner should be standardized.

Sampling sites should be consistent and the following preferred sites should be sampled separately:

1) The upper mattress surface (single mattress, 2 m^2) should be vacuum cleaned for 2 min after the bedding has been removed. The entire surface should be sampled;
2) Floor samples should be collected in the living-room and bedroom. An area of 1-3 m^2 should be sampled depending on the type of flooring. Each square metre should be sampled for 2 min. In the bedroom an area immediately underneath and beside the bed should be sampled. The type of flooring and the total area sampled should be recorded.

Samples can also be obtained from upholstered furniture. However, the results may be different from those in floor dust. Alternative techniques of collecting dust samples include shaking blankets in a plastic bag and scraping flat surfaces higher than floor level with a piece of firm card. However, these techniques are less effective than collection by vacuum cleaner and not standardised. All large particles, e.g. stones, leaves and paperclips, should be removed from the dust samples before analysis.

2.4. Available methods of analysis

There are three types of method for estimating the concentrations of house dust mites or their allergens in (airborne) dust samples: mite counts, immunochemical assays of mite allergen, and guanine determinations. The choice of a particular method depends on the specific purpose of a study.

Mite counts

The prevalence of mites can be determined by counting under a microscope after separation from the dust sample by flotation or suspension. This technique permits the identification of the predominant mite species and the recognition of live, dead, larval or

adult types. However, the disadvantages of this method include (1) the need for training and development of skill in determining different mite species; (2) the failure to quantify faecal pellets and disintegrated mite bodies and therefore to reflect the true extent of exposure to mite allergen levels; and (3) the unsuitability for large-scale (epidemiological) studies, owing to the time-consuming nature of the work (Platts-Mills and de Weck 1988).

Immunochemical assays

For immunochemical analysis, the normal extraction ratio for dust samples is 100 mg dust to 2 ml buffered saline (or 50% glycerine). The extraction is facilitated by rotation or agitation for 4 h. The extract is then stored frozen or in 50% glycerin at -20 °C. Lyophilization or repeated freezing and thawing should be avoided (Platts-Mills and de Weck 1988).

Mite allergens can be quantified in extracts of house dust by several immunochemical techniques. General information on different immunochemical analytical techniques can be found in Kemeny (1987).

1) *Assessment of total mite allergen content by inhibition radioallergosorbent testing (RAST inhibition).*

   This method provides a good estimate of the relative potency of different allergen extracts but cannot be used for absolute quantitation of mite allergen levels. An advantage of the method is that it measures "relevant" antigenic determinants that have elicited a response in allergic subjects, since human IgE is used. However, RAST inhibition results are difficult to reproduce over an extended period of time, because the results vary with the composition of the extract used on the solid phase and with the composition of the serum pool used for detecting bound allergen. Furthermore, the sensitivity is rather low. This method is not recommended for routine purposes because the availability of specific human serum samples needed for this analysis may be a problem (Platts-Mills and Chapman 1987).

2) *Assays that measure individual mite allergens, including radial immunodiffusion , rocket electrophoresis, enzyme-linked immunosorbent assay (ELISA) and inhibition radioimmunoassay (RIA).*

   Sandwich radio- or enzyme immunoassays employ either rabbit polyclonal or mouse monoclonal antibody for capture, and affinity- purified antibody or a second monoclonal

antibody for detection. These assays are more sensitive than RAST inhibition and those using monoclonal antibodies in particular have the great potential advantage of long-term reproducibility. Other advantages of immunochemical assays are their specificity, the fact that the results can be expressed in absolute units (ng or µg) of a defined protein, and their suitability for large-scale surveys because they can be automated. A disadvantage is that they require trained laboratory technicians and sophisticated laboratory equipment (Platts-Mills and Chapman 1987).

The detection limits for these assays vary from about 10 ng/g dust for Der f I and Der f II to 20 ng/g dust for Der p I, Der p II, Der m I and Der m II.

Guanine determination

Guanine is a nitrogenous excretory product of arachnids, and arachnid excreta are the major source of guanine in house dust. Since mites are predominant among arachnids in house dust, determination of the guanine content of house dust is an indirect method for assessing faecal pellets (Platts-Mills and de Weck 1988). Analysis of guanine is based on a colour reaction between guanine and an azo-compound. The amount of guanine can be measured quantitatively on a w/w basis using a spectrophotometer, or semi-quantitatively using a commercially available test-kit. This method is simple and economical, but does not identify the source, i.e. mite species. In addition, the test-kit only provides semi-quantitative results, and occasional false-negative and false-positive results are obtained. Lau *et al.* (1990) compared the results obtained with the test-kit with those obtained with the ELISA for Der p I and Der f I. There was a good correlation between the results of the two methods (r_s = 0.7), but this was mainly due to the correlation between very low and very high guanine levels and the corresponding Der p I and Der f I concentrations. They concluded that the guanine test was less sensitive than the ELISA and that its reliability was insufficient. Thus, the guanine test-kit may be used to obtain semi-quantitative information on the presence of house dust mite allergens.

2.5. Recommendations for different studies

A. Private homes

- Case studies

For case studies the following methods of sampling and analysis are recommended:

- Dust sampling, as described in section 2.3. Dust samples should be obtained from the upper mattress surface, the bedroom floor and the floor of the living-room. The type of flooring and mattress, as well as the area sampled should be recorded;
- If information on the prevalence of different mite species is required, mite counts can be obtained as described in section 2.4. If sampling is performed in order to obtain information about the presence of mite allergens, the dust samples should be analyzed using an immunochemical assay, preferably ELISA for Der p I (see section 2.4).

Additional information about building characteristics and the behaviour of the occupants which may be major influences on the prevalence of house dust mites and their allergens should be recorded, according to the checklist in Appendix II.

- Epidemiological studies

For epidemiological investigations of the relationship between the exposure to house dust mites and health effects the following methods of sampling and analysis are recommended:

- Dust sampling as described in section 2.3. Dust samples should be obtained from the upper mattress surface, the bedroom floor and the floor of the living-room. The type of flooring as well as the material of the mattress should be recorded;
- The dust samples should be analyzed using an immunochemical assay, preferably ELISA (see section 2.4). The analysis should be performed for at least Der p I, but - depending on the study - other mite allergens might also be included.

Additional information about building characteristics and the behaviour of the occupants - as far as they can influence the concentrations of mite allergens in settled house dust - should be recorded, according to the checklist in Appendix II.

B. Other non-industrial indoor environments

As house dust mites seem rarely to be found in either industrial or non-industrial work places, measurement of house dust mites allergens in these environments is not considered to be necessary.

Low amounts of house dust mite allergens can, however, be found in dust deposits in schools, day-care centres and hotels. Therefore, assessing the concentrations of house dust mite allergens in these environments might be relevant.

The following methods of sampling and analysis are recommended:

- Dust sampling as described in section 2.3. Dust samples should be obtained from the floor, and, if beds are present, from the upper mattress surface. The type of flooring and the material of the mattress should be recorded;
- The dust samples should be analyzed using an immunochemical assay, preferably ELISA for Der p I (see section 2.4.).

2.6. Observed values and evaluation of the results

The levels of house dust mite allergens in house dust vary widely. Appendix I summarises the results of some recent studies in Europe and the USA on house dust mite allergens in house dust.

According to WHO (Platts-Mills and de Weck 1988), at present there seem to be sufficient data to propose the following hygienic threshold limits:

- 2 µg Der p I/g dust should be regarded as representing a risk for genetically predisposed individuals in the development of specific IgE to house dust mite allergen;
- 10 µg Der p I/g dust should be regarded as a risk factor for acute attacks of asthma; at this level most mite-allergic patients will experience symptoms.

Table 1 presents categories for the evaluation of the concentrations Der p I and Der f I measured by ELISA after extraction of 100 mg dust with 2 ml buffered saline. It should be stressed that these categories are based on the range of values obtained in houses and not on a health risk evaluation. Furthermore, the seasonal influence on the expected concentrations of house dust mite allergens, should be taken into account.

Table 1 : Categories of Der p I and Der f I in house dust.

category	Der p I (µg/g dust)	Der f I (µg/g dust)
very low	< 0.5	< 0.5
low	< 5	< 5
intermediate	< 15	< 15
high	< 20	< 20
very high	> 20	> 20

N.B. : These categories are based on the range of values obtained in houses and not on a health risk evaluation

3. DANDER FROM FURRED ANIMALS (PETS)

3.1. Health effects and occurrence of allergens

Dogs and cats are likely to be the most common animal species with which humans have close domestic contact, but the keeping of other pets such as guinea pigs, rabbits, hamsters and birds is also widespread in Western society. Most pets produce allergens which can be inhaled (Lowenstein *et al.* 1986) and larger animals such as horses, goats, cows, chickens and ducks, even although kept outdoors, can also cause problems. In addition, rats, mice and insect vermin, such as cockroaches, may be sources of allergens in indoor environments (Reed and Swanson 1986). Furthermore, there is an increasing prevalence of allergy among laboratory workers handling large numbers of experimental rats and mice.

The presence of pet-derived allergenic material is of course independent of environmental factors such as humidity, temperature, altitude or the quality of the building. The major factor of importance is the thoroughness of the cleaning carried out in the house, i.e. the removal of the allergenic material both produced in the house and brought in from outside. The allergens produced by pets are mostly associated with dander, hair, saliva and/or urine (Lowenstein *et al.* 1986).

The most important source of cat allergens is dander. The major allergen of the cat (Fel d I) has been isolated, characterized and standardized. This allergen is released into indoor air and house dust in dander and the shed hair to which the allergens are attached (Wentz *et al.* 1990). The allergen becomes airborne as particles 1-10 μm in diameter, presumably after it dries and flakes off the fur (Reed and Swanson 1986). The recent indication by Luczynska *et al.* (1990) that airborne Fel d I is associated with small particles which remain airborne for long periods might explain the distinctive rapid onset of asthma or rhinitis in patients allergic to cats on entering a house with a cat. Lopes da Mata *et al.* (1990) suggested that cats are the most important pets causing allergy in the home, owing to their widespread occurrence and the allergenic potency of the allergens they produce.

Fel d I can be detected in indoor air, house dust and also in mattress dust. In air samples taken with cascade impactors, impingers or high volume samplers in houses where at least one cat was present the reported allergen concentrations ranged from 250 to 1140 ng/m^3

air. In houses without cats, the concentration of Fel d I present in house-dust ranged from amounts below the limit of detection to 100 000 ng/g of dust, but in houses where cats were present, the concentrations ranged from below the limit of detection to around 300 000 ng/g (e.g. Lowenstein *et al.* 1986; Wood *et al.* 1988; Luczynska *et al.* 1990). In houses where cats are present, dust samples usually contain more than 8 µg Fel d I/g dust. Cat allergens have also been demonstrated in dust samples from carpeted and smooth floors in schools (Dybendal *et al.* 1989a,b).

Prevalence studies of hypersensitivity to cat allergens in unselected populations have shown a frequency of positive skin reactions of around 15%. Hader *et al.* (1990) found in a cross-sectional study with 704 unselected schoolchildren (7-16 years) that 10.5% had a positive skin test against cat dander. Sears et al. (1989) followed a birth cohort of New Zealand children for 13 years. Of 714 children skin-tested, 13.3% were sensitive to cat dander. Lelong *et al.* (1990) reported for a group of allergic children a frequency of sensitization to cats of 30%. Among atopic individuals who are not sensitive to house dust mite allergens, a prevalence of 80% is reported (Pauli *et al.* 1979; Ammann and Wütrich 1985).

Adverse reactions to dog dander are considered to be species specific (Blands *et al.* 1977), although evidence of breed specific allergens has been presented (Lindgren *et al.* 1988). Furthermore, cross-reactivity between allergens of cats and dogs has been found. Isolation and standardization of the major allergen(s) produced by dogs is still a problem, although recently the first dog-hair and dander specific allergen, termed Can f I, was isolated (Schou *et al.* 1991). These authors suggested that Can f I could be used as a marker allergen for future studies of the environmental exposure to dog-hair and dander allergens, and for the study of the relationship between exposure and sensitization.

The concentrations of dog allergens found in house dust vary widely. In house dust sampled in houses without dogs, concentrations of 110 - 82 500 IU/g (International Units/g) have been found and in houses where dogs are present, 1 100 - 585 000 IU/g have been reported (Wood *et al.* 1988). Dog allergens have also been demonstrated in mattress dust (Lind *et al.* 1987b). In houses where dogs are present, dust samples contain generally more than 10 µg Can f I/g dust.

The prevalence of dog allergy in unselected populations is reported to be 4-15%. Lelong *et al.* (1990) showed among a group of allergic children a frequency of sensitization of 17%

to dogs. In a study among 203 asthmatic children Vanto and Koivikko (1983) found a relatively high prevalence (56%) of positive skin prick tests to dog dander.

3.2. Available sampling methods

Air sampling

For the sampling of airborne pet-derived allergens, the same methods can be used as for the sampling of airborne mites and/or their allergens (see Section 2.3). Pet-derived allergens in air have been sampled using cascade impactors, high volume samplers in combination with membrane filters and liquid impingers. These techniques have the advantage that they sample airborne pet-derived allergens and may be more representative of exposure than assays of settled dust. However, at present there is no reliable information available which will support adoption of a standard method for air sampling of pet-derived allergens. Therefore, further research to compare the usefulness of air and dust sampling is recommended. If air sampling is presently undertaken, dust samples should also be collected.

Dust sampling

In principle, the sampling of house dust to investigate the presence of pet-derived allergens can be conducted exactly as for house dust mites and their allergens (see Section 2.3).

3.3. Available methods of analysis

Immunochemical assays are used for the detection of pet-derived allergens in air samples or house dust samples (Lowenstein *et al.* 1986). The techniques used include CCIE (counter current immunoelectrophoresis), RIE (rocket immunoelectrophoresis) and ELISA. In all cases the extracted material is analyzed by the use of species-specific rabbit antibodies raised against the various pet-derived allergens. RAST-inhibition is also used. However, this technique cannot be used for absolute quantitation of the allergen levels. Moreover, the results vary with the composition of the extract used on the solid phase and with the composition of the serum pool used for detecting bound allergen. Thus, for

routine purposes, RAST inhibition is not recommended.

A variety of methods have been described in the literature for the extraction of the dust samples prior to the analysis. For example, extraction has been conducted with 0.1 *M* phosphate buffer (pH 7.4); 0.1 *M* phosphate buffer containing 0.2% bovine serum albumin (BSA); 1% BSA phosphate-buffered saline containing 0.05% Tween 20; and 1 : 10 w/v with 0.125 mol/l ammonium bicarbonate buffer. As far as is known, no comparative studies have been conducted with regard to the use of different extraction methods. Therefore, at present no recommendation can be given. However, the use of the 0.125 mol/l ammonium bicarbonate buffer is convenient.

Further standardisation of the relevant pet-derived allergens is needed and should take priority over standardisation of the analytical techniques for (airborne) dust samples. For Fel d I a standardized ELISA technique is available (Wood *et al.* 1988; De Blay *et al.* 1991). In the near future a standardized ELISA technique will also become available for Can f I which will be more sensitive and specific than the CCIE currently used.

3.4. Recommendations for different studies

A. Private homes

- Case studies

For case studies the following methods of sampling and analysis are recommended.

- Dust sampling, as described in section 2.3. Dust samples should be obtained from the floor of the living-room and the bedroom. The type of flooring as well as the presence of pets (species and number) should be recorded. Pet-derived allergens can also be found in mattress dust. Therefore, the sampling of the upper mattress surface might be relevant as well.
- Dust samples should be analyzed by immunochemical assays (ELISA) for Fel d I and Can f I (see section 3.3).

- *Epidemiological studies*

For epidemiological studies on the relationship between the exposure to pet-derived allergens and health effects, the following methods of sampling and analysis are recommended.

- Dust sampling as described in section 2.3. Dust samples should be obtained from the floors of the living-room and bedroom, as well as from the upper mattress surface. The type of flooring should be recorded. Furthermore, information about the presence of pets - in the past and at present - should be recorded (species and number).
- Dust samples should be analyzed by ELISA, for Fel d I and Can f I (see section 3.3).

B. Other non-industrial indoor environments

As far as is known, allergens derived from furred animals are rarely found in office buildings, schools and day-care centres, since animals such as cats, dogs, guinea pigs, hamsters, desert-rats or mice, are not regularly kept in these environments. However, if pets are present, e.g. in school classrooms, the measurement of pet-derived allergens might be relevant. It should also be remembered that allergens (mainly from cats, dogs and horses) deposited on (children's) clothing, can be brought into such environments from the home, as indicated by Dybendal *et al.* (1989a,b). For investigations of these non-industrial indoor environments, the same sampling and analytical methods would be adopted as for private homes.

3.5. Observed-values and evaluation of results

Appendix I summarises the results of a limited number of recent studies on the presence of pet-derived allergens in indoor environments in Europe and the USA. At present no threshold limit values for the exposure of occupants to pet-derived allergens can be given.

Table 2 presents categories for the evaluation of the concentrations of Fel d I and Can f I, measured by ELISA. It should be stressed that these categories are based on the range of values obtained in houses and not on a health risk evaluation.

Table 2 : Categories of Fel d I and Can f I in house dust

category	Fel d I (ng/g dust)	Can f I (ng/g dust)
very low	< 100	< 300
low	< 1 000	< 10 000
intermediate	< 10 000	< 100 000
high	< 100 000	< 1 000 000
very high	> 100 000	> 1 000 000

N.B.: These categories are based on the range of values obtained in houses and not on health risk evaluation

4. FUNGI

4.1. Health effects

Although the causative role of fungi in individual cases of respiratory allergy and asthma has been known since the 18th Century, their overall significance in respiratory health is still debated. Reported allergic reactions to single or clustered spores and hyphal elements of fungi in air include rhinitis, asthma and extrinsic allergic alveolitis (Tobin *et al.* 1987). Allergic reactions to fungi may be either immediate, developing within minutes of exposure (Type I), or delayed, occurring 4 - 8 h later (Type III). Rhinitis and asthmatic episodes fall into the Type I category, and the much rarer instances of extrinsic allergic alveolitis caused by exposure to high concentrations of specific allergens are Type III reactions. The latter are most often associated with high levels of actinomycete or fungal spores (10^6 -10^{10} spores/m^3 air) released from moulded agricultural materials and causing occupational diseases such as farmer's lung.

The literature on the relationship between inhaled fungal spores and induction of respiratory allergy is still inadequate and controversial in many respects. However, in the last ten years there has been considerable progress in the identification, purification and characterization of at least some allergens produced by fungi, especially by *Alternaria* (Alt a I) and *Cladosporium* (Cla h I). However, much still needs to be done in standardizing allergens used in testing; there is still a need for highly purified and standardized extracts (Burge 1985).

Traditionally, allergologists have assumed that mould-induced asthma was entirely due to an allergic reaction. However, it is highly likely that toxigenic properties of fungi may be involved as well as their allergenic properties. In the case of pathogenic species of genera such as *Aspergillus*, all of these contribute to the overall damage which results from invasion of lung tissue (mycosis). The metabolites of such fungi probably contribute very significantly to the lung damage observed (Tobin *et al.* 1987).

Among patients with respiratory allergy, 2 - 30% are allergic to fungi (Gravesen 1979). A multi-centre study in Denmark revealed that the prevalence of fungal allergy in a atopic population was 8% in adults and 23% in children, 44% of the children having asthma and

23% rhinitis (Osterballe 1981).

The mycotoxins associated with fungi are secondary metabolites (Miller, 1990) with molecular weights generally greater than 200 but considerably smaller than allergens. It is well established that ingested mycotoxins can cause illness and death in humans and animals, but it has also been demonstrated in experimental animal studies that inhalation challenge with trichothecenes is 20-50 times more hazardous than intravenous injection (Miller 1990). The trichothecenes, produced by *Stachybotrys atra* and *Fusarium* spp., and patulin and penicillic acid produced by a number of penicillia, demonstrate acute toxicity to pulmonary alveolar macrophages (Sorenson 1989). Trichothecenes and a number of other mycotoxins are also immuno-suppressive. Therefore, although there is no definitive proof, it is nevertheless possible that inhalation of high concentrations of mould spores may deleteriously affect macrophage functions such as phagocytosis of living and non-living particles in the lung, and therefore affect respiratory health. The effects on the immune system could also compromise health by reducing resistance to other micro-organisms, perhaps resulting in chronic health problems such as those encountered in the household mentioned on page 3 (Croft *et al.* 1986). However, toxicological data for many mycotoxins are limited, particularly for the dermal or inhalation route. Thus, the significance of mycotoxins in indoor environments is not yet clear (Miller 1990; Flannigan *et al.* 1991).

Like endotoxin in Gram-negative bacteria (see Chapter 5), 1,3-β-glucan is a wall component in fungi. Glucan can be extracted from the walls of fungal hyphae and spores. It acts as an inflammatory agent and has been implicated in increased reporting of mucous membrane irritation and fatigue by occupants of buildings about which indoor air quality complaints had been made (Rylander *et al.* 1991). Glucan can be assayed by a modification of the procedure used for bacterial endotoxin and can be used to give an overall indication of airborne fungal matter. Although 1,3-β glucan analysis appears to be a promising technique, further evaluation of its use is necessary before it can be recommended for application to problems of indoor air quality.

As mentioned in Chapter 1, the volatiles which are produced by fungi are frequently evident as "mouldy smells". The volatiles are complex mixtures of alcohols, esters, aldehydes, various hydrocarbons and aromatics, and a large number have been identified. There is considerable variation in the production of volatiles, even between closely related genera or species. Symptoms like headaches, eye, nose and throat irritation or fatigue have been

associated with volatile compounds produced by fungi (Tobin *et al.* 1987; Flannigan *et al.* 1991).

4.2. Occurrence

Among the approximately 100 000 known species of fungi, those of interest in indoor environments belong to the class of Deuteromycetes or Fungi Imperfecti, with a few exceptions (e.g. Mucorales, Ascomycetes, wood-rotting Basidiomycetes and some yeasts).

The majority of fungi are saprotrophic utilizing dead organic material for food. Providing temperature and moisture conditions are met, many species can utilize a wide range of organic materials. This ranges from plant or animal remains to materials such as cellulose, paint, or stored products (Gravesen, 1979). Although some fungi will grow at 2 to 5°C and others at temperatures as high 55 to 60°C (thermophiles), the majority of the fungi in indoor environments grow at temperatures between 10 and 35°C. The water content of these materials is the most critical factor in the development of fungi. The minimum water activity (a_w, the ratio of the vapour pressure above a substrate to the vapour pressure above pure water under the same conditions of temperature and pressure) needed for the growth of fungi on building related substrates varies from 0.75 to more than 0.98 for different mould species (Grant *et al.*, 1989).

Interior environments, unless scrupulously clean, offer a wide variety of substrates for growth. Damp, non-living organic material can be quickly colonized (Burge, 1985). Condensation is the principal source of the moisture needed for growth of fungi on the internal surfaces of domestic dwellings. Besides superficial condensation, interstitial condensation within porous building materials such as concrete, brick and plaster, may provide a reservoir allowing fungal growth to continue in circumstances under which the surface would otherwise dry out. Interior dampness problems are usually related to construction faults, such as inadequate insulation or "cold-bridging", in combination with inadequate ventilation and/or the pattern of usage of these houses. Condensation and mould problems may also be encountered in "tight" houses built to conserve energy, particularly when measures to prevent excessive generation of moisture (e.g. in cooking or laundering) are not taken.

The presence of fungi shows wide seasonal differences (Gravesen 1972; Wilken-Jensen and Gravesen 1984). For most genera, the highest numbers in the outdoor air are found during summer and autumn. During these seasons the outdoor air is the main source of fungi in the indoor air. Recent studies indicated that the outdoor air spora influences the presence of fungi in indoor environments, but the indoor air spora is not a simple reflection of the presence of fungi in outdoor air (Fradkin *et al.* 1987; Verhoeff *et al.* 1992). Indoor sources of spores may also be present.

A large variety of fungal species can be found in indoor air. The most common species are likely to belong to the genera *Cladosporium, Penicillium, Alternaria, Aspergillus, Eurotium,* and *Wallemia*. However, the viable fungal propagules may only comprise 1- 2% of the total number of propagules. Again, the number of colony forming units (CFU)/m^3 varies widely, from <10 to more than 20 000 and even as high as 400 000 in exceptional cases (Hunter *et al.* 1988).

The presence of fungi in house dust seems to be an important source of airborne spores in indoor environments also (Burge, 1985). In house dust, species belonging to the following genera are commonly isolated: *Cladosporium, Penicillium, Alternaria, Aspergillus, Eurotium, Mucor* and *Wallemia*. The number of CFU/g dust ranges from $<10^2$ to $>10^6$.

4.3. Available sampling methods

Air sampling

Viable and non-viable fungal particles are both important in relation to health effects. Thus, in order to optimize the information available from air sampling for fungal particles, both types of particle should be sampled. However, even using ideal methods, a large number of airborne spores will not grow in culture *and* cannot be visually identified using currently available methods. Furthermore, the reproducibility of the measurements of viable fungal propagules in terms of CFU/m^3 and in terms of species isolated is low. The coefficients of variation of parallel duplicate samples are high. Also the coefficients of variation of sequential duplicates taken within minutes of each other are high (Verhoeff *et al.* 1990).

In view of these problems, air sampling of fungal particles cannot satisfactorily be used to assess the exposure of occupants to fungi in indoor environments. Air sampling can only provide information about the presence of fungi in indoor air at the time of sampling.

Several techniques have been described for volumetric sampling of fungi in indoor environments. Table 3 presents an overview of the techniques most commonly used for the sampling of fungi in aerobiological studies. Some of the techniques give total counts of all airborne particles, viable and non-viable, whereas others only give counts of viable fungal particles, i.e. propagules or CFU. Furthermore, no samplers collect all particles with equal efficiency. Samplers differ in cut-off size, i.e. the particle size above which 50% or more of the particles are collected (see Table 3). At present, there is no standardized method for the sampling of airborne fungi, although a proposal was published by the American Conference of Industrial Hygienists (ACGIH) in 1987 (Burge *et al.* 1987). However, the ACGIH recommends several air sampling devices for the sampling of airborne viable fungal particles. At present, the ACGIH is updating these recommendations.

Table 3 : Overview of sampling techniques for airborne fungal propagules.

Method with examples	Sampling rate and time	Remarks
A. Non-viable, non-volumetric		
* gravity slide	-	semi-quantitative, overrepresentation of larger particles
A. Non-viable, volumetric		
* Burkard trap	10 l/min; 7 days	cut-off unknown
* rotating arm impactors	47 l/min; 15-60 sec.	cut-off unknown
* filter methods	1 - 4 l/min; hours	
B. Viable, non-volumetric		
* open petri dish	-	semi-quantitative, overrepresentation of larger particles
B. Viable, volumetric		
* Andersen 6-stage impactor	28.3 l/min; 1 - 30 min	cut-off 0.65 µm[a]
* Andersen 2-stage impactor	28.3 l/min; 1 - 30 min	cut-off 0.65 µm[a]
* Andersen 1-stage impactor (N6)	28.3 l/min; 1 - 30 min	cut-off 0.65 µm[a]
* Surface Air System impactor (SAS)	180 l/min; 20 s - 6 min	cut-off 1.9 µm[b]
* Reuter Centrifugal impactor (RCS)	ca. 40 l/min; 30 s - 8 min	cut-off 3.8 µm[c]
* Reuter Centrifugal Plus impactor (RCS Plus)	ca. 50 l/min; 30 s - 8 min	cut-off unknown
* slit samplers	10 - 30 l/min	cut-off 0.7 µm[d]
* liquid impingers	12.5 l/min	cut-off 0.3 µm[d]

[a] Andersen 1958, [b] Lach 1985, [c] Macher and First 1983, [d] Nevalainen *et al.* 1992

A. Total counts of viable and non-viable fungal particles

The techniques available for sampling the total number of fungal particles cannot be recommended as giving a good assessment of the composition of the air spora because only fungi with distinctive spores can be identified. However, filter methods can be used not only to give total counts, e.g. by direct epifluorescence microscopy, but also to obtain viable counts by plating washings from the filter (Palmgren *et al.* 1986).

B. Viable fungal particles

In terms of CFU/m^3 and species isolated, the results obtained depend largely on the sampling device used because of differences in cut-off point, sampling time and sampling volume (Verhoeff *et al.* 1990) and on the level and type of activity in the room during sampling (Hunter *et al.* 1988). For internal comparisons, in principle any of the available sampling devices can be used. However, in order to make proper comparisons between the results of different studies standardization is needed.

Most published data on the presence of viable fungal particles in indoor air have been obtained using the Andersen 6-stage impactor (Andersen 1958). But, high correlations have been found between the results in terms of CFU/m^3 obtained with the Andersen 2-stage impactor and the N6-Andersen 1-stage impactor (Jones *et al.* 1985).

In a correctly operated all-glass impinger, clusters of spores are broken up into smaller units of individual spores. Therefore, counts may be higher than with an impactor sampler.

- *Settle plates*

The use of settle plates or open petri dishes (OPD) is generally considered the least reliable. However, two recent studies, in which the results obtained with the N6-Andersen were compared with those obtained with settle plates, reported high statistically significant correlation coefficients between the results obtained with both methods in terms of CFU (Verhoeff *et al.* 1990; Verhoeff *et al.* 1992). Thus, settle plates may be used to obtain semi-quantitative information on the presence of viable fungi in indoor air. However, the number of different fungal species sampled with settle plates appears to be significantly lower than the yield in terms of number of species isolated with the N6-Andersen

(Verhoeff *et al.* 1992).

- *Collection media*

The choice of the collection medium is important, because the results obtained depend on the collection medium used. It should be stressed that no single medium will enable the entire range of fungi in the air to be isolated. V8 agar, in which the source of nutrients is vegetable juice and on which sporulation is favoured, can be used (Gravesen 1972), but nutrient-rich media such as Sabouraud agar which favour vegetative growth are not suitable for aerobiological studies. Although ACGIH (Burge *et al.* 1987) advocate the use of a malt extract agar (MEA [pH 4.5 - 5.0]) and it is widely used, it is a relatively rich medium, containing 20 g malt extract, 20 g dextrose, 1 g peptone and 20 g agar per litre distilled water and its use as an isolation medium in sampler plates cannot therefore be recommended. Such rich media allow a wide range of fungi to grow, but the colonies of fast growing fungi rapidly overgrow slow growing fungi on plates. Omission of the dextrose and peptone from MEA gives a less rich but nutritionally adequate medium on which most fungi can be isolated and enumerated (Hunter *et al.* 1988; Miller *et al.* 1988) and reduces the risk of overgrowth.

Another limitation of MEA is that xerophilic fungi like *Eurotium* and *Wallemia* may not be isolated. Recently, Dichloran:18% Glycerol Agar (DG18), developed by Hocking and Pitt (1980), has proved to be useful in aerobiological studies (Verhoeff *et al.* 1990). It is intended to be a selective medium with a low water activity for isolation of xerophilic fungi, but many of the common fungal species in indoor air can also be isolated, although some hydrophilic fungi may not occur. The dichloran restricts the growth of the colonies, facilitating the counting and isolation of all species present.
In general, it is recommended that antibiotics such as penicillin and streptomycin, or chloramphenicol, are added to the collection medium to prevent bacterial growth.

Dust sampling

Settled house dust can be sampled for viable fungi in exactly the same way as for house dust mites and/or their allergens (see Section 2.3). The dust samples can be stored at room temperature, but the analysis should be performed within a few days.

Surface sampling

Surfaces with or without visible fungal growth can be sampled to obtain qualitative information about the fungi present in one of the following ways:

1) Sellotape samples, used for direct microscopic examination of the presence of fungal spores and mycelium. A piece of transparent adhesive tape is pressed gently on the surface and then transferred onto a microscope slide (adhesive side downwards). Before microscopical examination, a drop of lactophenol cotton blue is added.
2) Swab samples. A sterile swab is swept gently over the surface. Where fungal growth is visible dry swabs can be used. Otherwise, the swabs can be wetted with sterile 0.1% peptone solution. Then the sample is either directly plated onto an agar medium for fungal growth (see under "Collection media") by streaking the swab over the agar surface. Alternatively the swab may be washed in 0.1% peptone solution and the washings and/or dilutions plated out. After incubation, the colonies can be transferred onto appropriate media for identification. A disadvantage of swab samples is that they over emphasise heavily sporing moulds.
3) Contact or pressure (Rodac) plates. A contact or pressure plate containing medium for fungal growth (see under "Collection media") is pressed gently onto the surface. After incubation, the colonies can be transferred onto appropriate media for identification.

4.4. Available methods of analysis

Air samples

- *Total counts of viable and non-viable fungal particles*

Counting and identification of the spores is performed using a microscope. However, as indicated above, it is often difficult to identify the fungal spores to species or even genus. Filter methods can also be used to obtain viable counts by plating washings from the filter (or dilutions) on agar media.

- *Viable fungal particles*

Samples should be incubated at 25°C for 4 days if MEA, V8 or DG18 is used as collection medium. Then the number of colonies is counted by the naked eye and expressed as CFU/m^3. After colony counting, fungal spores and/or mycelium can be transferred onto appropriate media for identification to species level, according to standard mycological procedures.

Dust samples

House dust samples can be plated directly onto an appropriate agar medium for cultivation by spreading duplicate representative samples of dust (30 mg) over the agar using a sterile glass spreader. The same media can be used as for air sampling. After incubation for four days at 25°C, the colonies are counted and then isolated for identification up to species level, according to standard mycological procedures. With direct plating, counting and isolation of the colonies might be difficult or even impossible due to a high density of the colonies on the agar plates. Therefore, the house dust samples may also be suspended in a peptone or sucrose solution prior to plating on the medium. An advantage of this method is the possibility of stepwise dilution. Generally, 100 mg of dust is used with this method. The counts obtained by this method will be higher than by direct plating, due to breaking up of aggregates of spores during suspension and dilution, similar as for the dilution plate count technique for the enumeration of fungi in soil (Jensen 1962).
At present, research comparing the different methods available for determining viable moulds in house dust is being undertaken, but present experience suggests that a wider range of mould species may be obtained by plating the dust directly onto an agar medium, although the counts will be lower.

4.5. Recommendations for different studies

A. Private homes

- Case studies

The first step in a case study - performed in order to explain cases of disease caused by fungi in the home environment - is to conduct a "walk-through" of the home for visual and olfactory assessment of factors having a possible bearing on fungal problems, e.g. presence of visible dampness (including on furnishing), mouldy spots, condensation, leakage of pipes, penetration of rainwater, rising dampness and the presence of (local) HVAC systems.
If dampness problems are observed, remedial action should be taken in order to solve the problems and to reduce the exposure of the occupants to fungi. In such cases, sampling of fungi is not necessary, since it is clear that problems involving fungi are present. Furthermore, the results of air-, dust- or surface-sampling for fungi cannot be used to obtain a cause-effect relationship between the level of exposure and disease.

Sampling of fungi might be useful in the following cases:

1. If (local) HVAC systems are present, sampling of fungi is recommended to evaluate whether the system is a possible source of bioaerosols.
2. If filters within the ventilation system are damp, sampling of fungi might be useful to evaluate whether these filters are a possible source of fungi for the indoor environment.
3. If damp spots and/or mould growth is not visible but the typical odour of moulds, described as "earthy", "mouldy" or "musty" is detected, sampling of fungi is recommended since the observed odour indicates the presence of fungi.
4. In order to evaluate whether a specific organism is present in the indoor environment, when that particular organism is indicated as a cause of observed allergic reaction(s) among occupants.
5. Sampling of fungi might be considered in a suspected case of extrinsic allergic alveolitis (EEA) (Fergusson *et al.* 1984) and recurrent episodes of rhinitis and/or asthma or other recurrent respiratory problems (Kozak *et al.* 1979).
6. Sampling of fungi might also be considered if no physical and/or chemical indoor air factors can be found which may be or have been the cause of the complaints.
7. Sampling of fungi may also be used to evaluate the effects of remedial action.

If sampling of fungi as part of a case study is performed, the following sampling and analytical methods are recommended:

- Surface sampling, to obtain qualitative information about the fungi present. Sellotape, swab and/or contact plate samples can be taken, as described in section 4.3. Swab and contact plate samples allow for species identification;
- Air sampling, to investigate whether specific mould species are abundant in the indoor air. For air sampling, any air sampling device can be used if properly and consistently used. As collection media, V8-agar, MEA (2%, without dextrose and peptone) or DG18 can be used. It is important to stress that results obtained with different sampling devices and collection media cannot be compared. Parallel duplicate samples should be taken under normal living conditions. Furthermore, parallel outdoor samples should be taken.

  The samples should be incubated at 25°C for 4 days. The number of colonies should then be counted by naked eye. Fungal spores and/or mycelium can be transferred onto appropiate media for identification. If specific fungal species are of interest, a selective identification medium may be used for collection, e.g. AFPA agar for *Aspergillus flavus/A. parasiticus*.

If sampling is performed in order to determine whether a (local) HVAC system or damp filters within the ventilation system are possible sources of fungi, samples should be taken at the inlet supply for the outdoor air and at the point of ingress of air into a room, both when the ventilation system is and is not operating.

If sampling is carried out because typical mould odours are present, samples should be taken at those locations where the odour is detected and preferably in a non-odorous reference room (or house).

To evaluate the effects of remedial action, sampling should be performed before and after the action has been taken.

In any other case, sampling should be performed in at least the living room and one bedroom.

Additional information about building characteristics and the behaviour of occupants,

which are the principal influences on the presence of fungi in the home, should be recorded, following the checklist in Appendix II.

- *Epidemiological studies*

In view of the problems arising with air sampling, dust sampling might be considered for large scale epidemiological studies of the relationship between the exposure to fungi and health effects.

- Dust sampling can be performed as described in section 2.3. Dust samples should be taken from the upper mattress surface, the bedroom floor and the floor of the livingroom. The type of flooring as well as the material of the mattress should be recorded.
- If information is needed on total viable counts/g dust and on the presence of mould genera, the house dust samples can be plated directly (in duplicate) onto V8, MEA or DG18 by spreading representative samples of 30 mg dust over the agar using a sterile glass spreader (see section 4.4).

  If information on total viable counts/g dust and on mould species present is required, the house dust samples (100 mg) should be suspended in a peptone solution (0.1% peptone + 0.85% NaCl + 0.02% Tween 80 per litre distilled water) prior to plating on the medium. After thorough mixing 0.1 ml is plated out in duplicate. After four days of incubation at 25°C, count the colonies and then make isolations for identification to genus or species.

B. Other non-industrial indoor environments

Specific indoor air quality problems related to mechanical ventilation systems, air conditioning systems and humidifiers have been described in Report No. 4 of these series (Commission of the European Communities 1989).

In order to evaluate the presence of fungi in buildings with central or local heating, ventilation and air conditioning systems (HVAC's), the following approach should be adopted.

1) Conduct a "walk through" of the building for visual and olfactory assessment of factors having a possible bearing on microbiological problems;
2) Determine whether the ventilation system involves recirculation or not. It is known that within recirculation systems, dust may accumulate and microbial growth may be more likely to occur;
3) Determine whether condensation within the system occurs, as this will promote the growth of micro-organisms;
4) Surface-sample dust deposits and/or visible fungal growth in order to evaluate whether these may be sources of micro-organisms in the indoor air;
5) Pay particular attention to humidifiers; inspect such devices and collect water and surface samples for microbiological evaluation;
6) If sources of micro-organisms within the system are found, sample air and/or surfaces in rooms supplied by the system, to determine whether spread of the organisms from the system is actually occurring.

It should be stressed that no general sampling strategy for these kinds of indoor environments can be given. Each problem demands its own sampling strategy.

4.6. Observed values and evaluation of results

As stated before, both the qualitative and quantitative results of air sampling for fungal propagules are highly dependent on the method used, on the activity and other circumstances during sampling, the season and on the geographical area. For these reasons reference values are difficult to establish. Data on fungi in settled house dust are limited. Appendix I summarises the results of some recent studies in Europe, Canada and the USA on the presence of viable fungal particles in indoor air and house dust. An extended review of the literature up to 1985 is presented by Beaumont (1985).

At present not enough is known to permit setting of threshold limit values for fungi in non-industrial indoor environments.

The observed values in tables 4 and 5 can be used to evaluate results of air- and dust-sampling. Since the results are highly dependent on the methods used, the activities during sampling and the season, these should be taken into account. It should be stressed that

these categories are based on the range of values obtained in houses and non-industrial indoor environments, and not on a health risk evaluation. With regard to air-sampling, the results should be considered in relation to the outdoor samples taken.

Table 4 : Categories of CFU/m³ (mixed populations of fungi) obtained with the Andersen six-stage sampler in combination with MEA, and with the N6-Andersen one-stage sampler in combination with MEA and DG18.

category	houses (CFU/m³)	non-industrial indoor environments (CFU/m³)
very low	< 50	< 25
low	< 200*	< 100
intermediate	< 1 000	< 500
high	< 10 000	< 2 000
very high	> 10 000	> 2 000

* : 500 CFU/m³ for DG18

N.B.: These categories are based on the range of values obtained in indoor environments and not on a health risk evaluation.

Table 5 : Categories of CFU/g dust (mixed populations of fungi) obtained by direct plating on V8 or DG18, and by suspension in peptone followed by plating on V8 or DG18, for houses and other non-industrial indoor environments

category	method			
	V8/direct[a]	DG18/direct[a]	V8/peptone[b]	DG18/peptone[b]
very low	< 1 000	< 2 000	< 10 000	< 10 000
low	< 2 500	< 6 000	< 20 000	< 20 000
intermediate	< 5 000	< 9 000	< 40 000	< 50 000
high	< 10 000	< 15 000	< 100 000	< 120 000
very high	> 10 000	> 15 000	> 100 000	> 120 000

[a] : direct plating of 30 mg dust
[b] : 100 mg dust suspended in a pepton solution (1 : 50), 0.1 ml of the solution plated on the medium

N.B. : These categories are based on the range of values obtained in indoor environments and not on a health risk evaluation.

5. BACTERIA (INCLUDING ACTINOMYECETES)

5.1. Health effects

It is well established that bacteria are agents of important infectious diseases, and occasionally such bacteria in droplet nuclei from individuals shedding pathogenic agents, e.g. *Mycobacterium tuberculosis*, can be rapidly and evenly dispersed throughout a closed environment with a recirculation system (Riley 1979). Of course, other medically important bacteria such as *Legionella* (see Chapter 6) may also be transmitted via aerosols and can cause respiratory problems.

Together with fungi, bacteria, (including their antigens and endotoxins) in humidifiers are implicated in "humidifier fever", a disease with elements of both toxic and allergic manifestations. Infrequently, bacteria in buildings may also be the cause of extrinsic allergic alveolitis in occupants. Bacteria in the air of houses or offices reported to have caused extrinsic allergic alveolitis among occupants are *Bacillus subtilis* and *Pseudomonas aeruginosa*, and the thermophilic actinomycetes *Faenia rectivirgula* (*Micropolyspora faeni*) and *Thermoactinomyces vulgaris* (Flannigan *et al.* 1991).

Research on bacterial endotoxins indicates that endotoxins in inhaled Gram-negative bacteria may have a role in the development of humidifier disease. Pseudomonads and other Gram-negative bacteria are well adapted for growth in humidifier reservoirs, which consequently may become heavily contaminated. However, they are also present in relatively large numbers in stagnant water and other wet environments, being able to grow in water containing very low levels of mineral salts and other nutrients. A variety of functions in alveolar macrophages, which appear to be the primary target, are affected by the toxins (cell-wall lipopolysaccharides); they cause increased metabolic activity and phagocytosis and secretion of lysosomal enzymes and membrane-derived mediators. Symptoms of humidifier fever include fever, chills, tightness in the chest and breathing difficulty. Respiratory symptoms equal to those found in typical acute episodes of this building-related illness have been observed in human volunteers on inhalation challenge with extracted endotoxin or whole cells of *Serratia marcescens*, *Enterobacter agglomerans* and *Escherichia coli*, and isolates of *Cytophaga*, *Flavobacterium* and *Pseudomonas* (Rylander 1986). Although bacterial endotoxins have been implicated in a number of cases of such

illness, numbers of airborne of airborne Gram-negative bacteria in homes and non-industrial workplaces are generally low. Although there is evidence that whole bacteria, endotoxin and other cell-wall components, potentiate histamine release and could therefore aggravate bronchial asthma (Clementsen *et al.* 1990), the general perception is that only in exceptional circumstances will numbers be high enough to be of potential significance in respiratory health.

Endotoxin has also been measured in settled house dust. Michel *et al.* (1991) reported endotoxin concentrations in house dust sampled in the homes of 28 patients with perennial asthma ranging from 0.12 to 20 ng endotoxin/mg dust.

5.2. Occurrence

Survival of bacteria in air has been studied experimentally, but the determinants are still rather obscure. Air is an extreme environment for bacteria, where their survival is limited by environmental stress. Generally speaking, low temperatures permit microbial survival. Genera of bacteria differ in their ability to withstand different humidities. For example, *Escherichia coli* survives best at high humidities (up to 90%), whereas *Serratia marcesens* and staphylococci survive best at a relative humidity below 40% (Nevalainen 1989).

In indoor air, the main sources of bacterial aerosols are usually humans and animals, but bacterial aerosols may also be created by disturbing previously settled dust. Furthermore, air humidifiers are potential sources of airborne bacteria.

There is very little published work on the numbers and types of bacteria present in the air of houses, but the numbers of bacteria may be greater than those of airborne fungi. A century ago in Scotland, the highest counts obtained by Carnelley *et al.* (1887) in crowded homes was approximately 120 000 viable bacteria/m^3 air. However, in a more recent investigation the maximum was 22 000/m^3 (Flannigan *et al.* 1989). Nevalainen *et al.* (1988) isolated 60-12 200 viable bacteria/m^3 air, including 0-154 actinomycetes/m^3, from Finnish houses which were damp/mould-affected or the subject of building-related health complaints. However, similar levels were obtained in non-problem houses. Compared with an outside maximum of 652 bacteria /m^3, Austwick *et al.* (1989) recorded an indoor maximum of 512 /m^3 in naturally ventilated offices and 244 /m^3 in air-conditioned offices.

The bacteria in the indoor air of houses are dominated by micrococci (Flannigan *et al.* 1989, 1991; Nevalainen 1989), with *Micrococcus luteus* and *Staphylococcus epidermidis* (associated with shed skin scales) likely to be the most prominent, and *Staphyl. aureus* less abundant (Flannigan *et al.* 1989). In contrast to outdoor air, where *Pseudomonas* spp. predominate (and micrococci are a minor component), within Finnish houses *Pseudomonas* spp. are much less common (Table 14, Appendix I). However, it has also been reported that in houses in Scotland pseudomonads are the next most abundant category to micrococci, with gram-negative oxidase-positive cocci following (Flannigan *et al.* 1989). A wide range of other bacteria, including Actinomycetes, may be found in houses. A number of these have also been isolated from surfaces (Table 14, Appendix I) and from mould patches on walls where micrococci and pseudomonads are the most frequently encountered types. A similar wide range of bacteria has been found in aerosols from humidifying and air-conditioning equipment or in the air of "sick" buildings (Austwick *et al.* 1986, 1989), e.g. *Enterobacter*, *Acinetobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* (including *Ps. aeruginosa*), *Staphylococcus aureus* and *Staph. epidermidis*, *Streptomyces* and various thermophilic actinomycetes and coryneforms.

5.3. Available sampling methods

Air sampling

Most of the samplers used for fungi (see Section 4.3, Table 3) can also be used for bacteria, but with the same limitations. The Andersen six-stage sampler, the slit-sampler and the Reuter Centrifugal Sampler (RCS) are those most commonly used for the sampling of bacteria. Because many bacteria are rather susceptible to desiccation it is widely held that sampling times should not exceed 10 min. No satisfactory data to support this assumption has been published, but desiccation may be particularly important in filter sampling.

The collection medium used depends on the type of bacteria to be enumerated. For general purposes, Tryptone Soya Agar (TSA) or Tryptone Yeast Glucose Agar (TYGA) with a suitable antimycotic to prevent fungal growth, e.g. cycloheximide, may be used. For specific groups of bacteria, selective media could be employed, e.g. Pseudomonas agar base supplemented with cetrimide and antibiotics for isolation of *Ps. aeruginosa* only and for

thermophilic actinomycetes half-strength nutrient agar can be used. However, it is worth noting that highly selective media may be inhibitory to sublethally injured bacteria. As far as known, no studies have been conducted to evaluate the selective media in terms of recovering aerosolized organisms. When a specific bacterium is suspected the medium used to recover that organism from water can be tried.

If a liquid impinger is employed, the liquid used for trapping bacteria should neither be unfavourable to them nor be sufficiently nutritive to support quick multiplication. Such a liquid may be 0.1% peptone solution with or without sodium chloride. Aliquots of the collection liquid should be plated on agar medium or added to enrichment medium within 1 h of sampling. If this is not possible, e.g. owing to transportation distance, the liquid should be kept at 3-5 °C until plating out, but for no longer than 24 h.

In a correctly operated all-glass impinger, clusters of cells are broken up into smaller units or individual cells. Therefore, counts may be higher than with an impactor sampler.

As for fungi, settle plates can also be used, in order to obtain semi-quantitative information on the presence of bacteria in indoor air (see Section 4.3.).

Dust sampling

In principle, the sampling of house dust for bacteria can be conducted in exactly the same way as for house dust mites and their allergens (see Section 2.3).

Surface sampling

Surfaces may be sampled using swabs or contact plates as described for fungi (see Section 4.3), using appropriate agar media for bacteria.

5.4. Available methods of analysis

Air samples

Although TSA or TYGA plates are often incubated at 37 °C for 48 h, incubation at lower temperatures may recover a greater number of species and give improved resuscitation of stressed bacteria (Hyvärinen *et al.* 1991). It is therefore recommended that plates should be incubated at 20 - 25°C and examined daily for several days. For the isolation of human pathogenic organisms, plates should be incubated at 37°C. For thermophilic actinomycetes plates should be incubated at 55°C.

Dust samples

The analysis of dust samples for bacteria is performed as described for fungi (Section 4.4), using appropriate agar media.

Surface samples

Swabs and contact plates are treated in the same way as for fungi (Section 4.4), using appropriate agar media.

5.5. Recommendations for different studies

A. Private homes

- *Case studies*

The first step in a case study - performed in order to explain cases of disease caused by bacteria originating in the home environment - is to conduct a "walk-through" of the home for visual and olfactory assessment of factors having a possible bearing on bacterial problems , e.g. presence of visible dampness (including on furnishing), mouldy spots, condensation, leakage of pipes, penetration of rainwater, rising dampness and the presence of (local) HVAC systems.
If dampness problems are observed, remedial action should be taken in order to solve the

problems and to reduce the exposure of the occupants to bacteria. In such cases, sampling of bacteria is not necessary, since it is clear that problems involving bacteria are present. Furthermore, the results of air-, dust- or surface-sampling for bacteria cannot be used to obtain a cause-effect relationship between the level of exposure and disease.

Sampling of bacteria might be useful in the following cases:

1. If (local) HVAC systems are present, sampling of bacteria is recommended to evaluate whether the system is a possible source of bioaerosols.
2. If filters within the ventilation system are damp, sampling of bacteria might be useful to evaluate whether these filters are a possible source of bacteria for the indoor environment.
3. If damp spots and/or mould growth is not visible but the typical odour of actinomycetes and moulds, described as "earthy", "mouldy" or "musty" is detected, sampling of bacteria is recommended since the observed odour indicates the presence of actinomycetes.
4. Sampling of bacteria might be considered in order to explain allergic conditions caused by a known bacterium in order to evaluate if the specific organism is present in the indoor environment.
5. Sampling of bacteria might be considered in a suspected case of extrinsic allergic alveolitis (EEA) and recurrent episodes of rhinitis and/or asthma or other recurrent respiratory problems.
6. Sampling of bacteria might also be considered if no physical and/or chemical indoor air factors can be found which may be or have been the cause of the complaints.
7. Sampling of bacteria may also be used to evaluate the effects of remedial action.

If sampling of bacteria as part of a case study is performed, the following sampling and analytical methods are recommended:

- Surface sampling and/or sludge, slime or sediments within humidifiers, to obtain qualitative information about the bacteria present. Swab and/or contact plate samples can be taken, as described in section 4.3;
- Air sampling, to investigate whether specific bacteria are abundant in the indoor air. For air sampling, any air sampling device can be used, if properly and consistently used. As collection media, TSA or TYGA are recommended. It should be stressed that results obtained with different sampling devices and media cannot be compared. Parallel duplicate samples should be taken. Furthermore, parallel outdoor samples should be taken. Since human beings are important sources of bacteria, sampling of bacteria

originating in the home environment should be performed without occupants in the room to be sampled.

If sampling is performed in order to determine whether a (local) HVAC system or damp filters within the ventilation system are possible sources of bacteria, samples should be taken at the inlet supply for the outdoor air and at the point of ingress of air into a room, both when the ventilation is and is not operating.

If sampling is carried out because the typical odours of moulds or Actinomycetes are detected, samples should be taken at those locations where the odour is detected and preferably in a non-odorous reference room (or house).

To assess the effects of remedial action, sampling should be performed before and after the action has been taken.

In any other case, sampling should be performed in at least the livingroom and one bedroom.

Additional information about building characteristics and the behaviour of occupants which are the principal influences on the presence of bacteria in the home should be recorded, following the checklist in Appendix II.

- *Epidemiological studies*

For large scale epidemiological studies on the relationship between the exposure to bacteria and health effects, a standardized sampling protocol should be used throughout.

B. Other non-industrial indoor environments

Specific indoor air quality problems related to mechanical ventilation systems, air conditioning systems and humidifiers have been described in Report Number 4 of this series (Commission of the European Communities, 1989).

In order to evaluate the presence of bacteria in buildings with central or local heating ventilation and air conditioning systems (HVAC's), the same approach should be adopted

as for fungi (see Section 4.5.).

5.6. Observed values and evaluation of results

Appendix I summarises the results of some recent studies in Europe. The presently available knowledge does not permit setting threshold limit values for the presence of airborne bacteria in non-industrial indoor environments.

The observed values in table 6 can be used to evaluate results of air sampling. For settled house dust, insufficient data are available. Since the results depend on the methods used, the activities during sampling and the season, these should be taken into account. The categories are based on the range of values obtained in houses and non-industrial indoor environments, and not on a health risk evaluation. Furthermore, the results should be considered in relation to the outdoor sample taken.

Table 6 : Categories of CFU/m^3 (mixed populations of bacteria) obtained with the Andersen six-stage sampler or slit sampler (sampling time 10 - 15 min., incubation at 20 - 25°C for 3 - 5 days), for houses and non-industrial indoor environments.

category	homes	non-industrial indoor environments
very low	< 100	< 50
low	< 500	< 100
intermediate	< 2 500	< 500
high	< 10 000	< 2 000
very high	> 10 000	> 2 000

N.B.: These categories are based on the range of values obtained in indoor environments and not on a health risk evaluation

6. LEGIONELLAE

6.1. Health effects

Legionellae are Gram-negative bacteria which can affect the human respiratory system. Severe health effects caused by these bacteria were recognized for the first time in 1976. In Philadelphia, USA, more than 200 people, attending a meeting of the US-American Legion, developed pneumonia like symptoms; 34 people died. The disease was called "Legionnaires' disease". The causative agent was a bacterium, named afterwards *Legionella pneumophila* (Fraser *et al.* 1977; Brenner *et al.* 1979). At present, the genus contains more than 40 described species. *Legionella pneumophila* is epidemiologically the most important species and contains at the moment 14 serogroups. *L. pneumophila* serogroup 1, is considered to be the most important.

The health effects of *Legionella* (legionellosis) vary markedly. Firstly, the very severe infection of the respiratory system as described above. The incubation period ranges from 2 to 13 days (average 5-6 days). Legionnaires' disease may affect other organs as well and requires special antibiotic treatment. Pneumonia caused by *Legionella pneumophila* has a considerable mortality rate and appears mostly as sporadic or hyperendemic cases. Epidemics are rare. The frequency of sporadic cases is estimated to be 2% of all hospitalized pneumonias in the United Kingdom, and 10% of community-acquired pneumonias in France and Germany (WHO 1990a). Less than 5% of those exposed appear to develop the illness, in 10-15% of these the illness is fatal (Dennis 1990). Secondly, there is Pontiac-fever, named after an outbreak in 1968 in Pontiac, USA. This type of disease, caused by a number of the *Legionella* species, is milder and appears as a non-pneumonic fever. The incubation period ranges from several hours up to 48 hours. The illness resolves spontaneously in 2-5 days. No fatal cases have been reported in relation to Pontiac fever. This disease mainly appears as epidemics. Of those exposed to the aerosol 95% will become ill (Dennis 1990). However, the incidence of Pontiac-fever in the general population is unknown and reports of sporadic cases are unlikely to be made even if they are recognized (WHO 1990a).

There is no evidence for transmission of these diseases from person to person or by ingestion. The infective transmission of *Legionella* occurs via fine water droplets inhaled

as aerosols from the environment only. *L. pneumophila* survives in aerosols only for a limited period of time. Laboratory experiments have shown a survival under different conditions of relative humidity up to about 15 min (Thornsberry *et al.* 1984; WHO 1990a). Epidemiological studies suggest that the virulence of strains is an important survival factor in aerosols. The virulence of *Legionella* for human beings varies. Highly virulent strains and less virulent or avirulent strains may be present in the (same) environment (Thornsberry *et al.* 1984; WHO 1990a). The infective dose for humans is still unknown.

Indoor transmission of legionellosis has been reported via hot water supplies in hospitals, hotels and other public buildings, respiratory therapy equipment, jacuzzis, spas (including natural warm spas) and air-humidifiers (Thornsberry *et al.* 1984; WHO 1990a). Two earlier working groups of WHO have described Legionnaires' disease (WHO 1982) and environmental aspects of its control (WHO 1986).

6.2. Occurrence

Legionellae are probably present in all types of fresh water, including tap water since they form a part of the natural aquatic bacterial population of lakes and rivers from which a lot of our water for domestic as well as industrial use is drawn. Aquatic bacteria can survive the traditional water treatment. They can multiply in water, using other microorganisms like bacteria, algae and protozoa (Thornsberry *et al.* 1984). Their concentration in fresh water varies and is influenced mainly by the temperature. In general, Legionellae are isolated more frequently and in higher concentrations from warm water, with temperatures ranging from about 30 to 50 °C, but they can survive at much lower temperatures indoors as well as outdoors. At temperatures above 60°C Legionellae can not survive. It should be stressed that Legionellae are only one category in a diverse population of microorganisms that find their way into and colonize water systems. Since most aquatic bacteria are harmless to humans, the presence of such bacteria in potable water is acceptable.

6.3. Sampling and analysis

Sampling of *Legionella* in indoor air or water on a routine basis is not recommended. It is more important that legionellosis is rapidly and correctly diagnosed so that the correct antibiotic treatment is given. However, sampling is useful in explaining outbreaks of legionellosis and may be used to check the effectiveness of maintenance practices and control measures for, and to guarantee the safe use of hot water supplies and humidified ventilation systems.

Water supplies

When investigating the water services within a building to determine whether they are colonized by Legionellae, it is essential to prepare or obtain a schematic diagram of these services. The route of the service should be traced. The condition of pipes, the joining methods used, the presence of lagging, sources of heat, and the standard of protection afforded cisterns should be noted, as well as disconnected fittings, 'dead-ends', and cross-connections with other services (Dennis 1990).

Water samples should be taken from:
1) the incoming supply;
2) cisterns and calorifiers;
3) an outlet close to, but downstream of, each cistern and calorifier;
4) the distant point of each service;
5) the water entering and leaving any fitting under particular suspicion.
Samples should be collected in sterile autoclavable plastic containers (Dennis 1990).

Swab samples should be taken of shower heads, pipes and taps. Furthermore, sludge, slime or sediments within building water services or humidifiers can also be collected, particularly where accumulation occurs.

Samples should be stored at room temperature (20 ± 5°C) in the dark and should be processed within 2 days. Aliquots of 0.1 ml can be plated directly on e.g. 0.3% glycine supplemented BCYE-alpha-Agar, containing 5 mg Vancomycin/litre, 10 mg Polymixin B/litre and 80 mg Cycloheximide/litre (Seidel *et al.* 1987).

Air sampling

The presence of *Legionella* in indoor air has occasionally been investigated using the six-stage Andersen sampler and liquid impingers. Sampling times which have been used varied from 5 to 30 min (Thornsberry *et al.* 1984; Seidel *et al.* 1987; Breiman *et al.* 1990).

The recommended collection agar at present is BCYE-agar, a semi-selective medium based on charcoal-yeast extract medium with different types of supplements (Thornsberry *et al.* 1984; WHO 1990a). When a liquid impinger is used, distilled water or a low ionic strength saline (Pages saline) are recommended as collection fluids (Dennis 1990).

7. CONCLUSIONS

1. All groups of biological particles dealt with in this report (i.e. house dust mites, dander from furred animals, fungi and bacteria) can be of significance for indoor air quality in homes, but in non-industrial indoor environments only fungi and bacteria are likely to be of significance. However, in schools and day-care centres allergens from house dust mites and pets may be of significance also.

2. Currently available methods for sampling and analysis of biological particles provide information on their relative abundance in air and/or dust, and may enable sources and their relative importance to be identified.

3. The levels of biological particles in air vary widely in time and space, both qualitatively and quantitatively. The levels are strongly influenced by the characteristics of the organisms and by environmental factors. Therefore, reports should provide detailed information concerning the conditions during sampling.

4. Neither sampling techniques nor analytical methods for biological particles in air and dust samples from indoor environments are well standardized. Therefore, any report of investigations must include detailed information of the sampling and analytical techniques used.

5. At present no methods are available which adequately assess the exposure of individuals to biological particles. For non-infectious microorganisms, no quantitative health risk assessment can be made, but visible mould growth in buildings should be regarded as unacceptable. Although the WHO has made a proposal with regard to house dust mite allergens, no health guideline values can be adequately set for biological particles on the basis of current knowledge. However, representative observed values are presented in Appendix I.

6. There is no standard procedure which can be applied to all case studies. Each case must be treated individually. A walk through and sensory inspection should always be made in order to decide whether further investigation is needed. For large scale (epidemiological) investigations the same sampling protocol should be used throughout.

7. It must be emphasised that investigation of biological particles in air and dust demands skill and experience. Interpretation of results can only be made and reliable advice concerning remedial actions given by investigators with specialised knowledge and experience of the area.

8. There is a need for international collaborative studies on sampling and analytical methods, to provide information for future standardization.

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ABBREVIATIONS

BCYE	buffered charcoal yeast extract
BSA	bovine serum albumin
CCIE	counter current immunoelectrophoresis
CFU	colony forming unit
DG18	dichloran 18% glycerol agar
EEA	extrinsic allergic alveolitis
ELISA	enzyme-linked immunosorbent assay
HVAC	heating, ventilation and air conditioning systmes
IU	international unit
MEA	malt extract agar
MOD	macromolecular organic dust compounds
N6	Andersen 1-stage sampler
ODTS	organic dust toxic syndrome
OPD	open petri dish
RAST	radio-allergosorbent test
RCS	Reuter Centrifugal impactor
RIA	radioimmuno assay
RIE	rocket immunoelectrophoresis
SAS	Surface Air System impactor
SBS	sick building syndrome
SMEA	malt extract agar containing 20% sucrose
TSA	tryptone soya agar
TYSA	tryptone yeast glucose agar
V8	vegetable-juice containing 8 different vegetables

APPENDIX I

OBSERVED VALUES

Allergens from house dust mites

The following 4 Figures and 2 Tables indicate recently reported values of house dust mite major allergens (Der p I, Der f I and Der m I) obtained with immunochemical assays.
The observed values from Europe and USA range from 20 to 200 000 ng major allergens/g dust from house dust samples in private homes. The detection limits for these assays vary from 10 to 20 ng/g dust.
For detailed information on the data in the Figures presented, consult the articles in the references.

Lind (1986):

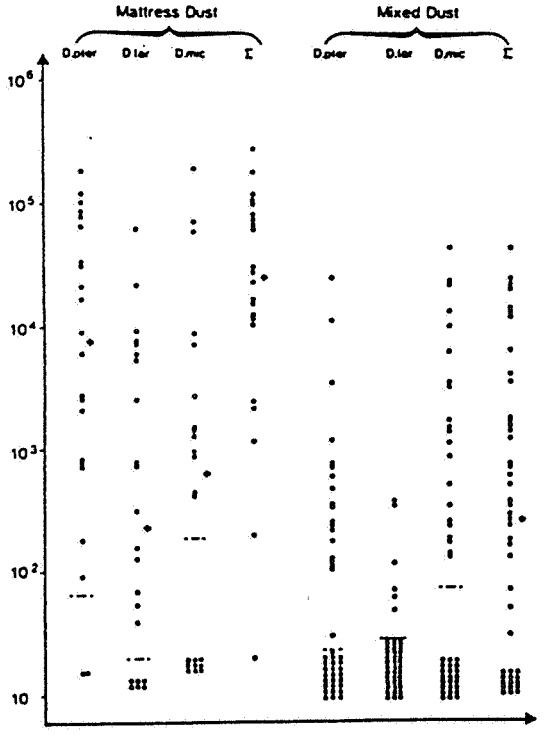

Fig. 1. Levels of *Dermatophagoides* allergen measured by ELISA in 22 matress dust samples and 45 mixed dust samples. Summed figures for three species appear in columns 4 and 8. Figures on vertical axis denote ng Dp-42/Df-6/Dm-6/g dust. Arrows in columns 1-4 and 8 point to median value. Observations positioned under the broken lines were below limit of detection.

Lind *et al.* (1987a):

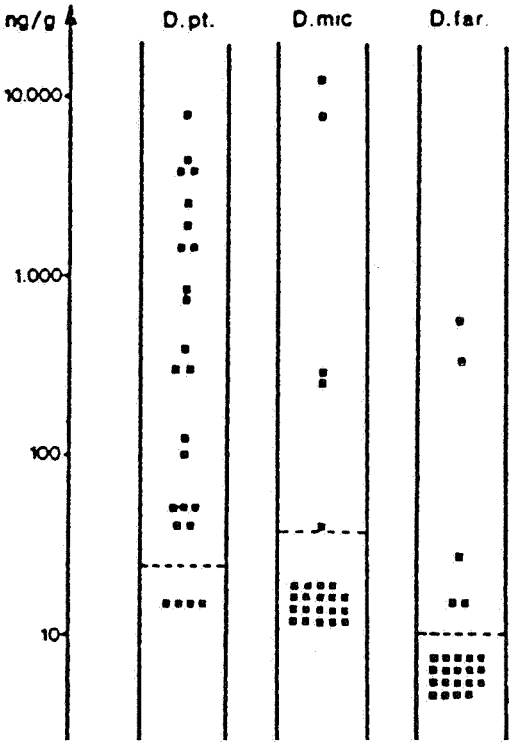

Fig. 2. Dust levels of major allergens Dp-42, Dm-6 and Df-6 in 24 Belgian homes, as determined by species specific ELISA. Vertical exis: major allergen concentration in ng/g dust. Broken horizontal lines indicate limits of detection.

Wood *et al.* (1988):

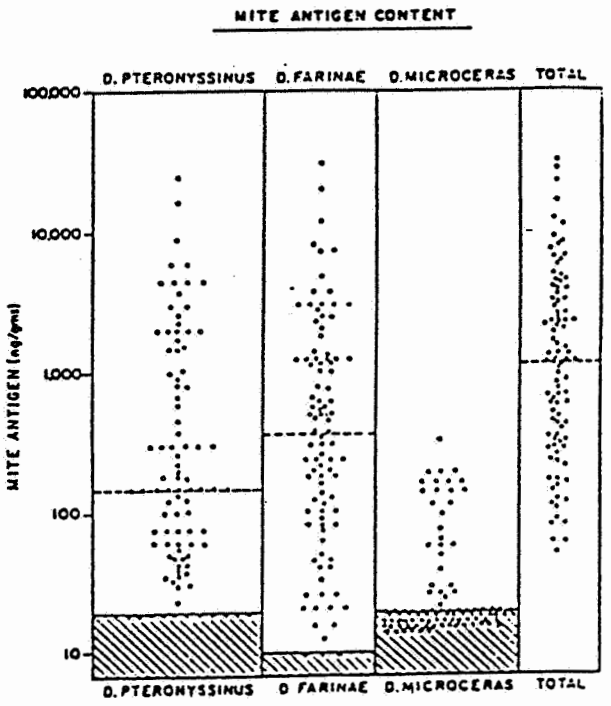

Fig. 3. Mite antigen content (ng/g) as determined for 97 homes by ELISA for 3 mite species (*Dermatophagoides, pteronyssinus, D. farinae* and *D. microceras*) and total mite content (determined as the sum of the 3 individual mites). Dotted lines indicate medians. Hatched areas below solid lines indicate lower limits of sensitivity of the assays (20 ng/g for *D. pteronyssinus* and *D. microceras*, 10 ng/g for *D. farinae*).

Table 1 : Overview of 5 studies (after Schwartz *et al.* 1987).

Study no.	Samples (n)	Allergens	Ranges (ng/gr dust)	Location	Home of allergics
1	48	Der p I Der f I Der m I	$<20 - 10^5$ $<20 - 5x\ 10^4$ $<45 - 8x\ 10^3$	Austria, Europe	yes
2	24	Der p I Der f I Der m I	$<20 - 8x\ 10^3$ $<10 - 5x\ 10^2$ $<20 - 10^4$	Belgium, Europe	yes
3	42	Der p I Der f I Der m I	$<60 - 10^4$ $<45 - 1.5x\ 10^4$ <80	Maryland, USA	no
4	18	Der p I Der f I Der m I	<20 - 5x 10 <20 - 5x 10 <20 - 5x 10	Stockholm, Sweden	yes
5	22	Der p I Der f I Der m I	$<30 - 2x\ 10^5$ $<10 - 6x\ 10^4$ $<60 - 2x\ 10^5$	Denmark, Europe	no

Table 2 : Der p I concentrations in house dust (µg/gr dust), The Netherlands (after Brunekreef *et al.* 1992).

Sample type	Geometric Mean	Range	n
living room, uncarpeted	0.19	0.03 - 1.30	29
living room, carpeted	6.29	0.03 - 5.91	68
couch, smooth surface	(a)		39
couch, upholstered	12.84	0.03 - 93.17	58
bedroom, uncarpeted	0.27	(b) - 6.30	34
bedroom, carpeted	3.89	0.05 - 66.55	63
mattress	6.83	0.10 - 78.10	97

(a) From couches with smooth surfaces (like leather), not enough dust was collected to permit Der p I determination
(b) From two uncarpeted floors, not enough dust could be collected to permit Der p I determination

Allergens from furred animals (pets)

Cat

The Figures below indicate observed levels of cat allergen (Fel d I) in ng/g dust from samples in Europe and USA. Range: 30 - 100 000 ng Fel d I/g dust.

Schwartz *et al.* (1987):

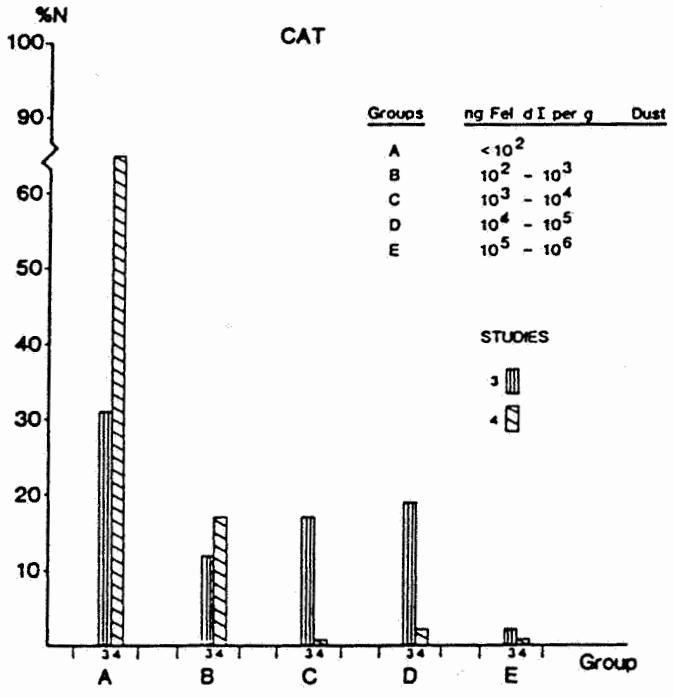

Fig. 4. The content of Fel d I in dust samples collected at two different locations: one from homes of allergics (3) and one from homes of non-allergics (4).

Wood *et al.* (1988):

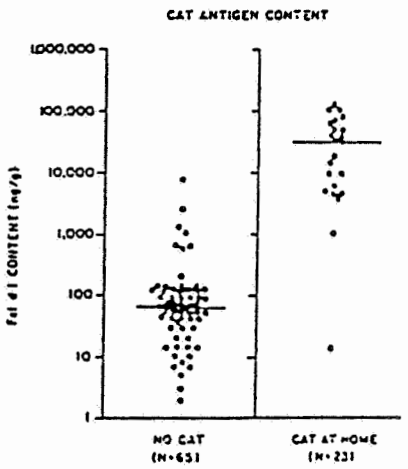

Fig. 5. Cat antigen concent (ng/g) as determined for 90 homes by ELISA specific for Fel d I. Homes without a cat represented on left, homes with a cat on the right. Lower limit of sensitivity is 0.5 ng/g. Difference between median antigen concent (solid lines) of homes with and without a cat statistically significant (medians 63 versus 18000 ng/g; $p < 0.001$).

Fungi

Viable fungal particles in indoor air of houses

Table 3 presents data from a study where airborne fungi were sampled in 27 homes in Toronto, Canada, during July-August. A two-stage Andersen sampler was operated at 28.3 l/min for up to 10 min, approximately 150 cm above the floor. Rose-bengal-agar was used as collection medium. The mean number indoors was 742 CFU/m^3 and outdoors 1131 CFU/m^3 (Fradkin *et al.* 1987)

Table 3 : Characteristics of fungal species in the air of indoor and outdoor environments (after Fradkin *et al.* 1987).

Mold	Number of homes with indoor/outdoor ratio			Colony Forming Units/m^3 (mean of 27 samples)	
	<1	1-2	>2	Outdoor	Indoor
Total (respirable fraction)	16	8	3	1131	742
Cladosporium	11	14	2	696	456
C. cladosporioides	16	8	3	334	211
C. sphaerospermum	19	5	3	232	111
C. herbarum	9	6	10	96	96
C. macrocarpum	10		10	34	30
Alternaria	19	4	2	124	52
A. tenuis	16	2	6	102	41
A. alternata	12	1	8	23	11
Epicoccum purpurascens	13	1	9	40	26
Candida spp.	8	1	9	45	19
Penicillium	13	1	12	79	108
P. chrysogenum	15	1	4	34	22
Other penicillia			14	<7	30
Aspergillus	5	1	14	45	22
A. fumigatus		1	9	23	11
Other species	15	6	5	102	59

The data in Tables 4 and 5 are from indoor air samples in 15 dwellings in England and 47 in Scotland obtained during 3 winters from early November to early April. Sampling was performed with a six-stage Andersen sampler. Sampling time varied from 10 sec to 3 min at 37 cm above floor level. Malt- extract-agar with antibiotics was used as collection medium. The range was <12 to 449 800 CFU/m^3 (Hunter *et al.* 1988).

Table 4 : Range of numbers of fungal propagules (cfu/m^3)in the air spora of different rooms in dwellings in the London area and Central Scotland (after Hunter *et al.* 1988).

Mould growth	n	Range	Median	Interquartile range	Modal class (n)
Living room					
Entirely absent	177	<12- 23070	236	118- 463	<12- 200 (44)
Present in other rooms only	144	<12- 48000	360	165- 759	<12- 200 (31)
Present in the room itself	61	424- 21790	2673	1165-4944	1001-2000 (21)
Bedroom					
Entirely absent	158	<12- 8950	177	95- 353	<12- 200 (55)
Present in other rooms only	103	12- 6300	247	118- 522	<12- 200 (46)
Present in the room itself	163	35-449800	1165	406-5615	<12- 200 (15)
Kitchen					
Entirely absent	9	12- 1170	212	115- 507	<12- 200 (44)
Present in other rooms only	7	12- 1860	180	59- 230	<12- 200 (57)
Present in the room itself	41	71- 20320	2296	1107-5862	1001-2000 (23)
Bathroom					
Present in other rooms only	12	212- 19990	829	598-1036	1001-2000 (33)
Present in the room itself	13	71- 19070	671	388-5615	201- 400 (23)

Table 5 : Fungi isolated most frequently from air of 47 dwellings in Central Scotland (after Hunter *et al.* 1988).

Type	Percentage frequency in: Dwellings	Samples
Penicillium spp.	95.7	90.2
Yeasts	93.6	82.1
Cladosporium spp.	88.7	73.7
Aspergillus spp.	74.5	52.0
Ulocladium spp.	61.7	18.2
Geomyces pannorum	56.7	20.3
Sistotrema brinkmannii	51.1	55.5
Aureobasidium pullulans	46.8	13.8
Acremonium strictum	42.6	4.2
Mucor spp.	38.3	8.4
Phoma herbarum	36.2	6.1
Oidiodendron griseum	23.4	4.4
Scopulariopsis brevicaulis	21.4	3.6
Alternaria alternata	17.0	2.6
Cunninghamella elegans	14.9	2.8
Stachybotrys atra	12.8	4.5
Fusarium spp.	10.6	1.0
Botrytis cinerea	8.5	1.3
Papulospora spp.	8.5	0.9

The difference in the number of airborne propagules obtained by different sampling methods is illustrated in Table 6. Samples were taken 150 cm above the floor under normal living conditions in private Dutch homes with visible mould growth during the period November to March. Sampling periods: Slit-sampler, 2 min; N6-Andersen sampler, 2 min; SAS, 20 sec; RCS, 1.5 min. (Verhoeff *et al.* 1990).

Table 6 : Geometric mean number of CFu/m^3 obtained with different combinations of air sampling device and culture medium (after Verhoeff *et al.* 1990).

Medium	Sampler				GM per medium
	Slit	SAS	N6	RCS	
MEA	545	164	508	721	424
DG18	713	478	518	327	493
DRBC	455	147	361	193	260
GM per sampler	561	226	455	358	

GM: geometric mean

Table 7 gives the number of viable moulds obtained in 46 Dutch houses during the autumn with the N6-Andersen sampler operated for 2 min (part I), and in 84 Dutch houses during May with both the N6-Andersen sampler (2 min) and the Open Petri Dish (OPD) operated for 60 min (part II). In both parts of the study sampling was performed 150 cm above floor level and DG18 was used as collection medium. In part I of the study, sampling was repeated after 4 weeks (period 1 and 2) (Verhoeff *et al.* 1992).

Table 7 : Concentrations of viable mould propagules in CFU/m³ and CFU (geometric mean, geometric standard deviation and range), found in 46 Dutch homes (part I) and 84 Dutch homes (part II) (after Verhoeff *et al.* 1992).

method	location	period	n	GM	GSD	range
part I						
N6	livingroom	1	40	645	2.6	62 - 7332
		2	34	640	2.0	159 - 1703
part II						
N6	livingroom		84	807	3.1	96 -43045
	bedroom		84	822	3.2	69 -23860
	outdoors		84	882	2.5	124 - 6248
OPD	livingroom		84	14	2.9	1 - 518
	bedroom		84	11	2.8	0 - 206

Table 8 presents a comparison between the presence of viable moulds in the indoor air of 18 Dutch houses and parallel outdoor samples. Sampling was performed in May with the N6-Andersen sampler for 2 min, in combination with DG18, 150 cm above floor level (Verhoeff *et al.* 1992).

Table 8 : Comparison (paired t-test) between the concentrations in CFU/m³ (geometric mean and geometric standard deviation) of seventeen different mould species in the indoor air of 18 Dutch homes and parallel outdoor samples (after Verhoeff *et al.* 1992).

Species	Indoors GM	Indoors GSD	Outdoors GM	Outdoors GSD	T	p
total number of CFU/m3	669	2.5	941	2.4	-1.27	0.22
Aspergillus (total)	24	6.6	8	6.0	1.70	0.11
A. penicillioides	6	6.6	2	4.5	1.80	0.09
A. versicolor	4	5.4	2	2.3	1.69	0.11
Aureobasidium pullulans	1	2.2	3	4.4	-2.19	0.04
Botrytis cinerea	2	2.7	6	4.0	-2.54	0.02
Cladosporium (total)	245	2.8	501	3.0	-3.08	0.01
C. cladosporioides	65	5.5	72	8.4	-0.18	0.86
C. herbarum	59	7.4	224	3.8	-3.49	<0.01
C. macrocarpum	2	2.3	1	1.6	1.86	0.08
C. sphaerospermum	3	5.3	3	3.9	-0.03	0.98
Eurotium amstelodami	2	3.1	2	3.5	-0.45	0.66
E. herbariorum	4	4.3	6	4.2	-0.88	0.39
Penicillium (total)	154	3.6	65	4.4	2.04	0.06
P. aurantiogriseum	2	2.8	3	5.6	-0.72	0.46
P. brevicompactum	15	7.6	6	4.8	1.85	0.08
P. glabrum	6	12.1	2	4.0	1.34	0.20
P. jensenii	2	4.5	2	2.4	0.87	0.40
P. olsonii	8	8.4	3	3.3	1.88	0.08
Ramularia deusta	1	2.0	1	2.1	-0.35	0.73
Wallemia sebi	5	5.3	4	5.3	0.55	0.59

Viable fungal particles in house dust in homes

Table 9 presents the results of sampling undertaken in urban areas throughout Canada during December and January (snow covered). The home-owners' vacuum cleaner was used with a new collection bag and 1 g dust was diluted and 1 ml aliquots spread over malt-extract-agar (MEA) and malt-extract agar containing 20% sucrose (SMEA).
Average values on MEA: $4.3x10^5$ moulds and $3.7x10^5$ yeasts/g dust. Average values on SMEA: $1.9x10^5$ xerophilic/osmophilic moulds and $3.1x10^5$ yeasts /g dust (Miller *et al.* 1988).

Table 9 : Fungi found in dust and air samples in Canadian homes (after Miller *et al.* 1988).

Species	Dust samples % of houses	Dust samples CFU/gr dust x 10^4	Air samples % of houses
Penicillium spp.	80	8.1	47
Rhizopus spp.	73	4.7	
Cladosporium cladosporioides (Fresen.) de Unes	67	5.6	8
Alternaria alternata (Fr.) Keissler	57	1.5	x
sterile isolates	55	2.0	
Aspergillus niger van Tieghem	53	0.7	6
Penicillium viridicatum Westling	39	25.3	6
Mucor spp.	31	1.5	x
Trichoderma viride (Pers.) ex Gray	25	4.2	6
Ulocladium botrytis Preuss	22	4.9	4
Penicillium fellutanum Biourge	20	8.2	14
Penicillium decumbens Thom	18	4.8	10
Cladosporium herbarum (Pers.) Link	16	1.9	
Paecilomyces variotii Bainier	10	3.8	
Phoma spp.	10	2.3	
Arthrinium spp.	8	3.0	
Aspergillus fumigatus Fresen.	6	0.4	
Aureobasidium pullulans (deBary) Arnaud	6	0.8	
Monilia sitophila Sacc.	6	0.4	
Paecilomyces spp.	6	0.1	
Periconia spp.	6	0.4	
Aspergillus candidus Link.	4	1.5	
Aspergillus ochraceus Wilhelm	4	5.3	x
Aspergillus spp.	4	0.4	8
Phialophora melinii (Nannf.) Conant	4	5.3	
yeasts	82	59.2	6

x: indicates found in one house only

The following Figure presents the number of colonies from dust collected in the Baltimore area, USA, from January to March in 84 private homes. A Douglas ReadyVac vacuum cleaner equipped with a small filter-bag was used. Sampling period: 9 min (bedroom, 4 min; bathroom, 2 min; TV-area, 2 min; basement floor, 1 min). 30 mg dust was spread directly on V8-agar with antibiotics.

Wood *et al.* (1988):

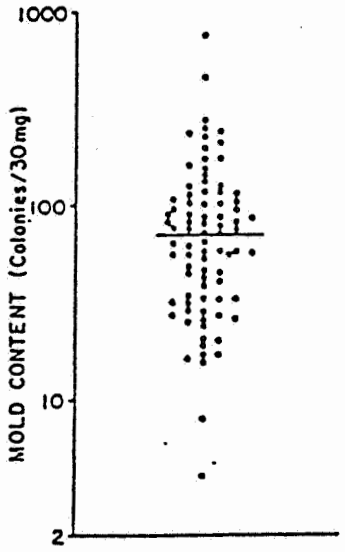

Fig. 6. Total mold content (colonies per 30 mg of dust) as determined by culture of samples from 84 homes (range 4 to 761 colonies/30 mg, median 72 colonies/30 mg).

This Figure presents percentage frequency of mould genera in house-dust samples collected in private homes with the home-owners' vacuum-cleaner throughout the year in Denmark.

Gravesen (1978):

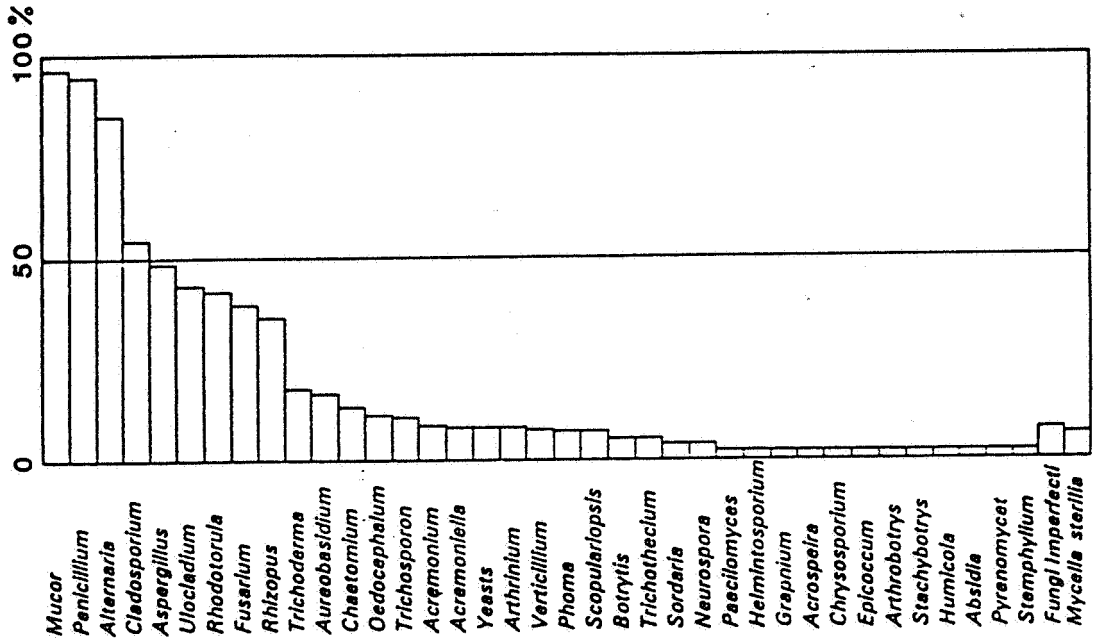

Fig. 7. Prevalence of microfungi cultivated on V8-agar from dust samples collected in 100 Danish homes

Viable fungal particles in indoor air and settled dust of non-industrial premises such as schools, kindergartens and offices

Table 10 presents the results of air sampling throughout the year in Denmark in schools and offices. The BIAP Slit-sampler was operated for 10 min at 33.3 l/min, using V8-agar with antibiotics as collection medium (Gravesen *et al.* 1983).

Table 10 : Number of airborne micro-organisms m^{-3} air collected with a BIAP Slit-sampler in schools and public institutions (average values) (after Gravesen *et al.* 1983).

	Microfungal CFU/m^3		Bacterial CFU/m^3	
	mean	range	mean	range
Schools				
Carpets (n=25)	291	12 - 2000	1538	15 - 6000
No carpets (n=7)	155	36 - 309	840	105 - 3000
Offices				
Carpets (n=61)	73	3 - 904	544	3 - 6000
No carpets (n=12)	42	6 - 111	253	3 - 798

Data for airborne moulds in Table 11 were obtained as described above. Dust samples were collected throughout the year with a vacuum cleaner from 12 m^2 over 4 min and 30 mg floor dust was also spread on V8-agar (Gravesen *et al.* 1986).

Table 11 : Frequency of airborne and dust-borne genera of microfungi in schools and offices (air: n=143; dust: n=98) (after Gravesen *et al.* 1986).

	Air	%	Dust	%
Schools	*Penicillium*	81	*Penicillium*	81
	Cladosporium	64	*Fusarium*	74
	Aspergillus	52	*Alternaria*	56
	Alternaria	29	*Cladosporium*	52
	Rhodotorula	24	*Mucor*	40
	Trichosporon	24	*Trichosporon*	30
	Arthrinium	19	*Aspergillus*	25
	Mucor	17	*Rhodotorula*	25
Offices	*Penicillium*	66	*Penicillium*	78
	Cladosporium	57	*Alternaria*	76
	Aspergillus	52	*Aspergillus*	55
	Yeasts	24	*Cladosporium*	45
	Trichosporon	15	*Fusarium*	45
	Alternaria	13	*Trichosporon*	45
	Rhodotorula	13	Yeasts	33
	Paecilomyces	9	*Mucor*	29

The data in Table 12 are from air and dust samples taken under normal working conditions during January to May in representative offices in 14 town halls in Denmark. Air samples were taken with a BIAP Slit-sampler for 10 min at 33.3 l/min, using V8-agar as collection medium. Dust samples were collected with a vacuum cleaner from 12 m^2 over 4 min and 30 mg floor dust was also spread on V8-agar (Skov *et al.* 1990).

Table 12 : Indoor climate measurements in 14 town halls. (cfu=colony forming units)

Measurement	Mean	Range
Airbone dust (mg/m^3)	0.201	0.382-0.086
Airborne microfungi (cfu/m^3)	32	111-0
Airborne bacteria (cfu/m^3)	574	2100-120
Airborne actinomycetes (cfu/m^3)	4	15-0
Vacuumed dust[a] ($g/12\ m^2$)	3.67	11.56-0.32
Vacuumed dust[b] ($g/12\ m^2$)	6.14	17.04-0.66
Macromolecular content in the dust (mg/g)	1.53	5.24-0
Microfungi in the dust[a] (cfu/30 mg)	33	90-11
Microfungi in the dust[b] (cfu/30 mg)	32	192-6
Bacteria in the dust[a] (cfu/30 mg)	199	380-41
Bacteria in the dust[b] (cfu/30 mg)	296	680-160

[a] In the office where all the measurements were performed

[b] In an office with a considerable load of clients during the day

Viable bacteria

Table 13 shows the levels of airborne bacteria in suburban townhouses in Finland. Sampling was conducted with the six-stage Andersen sampler with tryptone-glucose-yeast extract agar. The sampling period varied from 10 to 20 min, at a sampling height of 150 cm above the floor. The sampling volume flow was 28 l/min. Sampling was repeated 4 times within a period of 3 years (Nevalainen 1989).

Table 13 : Levels of airborne bacteria (geometric mean (GM), geometric standard deviation (GSD) and range) in suburban townhouses and in outdoor air during the three first years of occupancy (after Nevalainen 1989).

Group of samples	n	Bacteria levels CFU/m^3		
		GM	GSD	range
total 1986-88				
indoors	183	550	1.32	0 - 11900
outdoors	89	110	1.38	2 - 2200
winter 1986				
indoors	40	140	1.45	0 - 2400
outdoors	17	16	1.07	0 - 70
spring 1986				
indoors	36	680	0.91	100 - 5900
outdoors	18	145	1.69	2 - 3100
fall 1986				
indoors	36	520	0.90	30 - 2700
outdoors	18	120	1.03	20 - 850
fall 1987				
indoors	36	1200	0.80	200 - 11900
outdoors	18	150	0.75	30 - 560
fall 1988				
indoors	35	1100	1.12	20 - 11800
outdoors	18	130	1.14	30 - 2200

Table 14 presents a list of bacteria identified from the indoor air of houses. Air sampling was conducted using the Andersen six-stage sampler in combination with TYGA running for 15 min (Nevalainen 1989) and with the Andersen six-stage sampler in combination with TSA running for 10 min (Flannigan *et al.* 1989, 1991).

Table 14 : Bacteria identified in the indoor air of houses (after Nevalainen 1989, and Flannigan *et al.* 1989, 1991).

Gram-positive cocci

Aerococcus viridans (*)
Micrococcus spp. (*M. luteus* (*), *M. roseus*, *M. varians* and *M. viridans*,
Staphylococcus aureus (*) and *Staph. epidermidis* (*)
Streptococcus spp.

Gram-positive rods

Arthobacter sp.
Bacillus spp.(*) (*B. licheniformis*, *B. megaterium* and *B. subtilis*)
Corynebacterium spp. (*)
Erysipelothrix sp. (*)
Kurthia sp.
Lactobacillus sp.
Mycobacterium sp.

Gram-negative rods

Achromobacter spp.
Acinetobacter sp. (*)
Aeromonas hydrophila (*)
Agrobacterium sp. (*)
Alcaligenes denitrificans
Enterobacter agglomerans
Flavobacterium sp. (*)
Klebsiella spp.(*)
Moraxella lacunata (*)
Proteus sp.
Pseudomonas spp. (*) (*Ps. fluorescens*, *Ps. luteola*, *Ps. mallei*, *Ps. maltophilia*, *Ps. mendocina*, *Ps. oryzihabitans*, *Ps. paucimobilis*, *Ps pickettii*, *Ps. pseudomallei*, *Ps. putida*, *Ps. stutzeri* and *Ps. vesicularis*)

Actinomycetes

Actinomyces ochroleucus
Streptomyces spp. (*S. lepmarii*, *S. flavogriseus*, *S. parvus*, *S. rishiriensis*, *S. tetanusenus*, *S. roseus*, *S. wilkmorei* and *S. nitrosporeus*)

(*) also detected on surfaces by swabbing or by contact plates

Table 15 presents a summary of two surveys on the bacterial flora on domestic surfaces (in: Nevalainen (1989).

Table 15 : Bacterial flora on domestic surfaces (after Nevalainen 1989).

Bacterial group	Isolation frequency	Surface sampled	Ref.
Enterobacteriaceae			
E.coli	+ + +	dishcloth, sink, drain board	1
	+ +		2
Klebsiella	+ +	ditto	1
	+		2
Citrobacter	+ +	ditto	1
	+		2
Enterobacter	+ +	ditto	1,2
Pseudomonas sp.	+ +	dry and wet surfaces	2
Ps. aeruginosa	+ +	drain board, window sill	1
	+	ditto	2
Micrococcaceae	+ + +	dry and wet surfaces	1
Staphylococcus aureus	+ +	towels	1
	+	dry and wet surfaces	2
Bacillus sp.	+ + +	ditto	1
B. cereus	+	ditto	2

symbols: + + + a major genus, isolated in 40% of the samples
+ + found frequently, in 10-40% of the samples
+ found regularly, in <10% of the samples
References: (1) Finch *et al.* (1978), (2) Scott *et al.* (1982)

Table 16 presents data for viable airborne bacteria sampled in houses and non-industrial premises in France. Sampling was performed with a Slit sampler or RCS sampler using Tryptocasein-soy agar as collection medium. The samples were incubated at 37 °C for 48 h (Mouilleseaux and Squinazi 1991).

Table 16 : Concentrations of bacteria in houses and non-industrial premises in France (after Mouilleseaux and Squinazi 1991).

location	n	cfu/m^3 (pct)		
		5 pct	50 pct	90 pct
air conditioned offices*	918	95	280	900
hospitals **	50	60	260	860
class room (empty) **	22	300	1050	4900
class room (active) **	118	500	2600	8300
day care centres **	397	1000	5200	16000
old homes **	104	1750	4625	14000
outdoor	296	113	275	775

* Slit sampler ** RCS sampler

Table 17 presents data for viable airborne bacteria sampled in two air conditioned lecture theatres (type of humidifiers: (A) air washers, (B) evaporating type). The lecture theatres were not occupied during sampling. Sampling was performed with a slit sampler using Plate Count Agar as collection medium. The samples were incubated at 37°C for 2 days or at 22°C for 5 days (Wanner and Wirz 1974).

Table 17 : Concentrations of viable bacteria in two air conditioned lecture theatres (after Wanner and Wirz 1974).

Test	Lecture theatre A (cfu/m^3)		Lecture theatre B (cfu/m^3)	
	A	B	A	B
I	90	692	222	366
II	42	811	100	110
III	28	842	27	109
IV	55	1495	-	140

A: incubation at 37 C for 2 days B: incubation at 22 C for 5 days

APPENDIX 2

CHECKLIST

In this Appendix a list of some of the most important factors - related to building characteristics and the occupants behaviour - is presented, about which information should be recorded if sampling of biological particles in indoor environments is conducted. Furthermore, detailed information on the sampling- and analytical methods used, is required.

A. General information

* Type of house
* Location (urban/rural)
* No. of rooms within the house
* Type of heating
* Use of heating facilities
* Hot water supply
* Presence of humidifier(s) and type of humidifier(s)
* Type of ventilation
* Use of ventilation facilities
* Lowest level of the home
* Insulation of the home
* Visible dampness
* Visible mould growth
* Condensation on windows
* Odour (mouldy, musty, earthy, sulphury and the like)
* Type of floor covering (+ age of carpets)
* Presence of soft furnishings
* No. of occupants
* Age of the occupants
* Cleaning regime
* Presence of pets (types and number)
* Presence of plants (types, size and number)
* Temperature and humidity indoors and outdoors over a longer period (several weeks)

* Season
* Weather conditions
* Possible sites of external contamination

B. Air sampling

* Type of air sampler
* Type of collection medium
* Sampling rate
* Sampling time
* Sampling location(s)
* No. of samples per location
* Analytical method(s)

* Activities before and during sampling:
  - Number and age of persons present
  - Ventilation
  - Household activities

C. Dust sampling

* Type of vacuum cleaner
* Type of dust-sampler
* Suction rate
* Sampling time
* Area sampled
* Sampling location(s)
* Analytical method(s)

* Type and age of flooring
* Type and age of mattress
* Anti-mite measures undertaken

APPENDIX III

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EN

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This report is concerned with the strategy and methodology for investigating four major categories of biological particles in the indoor air of private houses, non-industrial workplaces and public buildings (excluding hospitals). These particles are mites and their faeces; dander from pets and other furred animals; fungi, including moulds and yeasts; and bacteria, including actinomycetes. For each of these categories the following items have been considered: health effects; occurrence; available sampling methods; available methods of analysis; recommendations for different studies; and observed values and evaluation of results. Health effects, occurrence and sampling and analysis of Legionella are also briefly discussed.