

Repeated measurements of allergens and endotoxin in settled house dust over a time period of 6 years

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Summary

Background Studies investigating the effect of exposure to indoor allergens and endotoxin on human respiratory health usually take dust samples only at one single point of time and consider them to be representative for the indoor biocontaminant burden during a time period of several years. This singly measured biocontaminant concentration is related to adverse respiratory outcomes such as asthma, wheeze or others.

Objective We analysed two repeated measurements of mite and cat allergens in mattress and living room floor dust as well as endotoxin concentrations in living room floor dust. The repeated samples were taken over a time period of about 6 years. We investigated the repeatability over time of their concentrations by determining correlation coefficients and computing within- and between-home variance components.

Methods Our analysis was based on the population of a study on Indoor Factors and Genetics in Asthma (INGA) being carried out in 1995/96 and followed up in 2000/01. Complete data were available from 152 participants.

Results The measured allergen concentrations were low and a considerable percentage of the values was below the limit of detection. The crude Spearman's rank correlation coefficients between the two measurements taken in 1995/96 and 2000/01 ranged from 0.32 to 0.61 for the dust mites allergens, from 0.21 to 0.44 for cat allergen and from 0.35 to 0.51 for endotoxin. Correlations were higher if measurements were performed on the same floor or the same mattress at both sampling time-points. The within-home variance for all measured biocontaminant concentrations was of about the same order as the between-home variance.

Conclusion For studies like ours with low allergen and endotoxin concentrations, the repeatability of these concentrations over a time period as long as 6 years is low and a single measurement does not accurately reflect the true long-term exposure in the homes.

Keywords biocontaminants, cat allergen, correlation, endotoxin, exposure, floor dust, mattress dust, mite allergen, repeated measurements, settled house dust

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Introduction

Dust mites are an important source of allergens in the indoor environment. The major mite allergens, Der p 1 and Der f 1, have been well characterized in the past [1–3]. Mite allergens can cause inflammatory allergic responses in sensitized individuals and are thought to be one of the most important allergic causes of childhood asthma [4]. Exposure to cat allergen (Fel d 1) is considered to be responsible for acute asthma attacks and prolonged bronchial hyper-responsiveness [5, 6].

Endotoxin is a lipopolysaccharide that forms the outer layer of the cell membrane of all Gram-negative bacteria. It is ubiquitous in the environment and is present indoors in house dust as well as outdoors in dirt. Endotoxin can have either a

protective or inducing effect on the immune response, depending on the time and dose of exposure [7–13].

When designing studies to assess the effects of exposure to house dust allergens or endotoxin, dust samples are usually taken at one point of time during these studies. However, the reliability of the conclusions drawn from these studies depends very much on the knowledge about the representativeness of the measurements based on this single sample. However, the variability over time of the indoor levels of house dust mites and endotoxin has not been investigated extensively, and most of the studies consider only time periods of less than 1 year [14–19]. To our knowledge, there is only one published study on repeated measurements of allergens over a time period of several years. Peterson et al. [20] reported a high variability of allergen concentrations within households and concluded that a single observation was inadequate to reflect accurately the average indoor allergen burden of a home over time. With respect to endotoxin, there does not exist any study reporting on the repeatability of its concentrations in settled house dust.

In this study, we investigated the variability of Der p 1, Der f 1 and Fel d 1 concentrations, and for the first time also the endotoxin concentrations, over a time period as long as 6 years. We determined the correlation between the two repeated measurements and analysed within- and between-home variance components.

Materials and methods

Study population

The data used in this analysis are based on the study population of the German Centers Erfurt and Hamburg, which participated in the European Community Respiratory Health Survey (ECRHS) [21]. In 1995/96, a nested case–control study (INGA I) was conducted that comprised 437 randomly chosen persons from the ECRHS. Out of these, 405 persons agreed to a home visit. In 2001, 355 of these 405 persons were contacted again and 316 finally participated in this second study, INGA II (26 could not be traced, one died, nine had moved away, one was temporarily away and two refused), meaning that they were available for medical examinations. Out of the 316 participants, 152 agreed additionally to a second home visit.

Our analysis is based on the data of those homes where repeated measurements for Der p 1, Der f 1 and Fel d 1 concentrations in living room floor and mattress dust as well as endotoxin concentrations in living room floor dust were available from both studies ($N = 152$). Furthermore, we conducted a sensitivity analysis using the data of the following subpopulations: for the house dust mite allergens and endotoxin, we separately analysed living room dust samples of those homes in which people were living for at least 5 years. Similarly, we separately analysed mattress dust from only mattresses of at least 5 years of age. For the cat allergen, we separately analysed dust samples that were collected from the living room floor or mattress of those person who either had or had not kept a cat in both INGA I and INGA II. This resulted in 98 and 81 dust samples, respectively, available for the sensitivity analysis of living

room dust and mattress dust when considering Der p 1, Der f 1 and endotoxin and 121 samples for the sensitivity analysis for Fel d 1 from both mattress and living room floor dust.

Dust sampling and extraction

The visits were conducted by trained personnel from June 1995 to October 1996 for INGA I, and from January 2001 to December 2001 for INGA II. The dust samples were taken uniformly over the months, so we can exclude a seasonal bias in the collected samples (data not shown). Dust sampling and extraction procedures followed general recommendations [22] and have been described in detail elsewhere [23]. Briefly, in each apartment a dust sample was taken from the living room floor and the bedroom mattress according to a standardized protocol [23]. All dust samples were taken using the same type of vacuum cleaner (Philips, Hamburg, Germany, Flüsterjet Vitall 371) and vacuuming an area of 1 m² for 2 min. 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. The allergens were extracted from the sample dust plus filter paper with 0.125 M NH_4HCO_3 plus 0.05% Tween 20 (v/v) for 2 h at room temperature under constant shaking, with an extraction ratio of 1 : 10–1 : 100 (w/v), depending on the amount of dust. Extracts were stored in pyrogen-free glassware at -20°C until analysis. Repeated home visits to collect house dust were conducted mostly by the same fieldworker, and the same technicians extracted and analysed the dust samples for all visits in the two study regions.

Allergen and endotoxin content in house dust

Sample allergen contents of house dust mite allergens (Der p 1 and Der f 1) and cat allergen (Fel d 1) were measured by means of monoclonal antibodies and reference samples from Indoor Biotechnologies (Clwyd, UK) [23]. The inter-assay variation for repeated assays was 10–16% and the intra-assay variation was approximately 3.5–8%. The lower limit of detection was 1 ng/mL for both Der p 1 and Der f 1, and 1.5 ng/mL for Fel d 1. All concentrations were expressed as both nanogram per gram of dust and per square metre sampled area.

Endotoxin was assayed with a quantitative kinetic chromogenic *Limulus Amebocyte Lysate* (LAL) method (INGA I [INGA II] Kinetic-QCL no. 5L3480 [OL4940]; Bio Whittaker, Walkersville, UK; LAL lot no. 5L3000 [OL449G]) at 37°C . Analyses were performed with an automated microtiter plate reader (anthos ht III, Anthos Labtec Instruments, Salzburg, Austria) and WinKQCL 1.1 software. *Escherichia coli* endotoxin (Bio Whittaker, lot no. 5L1570 [OL3200]) was used as standard endotoxin having a potency of 14.5 EU/ng [12]. Diluted samples were analysed in the inhibition/enhancement mode. Analyses were repeated if the difference between sample duplicates exceeded 10%. Endotoxin was assayed only for living room floor dust and concentrations were expressed as endotoxin units per milligram dust (EU/mg) and per square metre (EU/m²).

Statistical methods

As the distribution of all measured concentrations was markedly skewed to the right, we used log-transformed values in all analyses. Allergen concentrations less than the limit of detection were assigned a value of one-half the detection limit.

As a measure of stability over time, we used both Spearman's rank correlation coefficient and scatterplots of the log-transformed biocontaminant concentrations of the first (INGA I) and second (INGA II) visit.

We used mixed-effect models similar to those described in Park et al. [17] to examine to what extent the correlations of all assayed biocontaminants were influenced by seasonal and regional differences. We modelled the log-transformed concentrations as the sum of fixed region effects (Erfurt and Hamburg), fixed seasonal effects (January–March, April–June, July–September, October–December), 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 the square root of the variance). We used the ratio of the between-home variance to the sum of both variance components to express the correlation between the measurements of the first and second visit, adjusted for seasonal and

regional differences. All analyses were carried out using SAS V8 (SAS Institute, Cary, NC, USA).

Results

Table 1 shows the distribution of house dust allergen and endotoxin concentrations in living room floor dust (in both visits about 95% of the floors in the living rooms were carpets) and mattress dust. For living room floor dust, more than 60% of the Der p 1 values in the first visit and more than 80% of the values in the second visit were below the limit of detection. For Der f 1, this was true for about 30% of the values in the first visit and almost 60% of the values in the second visit. The percentage of Fel d 1 concentrations below the limit of detection was 9% in the first and 11% in the second visit. Endotoxin concentrations were detectable in all collected dust samples. The distribution of the endotoxin concentrations was slightly higher in the first visit, whereas the values of the Fel d 1 concentrations were generally higher in the second visit.

With respect to mattress dust, the percentage of undetectable values was 25% and around 60% for the Der p 1 concentrations and around 20% and 30% for Der f 1

Table 1. Distribution of mite and cat allergen and endotoxin concentrations in living room floor and mattress dust sampled in 1995/1996 and 2001

	N	% < LOD	Minimum	25th percentile	Median	75th Percentile	90th Percentile	Maximum
<i>Living room floor dust</i>								
<i>Der p 1 (ng/g)</i>								
1995/96	152	63.8	< 10	< 10	< 10	101	598	36 480
2001	152	82.2	< 10	< 10	< 10	< 10	987	24 055
2001 (same home)	98	80.6	< 10	< 10	< 10	< 10	995	17 693
<i>Der f 1 (ng/g)</i>								
1995/96	152	33.6	< 10	< 10	120	506	2004	50 496
2001	152	59.2	< 10	< 10	< 10	731	3957	107 306
2001 (same home)	98	56.1	< 10	< 10	< 10	1251	4515	57 482
<i>Fel d 1 (ng/g)</i>								
1995/96	152	5.3	< 15	103.5	246	1768	159 898	5 273 900
2001	152	10.5	< 15	305	727	5905	196 761	16 788 007
2001 (same cat-keeping status)	102	11.6	< 15	250	615	2231	16 585	16 788 007
<i>Endotoxin (EU/mg)</i>								
1995/96	152	0	1.8	14.7	30.4	65.2	121.8	1476.3
2001	152	0	1.7	7.8	19.7	50.2	146.1	2510.7
2001 (same home)	98	0	1.7	8.0	19.8	49.7	145.9	2510.7
<i>Mattress dust</i>								
<i>Der p 1 (ng/g)</i>								
1995/96	152	25.7	< 10	< 10	164	1294	19 353	166 717
2001	152	59.9	< 10	< 10	< 10	1107	10 248	131 856
2001 (old mattress)	81	54.3	< 10	< 10	< 10	2339	10 248	131 856
<i>Der f 1 (ng/g)</i>								
1995/96	152	23.03	< 10	27.5	677	10 161	51 207	653 233
2001	152	32.2	< 10	< 10	1095	8278	37 491	267 752
2001 (old mattress)	81	28.4	< 10	< 10	1354	11 840	48 918	267 752
<i>Fel d 1 (ng/g)</i>								
1995/96	152	9.2	< 15	58	146	1933	53 889	3 232 823
2001	152	17.8	< 15	155	397	3893	137 485	3 650 904
2001 (same cat-keeping status)	121	21.5	< 15	103	301	1716	44 507	2 631 227

LOD (limit of detection): Der p 1, 10 ng/g; Der f 1, 10 ng/g; Fel d 1, 15 ng/g.

Table 2. Spearman's correlation coefficients between the log-transformed allergen concentrations in living room dust and mattress dust in 1995/96 and 2001 ($N = 137$)

Living room dust	Mattress dust					
	1995/96			2001		
	Der p 1	Der f 1	Fel d 1	Der p 1	Der f 1	Fel d 1
Der p 1 (g)	0.54			0.51		
Der p 1 (m ²)	0.49			0.39		
Der f 1 (g)		0.62			0.54	
Der f 1 (m ²)		0.59			0.50	
Fel d 1 (g)			0.82			0.65
Fel d 1 (m ²)			0.76			0.66

concentrations in the first and second visits, respectively. For the Fel d 1 concentrations, 9% and 18% of the values were below the limit of detection in the first and second home visits, respectively. Fel d 1 and Der f 1 concentrations were higher in the second visit. The higher Fel d 1 concentrations in both mattress and living room dust could not be explained by a higher number of cat-keeping households as there were 20 of them in both INGA I and INGA II.

Table 2 shows the correlation coefficients between the log-transformed allergen concentrations in living room dust and mattress dust in both visits. It can be seen that the Fel d 1 concentrations correlate best. When considering only those persons who did not keep a cat in both visits, the correlations between the Fel d 1 concentrations in living room and mattress dust were somewhat lower: in 1995/96, it was 0.70 [0.61] and in 2000/01, it was 0.34 [0.40] when expressed per gram [per m²].

Correlations between the first and second measurement

For living room floor dust, and considering only those persons living at least 5 years in their current home (at the beginning of INGA II), the crude Spearman's rank correlation coefficient for the log-transformed Der p 1, Der f 1 and endotoxin concentrations of the two visits were 0.46, 0.53 and 0.43, respectively, when expressed per gram sampled dust (Table 3). Similar results were obtained when the concentrations were expressed per square metre sampled floor, only the correlation coefficient for endotoxin increased slightly to 0.51 (Table 3). In the sensitivity analysis for Fel d 1, that is when analysing the data of only those persons with unchanged cat-keeping status, Spearman's correlation coefficient was 0.21 [0.36] when the concentration was expressed per gram [per m²]. The results from the analysis of all available dust samples resulted in similar correlations for all assayed biocontaminants; only for endotoxin and Der p 1, there were better correlations in the sensitivity analysis (Table 3).

For mattress dust, and considering only those persons having a mattress aged at least 5 years (at the beginning of INGA II), the crude Spearman's rank correlation coefficients for the Der p 1 and Der f 1 concentrations expressed per gram in the two visits were 0.46 and 0.61, respectively (Table 3). Also including those persons with a newer mattress yielded

Table 3. Crude correlation coefficients between log-transformed data of first and second visits expressed per gram dust and per m²

	Living room floor dust		Mattress dust	
	Same living conditions*	All persons ($N = 152$)	Same living conditions*	All persons ($N = 152$)
Per gram dust				
Der p 1	0.46	0.32	0.46	0.45
Der f 1	0.53	0.52	0.61	0.58
Fel d 1	0.21	0.25	0.38	0.44
Endotoxin	0.43	0.35	n.a.	n.a.
Per m ²				
Der p 1	0.46	0.34	0.42	0.44
Der f 1	0.56	0.55	0.58	0.53
Fel d 1	0.36	0.34	0.37	0.41
Endotoxin	0.51	0.44	n.a.	n.a.

n.a.: no endotoxin assayed.

*This means for Der p 1, Der f 1 and endotoxin: living in the current home for at least 5 years ($N = 98$)/having the same mattress for at least 5 years ($N = 81$) and for Fel d 1: keeping/not keeping a cat in both INGA I and INGA II ($N = 121$).

similar results for Der p 1 and Der f 1. The correlation coefficients for the Fel d 1 concentrations in both visits were 0.38 [0.37] in the sensitivity analysis and 0.44 [0.41] in the general analysis when expressed per gram dust [per m² sampled area].

Considering only those households that did not keep a cat in both home visits ($N = 111$) and recalculating Spearman's correlation coefficient revealed that the correlations between the Fel d 1 concentrations of the two visits were mostly driven by the cat keepers: the correlation coefficient in cat-free homes was -0.002 [0.19] for living room dust and 0.22 [0.20] for mattress dust when expressed per gram [per m²].

Figures 1 and 2 show scatterplots of the allergen and endotoxin concentrations for all data; similar results were achieved when considering only the data for the sensitivity analysis (graphs not shown). It can be seen that there was a good correlation between the values that were above the limit of detection in both visits. The low overall correlation is due to those data pairs constituting one value below the limit of detection in one visit and one value above the limit of detection in the other visit. A closer look at these data pairs reveals that the values of some of them were measured in different seasons. But there is about the same number of these data pairs constituting values measured in the same season so that the low correlation cannot be explained entirely by seasonality.

Table 4 shows the within-home correlations and GSD for living room floor dust adjusted for season and region. The within-home correlations for the allergen concentrations in the sensitivity analysis were all around 0.5, whereas they were lower in the general analysis. For the endotoxin concentrations, it could be seen again that the expression per square metre sampled floor yielded higher values. For the Fel d 1 concentrations, the adjustment had the strongest effect and raised the correlation coefficient to values between 0.40 and 0.52.

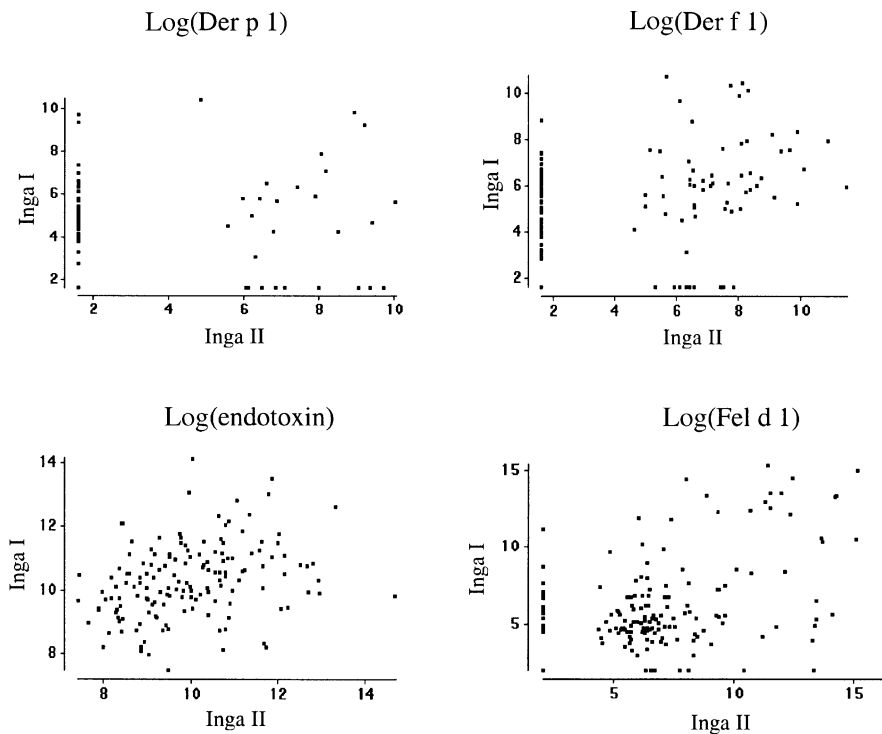


Fig. 1. Scatterplots of the log-transformed allergen and endotoxin concentrations of 1995/96 and 2001 in living room floor dust in ng/g (all samples).

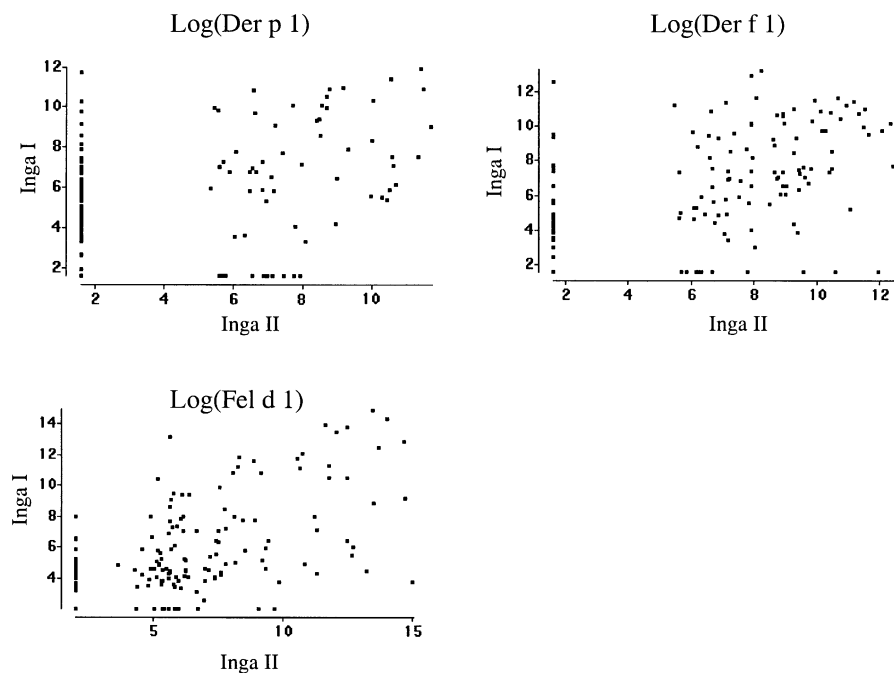


Fig. 2. Scatterplots of the log-transformed allergen and endotoxin concentrations of 1995/96 and 2001 in mattress dust in ng/g (all samples).

The between- and within-home variabilities were similar in the sensitivity analysis, but when all dust samples were analysed then the variability within homes was higher than that between homes (Table 4).

For mattress dust, correlations and within- and between-home variabilities of the Der p 1 concentrations were similar in both the sensitivity and general analyses (Table 5), and it

could be seen that the within-home GSD was markedly higher than the between-home GSD. In contrast, for the Der f 1 concentrations the between-home variability was markedly higher than the within-home variability (Table 5). The adjustment for season and region again had the strongest effect on the correlation of the Fel d 1 concentrations. Here, both GSD were of about the same order (Table 5).

Table 4. Within-home correlations and geometric standard deviations (GSD) for living room floor dust analysing only those who lived at least 5 years in the current home (Der p 1, Der f 1, endotoxin, $N=98$) or had the same cat-keeping status in both visits (Fel d 1, $N=121$) (analysing all, $N=152$)

	Within-home correlation	Between-home GSD	Within-home GSD	Ratio 'between vs. within'
Per gram dust				
Der p 1	0.50 [0.31]	4.65 [3.50]	4.69 [6.47]	0.99 [0.54]
Der f 1	0.49 [0.47]	7.15 [6.32]	6.96 [7.24]	1.03 [0.91]
Fel d 1	0.49 [0.40]	7.44 [7.30]	7.18 [11.63]	1.04 [0.63]
Endotoxin	0.33 [0.29]	2.94 [1.98]	2.13 [2.95]	1.38 [0.67]
Per m ²				
Der p 1	0.52 [0.37]	5.80 [4.69]	6.29 [7.57]	0.92 [0.62]
Der f 1	0.51 [0.50]	8.85 [8.45]	9.34 [8.51]	0.95 [0.99]
Fel d 1	0.52 [0.42]	8.01 [8.46]	8.54 [12.56]	0.94 [0.67]
Endotoxin	0.49 [0.44]	3.66 [3.14]	3.59 [3.62]	1.02 [0.87]

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

Table 5. Within-home correlations and geometric standard deviations (GSD) for mattress dust analysing only those who had a mattress aged at least 5 years (Der p 1, Der f 1, endotoxin, $N=81$) or had the same cat-keeping status in both visits (Fel d 1, $N=121$) (analysing all, $N=152$)

	Within-home correlation	Between-home GSD	Within-home GSD	Ratio 'between vs. within'
Per gram dust				
Der p 1	0.41 [0.38]	7.78 [6.90]	11.86 [11.58]	0.66 [0.60]
Der f 1	0.60 [0.55]	13.57 [11.36]	8.47 [8.94]	1.60 [1.27]
Fel d 1	0.54 [0.50]	10.64 [9.31]	7.84 [9.23]	1.36 [1.01]
Per m ²				
Der p 1	0.43 [0.41]	9.49 [8.67]	13.14 [13.05]	0.72 [0.66]
Der f 1	0.59 [0.53]	14.60 [12.66]	9.58 [10.84]	1.52 [1.17]
Fel d 1	0.50 [0.46]	9.02 [9.51]	8.99 [11.25]	1.00 [0.85]

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

Discussion

Knowledge about the repeatability over time of house dust allergen and endotoxin concentrations is important to design efficiently studies that want to investigate the role of these biocontaminants in the induction and maintenance of allergic diseases. Implications following from the results of these studies are valid only when taking into account the expected variability of the concentrations over time. In our study, we examined the representativeness of dust allergen and endotoxin concentrations using two repeated measurements. It is the first study reporting on the reproducibility of endotoxin measurements over a time period of several years.

Although there was a quite high drop-out rate of subjects from INGA I to INGA II, a comparison of the biocontaminant concentrations in the homes of those participants of INGA I who did and did not participate later in INGA II shows that the geometric mean concentrations were similar in both subpopulations (data not shown). Therefore, we do not expect selection bias for the study population of INGA II.

Mite and cat allergens

Two repeated dust samples were analysed with approximately 6 years between the collection of each sample. The two measurements were only moderately correlated for all house dust allergen concentrations. The results from the analysis

using mixed-effect models yielded higher adjusted correlations, especially for the Fel d 1 concentrations. The correlations for Der p 1 were generally lower than those for Der f 1, which may be explained by the higher sensitivity of *Dermatophagoides pteronyssinus* to climatic conditions [24]. The results indicate that in our setting, the repeatability of the measured allergen concentrations over 6 years is rather low and that one single measurement would not adequately describe the allergen exposure in a home over a time period of several years. This is illustrated by the scatterplots of the allergen concentrations, which show very clearly that the source of the low correlation between the two measurements were the many values that are below the limit of detection in one visit, but above the limit of detection in the other visit.

Our reported correlations were markedly lower than those reported in other German studies considering a time period of only 6–13 months between the sample collections [25, 19, 14]. This makes clear that the repeatability of allergen concentrations over 1 year is by no means comparable with the repeatability of these concentrations over a 6-year period as considered in our study. Figure 3 shows our results and those of Heinrich et al. [14], indicating that the ratios of the between- to within-home variances for the repeated measurements after 6 years lie markedly below those for after 6 months. Peterson et al. [20] analysed repeated measurements over a time period of 4 years, and although they reported quite high correlations for Der p 1, Der f 1 and Fel d 1

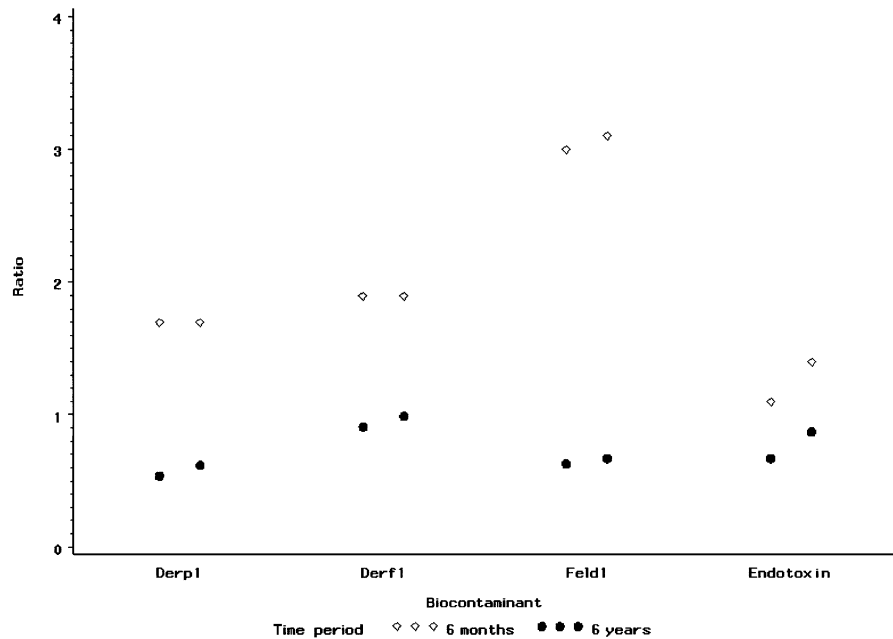


Fig. 3. Ratios of between- to within-home variance for repeated measures over 6 months and 6 years, respectively. Left dots: per gram dust, right dots: per square metre sampled surface. Six-month ratios were obtained from a larger German population comprising our participants as a subset, and are published in Heinrich et al. [14].

concentrations ranging from 0.65 up to 0.88, they also found a wide variability of the concentrations both within and between homes. One reason for the higher correlations in their study could be that the overall level of the allergen concentrations was markedly higher than in our study where the percentage of allergen concentrations below the limit of detection was considerable. In this context, it should be pointed out that at least our results for Der p 1 must be handled with care because of the low percentage of detectable concentrations.

There is still an ongoing discussion about the ideal spot in the house for dust sampling. Usually, dust samples are taken either from the kitchen floor, living room floor, sofa, bedroom floor or bedroom mattress. Some studies show that dust samples taken from bedroom mattresses are more stable in their allergen concentration over time than those coming from the kitchen or living room floor [18]. Also, samples taken from mattress dust seem to be of more relevance because of the person's closeness to the dust while sleeping and the relatively long time of exposure during the night. In our study, the results of the repeated measurement analysis also showed higher within-home correlations in mattress dust than in living room floor dust and furthermore revealed for mattress dust a more favourable ratio of the between- and within-home variability.

Endotoxin

The crude Spearman's rank correlation coefficient for the two endotoxin measurements from living room dust did not indicate a very high correlation of the two endotoxin concentrations. These results were confirmed by the mixed-model analysis. Studies analysing repeated endotoxin measurements over time periods of only a few months reported statistically non-significant differences in endotoxin concentrations [9, 14]. This again underlines the fact that the repeatability of biocontaminant concentrations over a short period of time is quite different from its repeatability over

several years, although the results of our study support the hypothesis that endotoxin concentrations vary less over time when they are expressed in EU/m² sampled surface instead of EU/g sampled dust.

In this context, it has to be stated that our results are only valid for regions with similarly low allergen and endotoxin levels such as those in our study.

Conclusions

Our results indicate that the reproducibility of house dust allergen and endotoxin concentrations over a time period as long as 6 years is not satisfactory. These findings contrast with those from studies analysing repeated measurements over shorter time periods up to 1 year and reporting a good reproducibility of allergen and endotoxin concentrations. Our study shows that over a longer period of time, a single measurement of the concentration of these biocontaminants cannot be assumed to be representative for the average burden in a home. Therefore, studies associating home characteristics or health effects with biocontaminant measurements taken at only one single point of time may find incorrect results because of biased estimates of the allergen exposure. As a consequence, multiple samples and a longer sampling period are required in order to improve the reliability of the estimated biocontaminant concentrations. This is especially recommendable if the population under consideration comprises persons who moved houses or used new mattresses during the study period.

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