

Presence of viable mould propagules in indoor air in relation to house damp and outdoor air

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The presence of viable mould propagules in indoor air was investigated using the N6-Andersen sampler in combination with DG18-agar, in relation to house damp (characterized with a checklist) and in relation to the presence of moulds in outdoor air. The first part of the study was conducted in 46 houses in the autumn of 1987, the second part in 84 houses in May 1989. Further, in the second part, the results obtained with settlement plates (OPD) were compared with those obtained with the N6-Andersen sampler. The number of CFU/m³ in the indoor and outdoor air varied widely. A large variety of mould genera and species was isolated. Species of *Cladosporium*, *Penicillium* and *Wallemia* predominated. The variability in time was high and the reproducibility of the measurements in terms of CFU/m³ and of species isolated was only moderate. The low predictive value of these measurements limits their use in epidemiological studies of the relationship between exposure to moulds and respiratory symptoms. Overall, the geometric mean concentration was somewhat higher outdoors than indoors. However, the clear differences found between the number of CFU/m³ belonging to different mould species in in- and outdoor air show that the presence of viable mould propagules in indoor air is not simply a reflection of the presence of moulds in outdoor air. The presence of moulds in indoor air was only weakly related to house damp as characterized by the checklist. High, statistically significant correlations were found between the CFU yield obtained with the OPD and the CFU/m³ yield obtained with the N6-Andersen sampler. However, the number of species isolated with the OPD was significantly lower than that obtained with the N6-Andersen sampler.

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Damp houses are common in the Netherlands; about 15% are estimated to be affected to some degree (17). Several epidemiological studies suggested a relation between living in damp houses and an elevated prevalence of respiratory symptoms experienced by the residents (5, 20, 25, 27, 29). In most of these studies, information about house damp and the prevalence of respiratory symptoms was obtained by questionnaires. Varekamp & Leupen (27) used a checklist to scale the degree of damp.

There are indications that moulds and house dust mites, which both encounter favourable conditions in damp houses, are a major cause of illhealth to people living in damp houses (1, 2). Prevalences of mould allergy ranging from 2–30% (10) and of house dust mite allergy up to 85% (21) are reported in subjects with respiratory allergy.

The development of allergic diseases in a healthy

individual is the result of an interaction between the genetic predisposition to react to antigens and exposure to environmental factors. In studies of twins, it has been found that in allergic diseases, environmental factors play an important role (18, 30).

The ability to assess human exposure to moulds and house dust mites may clarify the relation between living in damp houses and possible effects on health. The major allergens produced by the house dust mite are well characterized and standardized, making it possible to assess human exposure to these allergens by means of environmental monitoring as well as by measuring specific IgE antibodies in serum to these allergens (23). The results of environmental monitoring of moulds depend highly on the method used (28). Moreover, biological monitoring by measuring specific IgE antibodies to allergens produced by moulds is beset with difficulties (6, 19). The iso-

lation, purification and standardization of allergens produced by moulds are major problems.

In the present study, the presence of viable mould propagules in indoor air was investigated in relation to house damp. In the first part we investigated the variability in time of the presence of moulds in indoor air and in the second part, the relation between the presence of moulds in indoor and outdoor air.

Material and methods

Selection of houses

The first part of the study was conducted in 46 houses in the city of Amsterdam. The houses were obtained from the registry of the Housing Inspectorate of the Municipal Health Service of Amsterdam that also deals with complaints about damp. The selection of houses was based on the presence of visible mould growth, which is considered to be an indicator of house damp. About 80% of the houses had (some) visible mould growth. This part of the study (part I) was carried out in September – October 1987.

The second part (part II) was conducted in 84 houses in Wageningen during May 1989. They were randomly selected from approximately 220 homes in 4 neighbouring areas. According to the housing corporation damp houses were to be expected in these areas.

House damp

A checklist, developed by Varekamp & Leupen (27), was used to scale several building characteristics related to: 1) the likelihood of damp problems (e.g. date of construction, building materials, the presence of a crawl space), 2) the possibilities of eliminating moisture (e.g. ventilation and heating facilities) and 3) visible signs of damp (damp spots, mould growth, wet crawl space, silver-fish or sowbugs, stale odour and wood rot). For each room in the house, a damp score (scale 0–55) was calculated. The average damp score for all the rooms represents the dampness of the house; the higher the score, the damper the house. As a working hypothesis, a room or house with a damp score (DS) of 20 or more is considered “damp”. This cut-off point is based on the observation that the number of house dust mites was clearly increased in houses with an average $DS > 20$ (26), and on the DS distribution after inspection of approximately 1200 houses (16).

Airborne viable mould propagules

In parts I and II airborne viable mould propagules were sampled using the N6-Andersen sampler (15), in combination with DG18 agar, developed by Hocking & Pitt (11). In a previous study, this combina-

tion gave the highest yield and the best reproducibility in terms of number of colony forming units (CFU) per m^3 and number of mould species isolated compared with several other combinations of samplers and culture media (28). The sampling period was 2 min. The air volume sampled was recorded by a gasmeter (G1.6 Meterfabriek, Dordrecht, the Netherlands). Sequential duplicate samples were taken.

The samples were incubated at 25°C for 4 days. CFU were then counted and corrected according to the positive hole conversion table. In part II, sampling was also performed with the open petri dish (OPD), a method relying on the gravitational deposition of mould propagules. The OPD was used in combination with DG18 agar. This method was included because in our previous study (28) strong correlations were found between the number of CFU/ m^3 obtained with the N6-Andersen and the number of CFU obtained with the OPD. The OPD has the advantage of longer sampling periods, which may increase measurement reproducibility. Parallel duplicate samples were taken. The sampling period was 60 min.

In part I, sampling was conducted at a central point in the living-room, at a sampling height of 1.5 m, under normal living conditions. From the samples taken in 25 randomly selected houses, the colonies were transferred onto the appropriate media for identification up to *genus* level. To assess the variability in time, the measurements were repeated in the same houses after 5 weeks.

In part II sampling with the N6-Andersen was performed in the living-room, the master-bedroom and outside, near the house. With the OPD, sampling was performed in the living-room and master-bedroom. The residents were asked to close windows and doors at least 1 h before the measurements were taken. From the samples taken in the living-rooms of 18 randomly selected houses and the corresponding outdoor air samples, the colonies were identified up to *species* level. Isolation and identification of the fungi were performed according to standard mycological procedures.

Table 1 summarizes the sampling protocol for both parts of the study.

Statistical analysis

Statistical analysis of the data was performed with the Statistical Package for Social Sciences – PC (22) and -mainframe (13). Pearson correlation coefficients were calculated to assess the association between different variables. T-tests were performed for testing differences between means.

To obtain information on the variability in time of the concentrations of viable mould propagules, a

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Table 1. Sampling protocol of viable mould propagules

	Method	Location	<i>n</i>	Remarks
Part I	N6	Living-room	46	Sequential duplicate samples; sampling repeated after 5 weeks; identification up to genus level of samples taken in 25 randomly selected houses
Part II	N6	Living-room	84	Sequential duplicate samples; identification up to species level of samples taken in 18 randomly selected houses (living-room) and corresponding outdoor samples
		Master bedroom	84	
		Outdoors	84	
	OPD	Living-room	84	Parallel duplicate samples; identification up to species level for samples taken in 18 randomly selected houses (living-room)
		Master bedroom	84	

Table 2. Damp scores (mean and range) for four rooms separately and for the entire homes, and the number of "damp" rooms, cq houses for both parts of the study

Location	Part I (<i>n</i> =45) damp score			Part II (<i>n</i> =84) damp score		
	Mean	Range	Damp (<i>n</i>)	Mean	Range	Damp (<i>n</i>)
Living-room	20.0	7.8–38.0	19	11.0	7.0–16.9	0
Master bedroom	16.2	6.6–40.0	15	11.4	6.0–23.2	7
Kitchen	19.8	7.8–36.0	18	10.7	6.5–19.2	0
Bathroom	20.9	11.2–36.2	20	12.3	6.0–22.8	3
Entire home	18.7	10.6–33.5	13	11.5	7.1–22.1	1

analysis of variance was performed as described by Brunekreef et al. (4), which separates the within-home or "error" variance from the between-homes or "true" variance. The ratio of these components of variance is called the variance ratio or labda.

Results

House damp

Table 2 presents the damp scores (mean and range) for 4 rooms separately and averaged over the entire house. The damp scores of the entire house and those of the separate rooms were all normally distributed. DS in part I were higher than those in part II. In part I, 13 houses of 45 were classified as "damp", whereas only one of the houses in part II was classified as "damp".

Airborne viable mould propagules

Concentrations. The number of CFU/m³ (N6-Andersen) and CFU (OPD) in indoor and outdoor air varied widely. The distributions were right-skewed and became normally distributed after ln-transformation. Table 3 presents the number of CFU/m³ and CFU (geometric mean, geometric SD and range) found in both parts of the study.

The average coefficients of variation of the sequential duplicate samples taken in part I were 39% for the first sampling period and 19% for the second period. The average coefficients of variation of the sequential duplicate samples taken with the N6-Andersen in part II were 21% for living-room samples, 18% for bedroom samples, and 28% for the

Table 3. Concentrations of viable mould propagules in CFU/m³ and CFU (geometric mean, geometric SD and range), found in both parts of the study

Method	Location	Period	<i>n</i>	GM	GSD	Range	
Part I	N6	living-room	1	40	645	2.6	62–7,332
			2	34	640	2.0	159–1,703
Part II	N6	living-room	–	84	807	3.1	96–43,045
		bedroom	–	84	822	3.2	69–23,860
		outdoors	–	84	882	2.5	124–6,248
	OPD	living-room	–	84	14	2.9	1–518
		bedroom	–	84	11	2.8	0–206

outdoor samples. The average coefficients of variation of the parallel duplicate samples taken with the OPD were 30% for living-room samples and 27% for bedroom samples.

Table 3 shows that the geometric mean of CFU/m³ was somewhat higher outdoors than indoors (part II). However, the highest CFU/m³ were found in the indoor air of some homes. Pearson correlation coefficients were calculated between the numbers of CFU/m³ found in the living-room, bedroom and outdoors. A high correlation coefficient was found ($r = 0.73$; $p < 0.001$) between the concentrations in living-rooms and bedrooms. A correlation coefficient of only 0.13 was calculated between CFU/m³ in living-rooms and outdoors. The correlation coefficient was similarly low (0.19) between concentrations in bedrooms and the outdoor air. The latter two correlation coefficients were not statistically significant. No statistically significant differences

(paired t-test) were found between the geometric mean of CFU/m³ found in the living-room, bedroom and outdoors. However, high statistically significant correlations were found between the results of the measurements obtained with the N6-Andersen and the OPD in the living-room and master bedroom (Table 4). Fig. 1 presents a scattergram of CFU/m³ obtained with the N6-Andersen and the number of CFU obtained with the OPD in the living-room.

Mould genera and species. In part I the colonies were isolated and identified up to genus level from the samples taken during both periods in 25 randomly selected houses. However, for 22 of these samples, isolation and identification was impossible because the samples were overgrown by *Chrysomilia*, or because the medium was dehydrated. The qualitative results refer therefore to 78 samples taken in 25 houses.

In part II the fungi were isolated and identified up to species level, from the samples taken with the N6-Andersen sampler and the OPD in the living-rooms of 18 randomly selected houses and corresponding outdoor samples.

In total, 19 different mould genera were found in part I whereas 66 different mould species belonging to 24 genera were found in part II.

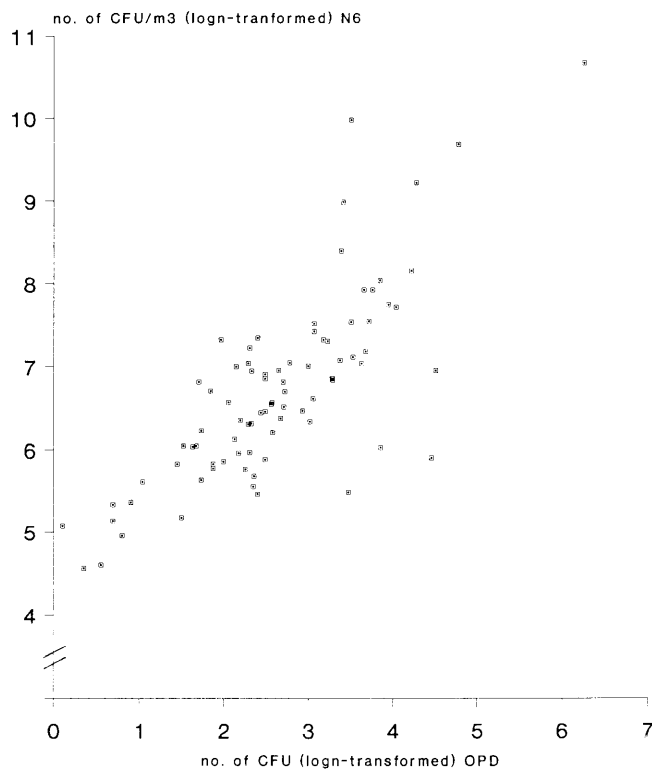


Fig. 1. Scatterplot of the number of CFU/m³ (ln-transformed) obtained with the N6-Andersen and the number of CFU (ln-transformed) obtained with the OPD in the living-room.

Table 4. Pearson correlation coefficients between the number of CFU/m³ (ln-transformed) obtained with the N6-Andersen and the number of CFU (ln-transformed) obtained with the OPD

N6-Andersen	OPD	
	Living-room	Master bedroom
Living-room	0.78**	0.65**
Master bedroom	0.70**	0.83**
Outdoors	0.28*	0.15

**p<0.001; *p<0.01

Table 5 presents the most frequently isolated mould genera (part I) and species (part II) in indoor and outdoor air samples. *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium* and *Wallemia* were found in almost all the investigated houses included in part I. In part II, *Cladosporium cladosporioides*, *C. herbarum* and *Penicillium brevicompactum* were found in almost all investigated houses. *Cladosporium herbarum* was found in all outdoor samples.

The reproducibility of the duplicate samples of mould genera or species isolated was calculated as the agreement rate: the ratio of the number of identical mould genera or species isolated in both samples and the total number of mould genera or species isolated from both samples (Table 6).

A comparison (t-test) was made between the yield of species isolated using the N6-Andersen and the

Table 5. Mould genera and species identified in 50% or more of the houses or corresponding outdoor samples

Genus/Species	Part I % of houses N6	Part II		
		% of houses		% outdoor samples N6
		N6	OPD	
<i>Alternaria</i>	84	12	6	23
<i>Aspergillus</i>	96	78	61	61
<i>A. penicilliioides</i>	—	56	33	28
<i>A. versicolor</i>	—	44	33	28
<i>Aureobasidium</i>	4	17	22	50
<i>A. pullulans</i>	—	17	22	50
<i>Botrytis</i>	80	39	50	78
<i>B. cinerea</i>	—	39	50	78
<i>Cladosporium</i>	100	100	100	100
<i>C. cladosporioides</i>	—	94	94	94
<i>C. herbarum</i>	—	89	78	100
<i>Eurotium</i>	64	56	44	72
<i>E. herbariorum</i>	—	56	44	72
<i>Penicillium</i>	100	100	83	94
<i>P. aurantiogriseum</i>	—	44	22	56
<i>P. brevicompactum</i>	—	89	50	67
<i>P. olsonii</i>	—	67	33	44
<i>Wallemia</i>	100	61	67	56
<i>W. sebi</i>	—	61	67	56

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Table 6. Reproducibility in terms of mould genera (part I) or species (part II) isolated from duplicate samples (mean and SD of the agreement rate in %)

	Method	Location	Period	n	Mean	SD
Part I	N6	Living-room	1	22	74.6	15.2
			2	12	51.7	23.7
Part II	N6	Living-room		18	42.3	20.1
		Outdoors		18	32.2	10.3
	OPD	Living-room		18	30.2	19.3

OPD. The yield obtained with the OPD (mean 5.6) was lower than that obtained with the N6-Andersen (mean 7.9). The mean agreement rate between the species isolated from the samples taken with the N6-Andersen and with the OPD was 26.0% (SD 10.2, $n = 18$).

In part I the concentrations (in CFU/m³) were calculated for the most frequently isolated mould genera, *Aspergillus*, *Cladosporium*, *Penicillium* and *Wallemia*. From 13 houses reliable duplicate samples were obtained during both sampling periods. Table 7 presents the concentrations of these genera for both sampling periods. In the first period the highest concentrations were found for *Penicillium*, followed by *Wallemia*, *Cladosporium* and *Aspergillus*. In the second period again the highest concentrations were found for *Penicillium*, whereas the lowest concentrations were found for *Wallemia*.

Table 8 presents the concentrations of the most frequently isolated mould species found in living-rooms and in the outdoor air in part II of the study. The highest concentrations were found for *Cladosporium*, indoors as well as outdoors.

Relation with house-damp. Pearson correlation coefficients were calculated between total CFU/m³ (ln-transformed) and damp scores of living-room, master bedroom, kitchen, bathroom and the entire house. The results for both parts of the study are presented in Table 9.

In part I statistically significant correlations were found between bathroom DS and total numbers of CFU/m³, for both sampling periods. The DS of

the entire home and total CFU/m³ correlated significantly only in the first sampling period. Pearson correlation coefficients were also calculated between numbers of CFU/m³ belonging to the most frequently isolated genera and DS. For *Aspergillus* and *Penicillium* the correlation coefficients were all positive (varying from 0.01 to 0.44), but not statistically significant. For *Cladosporium* negative correlation coefficients were found; in the first sampling period 3 of 5 correlation coefficients were statistically significant. For *Wallemia* the correlation coefficients were positive for the first sampling period, but negative for the second sampling period. However, these coefficients were not statistically significant.

The geometric mean concentrations of mould propagules in "dry" and "damp" houses were compared per sampling period (t-test). These comparisons were made for total CFU/m³, as well as for concentrations of the 4 most frequently isolated genera. In both sampling periods, the total geometric mean of CFU/m³ and the geometric mean of CFU/m³ for the different mould genera were generally higher in "damp" houses than in "dry" houses. For *Cladosporium*, however, the geometric mean of CFU/m³ for the first sampling period was higher in "dry" houses than in "damp" houses. None of these differences was statistically significant. Also, the concentrations found in houses where mould growth was visible in at most one room ($n = 19$) and those where mould growth was visible in 2 rooms or more ($n = 3$) were compared. The geometric mean concentrations of viable mould propagules were higher in houses with visible mould growth in at least 2 rooms for both sampling periods. However, the differences were small and not statistically significant. The number of houses with no visible mould growth was too small to make a proper comparison.

In part II, significant correlations were found only between living-room damp scores and numbers of CFU in living-rooms and bedrooms, as well as the numbers of CFU/m³ in bedrooms. No statistically significant correlations were found between CFU/m³ belonging to the most frequently isolated mould species and damp scores.

A comparison between the geometric mean concentrations of mould propagules in "dry" and "damp" houses was not possible, because only one of the investigated houses was classified as "damp". However, the numbers of CFU/m³ and CFU found in the living-rooms without visible mould growth ($n = 78$) could be compared with those found in the living-rooms with visible mould growth ($n = 6$). CFU/m³ and CFU were significantly higher ($t = 2.75$, $p = 0.01$ and $t = 2.12$, $p = 0.04$, respectively) in living-rooms with visible mould growth. However, the same comparison between master bedrooms showed no statistically significant differences.

Table 7. Concentrations in CFU/m³ (GM, GSD and range) of *Aspergillus*, *Cladosporium*, *Penicillium* and *Wallemia* for both sampling periods in 13 houses (part I)

Genus	Period 1			Period 2		
	GM	GSD	Range	GM	GSD	Range
<i>Aspergillus</i>	32	4.7	0-215	17	3.9	0-112
<i>Cladosporium</i>	63	2.8	8-545	61	5.0	0-412
<i>Penicillium</i>	127	2.6	18-1043	167	2.7	17-561
<i>Wallemia</i>	72	3.8	18-982	2	4.8	0-224

Table 8. Comparison between concentrations in CFU/m³ (GM, GSD) of 17 different mould species in indoor and outdoor air (t-test; n=18) (part II)

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

Table 9. Pearson correlation coefficients between the total number of CFU/m³ and CFU (ln-transformed) and the damp score of four different rooms and the entire homes for both parts of the study

	Damp score				
	Living-room	Master bedroom	Kitchen	Bathroom	Entire home
Part I (N6)					
Period 1 (n=40)	0.10	0.19	0.10	0.39**	0.22*
Period 2 (n=40)	-0.18	-0.02	-0.20	0.23**	0.13
Part II (N6)					
Living-room (n=84)	0.17	0.01	0.18	-0.04	0.02
Master bedroom (n=84)	0.26*	0.18	0.20	0.08	0.20
Part II (OPD)					
Living-room (n=84)	0.28**	0.06	0.19	-0.04	0.07
Master bedroom (n=84)	0.30**	0.20	0.22	0.06	0.20

* p<0.05

** p<0.01

Variability in time. In part I sampling was repeated after 5 weeks, to obtain information about the variability in time of the presence of viable mould propagules in the indoor air. There was no statistically significant difference between the average total number of CFU/m³ found in both periods (t = 1.43; p = 0.16). Further, there were no statistically significant differences between the concentrations found in both periods for the most frequently isolated mould genera, except for *Wallemia* (t = 6.43; p < 0.01).

An analysis of variance was performed to estimate the within-house and between-house components of

variance. The analysis of variance was conducted for the total number of CFU/m³, as well as for the concentrations of the most frequently isolated mould genera. The results are presented in Table 10.

The estimated variance ratios of "error" to "true" variance were generally high. This indicates that in this group of houses, the differences between houses regarding the presence of viable mould propagules were small compared with the variation in time within houses.

The mean agreement rate between the mould general isolated in both sampling periods was 50.8% (n = 11, SD 22.4%).

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Table 10. Estimated within-house and between-house components of variance and variance ratio for the total concentrations of viable mould propagules and for *Aspergillus*, *Cladosporium*, *Penicillium* and *Wallemia* separately (part I)

	Estimated within-house variance	Estimated between-house variance	Variance ratio
Total CFU/m ³	0.665	0.167	3.982
<i>Aspergillus</i>	2.133	-0.027	*
<i>Cladosporium</i>	1.283	0.335	3.829
<i>Penicillium</i>	0.503	0.426	1.181
<i>Wallemia</i>	2.019	0.107	18.794

* Variance ratio not calculated because of the estimated negative between-home variance

Comparison of mould species in in- and outdoor air. In total, 66 different mould species were isolated. Eighteen species were isolated only from indoor air samples, whereas 14 species were isolated only from outdoor samples. However, these species were not isolated more than twice out of 36 samples. The mean agreement rate between the species isolated from in- and outdoor samples taken with the N6-Andersen was 26.2% (SD 7.5, $n = 18$). The mean agreement rate between species isolated from indoor samples taken with the OPD and the outdoor samples was 23.5% (SD 7.1, $n = 18$).

A comparison (t-test) was made between the numbers of CFU/m³ found for the most frequently isolated species in the indoor and outdoor air.

Table 8 shows that the total number of CFU/m³ was somewhat higher in the outdoor air than in the indoor air of these 18 houses. However, the mean numbers of CFU/m³ belonging to *Aspergillus* (total), *A. penicillioides* and *A. versicolor* were higher in the indoor air, as also for most of the *Penicillium* species. For *Cladosporium* (total) as well as *C. herbarum*, the mean numbers of CFU/m³ were significantly higher in the outdoor air, whereas no differences were found for the remaining species belonging to the genus *Cladosporium*. The mean number of CFU/m³ of *Aureobasidium pullulans* was significantly higher in outdoor air than in indoor air; no differences were found for *Ramularia deusta* and *Wallemia sebi*.

Discussion

House damp

The number of "damp" houses found in part I was much higher than that found in part II. The selection of houses included in part I was based on the presence of visible mould growth, whereas those in part II were selected randomly. Further, the weather during part II was unseasonably dry and warm. This may have affected the results, because some of the checklist items apply to the actual presence of visible signs of dampness in the house.

In most epidemiological studies investigating the

relation between house damp and respiratory health, information was obtained by questionnaires (5, 20, 25, 29). However, as indicated by Strachan (25) and others, this method can incur potential responder bias. Yet also it is not clear whether the checklist used in this study, that scales several building characteristics in relation to house damp, is a better method for classifying houses as "damp" or "dry". Although a responder bias is avoided, the question remains whether the characteristics that favour the production of inhalatory allergens, such as moulds, are distinguished and weighted properly with this checklist. It is worthwhile pursuing this issue further, because a simple and validated checklist that enables a discrimination between "damp" and "dry" houses would be very useful in epidemiological studies.

Viable mould propagules

The geometric mean of CFU/m³ found in indoor air was higher than that reported by Holmberg (12) and Waegemaekers et al. (29), but comparable with that reported by others (3, 7, 14). In part I 19 different mould genera were isolated. *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Penicillium* and *Wallemia* were found in almost all investigated houses. In part II, 66 different mould species belonging to 24 different genera were isolated. *Cladosporium cladosporioides*, *C. herbarum*, *Eurotium herbariorum*, *Penicillium brevicompactum* and *Wallemia sebi* were isolated from most indoor and outdoor air samples. In part I the highest numbers of CFU/m³ were found for *Penicillium*, followed by *Cladosporium*. In part II the highest numbers of CFU/m³ were found for *Cladosporium cladosporioides*, followed by *C. herbarum* and some of the species belonging to *Penicillium*. These results agree generally with those reported by others (e.g. Gravesen (8); Fradkin et al. (7); Hunter et al. (14)), although in most studies the fungi were only identified up to genus level.

However, a proper comparison between the quantitative and qualitative results of different studies is difficult, because in most studies different methods were used for the sampling of airborne viable mould propagules. In our previous study it was shown that the results in terms of CFU/m³ and of species isolated depend on the combination of sampler and culture medium used (28). Also, the studies were performed in different seasons. It is well known that the presence of viable moulds outdoors as well as indoors varies widely between seasons (8, 14). This may also have contributed to the differences found between part I and part II of this study. Thirdly, the reproducibility of the measurements in terms of CFU/m³ and of moulds species isolated is low. The coefficients of variation of samples taken within min-

utes of each other were high, which is in agreement with results published previously (24, 28, 29). The reproducibility of mould genera or species isolated was not very good either. Another possible source of variation might have been the considerable influence of air disturbances caused by indoor activities on the number of CFU/m³, as reported by Hunter et al. (14).

The results of part I indicated that the within-house variation in the concentrations of mould propagules was much higher than the between-house variation. The ratios of "error" to "true" variance were generally high. Thus, the measurement of viable mould propagules in air with the techniques used in this study approximated the true exposure to only a limited extent (4).

High, significant correlations were found between the CFU/m³ obtained with the N6-Andersen in the living- and bedroom and the number of CFU obtained with the OPD. However, the mould species isolated with the OPD were significantly fewer than those isolated with the N6-Andersen. These findings are in agreement with our previous results (28). Thus, with the OPD gave only a semi-quantitative but less complete picture about the presence of viable moulds in indoor air may be obtained.

The geometric mean concentration of CFU/m³ found in the outdoor air during part II was somewhat higher than those found in living- and bedrooms. However, the differences were not statistically significant. Further, no statistically significant correlations were found between the concentrations in indoor and outdoor air, but, a statistically significant correlation was found between the concentrations in living-rooms and bedrooms.

A comparison, between the CFU/m³ concentrations of mould species in indoor and outdoor air showed clear differences. For *Aspergillus*- and *Penicillium*-species, the concentrations were higher indoors than outdoors. For most species belonging to *Cladosporium* and for *Aureobasidium pullulans* and *Botrytis cinerea*, the concentrations in outdoor air were higher than in indoor air. These results indicate that outdoor air may influence the presence of viable mould propagules in indoor environments, but that the presence of viable moulds in indoor air is not simply a reflection of the presence of moulds in outdoor air. Also, the high correlation found between the total numbers of CFU/m³ in the living-room and bedroom indicates that indoor sources of moulds are present. These results are in agreement with those reported by Fradkin et al. (7). This suggests that in aerobiological studies, attention should be given to the identification of colony forming units up to species levels.

Despite the high within-house variability, it is notable that the total mean numbers of CFU/m³ and

the mean number of CFU/m³ of the different genera were higher in "damp" houses than in "dry" houses during both sampling periods in part I of this study. However, these differences were not significant.

Further, the correlations between house damp and indoor viable mould concentrations in both parts of the study were generally positive but low. Thus, the presence of viable mould propagules was only weakly related to house damp as characterized by the checklist. Nevertheless, Hunter et al. (14) and Waegemaekers et al. (29) also reported higher numbers of CFU/m³ in houses which were classified as "damp".

It is concluded that, due to the large variability, the measurement of viable mould propagules in indoor air with the techniques used in this study does not provide a method to distinguish the exposure to moulds in "damp" and "dry" homes. The question thus remains whether house characteristics favourable for moulds are indeed properly distinguished and weighted with the checklist used in this study.

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