

Short communication

The effect of storage on allergen and microbial agent levels in frozen house dust

Background: House dust samples collected for exposure studies are often stored for variable time periods until analysis. However, there is currently no information on the effects of dust storage on the content of biocontaminants. Therefore, associations were analysed between the levels of mite allergens (Der p 1, Der f 1), cat allergen (Fel d 1) and microbial components (endotoxin, $\beta(1 \rightarrow 3)$ -glucan) on the one hand and the storage duration of dust samples at -20°C on the other hand.

Methods: Within the framework of a study on the influences of INdoor factors and Genetics on Asthma (INGA), dust samples were collected from living room floors between June 1995 and August 1998 and extracted according to a standardized protocol. The concentrations of Der p 1, Der f 1, Fel d 1 and $\beta(1 \rightarrow 3)$ -glucan were determined with specific enzyme immunoassays. Endotoxin content was quantified using a chromogenic kinetic Limulus amoebocyte lysate (LAL) test. All concentrations were expressed per gram of dust

Results: Dust samples ($n = 1236$) were obtained from 655 homes in Hamburg, Hettstedt, Zerbst and Bitterfeld. Storage duration (range 8–298 days) was grouped into four categories (≤ 60 d; 61–90 d; 91–120 d; > 120 d). After adjustment for city of residence and season of dust sampling, means ratios comparing categories 2–4 to the first category were not statistically significant for Der p 1, Der f 1, endotoxin and $\beta(1 \rightarrow 3)$ glucan). However, Fel d 1 concentrations significantly declined with increased storage times of dust samples.

Conclusions: Storage of house dust at -20°C for up to 10 months has no effect on mite allergen, endotoxin and $\beta(1 \rightarrow 3)$ -glucan levels. A potential loss of Fel d 1 during storage of frozen dust samples needs further investigations by repeated measurements of allergen in identical dust samples.

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Introduction

House dust has been recognized as a trigger factor for allergic diseases, such as asthma and allergic rhinitis for a long time. In addition to allergens (1, 2), microbial components such as bacterial endotoxins and mould glucans present in house dust are believed to be of importance (3). To establish the link between allergens or microbial contaminants and allergic diseases, many research laboratories measured allergens and microbial agents in settled dust samples. House dust can easily be collected at different locations of the homes (4). Standardized, reliable, and reproducible methods for measuring exposure are essential for the assessment of the relationship between exposure, sensitization, and asthma. Although there are several potential problems in assessing exposure using reservoir measurements (depending on sampling areas, collection devices and flow rates, as well

as gross composition of dust), standardized sampling techniques (5, 6) and extraction procedures have been developed in recent years and have provided data on allergen and microbial agent levels in house dust from different parts of the world. Nevertheless, some aspects of these procedures have not been well standardized. For example, in many large-scale epidemiological studies the collected dust samples are stored frozen (at -20°C) for variable periods of time before analysis, although the effects of storage on allergen and microbial agent levels in house dust are not known. Therefore a secondary analysis of an already existing data set was performed to assess the effect of storage duration (before extraction) on levels of mite allergen (Der p 1, Der f 1), cat allergen (Fel d 1) and microbial components (endotoxin, $\beta(1 \rightarrow 3)$ -glucan) in a large number of house dust samples that were collected as part of a study on influences of INdoor factors and Genetics on Asthma (INGA) (7).

Material and methods

House dust samples

We evaluated data collected within the framework of the INGA study. Study design and methods have been described in more detail elsewhere (7). Homes were randomly selected in two cities (Hamburg, West Germany, $n = 201$; and Erfurt, East Germany, $n = 204$) and in three areas of Sachsen-Anhalt (Zerbst, Bitterfeld and Hettstedt; $n = 454$). Out of these 859 homes, 777 (91%) were visited for a second time approximately 6 months after the first home visit.

Between June 1995 and August 1998, dust samples were taken from living room floors, bedroom floors and mattresses according to a standardized protocol (5), using the same type of vacuum cleaner (Philips, Typ Flüsterjet Vitall 371, 1000 watts, Hamburg, Germany) and the same sampling device (sampler and filter, ALK, Denmark). For the current study, we used only the living room floor samples, because only in these samples were endotoxin and $\beta(1 \rightarrow 3)$ -glucan measured in parallel with allergens. After vacuuming, the filter dishes were closed and stored in separate plastic bags at -20°C until extraction and analysis were performed. Since dust samples from Erfurt were stored for a much shorter period than the samples in the other centres (4–148 days, median 33 days), we excluded the samples from Erfurt from this analysis.

Dust samples (including paper filter) were extracted without prior sieving under pyrogen-free conditions in 0.125 M ammonium hydrogen carbonate buffer + 0.05% Tween (v/v) for 2 h at room temperature with continuous shaking. The dilutions ranged from 1 : 10 to 1 : 100 w/v, depending on the amount of sampled dust. Both the aliquoted supernatants and the residues were stored in pyrogen-free glassware at -20°C until analysis. The pellets were redissolved in distilled water + 0.05% Tween and extracted at 120°C to solubilize $\beta(1 \rightarrow 3)$ -glucans (8).

Allergen levels of house dust mites *Dermatophagoides pteronyssinus* (Der p 1) and *D. farinae* (Der f 1) and cat (Fel d 1) were measured by means of enzyme-linked immunosorbent assays (Indoor Biotechnologies, Clwyd, UK) (9). The lower limit of detection was 1 ng/ml for Der p 1 and Der f 1, and 1.5 ng/ml for Fel d 1.

Endotoxin content in living room floor dust was quantified using a chromogenic kinetic Limulus amoebocyte lysate (LAL) test (10). $\beta(1 \rightarrow 3)$ -glucan was assayed with a specific inhibition enzyme immunoassay (8) only in samples collected during the first home visit.

Data analysis

Because of the log-normal distribution of the allergen (ng/g dust), endotoxin (EU/g dust) and $\beta(1 \rightarrow 3)$ -glucan (ng/g dust) levels, mean concentrations were presented as geometric means (GM) with 95% confidence intervals (CI). Samples with concentrations below the limit of detection were assigned a value of one-half of the detection limit for the specific component determined. The time between the dust sampling and the dust analysis was grouped into four categories based on quartiles (see Table 1). Repeated-measures analysis of variance was performed to describe the relationship between allergen and endotoxin levels and the dust storage duration. A compound symmetry covariance-structure was chosen, assuming a constant correlation between repeated measurements within one home and independency between homes. In the case of $\beta(1 \rightarrow 3)$ -glucans, available only for the first home visit, analysis of variance for independent observations was performed. All associations are presented as means ratios (MR), that is the ratio of the geometric mean in the category of study vs. the reference category (storage of less than 60 days). We adjusted for study region and season of dust sampling, i.e. winter (January–March), spring (April–June),

Table 1. Number of stored dust samples based storage time for the four areas

	Number of dust samples from				
	Hamburg	Hettstedt	Zerbst	Bitterfeld	Total
Storage time					
≤ 60 days	43	90	59	28	220
61–90 days	129	97	62	58	346
91–120 days	101	43	77	93	314
> 120 days	98	122	90	46	356
Total	371	352	288	225	1236

summer (July – September), and autumn (October – December). Statistical significance was assumed at a conventional 5% level. All analyses were performed using the Statistical Analysis System sas for Windows version 8.2.

Results

Results were available from 655 homes visited in Hamburg (first sampling $n = 201$; second sampling $n = 170$), Hettstedt (first sampling $n = 183$; second sampling $n = 169$), Zerbst (first sampling $n = 152$; second sampling $n = 136$) and Bitterfeld (first sampling $n = 119$; second sampling $n = 106$). All 1236 dust samples were assayed for Der p 1, Der f 1, Fel d 1, and endotoxin concentrations; 648 dust samples of the first home visit were analysed for $\beta(1 \rightarrow 3)$ -glucan levels.

The time periods between dust sampling and dust analysis ranged from 8 days (Bitterfeld) to 298 days

Table 2. Geometric means and 95% confidence interval (CI) of Der p 1, Der f 1, Fel d 1, endotoxin and $\beta(1 \rightarrow 3)$ -glucan concentrations in living room floor dust in relationship to the storage time

Biocontaminants	Storage time	n	GM	95% CI	
				Lower	Upper
Der p 1 (ng/g)	≤ 60 days	220	36	25	52
	61–90 days	346	31	24	41
	91–120 days	314	41	30	56
	> 120 days	356	32	24	43
Der f 1 (ng/g)	≤ 60 days	220	159	105	241
	61–90 days	346	166	123	223
	91–120 days	314	206	150	282
	> 120 days	356	165	121	224
Fel d 1 (ng/g)	≤ 60 days	220	520	352	769
	61–90 days	346	764	540	1082
	91–120 days	314	515	372	712
	> 120 days	356	327	238	450
Endotoxin (EU/g)	≤ 60 days	220	26 386	22 867	30 445
	61–90 days	346	31 253	27 690	35 273
	91–120 days	314	27 847	24 426	31 747
	> 120 days	356	29 664	26 632	33 041
$\beta(1 \rightarrow 3)$ -glucan (ng/g)	≤ 60 days	63	2297	1973	2673
	61–90 days	156	2129	1927	2351
	91–120 days	184	2054	1870	2257
	> 120 days	245	2384	2146	2648

(Hettstedt) and were classified into four categories (Table 1). Crude descriptive analyses did not indicate any association between storage duration and levels of Der p 1, Der f 1, Fel d 1, endotoxin and $\beta(1 \rightarrow 3)$ -glucan (Table 2). Because dust sampling was performed in four different regions (Hamburg, Bitterfeld, Hettstedt, and Zerbst), we carefully excluded possible selection effects by enrichment of the data with samples from a specific region (Table 1).

The results of repeated measures analysis of variance between concentrations of biocontaminants in house dust and storage time are presented in Fig. 1. After adjustment for place of residence and season, no statistically significant association was observed between storage duration and the levels of Der p 1, Der f 1, endotoxin and $\beta(1 \rightarrow 3)$ -glucan. In contrast, there was a significant association between Fel d 1 concentrations and the storage duration of dust samples, i.e. dust samples stored for more than 90 days showed a decline in Fel d 1 levels of up to 40%.

Discussion

House dust samples collected for exposure assessment of indoor allergens and microbial agents in epidemiological studies are commonly stored at -20°C for variable time periods before analysis. Since little is known about the effects (e.g. condensation) of storage before extraction on the content of biocontaminants, associations were analysed between the levels of allergens or microbial components in house dust and the storage duration.

The observations of the present study indicate that the levels of Der p 1, Der f 1, endotoxin and $\beta(1 \rightarrow 3)$ -glucan in dust samples are stable at -20°C for up to 10 months. Concerning Der p 1, the results of the present study are in agreement with reports from Kort et al. (11), who did not observe any reduction of Der p 1 levels in house dust under simulated domestic conditions *in vitro* (storage at room temperature for 47 months) by means of RAST inhibition.

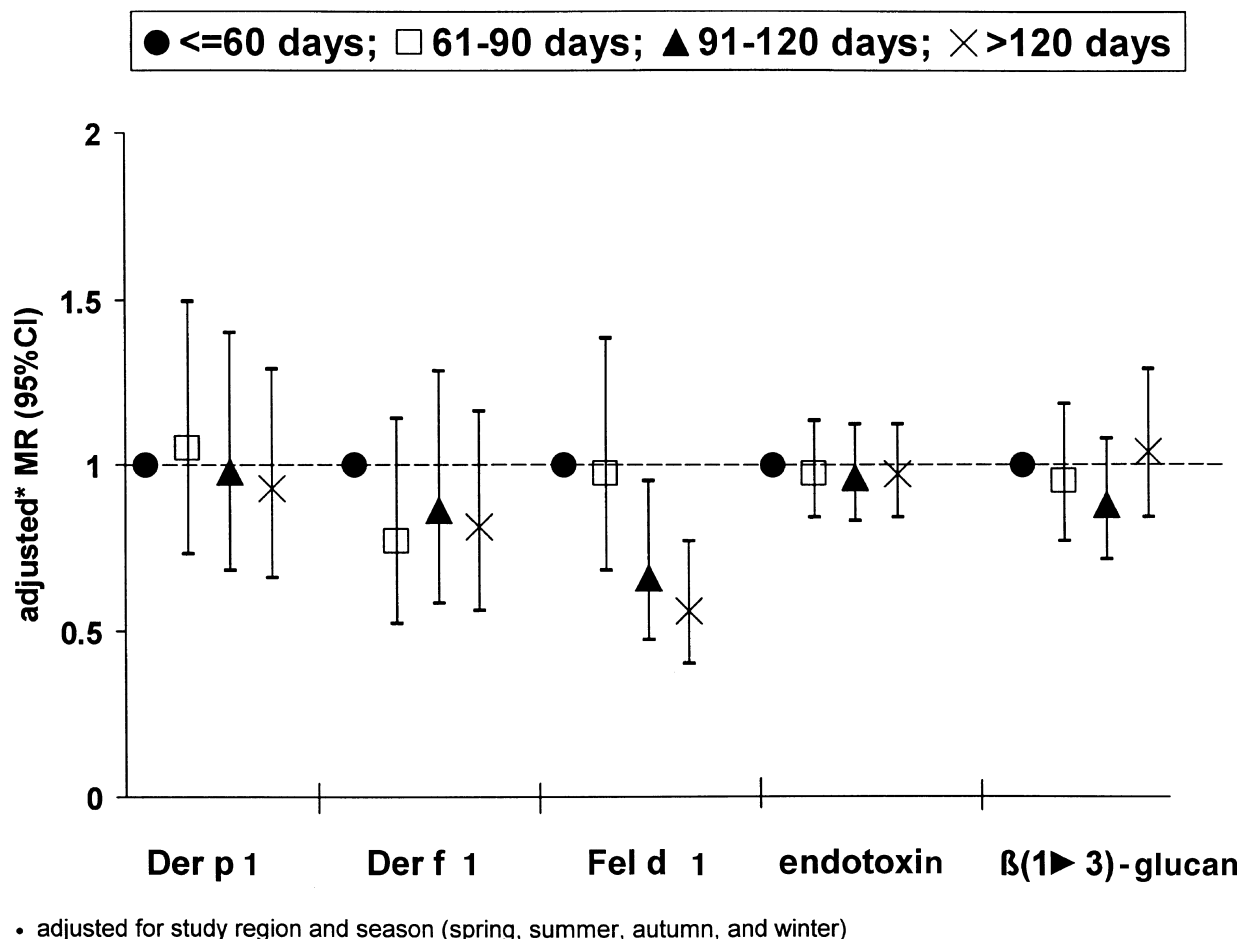


Figure 1. Results of (repeated measures) analysis of variance assessing the relationship between the concentrations of biocontaminants in house dust and the storage time. Data are represented as means ratios (MR) adjusted for study region and season and 95% confidence intervals for Der p 1 (ng/g), Der f 1 (ng/g), Fel d 1 (ng/g), endotoxin (EU/g) and $\beta(1 \rightarrow 3)$ -glucan (ng/g). □ : 61–90 days; ▲ : 91–120 days; × : > 120 days. Reference category: ● ≤ 60 days.

Conflicting results from previous studies using allergen extracts (12–14) show only limited suitability for comparisons with the results of the present study because (i) allergen extracts consist of a limited number of components, whereas dust samples contain a broad spectrum of potent allergens (from pollen, moulds, pets), microbial agents and proteolytic enzymes or other undefined substances; (ii) allergen extracts are stored in liquid form in a different buffer solution, whereas the dust samples probably contain variable degrees of humidity depending on the conditions in the sampling location (with a humidity range from 10–70%).

The decline of the Fel d 1 concentrations with increased storage time remains unexplained and controversial. In fact, Batard et al. (13) observed a time-related increase of Fel d 1 concentration in a concentrated cat hair extract stored at 4°C. They proposed that this finding may reflect a conversion of Fel d 1 with subsequent revelation of cryptic epitopes on the Fel d 1 molecule. Hypothetical explanations for the decline of Fel d 1 levels in the present study may also include protein unfolding, as well as protein degradation by proteolytic enzymes. Technical artifacts can be largely excluded, since the processing of all dust samples was performed by the same

laboratory technicians, strictly adhering to standardized extraction protocols (5, 6).

However, the present explorative study has some limitations. The first is the comparison of biocontaminant levels in samples differing in both sampling location and storage duration. Another limitation is the high variability of biocontaminants among homes, which is obviously much more pronounced than the potential influence of storage time. Furthermore, the results of this study do not justify any extrapolation to longer storage periods (> 1 year). Despite these limitations, this study used data of biocontaminants of a study with a rather large sample size of 655 homes. In order to exclude storage effects for sure, repeated analyses of biocontaminants are needed in identical dust samples which are investigated both directly after dust sampling and several months later.

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