

Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment

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Measurements of biocontaminants in settled house dust once a year are commonly used to assess long-term exposure. To examine stability over time and seasonal variation, we measured concentrations of mite and cat allergens, endotoxin and mold spores in living room floor dust in 745 German homes collected twice a year in two different seasons. The study population consisted of adults and children living in five different areas in Germany. All dust samples were collected in a standardized manner from the living room floor and taken during the years 1995 to 1998. The median interval between the two dust samplings was approximately 7 months. Mite and cat allergens were measured in settled house dust by monoclonal antibodies, endotoxin by the limulus amoebocyte lysate method, and total spore counts by cultural methods. Crude Pearson's correlation coefficients between log-transformed concentrations in the first and second dust samples ranged between 0.65 and 0.75 for allergens, 0.59 for endotoxin and only 0.06 for total spore counts. The strongest and most consistent seasonal effects were observed for fungi with highest levels in July–September. Cat allergen concentrations were found consistently to be increased in January–March. Mite allergens did not show a strong and consistent seasonal pattern. We conclude that repeated measurements of mite and cat allergens and endotoxin in settled house dust improve the estimate for annual mean concentrations. However, even a single observation of these biocontaminants may be a good proxy for a 1-year exposure since repeated measures were highly correlated. However, repeated measurements of fungi levels were only weakly correlated and thus repeated observations for assessment of annual means of total spore counts are needed.

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Introduction

Indoor allergens and fungi have been found to be associated with allergic sensitization and respiratory morbidity (Lau et al., 2000; Jacob et al., 2002). Bacterial endotoxin in indoor environment has also been suggested to play a role in the

development of atopy and asthma. However, it is currently not clear whether endotoxin has a protective or inducing effect, or both, depending on timing and dose of exposure (Martinez and Holt, 1999; Gereda et al., 2000; Gehring et al., 2001, 2002; Park et al., 2001; Douwes et al., 2002). Long-term exposure to allergens, fungi and endotoxin is generally assessed by measuring these agents in house dust at one point in time. However, only very few studies have been conducted to assess the reproducibility of those measurements over time. Thus, exposure assessment may be further improved by considering variability over time, including seasonal effects on indoor biocontaminant levels. Studies on repeated measurements of mite allergens in house dust samples consistently showed a correlation between repeated measurements (Kuehr et al., 1994; Marks et al., 1995; Hirsch et al., 1998; Petersen et al., 1999). However, different conclusions were drawn from those studies. Some authors (Hirsch et al., 1998; Petersen et al., 1999) conclude that repeated measure-

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ments are recommended, whereas others (Kuehr et al., 1994) concluded that allergen levels are relatively stable.

Chew et al. (2001) studied fungal extracellular polysaccharides (EPS), β (1,3)-glucans and culturable fungi in house dust that was sampled at six occasions in 23 Dutch homes during a period of 1 year. They showed that the within-home variation for EPS in house dust was smaller than the between-home variation suggesting that a one-time measurement of EPS (a general marker for fungal biomass in house dust; Douwes et al., 1999) is a good proxy for long-term fungal exposure. The within-home variability for β (1,3)-glucans and culturable fungi was large and in case of fungi even exceeded the between-home variability suggesting that a one-time measurement to assess long-term exposure to fungi may not be valid. Only few studies have been published on repeated measurements of endotoxin in house dust (Rizzo et al., 1997, Gereda et al., 2000, Park et al., 2000, Su et al., 2001). Therefore, we investigated seasonal variability of allergen, endotoxin and fungi in house dust in a large sample of German homes under temperate climatic conditions.

Materials and Methods

Study Population

A total of 777 homes in five study regions were visited twice for house dust collection within the framework of a study on indoor factors and genetics in asthma (INGA). The time between both home visits ranged from 6 to 13 months with a median time interval of 7 months, whereas three-quarters of all homes were revisited after 6–8 months. The study population consisted of two parts corresponding to subsets of participants from two other studies: 362 adults living in Erfurt or Hamburg who had participated within the German centers of the European Respiratory Health Survey (ECRHS) (Burney et al., 1994) and 415 school children living in three regions of former East Germany (Zerbst, Bitterfeld, Hettstedt) who participated in a study of air pollution effects and determinants of allergic diseases (Heinrich et al., 1999, 2000). Details of the selection of the study population have been described elsewhere (Richter et al., 2000; Heinrich et al., 2001). Subjects who had moved

between the first and the second home visit were excluded from the analyses ($n = 32$). Thus, the population consisted of 745 subjects (Erfurt: 182, Hamburg: 166, Zerbst: 127, Bitterfeld: 103, Hettstedt: 167).

Dust Sampling and Extraction

The visits were conducted by trained personnel from June 1995 to May 1997 for the adult population and from April 1996 to September 1998 for the children's population (see Table 1). The dust sampling and extraction procedures followed general agreed recommendations (Platts-Mills et al., 1992) and have been described in more detail elsewhere (Fahlbusch et al., 1999). Briefly, in each apartment a dust sample was taken from the living room floor according to a standardized protocol (Fahlbusch et al., 1999). All dust samples were taken using the same type of vacuum cleaner (Philips, Hamburg, Fluster jet Vitall 371) by vacuuming an area of 1 m² for 2 min. In the majority of homes, samples were obtained from carpets and in only very few homes, no carpets were available in the living room ($N = 28$). Noncarpeted smooth floors ($N = 28$) were vacuumed as a whole. Dust was collected on cellulose filters using ALK sampling nozzles (ALK Laboratories, Hørsholm, Denmark). The dust filters were weighed before and after vacuuming. The samples were stored at -20°C until extraction. Extracts were stored in pyrogen-free glassware at -20°C until analysis. Repeated home visits to collect house dust were conducted by the same fieldworker and the same technicians extracted and analyzed the dust samples for all visits in all study regions.

Allergen and Endotoxin Content in House Dust

Sample allergen content of house dust mite allergens (Der p 1 and Der f 1) and cat allergen (Fel d 1) was measured by means of a two-site monoclonal enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer with standards UVA 93/03, UVA 93/02 and UVA 94/01 (Indoor Biotechnologies, Clwyd, UK) (Fahlbusch et al., 1999). The allergen concentration was expressed as nanogram per gram of dust. The lower limit of detection was 10 ng/g dust for both Der p 1 and Der f 1, and 15 ng/g dust for Fel d 1.

Table 1. Time frame for first and second visits (numbers of visited homes) per quarters of the years from 1995 through 1998 stratified for the cities Erfurt/Hamburg and Zerbst/Bitterfeld/Hettstedt and the first and repeated second visits.

| | | 1995 | | 1996 | | | 1997 | | | 1998 | | | | |
|---------------------------------|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| | | Jul-Sep | Oct-Dec | Jan-Mar | Apr-Jun | Jul-Sep | Oct-Dec | Jan-Mar | Apr-Jun | Jul-Sep | Oct-Dec | Jan-Mar | Apr-Jun | Jul-Sep |
| Erfurt/Hamburg | 1st visit | 40 | 74 | 102 | 78 | 50 | 4 | | | | | | | |
| | 2nd visit | | | 4 | 76 | 80 | 71 | 105 | 12 | | | | | |
| Zerbst/Bitterfeld/ Hettstedt | 1st visit | | | | 50 | 81 | 33 | 24 | 59 | 51 | 47 | 52 | | |
| | 2nd visit | | | | | | 9 | 71 | 61 | 26 | 74 | 39 | 93 | 24 |

Endotoxin was assayed with the quantitative, kinetic chromogenic limulus amoebocyte lysate (LAL) method (Kinetic-QCL no. 50-650U; Bio Whittaker; LAL lot no. 5L3480) at 37°C. Analyses were performed with an automated microtiter plate reader (anthos ht III, Anthos Labtec Instruments) using the WinKQCL 1.1 software. *Escherichia coli* endotoxin (Bio Whittaker, lot no. 5L1570) was used as standard. The endotoxin potency of this standard was 14.5 EU/ng. The lower limit of detection of the test was 0.15 EU/g dust. Endotoxin concentrations were expressed as endotoxin units EU/g dust.

Culturable Mold Spore Counts (total fungi)

A second dust sample from the living room floor was collected for the measurement of culturable fungi. The dust samples were stored at room temperature and analyses were performed within 10 days after sampling. The collected dust was sieved (500 µm) for further analysis. A total of 30 mg sieved house dust was analyzed for identification and quantification of culturable fungi (Koch et al., 2000). Dust samples were diluted in 0.9% NaCl and plated on DG18 (dichloran-18% glycerol agar) with 0.100 g/l chloramphenicol added to prevent bacterial growth. Plates were incubated at 25°C for 10 days and all analyses were duplicated. The number of colony-forming units (CFU) was counted and expressed as CFU/g dust. The lower limit of detection of this method was 1000 CFU/g dust.

In addition to the expression of biocontaminant concentrations per gram dust or fine dust (for mold spore counts), all concentrations were also calculated per square meter sampled floor.

Statistical Methods

Since the distributions of all measured concentrations were markedly skewed to the right we used log-transformed values. Allergen concentrations less than the detection limit were assigned a value of one-half of the detection limit. For Der p 1 levels this was the case in more than 60% of the samples. Nevertheless, we treated the allergen levels as a continuous variable. In addition we analyzed the data using categorical variables and compared the results with those we had obtained when a continuous outcome was used in the analyses.

As a measure of stability of the concentrations over time, we calculated Pearson's correlation coefficients between the log-transformed measurements of the first and second visits. To compare the correlation in subgroups we used the difference of the Fisher-*z*-transformed correlation coefficients (Altman, 1991, p. 293) as a test statistic. Bootstrap estimates (Efron and Tibshirani, 1993) of the corresponding standard errors were calculated. The standard formula was distorted since more than half the values were below the detection limit.

To examine to what extent the correlations were influenced by seasonal and regional differences and also to characterize

the seasonal variation itself, we used mixed-effects models, similar to those described by Park et al. (2000). We modelled the log-transformed concentrations as the sum of fixed region effects (Erfurt, Hamburg, Zerbst, Bitterfeld, Hettstedt), fixed seasonal effects (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec), random home effects and random deviations within homes. The estimated between- and within-home variance components were expressed as geometric standard deviations (GSD = antilog of square root of the variance). The ratio of the between-home variance to the sum of both variance components is the correlation between measurements of the first and second visit, adjusted for seasonal and regional differences. To investigate the consistency of seasonal variations across different time intervals (July 1995–June 1996, July 1997–June 1998) interaction terms were added to the models. Additionally, separate analyses were conducted for the study regions of adults (Erfurt, Hamburg) and children (Zerbst, Bitterfeld, Hettstedt).

Since large fractions of the allergen levels were below the detection limit, we examined the seasonal variation of allergen concentrations additionally with logistic regression models, using the detection limit and the 75th percentile as cut-off points. We used the GEE methodology (Diggle et al., 1994) to account for repeated measurements.

To obtain a more precise seasonal adjustment of the correlations and to generate graphical displays of the seasonal effects we also fit cubic regression splines within the mixed-effect models. We used nine interior knots, which is equivalent to 12 degrees of freedom (see, e.g., Hastie and Tibshirani 1990). All analyses were carried out with SAS V8 (SAS Institute, Cary, NC, USA).

Results

Results on biocontaminant levels of the first home visit of the INGA study have been published previously (Fahlbusch et al., 1999, 2002; Koch et al., 2000; Heinrich et al., 2001; Bischof et al., 2002; Groß et al., 2001). Here we present the results on repeated measurements of biocontaminants collected in the same homes. Table 1 presents the number of visited homes by quarters of the years. The dust samples were collected uniformly distributed over the quarters of a year so we can exclude a seasonal bias in the collected samples.

Table 2 shows the distributions of dust amount collected on living room floors. Furthermore, the descriptive statistics are shown for allergens, endotoxin and total culturable fungi levels expressed per gram dust. More than 60% of Der p 1 and more than 35% of Der f 1 levels were below the limit of detection (LOD). Overall, the selected parameters of distributions of dust mass were similar for both visits. However, the percentage of values below the detection limit differed for mite and cat allergens and concentration of

Table 2. Distribution of allergen, endotoxin and fungi concentrations expressed per gram collected dust.

| | Visit | N | % < LOD | Min. | 25th perc. | Median | 75th perc. | 90th perc. | Max. |
|------------------------------------|-------|-----|---------|------|------------|--------|------------|------------|-----------|
| Der p 1 (ng/g) | 1 | 745 | 62.0% | < 10 | < 10 | < 10 | 214 | 1587 | 146,195 |
| | 2 | 742 | 68.1% | < 10 | < 10 | < 10 | 261 | 1291 | 84,088 |
| Der f 1 (ng/g) | 1 | 745 | 35.6% | < 10 | < 10 | 173 | 1075 | 5722 | 190,536 |
| | 2 | 742 | 40.6% | < 10 | < 10 | 176 | 1035 | 6639 | 175,809 |
| Fel d 1 (ng/g) | 1 | 745 | 20.4% | < 15 | 64 | 260 | 1478 | 49263 | 3,680,851 |
| | 2 | 742 | 14.7% | < 15 | 94 | 278 | 1575 | 48129 | 9,662,994 |
| Endotoxin (10 ³ × EU/g) | 1 | 745 | 0% | 1.5 | 13.5 | 27.8 | 57.3 | 107.2 | 1476 |
| | 2 | 742 | 0% | 0.2 | 14.2 | 27.5 | 58.3 | 111.1 | 1335 |
| Fungi (10 ³ × CFU/g) | 1 | 745 | 0% | 4 | 55 | 105 | 200 | 410 | 76,000 |
| | 2 | 743 | 0% | 5 | 50 | 90 | 175 | 350 | 2600 |
| Fungi dust (mg) | 1 | 745 | | 19 | 495 | 806 | 1302 | 1989 | 8063 |
| | 2 | 743 | | 58 | 446 | 734 | 1195 | 1831 | 6305 |

LOD = limit of detection: Der p 1 = 10 ng/g, Der f 1 = 10 ng/g, Fel d 1 = 15 ng/g, Endotoxin: 0.15 EU/g, Fungi: 1000 CFU/g.

Table 3. Crude correlation coefficients (Pearson's correlation coefficients) between log-transformed data of first and second visits expressed per gram dust and per square meter sampled surface

| | All homes (N = 742) | Erfurt/Hamburg (N = 348) | Zerbst/Bitt./Hett. (N = 394) | Changes* from 1st to 2nd visit in | | |
|----------------------------------|------------------------|-----------------------------|---------------------------------|-----------------------------------|--------------------------------------|-------------------------|
| | | | | Floor covering (N = 97) | Curtains, pillows, etc. (N = 110) | Pet keeping (N = 81) |
| Per gram dust | | | | | | |
| Der p 1 | 0.65 | 0.61 | 0.68 | 0.51 | 0.52 | 0.51 |
| Der f 1 | 0.67 | 0.66 | 0.65 | 0.49* | 0.52* | 0.56 |
| Fel d 1 | 0.75 | 0.80 | 0.74 | 0.67 | 0.78 | 0.68 |
| Endotoxin | 0.59 | 0.56 | 0.62 | 0.38* | 0.50 | 0.62 |
| Fungi | 0.06 | 0.03 | 0.07 | 0.11 | -0.03 | 0.07 |
| Per square meter sampled surface | | | | | | |
| Der p 1 | 0.65 | 0.61 | 0.68 | 0.48 | 0.53 | 0.50 |
| Der f 1 | 0.67 | 0.67 | 0.65 | 0.46* | 0.52* | 0.57 |
| Fel d 1 | 0.74 | 0.78 | 0.74 | 0.66 | 0.76 | 0.69 |
| Endotoxin | 0.67 | 0.65 | 0.70 | 0.57 | 0.63 | 0.66 |
| Fungi | 0.39 | 0.42 | 0.31 | 0.40 | 0.42 | 0.42 |

*significantly different from other homes ($P < 0.05$). ^aWe asked for replacement/removal of floor covering, and curtains, pillows, upholstered furnitures and sofas. Furthermore, we asked if the occupants gave pets away or became pet owners during first and second visits.

allergens, endotoxin, and total culturable fungi between the two visits.

A similar result was obtained when concentrations were expressed per square meter of sampled floor (data not shown). Again selected parameters of the distribution of concentrations for allergens, endotoxin, and fungi expressed as per square meter collected surface were similar between both visits (data not shown).

Associations between First and Second Measurements

The crude Pearson correlation coefficients between the log-transformed levels of the two visits were 0.65, 0.67, and 0.75 for the allergens Der p 1, Der f 1, and Fel d 1, respectively, and 0.59 for the endotoxin levels, and only 0.06 for total fungi (Table 3). Similar results were obtained for the Spearman rank correlations (data not shown). The correla-

tions differed only marginally between the two subpopulations of adults and children. Furthermore, the magnitude of the correlation was very similar across study regions.

For Der f 1 and endotoxin levels we observed significantly decreased correlations ($P < 0.05$) in homes of participants, who renovated or changed floor coverings between the first and second visits (Table 3). No dependence of the correlation with regard to the time interval between the visits was found. The concentrations of cat allergens were not affected by changes in pet keeping.

Sensitivity analyses in the subject's homes without any regular contact to cats resulted in lower correlation coefficients of 0.45 and 0.50 compared with 0.75 and 0.74 (Table 3).

Correlation coefficients were more or less the same for mite and cat allergen independent of whether the concentrations

Table 4. Within-home correlations of two repeated measurements in 742 German homes and geometric standard deviations (GSD)

| | Within-home correlation | Between-home GSD | Within-home GSD | Ratio ^a 'between vs. within' |
|----------------------------------|-------------------------|------------------|-----------------|---|
| Per gram dust | | | | |
| Der p 1 | 0.64 | 7.98 | 4.72 | 1.7 |
| Der f 1 | 0.65 | 9.85 | 5.32 | 1.9 |
| Fel d 1 | 0.75 | 13.80 | 4.56 | 3.0 |
| Endotoxin | 0.58 | 2.27 | 2.01 | 1.1 |
| Fungi | 0.15 | 1.43 | 2.31 | 0.6 |
| Per square meter sampled surface | | | | |
| Der p 1 | 0.64 | 9.01 | 5.20 | 1.7 |
| Der f 1 | 0.65 | 11.20 | 5.93 | 1.9 |
| Fel d 1 | 0.74 | 15.27 | 4.96 | 3.1 |
| Endotoxin | 0.67 | 3.27 | 2.28 | 1.4 |
| Fungi | 0.42 | 2.27 | 2.63 | 0.9 |

Mixed-effect models for log-transformed concentrations with a random home effect and fixed effects of region and season (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec).

^aThe ratio 'Between-home GSD versus within-home GSD' is measuring whether the variation of the measurements in different homes is greater than (ratio is greater than 1), is equal to (ratio is equal to 1) or is less than (ratio is below 1) the variation of the repeated measurements within a single home.

Table 5. Seasonal differences of biocontaminants expressed as per gram dust in all areas and stratified for the cities Erfurt/Hamburg and the small towns Zerbst/Bitterfeld/Hettstedt — means ratios^a for quarters of the year.

| | Mean ratios | | | | | | | | | | | | | | |
|-----------|-------------|---------|---------|---------|-----|----------------|---------|---------|---------|-----|---------------------------------|---------|---------|---------|-----|
| | All areas | | | | | Erfurt/Hamburg | | | | | Zerbst/Bitt./Hett. ^b | | | | |
| | Jan–Mar | Apr–Jun | Jul–Sep | Oct–Dec | P | Jan–Mar | Apr–Jun | Jul–Sep | Oct–Dec | P | Jan–Mar | Apr–Jun | Jul–Sep | Oct–Dec | P |
| Der p 1 | 1.00 | 0.77 | 0.99 | 1 | NS | 0.97 | 0.95 | 1.03 | 1 | NS | 1.04 | 0.67 | 0.95 | 1 | * |
| Der f 1 | 0.67 | 0.57 | 0.92 | 1 | *** | 1.06 | 0.88 | 0.98 | 1 | NS | 0.41 | 0.40 | 0.85 | 1 | *** |
| Fel d 1 | 1.21 | 0.72 | 0.72 | 1 | *** | 1.25 | 0.83 | 0.98 | 1 | NS | 1.22 | 0.65 | 0.56 | 1 | *** |
| Endotoxin | 1.13 | 1.01 | 0.95 | 1 | * | 0.96 | 0.91 | 0.81 | 1 | NS | 1.35 | 1.11 | 1.11 | 1 | ** |
| Fungi | 0.70 | 1.02 | 2.40 | 1 | *** | 0.62 | 1.04 | 2.18 | 1 | *** | 0.79 | 1.02 | 2.62 | 1 | *** |

^a(geometric) means ratios, adjusted for area and random home effects, reference group is Oct–Dec.

^bBitt. = Bitterfeld, Hett. = Hettstedt. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. NS = not statistical significant.

were expressed per gram dust or per square meter. However, the correlation coefficient for fungi strongly increased when total spore counts were calculated per square meter. Also correlation between the repeated endotoxin measurements slightly improved (Table 3). The increased correlations for fungi and endotoxin were consistently found in both the adults and children populations.

Table 4 shows within-home correlations and GSD adjusted for region and season (Jan–Mar, Apr–Jun, Jul–Sep, Oct–Dec). The adjustment had only minor effects on the correlations for allergens and endotoxins expressed per gram dust. All four correlations were above 0.5. Thus, the within-home variation was smaller than the between-home variance for those components. For total fungi we calculated an adjusted correlation of 0.15. Using a cubic regression spline to fit the seasonal effects more accurately changed the within-home correlation only slightly to 0.18. Only for total spore counts the within-home GSD exceeded the between-home GSD. However, when the concentrations of total fungi were

expressed per square meter sampled floor the ratio of between and within home-GSD increased from 0.6 to 0.9 (Table 4).

Seasonal Variability

Table 5 shows seasonal differences of the biocontaminant concentrations expressed per gram dust between the four quarters of the year. We calculated adjusted (geometric) means ratios for the quarters Jan–Mar, Apr–Jun, Jul–Sep in comparison to the arbitrarily chosen date group Oct–Dec. The means ratios were adjusted for fixed region effects and random home effects in the framework of mixed-effect models for the log-transformed concentrations. Strong and consistent seasonal effects were observed only for total fungi. This was found for both the adult and children population (data not shown). Figure 1 shows the corresponding fit of a cubic regression spline. For endotoxin, the data showed only a small significant seasonal effect with highest levels in dust samples collected between January and March. However, no consistent seasonal pattern was found, Seasonal differences in

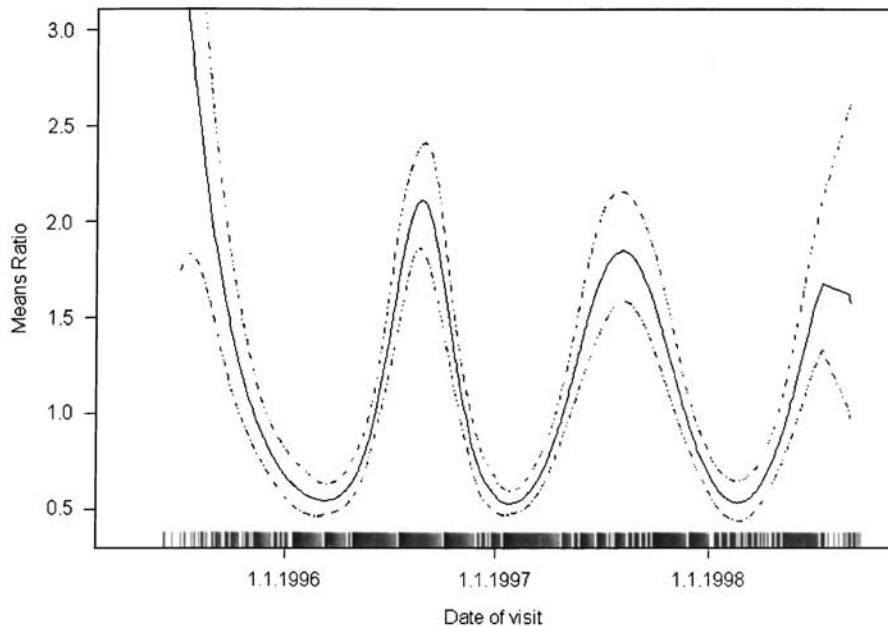


Figure 1. Seasonal variation of total fungi levels in living room floor dust of 743 German homes. The solid curve represents means ratios (adjusted for region and random home effects), dashed curves show pointwise ± 2 standard error bands, fitted with a cubic regression spline (12 degrees of freedom).

the Zerbst, Bitterfeld, and Hettstedt region (children population) could be found only in 1996/97, but not in the following 12 months period (data not shown). Cat allergen was found to be increased in winter (Table 5). This was consistent for all years that measurements were collected and particularly in the children's population (data not shown). Only small differences were observed for the adult population. A seasonal pattern for Der p 1 in house dust was not statistically significant. Der f 1 concentrations showed statistically significant ($P < 0.001$) seasonal pattern with slightly higher concentrations in autumn and lower levels in January through March. This seasonal effect was not seen consistently in all areas. The findings from the GEE models were very similar (data not shown).

In addition, seasonal variation of biocontaminants expressed per square meter sampled floor was modeled. Similar results were found (data not shown).

Discussion

Several epidemiological studies on determinants of asthma and allergies investigate long-term exposure to biocontaminants. Assessment of exposure to biocontaminants is often based on measurements in settled house dust sampled at one time point spread over the seasons. Information on repeated measurements of biocontaminants in several seasons and the timing of season-specific peak levels may be more useful for assessment of long-term exposures.

Associations between First and Second Measurements

Repeated dust sampling was conducted in different seasons. However, both measurements expressed per gram dust were highly correlated for all measured biocontaminants, with the exception of total mold spore counts. The higher correlation coefficient for Der f 1 compared to Der p 1 is biologically plausible, because *Dermatophagoides pteronyssinus* has been shown to be more sensitive to heat and low relative humidity than *D. farinae*. In addition, the level of *D. pteronyssinus* in house dust is more related to climatic conditions (Groß et al., 2000). Repeated measurements of Der p 1 and Der f 1 in 91 children's mattress dust showed generally higher correlations (0.76–0.95) compared with this study on floor dust measurements, but the reported correlation coefficient for Der f 1 was consistent with our study and higher than Der p 1 correlation (Peterson et al., 1999). Another study in Germany by Hirsch et al. (1998) showed only a weak correlation between two and four repeated measurements of Der p 1 and Der f 1 on carpets ($n = 46$) and mattresses ($n = 31$). The authors concluded that single observations of dust mite allergens in house dust give only limited information about long-term exposure. In contrast, another study also conducted in Germany showed a high correlation ($r = 0.82$ and 0.72 , respectively) between Der p 1 and Der f 1 levels in mattresses of more than 1000 school children measured twice over a period of 1 year (Kuehr et al., 1994). The authors concluded that mite antigen allergen levels in house dust were a stable proxy for exposure. Based on our study results we conclude that a single observation of mite

allergens in settled house dust may be a good proxy for long-term exposure during a period of 1 year.

Keeping a cat and second-hand cat exposure are very strong predictors for high concentrations of Fel d 1 in settled house dust (Fahlbusch et al., 2002). Since Fel d 1 is a relatively stable molecule and it is generally bound on particles of less than $2.5 \mu\text{m}$ in aerodynamic diameter keeping the cat allergen airborne, it is ubiquitously distributed in all rooms of the home (Fahlbusch et al., 2002). Thus, the high correlation between repeated measurements even if floor covering or curtains were replaced and pet ownership changed seems plausible. However, the magnitude of correlation is in large explained by the abundantly higher cat allergen concentrations in homes where a cat was kept.

In a study in the US (Peterson et al., 1999) the correlation coefficients for Fel d 1 ranged between 0.68 and 0.82 for repeated measurements (minimum six, maximum 36 measurements) in 91 children's mattress dust samples. Despite the quite high correlation, the authors conclude that a single observation would be inadequate to accurately represent the average burden of a home (Peterson et al., 1999). The high correlation between the two measurements of cat allergens in this study indicates that already a single measurement is a good proxy for long-term exposure.

Three studies in Brazil, the US and Taiwan measured endotoxin in more than 12 dust samples collected monthly during a year from in a relatively small sample of homes (Rizzo et al., 1997 ($n=20$ homes); Park et al., 2000 ($n=20$ homes); Su et al., 2001 ($n=35$ homes)). Only Park et al. (2000), reported correlation coefficients between the repeated measurements of 0.76 for bed dust and 0.40 for bedroom floor dust samples, which were statistically significant.

Our study results showed a high stability of endotoxin concentrations in settled house dust.

Total spore counts measured twice per home in one of these three studies were similar as in our study also not correlated (Su et al., 2001). Owing to high correlations between indoor and outdoor spore counts (Koch et al., 2000, Su et al., 2001) and the large variation in outdoor air concentrations repeated measures indoors in different seasons were not correlated. The concentration of total spore counts showed higher within-home GSD than between-home GSD, whereas this was reversed for all other biocontaminants, indicating that assessment of long-term exposure to molds requires repeated measurements (potentially more than two) in particular in late summer in temperate climates.

Summarizing our results, a single measurement of total spore counts is an unacceptable estimate for the annual average concentration of total spore counts in a temperate climate such as in Germany. However, the repeated measurements of other biocontaminants correlated fairly well.

Seasonal Variability

Allergen concentration and total mold spore counts The seasonal variation of allergen and mold concentrations in different climates has been discussed in detail in the literature, indicating allergen-specific seasonalities (Platts-Mills et al., 1987; Lintner and Brame, 1993; Munir et al., 1993; Chan-Yeung et al., 1995; Marks et al., 1995; Miyazawa and Suzuki, 1996; Hirsch et al., 1998; Koch et al., 2000; Chew et al., 2001; Su et al., 2001).

Consistent with studies in the US and Japan, our study showed highest Der f 1 levels in autumn and decreased through spring. Most studies showed similar seasonal pattern of Der p 1 allergen levels. However, building characteristics far outweighed the seasonal associations in most of the studies (Chew et al., 2001; Groß et al., 2000).

Also for cat allergen concentrations in settled house dust, seasonal influences are negligible compared to potent predictors such as cat ownership or transfer of cat allergens by second-hand cat exposure. The higher levels during winter were ascribed to cats spending more time inside during colder months (Munir et al., 1993), which may also explain the higher levels of cat allergen in dust sampled during January–March observed in this study.

Indoor mold concentrations showed a very strong seasonality consistently with several other studies (Gravesen, 1972; Koch et al., 2000; Chew et al., 2001; Su et al., 2001).

Endotoxin

Although our data showed higher endotoxin concentrations in dust samples collected during January–March, the seasonal pattern differed between study areas and years of examination. Therefore, we consider the reported statistical significant seasonality of endotoxin concentrations not as strongly convincing. The three studies on repeated measurements of endotoxin in settled dust collected in small numbers of homes showed inconsistent seasonal patterns. Repeated endotoxin measurements in 35 children's homes in Taiwan revealed statistically significant higher concentrations in spring and fall which were twofold the levels observed in winter and summer dust concentrations (Su et al., 2001). A study in 20 homes from the Greater Boston area did not suggest a consistent temporal pattern in endotoxin levels in settled dust (Park et al., 2000). Endotoxin concentrations in the kitchen floor dust was highest in spring and lowest in fall, whereas a seasonal influence on endotoxin levels in bed and bedroom floor dust was not observed. Finally, Rizzo et al. reported low endotoxin levels in winter in 20 homes sampled 13 times during 1 year in Sao Paulo, Brazil. Summarizing the results from studies performed under different climatic conditions, endotoxin levels showed only marginally seasonality, if any.

Although the correlation coefficients found in different repeatability studies are similar, different authors come to

contradictory recommendations with respect to the number of measurements necessary to assess long-term exposure. The different conclusions drawn by different authors from similar results might be related to a lack of knowledge of which correlation coefficients might be considered as high, medium or low to assess “long-term exposure” of a certain duration. Obviously, different authors have different lengths of exposure duration in their mind.

Strengths and Limitations

One strength of this study is the large number of homes, where the dust was repeatedly collected in a standardized manner for measurements of biocontaminants. The large number of visited homes outweighs the small number of only two repeated measurements. This study has collected data from five areas that allowed us to investigate both spatial and temporal differences in exposures. In particular, the few studies that published data on seasonal effects on endotoxin concentration in settled dust is based on repeated measurements in dust samples from only a small number of homes. Therefore, the evidence of low seasonality of endotoxin concentration derived from these studies is limited.

This study has the limitation that only once during the first home visit detailed questions on home characteristics were asked and changes between both visits were not addressed in great detail. Furthermore, dust was collected only twice per home. Short-term variability of dust content within a single home could not be assessed.

Finally, the findings of this study are strongly influenced by the moderate climatic conditions in Germany and could not be extrapolated to other climates.

Conclusion

We conclude that repeated measurements of mite and cat allergens and endotoxin in settled house dust improve the estimate for annual mean concentrations. However, even a single observation of these biocontaminants may be a good proxy for a 1-year exposure since repeated measures within 1 year even in different seasons were highly correlated. However, repeated measurements of fungi levels were only weakly correlated and thus repeated observations for assessment of annual means of total spore counts are needed.

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