

$\beta(1\rightarrow3)$ -Glucan in House Dust of German Homes: Housing Characteristics, Occupant Behavior, and Relations with Endotoxins, Allergens, and Molds

Ulrike Gehring,^{1,2} Jeroen Douwes,³ Gert Doekes,³ Andrea Koch,⁴ Wolfgang Bischof,⁴ Bärbel Fahlbusch,⁵ Kai Richter,⁶ H.-Erich Wichmann,^{1,2} and Joachim Heinrich¹ for the INGA Study Group

¹GSF - National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg, Germany; ²Ludwig-Maximilians-University of Munich, Institute of Medical Data Management, Biometrics and Epidemiology, Department of Epidemiology, Munich, Germany; ³Utrecht University, Environmental and Occupational Health Group, Utrecht, The Netherlands; ⁴Friedrich-Schiller University of Jena, Institute of Occupational, Social and Environmental Medicine, Erfurt, Germany; ⁵Friedrich-Schiller University of Jena, Institute of Clinical Immunology, Jena, Germany; ⁶Grosshansdorf Hospital, Hamburg, Germany

$\beta(1\rightarrow3)$ -Glucans are potent proinflammatory agents that have been suggested to play a role in indoor-related respiratory health effects. The aim of this study was to assess whether $\beta(1\rightarrow3)$ -glucan concentrations in house dust are correlated with levels of endotoxins, allergens, and culturable mold spore counts in house dust. Further, the associations of $\beta(1\rightarrow3)$ -glucan with housing characteristics and occupant behavior were assessed. $\beta(1\rightarrow3)$ -Glucan was measured in settled house dust from living room floors of 395 homes of two German cities, Erfurt and Hamburg, with a specific enzyme immunoassay. Concentrations ranged from below the limit of detection to 19,013 $\mu\text{g}/\text{m}^2$ (22,588 $\mu\text{g}/\text{g}$ dust). Concentrations per square meter were found to be correlated with endotoxins, mite and cat allergens, and culturable mold spores. Correlations were weaker when concentrations were expressed per gram of dust, indicating that variance in concentrations of all factors is largely determined by the amount of dust sampled. Associations between $\beta(1\rightarrow3)$ -glucan, housing characteristics, and occupant behavior were found for concentrations per square meter but not for concentrations per gram of dust. The following characteristics were associated with a significant increase in $\beta(1\rightarrow3)$ -glucan levels: carpets in the living room [means ratio (MR) = 1.9–2.1], keeping a dog inside (MR = 1.4), use of the home by four or more persons (MR = 1.4), use of the living room for > 180 hr/week (MR = 2.1), lower frequency of vacuum cleaning (MR = 1.6–3.0) and dust cleaning (MR = 1.2 and 1.4, respectively), and presence of mold spots during the past 12 months (MR = 1.4). We conclude that the amount of dust sampled can be used as a proxy for hygiene and that $\beta(1\rightarrow3)$ -glucan concentrations per square meter are related to the amount of dust sampled. **Key words:** $\beta(1\rightarrow3)$ -glucan, allergens, endotoxins, house dust, housing characteristics, indoor factors, molds, occupant behavior. *Environ Health Perspect* 109:139–144 (2001). [Online 19 January 2001] <http://ehpnet1.niehs.nih.gov/docs/2001/109p139-144gehring/abstract.html>

There is increasing evidence for the impact of dampness and mold growth in homes on respiratory symptoms in children and adults (1–4). Microorganisms and microbial components such as bacterial endotoxins and mold glucans present in house dust are believed to be important (5,6), but it is not yet clear which components primarily account for the observed effects.

$\beta(1\rightarrow3)$ -Glucans are glucose polymers with various molecular weights and degrees of branching that can elicit inflammatory reactions. They are present in the cell walls of most fungi and yeasts, some bacteria, most higher plants, and many lower plants. Rylander et al. demonstrated dose–response relationships between levels of airborne $\beta(1\rightarrow3)$ -glucan and eye and throat irritation, dry cough, and itching skin (7) as well as irritation of the nose, hoarseness, and tiredness (8). In a subsequent study, $\beta(1\rightarrow3)$ -glucans were found to be associated with an increased prevalence of atopy and a decrease in forced expiratory volume in 1 sec (FEV₁) over the number of years living in the building (9). Douwes et al.

(10) demonstrated an association between $\beta(1\rightarrow3)$ -glucan levels on living room floors and peak flow variability in symptomatic children, and in particular in atopic children with asthmatic symptoms.

$\beta(1\rightarrow3)$ -Glucan concentrations in settled house dust from 395 German homes were studied as part of a collaborative study on Indoor Factors and Genetics in Asthma (INGA). This is the first large-scale study in which relations between glucan levels and a large number of housing characteristics and occupant behavior have been assessed. Because the health effects described for β -glucans may also be caused by many other house dust-associated agents such as allergens, endotoxins, and molds, probably sharing (at least partially) the same determinants such as home dampness, for example, the first aim of this study was to assess the association between $\beta(1\rightarrow3)$ -glucan concentrations in house dust and levels of endotoxins, allergens, and culturable mold spores. The second aim of the study was to assess associations between β -glucan concentrations and housing characteristics and occupant behavior.

Materials and Methods

Study population. A total of 405 homes (204 in Erfurt and 201 in Hamburg) were visited within the framework of the INGA study. The study population consisted of a subset of the subjects who had participated in the European Respiratory Health Survey (11). Details of the selection of the study population are described elsewhere (12). The homes were visited from June 1995 to November 1996 by trained personnel. Every subject willing to participate answered a detailed questionnaire on building and housing characteristics and occupant behavior.

Dust sampling and extraction. In each apartment a dust sample was taken from the living room floor according to a standardized protocol following the recommendations of an international workshop on dust mites (13). All dust samples were taken using the same type of vacuum cleaner (Flüsterjet Vitall 371; Philips, Hamburg, Germany) by vacuuming an area of 1 m² for 2 min. After sampling in 100 homes, the vacuum cleaners were replaced by new ones to avoid a decrease of air flow during sampling. Ninety-five percent of living room floors had carpets, and 78% of these had wall-to-wall carpeting. Dust was collected on cellulose filters using ALK sampling nozzles (ALK Laboratories, Hørsholm, Denmark). The samples were stored at –20°C until extraction. Gravimetric measurements of filters were performed before and after vacuuming. Allergens and endotoxin were extracted from the whole dust sample including filter paper as described earlier (14). The dust was not sieved. A subsequent heat extraction to dissolve $\beta(1\rightarrow3)$ -glucan was performed on pellets after the first extraction (15). After

Address correspondence to U. Gehring, GSF-National Research Center for Environment and Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany. Telephone: +49-89-3187-2549. Fax: +49-89-3187-3380. E-mail: gehring@gsf.de

This study was supported by the Federal Ministry for Education, Science, Research, and Technology, grant 01 EE 93016.

Received 18 May 2000; accepted 11 September 2000.

extraction of dust samples, the extracts were stored in pyrogen-free glassware at -20°C until analysis.

$\beta(1\rightarrow3)$ -Glucan analysis. We assayed $\beta(1\rightarrow3)$ -glucan with a specific enzyme immunoassay, which was developed and described by Douwes et al. (15). Samples were analyzed in duplicate on different days, using one aliquot of each sample per analysis. The mean of the duplicates was used in the further analyses. The interassay variability, expressed as mean coefficient of variation, was 17%, which is similar to what is usually found for many immunoassays. The intra-assay variability was considerably lower, but the exact variability is unknown because in this study no intra-assay duplicates were included.

Blank paper filters used for dust sampling contained $\beta(1\rightarrow3)$ -glucan (mean concentration $830\ \mu\text{g}/\text{filter}$, SD $89\ \mu\text{g}/\text{filter}$, $n = 10$), which was, on average, 42% (range 4–86%) of the total amount of $\beta(1\rightarrow3)$ -glucan detected in each dust sample. Thus, a correction was applied by subtracting the mean concentration detected in the blank filters from the absolute $\beta(1\rightarrow3)$ -glucan level measured in the whole sample (including dust and filter). We do not expect this to bias our results, but using glass fiber filters for future studies will circumvent these problems with blank filters. The limit of detection, defined as $3 \times$ the SD of the blanks, was $267\ \mu\text{g}/\text{filter}$. $\beta(1\rightarrow3)$ -Glucan concentrations were expressed per square meter and per gram of house dust.

Allergen and endotoxin content in house dust. Endotoxin content was quantified using a chromogenic kinetic *Limulus* amoebocyte lysate test (16). *Escherichia coli* endotoxin (lot no 5L570, Bio Whittaker, Walkersville, UK) was used as standard endotoxin. The endotoxin potency of this standard was 14.5 EU/g. Concentrations were expressed as nanogram per gram of dust.

Analysis of dust mite allergens *Dermatophagoides pteronyssinus* (*Der p 1*) and *Dermatophagoides farinae* (*Der f 1*) and cat allergens (*Fel d 1*) was done using a monoclonal enzyme-linked immunosorbent assay (ELISA) (14) with standards UVA 93/03, UVA 93/02, and UVA 94/01 (Indoor Biotechnologies, Clwyd, UK). The allergen concentration was expressed as nanograms per gram of dust. The lower limit of detection derived from calibration curves was 1 ng/mL for both *Der p 1* and *Der f 1*, and 1.5 ng/mL for *Fel d 1*.

Culturable mold spore counts. We collected separate dust samples to measure molds. Sampling was performed as described for $\beta(1\rightarrow3)$ -glucan. Samples were stored at room temperature until analysis. Mold spore counts in house dust were performed as

described by Koch et al. (17). We analyzed a total of 30 mg of sieved housed dust ($500\ \mu\text{m}$) for identification and quantification of viable fungi. Dilutions of dust in 0.9% NaCl were plated on DG18 (dichloran-18% glycerol) agar. The plates were incubated at 25°C for 10 days. Mold spores were counted by naked eye and expressed as colony forming units (CFU) per gram of dust. The lower limit of detection of this method is 1,000 CFU/gram of dust. The following identified genera were counted separately among the cultured colonies: *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium*. Determination of the genus was performed by high-powered light microscopy (Carl Zeiss Jena, Ergaval, Germany).

Indoor climate. Relative humidity and temperature were measured with Tinytalk II Data Loggers, (Gemini Data Loggers Ltd., Chisester, UK) for 7 days starting on the day dust samples were taken. The data loggers were placed in the living room, roughly 2 m above the floor. We used the median relative humidity and median temperature of each measurement series in further analysis. We assume that these measurements are reasonable proxy variables for temperature and humidity for the time of dust sampling, but are not representative for a longer period.

Building characteristics and occupant behavior. A checklist on building characteristics and occupant behavior was developed, and a trained interviewer completed the checklist in each dwelling. From this checklist, housing characteristics and occupant behavior were assessed for further evaluation.

The heating system was classified either as central or noncentral heating; the latter category included gas heating, fireplace heating, and other noncontinuous heating systems such as coal or wood burning.

Ventilation was recorded during the week that humidity and temperature measurements were taken and classified as good, medium, and poor according to the number of hours per week the window was fully and/or partially opened. Hours were summed (number of hours the window was fully opened + $0.15 \times$ number of hours the window was partially opened), and tertiles were used as cutoffs.

Presence in the living room expressed as hours per day was recorded for up to four persons during the week when humidity and temperature measurements were taken. Presence was recorded for weekdays and weekends separately. Hours were summed ($5 \times$ weekday + $2 \times$ weekend), and use of the of the living room by the inhabitants was classified according to the total number of hours the living room was used. Cutoffs were set arbitrarily at 25 and 180 hr/week, which approximately corresponds to one person using the living room for 1 whole day and one person using the living room for 7 days, respectively.

Statistical analysis. Because $\beta(1\rightarrow3)$ -glucan levels in living room floor dust expressed as micrograms per square meter and as micrograms per gram of dust were best described by a lognormal distribution (skewness 3.8 and 7.1, respectively), $\beta(1\rightarrow3)$ -glucan concentrations were natural log transformed. We therefore expressed mean concentrations as geometric means (GM) with a geometric standard deviation (GSD). $\beta(1\rightarrow3)$ -Glucan concentrations below the limit of detection calculated from the field blanks (see " $\beta(1\rightarrow3)$ -Glucan Analysis") were assigned a value of one-half of the detection limit.

We used the Spearman rank-order correlation coefficient to analyze correlations between $\beta(1\rightarrow3)$ -glucan concentrations and indoor climate, the amount of dust sampled, and other agents in the dust (i.e., endotoxins, allergens, and fungi). Associations between $\beta(1\rightarrow3)$ -glucan concentrations on the one hand and housing characteristics and occupant behavior on the other hand were analyzed by means of *t*-tests based on ln-transformed data comparing the category of study with the respective reference category.

To select the most important factors with regard to their impact on $\beta(1\rightarrow3)$ -glucan concentrations, we performed stepwise multiple linear regression on $\beta(1\rightarrow3)$ -glucan concentrations per square meter and per gram of dust with variables that were found to be at least marginally associated ($p < 0.10$) with $\beta(1\rightarrow3)$ -glucans. Forward selection was done. A significance level of 0.10 was used for entry into the model. Finally, we used the same model for glucan concentrations per gram of dust and per square meter for comparability purposes, including any factor selected for either glucan per gram of dust or for glucan per square meter.

Associations between $\beta(1\rightarrow3)$ -glucan levels and explanatory variables are presented as the means ratio (MR), which is the ratio of the geometric mean in the category of study versus the reference category. Statistical significance was set at a conventional 5% level.

All analyses were performed using the SAS software, version 6.12 (SAS Institute Cary, NC, USA).

Results

$\beta(1\rightarrow3)$ -Glucan concentrations. $\beta(1\rightarrow3)$ -Glucan concentrations in living room floor dust were available for 395 of the 405 homes visited. Ten samples were not available because of insufficient handling in the laboratory. $\beta(1\rightarrow3)$ -Glucan concentrations in living room floor dust from 24 homes were below the limit of detection, and $\beta(1\rightarrow3)$ -glucan levels in living room floor dust from the remaining homes ranged from 271 to $19,013\ \mu\text{g}/\text{m}^2$ and from 415 to $22,588\ \mu\text{g}/\text{g}$

dust, respectively. Geometric means, GSDs, and percentiles are presented in Table 1.

Dust weight, endotoxin and allergen measurements, and culturable molds. The average amount of dust sampled from the 395 living room floors was 0.94 ± 0.83 g/m², ranging from 0.02 to 8.0 g/m². Endotoxin concentrations varied between 10 and 120,700 ng/g dust (median 2,200 ng/g dust). *Der f 1* levels in living room floor dust were higher than *Der p 1* levels (median 415 vs. 156 ng/g dust), ranging from nondetectable to 1,255,751 and 344,806 ng/g dust, respectively. *Fel d 1* concentrations ranged from below the limit of detection to 5,274 ng/g dust, with a median of 244 ng/g dust. Total culturable mold spore counts varied between 5,000 and 2,600,000 CFU/g dust with a median of 95,000 CFU/g dust.

Detailed presentations of the results for mite allergens and endotoxins are given by Gross et al. (18) and Bischof et al. (16). Results for *Fel d 1* and culturable mold spore counts will be presented in forthcoming publications.

$\beta(1\rightarrow3)$ -Glucan, dust weight, endotoxins, allergens, and culturable molds. Strong positive correlations were found between levels of $\beta(1\rightarrow3)$ -glucan, endotoxin, mite and cat allergens, and culturable molds per square meter on the one hand and the amount of dust sampled on the other hand. Correlations with the amount of dust sampled were stronger for $\beta(1\rightarrow3)$ -glucan, endotoxin, mite allergens *Der p 1* and *Der f 1*, and total culturable molds ($r = 0.78, 0.63, 0.52, 0.47,$ and 0.56 , respectively) than for *Fel d 1*, *Alternaria*, *Aspergillus*, *Cladosporium*, and *Penicillium* ($r = 0.34, 0.23, 0.22, 0.32,$ and 0.19 , respectively), but all were highly significant ($p < 0.01$).

Correlations between $\beta(1\rightarrow3)$ -glucan levels and concentrations of endotoxin, mite and cat allergens, and culturable molds are presented in Table 2. When all concentrations were expressed per square meter, strong correlations were found between $\beta(1\rightarrow3)$ -glucan levels and concentrations of endotoxins, mite allergens *Der p 1* and *Der f 1*, and total culturable molds. Weaker but also statistically significant correlations were found between $\beta(1\rightarrow3)$ -glucan concentrations and levels of *Fel d 1* and culturable mold spore counts of the various identified genera.

When all concentrations were expressed as micrograms per gram of dust instead of micrograms per square meter, no statistically significant correlation was found between $\beta(1\rightarrow3)$ -glucan levels and concentrations of endotoxins and *Fel d 1*, respectively. Correlations between $\beta(1\rightarrow3)$ -glucan concentrations and levels of *Der p 1* and *Der f 1* in living room floor dust were still statistically significant, but much weaker. Additionally,

there was a statistically significant correlation between $\beta(1\rightarrow3)$ -glucan levels and culturable mold spores counts from the two genera *Alternaria* and *Cladosporium*. Correlations between $\beta(1\rightarrow3)$ -glucan levels and total culturable mold spore counts and culturable mold spore counts from the other genera were not found when concentrations were expressed per gram of dust.

$\beta(1\rightarrow3)$ -Glucan concentrations and indoor climate. We found a marginal statistically significant negative correlation ($p = 0.09$) between $\beta(1\rightarrow3)$ -glucan concentrations per square meter and indoor temperature (Table 2). Additionally, a weak but statistically significant positive correlation between $\beta(1\rightarrow3)$ -glucan levels and relative humidity was found when $\beta(1\rightarrow3)$ -glucan concentrations were expressed per gram of dust (Table 2). Average indoor relative humidity ranged from 11% to 73%, with a median of 45%. Average room temperature ranged from 9°C to 28°C, with a median of 22°C. The average room temperature of 9°C was measured in December in an apartment that was being renovated, so the heating was turned off. All analyses were performed with and without this apartment. As our results were not influenced by this observation, we decided not to exclude it from the analysis. Average room temperature for the remaining apartments was at least 15°C.

$\beta(1\rightarrow3)$ -Glucan concentrations, season, housing characteristics, and occupant behavior. Geometric means and GSDs of $\beta(1\rightarrow3)$ -glucan concentrations in living room floor dust expressed as micrograms per square

meter and micrograms per gram of dust, respectively, related to place of residence, season, housing characteristics, and occupant behavior are presented in Table 3.

When $\beta(1\rightarrow3)$ -glucan levels were expressed per square meter, concentrations in Erfurt were only slightly lower than in Hamburg. We found $\beta(1\rightarrow3)$ -glucan concentrations marginally associated with type of the building, but not with any of the other building or housing characteristics. Elevated $\beta(1\rightarrow3)$ -glucan levels were observed in living rooms that had carpets and > 20 indoor plants. Furthermore, keeping a dog inside, use of the apartment, and cleaning habits were important for $\beta(1\rightarrow3)$ -glucan concentrations. Additionally, $\beta(1\rightarrow3)$ -glucan concentrations were slightly elevated in homes with mold spots during the past 12 months.

When $\beta(1\rightarrow3)$ -glucan levels were expressed per gram of dust, concentrations in Hamburg were significantly higher than in Erfurt. Furthermore, levels of $\beta(1\rightarrow3)$ -glucan were associated with building material and the number of indoor plants. Dose-response relationships were found between levels of $\beta(1\rightarrow3)$ -glucan and frequencies of vacuuming carpets, mopping of smooth floors, and dusting. No other building characteristic and occupant behavior was found to be of importance.

Neither concentrations expressed per square meter nor concentrations expressed per gram of dust were associated with season of dust sampling, year of construction of the building, story and size of the dwelling, type

Table 1. Distribution of $\beta(1\rightarrow3)$ -glucans in living room floor dust expressed per square meter and per gram of dust.

β -Glucans	GM (GSD)	Percentile				
		5th	25th	50th	75th	95th
$\mu\text{g}/\text{m}^2$	1,197 (2.5)	134	742	1,249	2,183	10,817
$\mu\text{g}/\text{g}$ dust	1,711 (1.9)	489	1,295	1,830	2,519	4,065

Concentrations less than the detection limit were assigned a value of one-half of the detection limit.

Table 2. Spearman rank-order correlations of β -glucans and endotoxins, indoor allergens, and fungi in living room floor dust, and indoor climate in the living room.

	$\beta(1\rightarrow3)$ -Glucan			
	$\mu\text{g}/\text{m}^2$		$\mu\text{g}/\text{g}$ dust	
	r	p -Value	r	p -Value
Endotoxins	0.55	< 0.01*	0.08	0.10
Indoor allergens				
<i>Der p 1</i>	0.46	< 0.01*	0.14	< 0.01*
<i>Der f 1</i>	0.42	< 0.01*	0.11	0.03*
<i>Fel d 1</i>	0.28	< 0.01*	0.06	0.26
Fungi				
Total fungi	0.50	< 0.01*	0.02	0.62
<i>Alternaria</i>	0.29	< 0.01*	0.13	< 0.01*
<i>Aspergillus</i>	0.18	< 0.01*	0.05	0.36
<i>Penicillium</i>	0.26	< 0.01*	0.03	0.57
<i>Cladosporium</i>	0.24	< 0.01*	0.18	< 0.01*
Indoor climate				
Temperature (°C)	-0.09	0.09	-0.06	0.28
Relative humidity (%)	0.04	0.43	0.12	0.02*

*Statistically significant.

of heating (central vs. noncentral), ventilation, keeping a cat indoors, use of humidifiers (active and passive), water damage, visible mold or mildew, and visible wet spots during the past 12 months (data not shown).

Multiple linear regression analysis. Means ratios and 95% confidence intervals for the resulting models for $\beta(1\rightarrow3)$ -glucan levels expressed as micrograms per square meter and as micrograms per gram of dust are presented in Table 4. Nineteen percent and 9%, respectively, of the variance of the $\beta(1\rightarrow3)$ -glucan concentrations were explained by the variables in the regression analysis.

$\beta(1\rightarrow3)$ -Glucan levels in Hamburg were somewhat higher than in Erfurt. The difference between the two cities was not significant when concentrations were expressed per square meter but was statistically significant when concentrations were expressed per gram of dust.

Carpets in the living room were associated with a statistically significant increase in $\beta(1\rightarrow3)$ -glucan concentrations per square meter. $\beta(1\rightarrow3)$ -Glucan concentrations measured in living rooms with a carpet were higher compared to those in living rooms without a carpet, with a tendency toward higher concentrations in carpets older than 5 years. $\beta(1\rightarrow3)$ -Glucan levels per gram of dust were not associated with carpets in the living room. Furthermore, there was an association between $\beta(1\rightarrow3)$ -glucan concentrations and the number of indoor plants in the living room. The highest $\beta(1\rightarrow3)$ -glucan levels per square meter were measured in living rooms with > 20 indoor plants, whereas concentrations per gram of dust were lower in dust samples from living rooms with > 10 plants compared to living rooms with ≤ 10 plants. In addition, keeping a dog indoors, use of the apartment by four or more persons, and use of the living room for > 180 person-hours per week was associated with elevated levels of $\beta(1\rightarrow3)$ -glucan per square meter.

Cleaning habits were of major importance for $\beta(1\rightarrow3)$ -glucan in living room floor dust expressed per square meter. Lower frequencies of vacuum cleaning and dusting were associated with elevated absolute $\beta(1\rightarrow3)$ -glucan concentrations (per square meter). Vacuum cleaning only a few times a week was associated with a statistically significant increase in $\beta(1\rightarrow3)$ -glucan levels per square meter compared to daily vacuum cleaning. Vacuum cleaning only once per week and once per month or less was associated with an even higher increase in $\beta(1\rightarrow3)$ -glucan levels compared to daily vacuum cleaning. $\beta(1\rightarrow3)$ -Glucan levels expressed as micrograms per gram of dust were not associated with cleaning habits. In homes with mold spots during the past 12 months, statistically significant higher levels of $\beta(1\rightarrow3)$ -glucan per square

meter were measured than in homes without mold spots, whereas concentrations per gram of dust were only slightly elevated in homes with mold spots.

To evaluate the effect of concentrations below the limit of detection on the results of the regression analyses, the final multiple linear regression models were recalculated excluding

Table 3. $\beta(1\rightarrow3)$ -Glucan in living room floor dust related to potential influencing factors: GM (GSD).

Factors	No.	$\beta(1\rightarrow3)$ -Glucan GM (GSD)	
		$\mu\text{g}/\text{m}^2$	$\mu\text{g}/\text{g}$
Place of residence			
Erfurt	201	1,117 (2.4)	1,578 (1.8)
Hamburg	194	1,287 (2.6)	1,862 (1.9)**
Season			
Summer	204	1,173 (2.6)	1,681 (1.9)
Winter	191	1,224 (2.4)	1,744 (1.8)
Building characteristics			
Building material			
Brick/clinker	217	1,202 (2.4)	1,843 (1.9)
Concrete	157	1,161 (2.6)	1,588 (1.8)**
Framework/clay	13	1,647 (1.9)	1,518 (1.4)*
Type of building			
One-/two-family house	114	1,381 (2.5)	1,781 (1.9)
Multiple-family dwelling	214	1,116 (2.5)**	1,696 (1.9)
Concrete slab house	67	1,174 (2.3)	1,646 (1.8)
Characteristics of living room			
Carpets			
None	19	719 (2.9)	1,741 (2.7)
Carpets < 5 years old	164	1,036 (2.5)	1,528 (1.9)
Carpets 5–10 years old	116	1,430 (2.3)**	1,913 (1.8)
Carpets > 10 years old	93	1,385 (2.5)**	1,791 (1.6)
Indoor plants			
≤ 10	201	1,199 (2.5)	1,887 (1.8)
11–20	121	1,055 (2.6)	1,511 (2.0)**
> 20	73	1,471 (2.4)*	1,609 (1.7)**
Use characteristics			
No. of persons living in the home			
1–3	261	1,087 (2.5)	1,714 (1.8)
≥ 4	134	1,444 (2.5)**	1,706 (1.9)
Use of the living room during sampling			
≤ 25 hr/week	23	1,075 (2.5)	1,596 (2.2)
25–180 hr/week	330	1,155 (2.5)	1,725 (1.9)
> 180 hr/week	42	1,687 (2.3)**	1,671 (1.5)
Residence time			
≤ 5 years	64	885 (2.6)	1,538 (1.9)
> 5 years	331	1,269 (2.5)**	1,747 (1.8)
Ventilation			
Poor	116	1,122 (2.3)	1,675 (1.9)
Medium	146	1,323 (2.3)	1,735 (1.8)
Good	133	1,135 (2.8)	1,717 (1.9)
Dog indoors			
No	356	1,156 (2.5)	1,724 (1.9)
Yes	34	1,685 (2.8)**	1,534 (1.7)
Cleaning habits			
Vacuum cleaning of carpets			
Daily	33	761 (2.6)	1,327 (2.3)
Several times a week	170	1,170 (2.5)**	1,744 (1.9)*
Once per week	158	1,280 (2.4)**	1,738 (1.7)*
Once per month or less	29	1,766 (2.5)**	1,834 (1.9)*
Mopping of smooth floors			
Daily/several times a week	104	983 (2.7)	1,557 (2.0)
Once a week	196	1,283 (2.3)**	1,717 (1.7)
Once a month or less	67	1,498 (2.6)**	1,987 (1.8)**
Dusting (dry)			
Daily/several times a week	100	977 (2.5)	1,557 (1.8)
Once a week	138	1,172 (2.3)	1,682 (1.9)
Once a month or less	156	1,395 (2.6)**	1,839 (1.8)**
Dusting (wet)			
Daily–once a week	130	1,065 (2.3)	1,713 (1.8)
Once a month or less	264	1,270 (2.6)*	1,706 (1.9)
Mold spots during past 12 months			
No	349	1,164 (2.5)	1,688 (1.9)
Yes	42	1,557 (2.5)*	1,940 (1.9)

^aConcentrations less than the detection limit were assigned a value of one-half of the detection limit. * $p < 0.10$. ** $p < 0.05$ refers to comparisons of the category of study with the first category of the respective factor by means of a t -test based on ln-transformed data.

$\beta(1\rightarrow3)$ -glucan concentrations below the limit of detection. Effect sizes tended to be a bit smaller, but effects did not disappear, and no additional effects were found.

Discussion

The aim of this study was to assess whether β -glucan concentrations in house dust are correlated with endotoxin, allergen, and culturable mold spore levels in house dust, and whether they are associated with housing characteristics and occupant behavior. When concentrations were expressed per square meter, $\beta(1\rightarrow3)$ -glucan levels were highly correlated with concentrations of endotoxin, mite allergens *Der p 1* and *Der f 1*, cat allergen *Fel d 1*, and culturable mold spore counts. When concentrations were expressed per gram of dust, correlations were weakened. Associations between $\beta(1\rightarrow3)$ -glucan levels and housing characteristics and occupant behavior were mainly found when concentrations were

expressed per square meter and not when concentrations were expressed per gram of dust. The following housing characteristics and occupant behavior were of major importance for $\beta(1\rightarrow3)$ -glucan levels per square meter: carpets in the living room, keeping a dog indoors, use of the apartment by four or more persons, and use of the living room for > 180 hr/week, lower frequencies of vacuum cleaning and dusting, and presence of mold spots during the past 12 months.

$\beta(1\rightarrow3)$ -Glucan levels. Mean $\beta(1\rightarrow3)$ -glucan concentrations measured in the present study are in accordance with mean $\beta(1\rightarrow3)$ -glucan concentrations in living room floor dust from the pilot study reported previously (19). $\beta(1\rightarrow3)$ -Glucan concentrations in settled house dust from approximately 125 homes were also reported by Douwes et al. (10). Mean $\beta(1\rightarrow3)$ -glucan concentrations measured by Douwes et al. (10) were much lower than mean $\beta(1\rightarrow3)$ -glucan

concentrations measured in the present study. One reason that the concentrations were lower in that study is that they included many homes with smooth floors not covered with wall-to-wall carpet, whereas 95% of the living room floors in this study had carpets, of which 78% had wall-to-wall carpeting. This makes a substantial difference, particularly for concentrations expressed per square meter. No other studies on $\beta(1\rightarrow3)$ -glucan concentrations in settled house dust were found in the literature. Rylander and colleagues (6,7,9,20,21) conducted studies on airborne $\beta(1\rightarrow3)$ -glucan concentrations, but a comparison of $\beta(1\rightarrow3)$ -glucan concentrations measured in settled house dust and airborne $\beta(1\rightarrow3)$ -glucan concentrations has not yet been done.

$\beta(1\rightarrow3)$ -Glucan concentrations and endotoxins, allergens, and culturable molds. When levels were expressed as micrograms per square meter, strong correlations were found between $\beta(1\rightarrow3)$ -glucan levels and endotoxins, mite

Table 4. Means ratios (MR) and 95% confidence intervals (CI) as determined by multiple linear regression ($n = 378$, $R^2 = 19\%$, 9%), based on ln-transformed data.

Factor	No.	$\beta(1\rightarrow3)$ -Glucan ($\mu\text{g}/\text{m}^2$)			$\beta(1\rightarrow3)$ -Glucan ($\mu\text{g}/\text{g}$)		
		MR	95% CI	p -Value ^a	MR	95% CI	p -Value ^a
Place of residence							
Erfurt	193	1			1		
Hamburg	185	1.16	(0.94–1.43)	0.1554	1.18	(1.01–1.37)	0.0333
Building characteristics							
Type of building							
One-/two-family house	111	1			1		
Multiple-family dwelling	202	0.84	(0.69–1.03)	0.1035	0.96	(0.83–1.12)	0.6371
Concrete slab house	65	1.05	(0.78–1.41)	0.7353	1.06	(0.85–1.31)	0.6107
Characteristics of living room							
Carpets							
None	15	1			1		
Carpets < 5 years old	158	1.85	(1.17–2.95)	0.0094	0.98	(0.70–1.36)	0.8875
Carpets 5–10 years old	114	2.36	(1.47–3.78)	0.0004	1.19	(0.85–1.68)	0.3120
Carpets > 10 years old	91	2.09	(1.30–3.34)	0.0023	1.08	(0.77–1.52)	0.6553
Indoor plants							
≤ 10	191	1			1		
11–20	117	0.85	(0.70–1.03)	0.1043	0.81	(0.70–0.94)	0.0045
> 20	70	1.19	(0.94–1.50)	0.1558	0.87	(0.73–1.03)	0.1033
Dog indoors							
No	344	1			1		
Yes	34	1.43	(1.05–1.94)	0.0247	0.92	(0.74–1.15)	0.4645
Use characteristics							
No. of persons living in the home							
1–3 persons	248	1			1		
≥ 4 persons	130	1.35	(1.12–1.64)	0.0022	1.03	(0.90–1.18)	0.6904
Use in person-hours per week							
≤ 25 hr/week	23	1			1		
25–180 hr/week	313	1.34	(0.92–1.95)	0.1319	1.17	(0.89–1.54)	0.2612
> 180 hr/week	42	2.06	(1.29–3.29)	0.0027	1.23	(0.87–1.72)	0.2389
Cleaning habits							
Vacuum cleaning the carpets							
Daily	31	1			1		
Several times a week	166	1.64	(1.17–2.29)	0.0040	1.23	(0.96–1.56)	0.0962
Once a week	154	1.84	(1.30–2.63)	0.0008	1.19	(0.92–1.53)	0.1871
Once a month or less	27	3.04	(1.88–4.93)	<0.0001	1.28	(0.90–1.81)	0.1666
Dusting (dry)							
Daily/several times a week	97	1			1		
Once a week	35	1.22	(0.96–1.55)	0.1043	1.09	(0.91–1.29)	0.3531
Once a month or less	146	1.35	(1.05–1.73)	0.0203	1.09	(0.91–1.30)	0.3626
Mold spots during past 12 months							
No	337	1			1		
Yes	41	1.35	(1.02–1.78)	0.0353	1.17	(0.96–1.43)	0.1277

Concentrations less than the detection limit were assigned a value of one-half of the detection limit.

^aComparisons of the category of study with the first category of the respective factor by means of a t -test based on ln-transformed data.

allergens, and total culturable mold spore counts. Weaker but also statistically significant correlations were found between $\beta(1\rightarrow3)$ -glucan levels and cat allergens and culturable mold spore counts of the identified genera of *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium*. When levels were expressed as micrograms per gram of dust, correlations were much weaker. Statistically significant correlations with $\beta(1\rightarrow3)$ -glucan concentrations were only found for levels of mite allergens *Der p 1* and *Der f 1* and mold spore counts of the two genera *Alternaria* and *Cladosporium*. The strong correlations among concentrations per square meter and the weak correlations and the lack of correlation, respectively, when concentrations were expressed as micrograms per gram of dust indicate that variance in levels per square meter of all the indoor factors considered was largely determined by the amount of dust sampled. Actually, correlations between the amount of dust sampled and glucans, endotoxins, mite allergens, and total culturable molds were very high. From the weak correlations between concentrations per gram of dust, we conclude that $\beta(1\rightarrow3)$ -glucan, endotoxin, mite and cat allergen levels, and culturable molds are (at least partially) determined by different characteristics.

We did not find an association between total culturable mold spore counts and $\beta(1\rightarrow3)$ -glucan concentrations expressed as micrograms per gram of dust. This is in accordance with previous findings (19). Possible reasons for this are a) only culturable molds were counted, whereas $\beta(1\rightarrow3)$ -glucan most likely represents total mold biomass, including nonculturable propagules; and b) $\beta(1\rightarrow3)$ -glucan is not completely specific as a marker for molds because it may also originate from plants and some bacteria. Interestingly, in our previous pilot study we also found a weak but statistically significant correlation between $\beta(1\rightarrow3)$ -glucan levels and culturable mold spore counts from *Alternaria* per gram of dust. The reason for this is not clear. Additionally, in this study $\beta(1\rightarrow3)$ -glucan was correlated with culturable mold spore counts from *Cladosporium* per gram of dust. The reason for this also is not clear.

$\beta(1\rightarrow3)$ -Glucan concentrations and housing characteristics and occupant behavior. There are no studies reported in the literature that identify building characteristics and occupant behavior associated with the level of $\beta(1\rightarrow3)$ -glucan concentrations in homes. We found $\beta(1\rightarrow3)$ -glucan concentrations per square meter in one- and two-family houses and in concrete slab houses to be higher than in multiple-family dwellings. The reason for this is not clear. Beyond this, carpets, presence of a dog, use of the apartment, and cleaning habits were of major

importance for concentrations per square meter but not for concentrations per gram of dust. Because $\beta(1\rightarrow3)$ -glucan concentrations per square meter as well as the housing characteristics and occupant behavior (carpets, presence of a dog, use of the apartment, cleaning habits) are associated with the amount of dust, the associations with concentrations per square meter and the lack of association when concentrations were expressed per gram of dust are plausible. With these results, the amount of dust sampled can be used as a proxy for hygiene, and $\beta(1\rightarrow3)$ -glucan concentrations expressed per square meter are related to the amount of dust sampled.

The association between $\beta(1\rightarrow3)$ -glucan concentrations and mold spots during the past 12 months is in accordance with the results of other studies where $\beta(1\rightarrow3)$ -glucan concentrations were found to be higher in buildings with problems of mold growth than in buildings without those problems (6,9,20,21).

$\beta(1\rightarrow3)$ -Glucan concentrations and indoor climate. When $\beta(1\rightarrow3)$ -glucan concentrations were expressed per square meter, an association between indoor climate in terms of temperature and relative humidity was lacking. When concentrations were expressed per gram of dust, a weak positive correlation was found with relative humidity, indicating that house dust from damper rooms contains higher $\beta(1\rightarrow3)$ -glucan levels. This weak correlation and the lack of correlation, respectively, may be due to the short period of only 1 week in which temperature and humidity measurements were taken. Temperature and humidity may not be representative for a longer period.

Conclusion

In conclusion, levels of $\beta(1\rightarrow3)$ -glucan, endotoxin, mite and cat allergens, and culturable mold expressed per square meter are largely determined by the amount of dust sampled. $\beta(1\rightarrow3)$ -Glucan concentrations per square meter can be determined by housing characteristics and occupant behavior, whereas $\beta(1\rightarrow3)$ -glucan concentrations per gram of dust cannot. Our results indicate that the amount of dust sampled can be used as a proxy for hygiene and that $\beta(1\rightarrow3)$ -glucan concentrations expressed per square meter are related to the amount of dust sampled. Further investigations on health effects are needed to determine whether it is sufficient to simply weigh the dust sampled (which would be more cost effective) instead determining $\beta(1\rightarrow3)$ -glucan concentrations in house dust.

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