International Journal of Occupational Medicine and Environmental Health 2013;26(6):870–889
DOI 10.2478/s13382-013-0156-5

CHILDREN'S RESIDENTIAL EXPOSURE TO SELECTED ALLERGENS AND MICROBIAL INDICATORS: ENDOTOXINS AND (1→3)-β-D-GLUCANS

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Abstract
Objectives: The study was aimed at assessment of exposure to endotoxins, (1→3)-β-D-glucans and mite, cockroach, cat, dog allergens present in settled dust in premises of children as agents which may be significantly correlated with the occurrence of allergic symptoms and diseases in children. **Materials and Methods:** The study covered 50 homes of one- or two-year-old children in Poland. Samples of settled dust were taken from the floor and the child's bed. The levels of (1→3)-β-D-glucans (floor), endotoxins (floor) and allergens of mite, cat, dog and cockroach (floor and bed) were analyzed. **Results:** Average geometric concentrations (geometric standard deviation) of endotoxins, (1→3)-β-D-glucans, Der p1, Fel d1, Can f1 and Bla g1 in children homes were on the floor 42 166.0 EU/g (3.2), 20 478.4 ng/g (2.38), 93.9 ng/g (6.58), 119.8 ng/g (13.0), 288.9 ng/g (3.4), 0.72 U/g (4.4) and in their beds (only allergens) 597.8 ng/g (14.2), 54.1 ng/g (4.4), 158.6 ng/g (3.1) 0.6 U/g (2.9), respectively. When the floor was covered with the carpet, higher concentrations of endotoxins, (1→3)-β-D-glucans and allergens (each type) were found in the settled dust ($p < 0.05$). The trend was opposite in case of allergens (except dog) analyzed from bed dust and significantly higher concentrations were found in the rooms with smooth floor ($p < 0.05$). **Conclusions:** Among the analyzed factors only the type of floor significantly modified both the level of biological indicators and allergens. The results of this study could be the base for verifying a hypothesis that carpeting may have a protective role against high levels of cockroach, dog and cat allergens.

Keywords:
Children exposure, Residential exposure, Allergens, Settled dust, Endotoxins, Glucans

INTRODUCTION

Epidemiological studies indicate that asthma is one of the most frequent diseases among children in highly industrialized countries [1,2]. These studies demonstrated in recent decades a significant increase in the incidence of allergic diseases and, at the same time, a decrease in the incidence of bacterial and viral contagious diseases [2,3]. Many public health experts account for a high incidence of allergies and atopy among children in developed countries by pointing to a higher standard of life and hygiene,

Source of financial support: This study was performed under the project "Prenatal and postnatal exposure to tobacco smoke, PAHs and heavy metals and the risk of respiratory diseases, allergy and poor mental and physical development" supported by the grant PNRF-218-AI-1/07 from Norway through the Norwegian Financial Mechanism within the Polish- Norwegian Research Fund.
Received: September 16, 2013. Accepted: October 23, 2013.
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and consequently a lower contact with microorganisms, especially in the prenatal period or a very early period in life. A number of studies providing potential confirmation of this theory, called the "hygiene hypothesis", were published in recent years in articles written by many authors [1,4–11].

Although many scientific studies confirmed a significant role of genetic basis in the process of allergy induction, yet this is not the only risk factor [12]. Apart from genetic susceptibility to asthma and atmospheric air pollution mainly related to road transport, risk factors for childhood asthma are also qualified as chemical agents and biocontaminants affecting the indoor air quality. Domestic animals and their allergens, mites and their allergens, home dampness and the excessive fungal and bacterial growth caused by this as well as compounds of microbial origin (endotoxins, (1→3)-β-D-glucans, the extracellular polysaccharides) [1] are classified as factors of biological origin.

Endotoxins (lipopolysaccharides, LPS), cell wall components of Gram-negative bacteria, are biologically active and have inflammatory properties on live organisms, including irritation of the airways and stimulation of the immune system [13]. As regards the activity of endotoxins in allergic processes, there exist two different hypotheses. According to one of them, this factor plays an important role in the induction of allergy (as well as (1→3)-β-D-glucans) [14]. However, according to the other ("hygienic hypothesis"), the environmental exposure to elevated levels of endotoxins during the neonatal period and early childhood can be protective against atopic asthma and allergic diseases at school age [15,16]. However, in the case of children with atopy, Gent et al. [17] demonstrated that limitation of exposure to allergens of dog (Can f1), cat (Fel d 1), mites (Der p1) and molds reduces the incidence of asthma in this population.

Taking into account the results of numerous studies, it is important to assess children's exposure to factors that could potentially influence the process of induction of allergy to evaluate also the concentration of allergens and factors which are considered as modulators impacting the immune response of human organism.

The study was aimed at assessment of exposure to endotoxins, (1→3)-β-D-glucans and common allergens (mite, cockroach, cat, dog) present in settled dust in premises of 1- and 2-year-old children as agents which may be significantly correlated with the occurrence of allergic symptoms and diseases in children.

MATERIALS AND METHODS

Homes characteristics

The study covered 50 homes (flats and houses) situated in central Poland (Łódź), occupied by mothers with one- or two-year-old children (from The Polish Mothers and Child Cohort). Data on the dwellings' characteristics were collected using a specially prepared questionnaire in accordance with the methodology described by Polańska et al. [18]. The factors included in the analyses were: type of building, number of inhabitants, condensation of water vapor during the winter on the windows and/or other surfaces, visible signs of moisture, visible signs of mold, frequency of cleaning floors, animals at home (contact with a dog and cat), contact with animals outside the home and type of floor.

Sampling strategy

Sampling was carried out in a heating season. All mothers were asked not to clean the home for three days prior to sampling. In each home, the settled dust from the floor in the living room and the child's room or in one room (when the living room = the child's room) and from the child's bed (mattress and bedding) were sampled. The samples of settled dust were collected by vacuuming two fields, each of which had a surface of 1 m^2 of the floor (carpet, smooth floor or smooth floor with rug(s)) for 2 min each, using a commercial vacuum cleaner (Panasonic, MC-E6003, 1600 W) equipped with a special nozzle (ALK, Horshølm, Denmark), loaded for the first field with

a glass fiber filter of a 70-mm diameter (Whatman International Ltd., UK), and for the second one with a paper filter of a 70-mm diameter (Whatman International Ltd., Maidstone, Kent, UK). The bed dust samples were collected by vacuuming the whole area of the mattress and bedding for 2 min, using the same vacuum cleaner with a nozzle (ALK, Horshølm, Denmark) fitted with a paper filter of a 70-mm diameter (Whatman International Ltd., Maidstone, Kent, UK).

Settled dust analysis

The gravimetric method was applied for assessing the total settled dust mass. Each filter was weighed before and after sampling with the accuracy equal to 0.01 mg using CP 225D scales (Sartorius AG, Goettingen, Germany). The settled dust loading (g/m^2 or g/bed) was calculated for each sample. The results for two filters used in the case of the floor of one room were averaged.

After weighing, the filters with the dust were transferred to sterile and pyrogen-free tubes and stored frozen at –20°C until the extraction and analysis. The concentrations of (1→3)-β-D-glucans were determined in the dust collected onto the glass fiber filters, the concentrations of endotoxins and allergens were determined in the dust collected onto the paper filters.

Analysis of endotoxins and allergens

For the analysis of endotoxins and allergens, the frozen filters (paper filter) were extracted in a volume of 5–40 ml of water LAL (Limulus Amebocyte Lysate) (Lonza, Walkerswille, MD, USA) with an addition of 0.05% Tween 20 (Sigma, Poland). The amount of water was determined by the net dust weight (5 ml: $m_{dust} < 500$ mg; 10 ml: 500 mg $\leq m_{dust} < 1000$ mg; 20 ml: 1000 mg $\leq m_{dust} < 2000$ mg; 40 ml: $m_{dust} \geq 2000$ mg). The samples were shaken on a platform shaker (Multi-Purpose Shaker KL-2, Edmund Bühler GmbH, Germany) for 60 min at room temperature, and then they were centrifuged (Laboratory Centrifuge 2-16K, Sigma, Germany) at 1000×g for 15 min at 4°C. After centrifugation, the upper 10% of supernatants were deposited into a 2-ml sterile pyrogen free tube and stored in –20°C for endotoxin analysis. The removed supernatants (for allergens analysis) were replaced with the same volume of 10 concentrated phosphate-buffered saline (PBS) (BTL, Poland), thus changing the extraction medium into PBS-0.045% Tween-20. The prepared samples were shaken for 60 min at room temperature, then again centrifuged at 2000×g for 15 min at 4°C. The obtained supernatant was used for the determination of pet and mite allergens: Der p1 (mite), Can f1 (dog), Fel d1 (cat) and Bla g1 (cockroach). For this purpose, about 1.5 ml of eluate was transferred into a 2-ml sterile and pyrogen-free tube.

Endotoxins were assayed with the quantitative kinetic chromogenic Limulus Amebocyte Lysate (LAL) test (Kinetic-QCL, Lonza, Walkersville, MD,USA). *Escherichia coli* endotoxin (bacterial strain *E. coli* 055:B5, Lonza, Walkersville, MD,USA) was used as the standard endotoxin. The endotoxin potency of this standard was 11 EU/ng. The concentrations of the endotoxin were measured using spectrophotometer SpectraMax Plus384 (Molecular Devices, USA) with light waves of 405 nm length and constant temperature of 37°C. The results were obtained by comparing the samples with the standard curve within 0.049–100 EU/ml, which was generated from twofold serial dilutions of the endotoxin standard. The concentration values were presented as both the unit of endotoxin per gram of dust (EU/g) and per square meter of the sampled area (EU/m^2) and as both nanogram per gram of dust (ng/g) and per square meter of the sampled area (ng/m^2).

The levels of Der p1, Can f1, Fel d1 and Bla g1 allergens were determined with a monoclonal antibody-based enzyme immunoassay from Indoor Biotechnologies (Charlottesville VA, USA). The concentrations of allergens were measured using spectrophotometer SpectraMax Plus384 (Molecular Devices, Sunnyvale, USA). The lower limit of detection was 2 ng/ml for Der p1 and Can f1, 0.8 ng/ml for Fel d1 and 0.008 U/ml

for Bla g1. Allergen concentrations were expressed as nanogram per gram of dust (ng/g) (settled dust from the floor and the child's bed) and nanogram per square meter of the sampled area (ng/m^2) (settled dust from the floor).

Analysis of (1→3)-β-D-glucans

For all samples, both water-soluble and alkali-soluble fractions of (1→3)-β-D-glucans were determined. For the analysis of (1→3)-β-D-glucans, the frozen filters (glass fiber filter) were extracted with 10 ml of water LAL (Limulus Amebocyte Lysate) (Lonza, Walkerswille, MD, USA) with an addition of 0.05% Tween 20 (Sigma, Poland). The samples were shaken on a platform shaker (Multi-Purpose Shaker KL-2, Edmund Bühler GmbH, Germany) for 60 min at room temperature, and then centrifuged (Laboratory Centrifuge 2-16K, Sigma, Germany) at 1000×g for 15 min at 4°C. From the obtained supernatant, 1.8 ml of eluate was deposited into a 2-ml (1→3)-β-D-glucan-free tube for the determination of the water-soluble fraction of (1→3)-β-D-glucans. To the remaining part of the supernatant, 10 M NaOH (Sigma, Poland) was added to obtain a solution with the concentration equaling 0.3 M NaOH. The prepared samples were shaken for 10 min at 4°C, then again centrifuged at 1000×g for 15 min at 4°C. The obtained supernatant was used for the determination of the alkali-soluble fraction of (1→3)-β-D-glucans. For this purpose about 2 ml of eluate was transferred into a 2-ml (1→3)-β-D-glucan-free tube.

The analysis was carried out using Glucatell assay in the kinetic version (Associates of Cape Cod Inc., USA). The concentrations of (1→3)-β-D-glucans were determined using a spectrophotometer SpectraMax Plus384 (Molecular Devices, USA), with light waves of 405 and 490 nm length and constant temperature of 37°C. The results were obtained by comparing the samples with the standard curve within 1.563–800 pg/ml, which was generated from twofold serial dilutions of the (1→3)-β-D-glucan standard. The final concentration of (1→3)-β-D-glucans was the sum of the two determined fractions. The concentration values were presented as both nanogram per gram of dust (ng/g) and per square meter of the sampled area (ng/m^2).

Measurement of microclimate parameters

During settled dust sampling, simultaneously, the basic microclimate parameters, such as: temperature (°C), relative humidity (%), CO_2 concentration (ppm) and airflow velocity (m/s) were measured in the rooms under study. The measurements were carried out using the microclimate multifunction meter Testo 435-2 (Testo AG, Germany) at a height of 1.5 m over the floor for 10 min. The values of individual parameters were read out every minute, then the results were averaged for a given measurement point.

Statistical analysis

Descriptive statistics including arithmetic means (AM), geometric means (GM), standard deviations (arithmetic SD and geometric GSD) were calculated. One way ANOVA was used to analyze the associations between selected predictors and concentrations of dust, glucans, endotoxins and allergens. T-test for paired observations was used to compare allergens in beds versus floors and carpeted floor versus uncovered floor. Dust, glucans, endotoxins and allergens were log transformed because the distribution of those variables was not normal. The normality of distribution was assessed using quantile-quantile plots. Spearman's rank correlation coefficient the were determined to analyze the association between the measured factors. For all calculations, a p-value less than 0.05 was considered as significant. All calculations were carried out using STATISTICA v. 7.0 software package (Stat-Soft Poland, Poland).

RESULTS

The average values, standard deviations and ranges of microclimate parameters' values determined in the examined homes are presented in Table 1. The microclimate inside the children's homes under study was characterized,

on average, by moderate temperature (AM: 22.4°C; range: 17.0–27.7°C) and relatively moderate humidity (AM: 45.1%; range: 28.2–67%), high concentration of CO_2 (AM: 1.223.4 ppm; range: 522.0–2570.0 ppm) and very low airflow velocity (AM: 0.001 m/s; range: 0.000–0.020 m/s). The statistical analysis showed the significant negative correlation between temperature and relative humidity in the examined homes ($p < 0.05$).

The amount of the total settled dust sampled from the examined room floors ranged from 0.01 g/m^2 to 1.8 g/m^2 with a geometric mean of 0.3 g/m^2. The settled dust load of children's beds differed from 0.03 to 1.2 g/bed with a geometric mean of 0.2 g/bed. The analysis of the amount of settled dust expressed per m^2 of the floor, depending on the type of the floor in the investigated rooms, showed a significantly higher dust load of the carpeted floors, as compared to non-covered floors ($p < 0.05$).

The endotoxin levels in room floor dust ranged from 235.3 to 152 126.2 EU/m^2 with a geometric mean of 11 011.7 EU/m^2. The (1→3)-β-D-glucan concentrations in floor settled dust ranged from 382.8 to 33 139 ng/m^2 with a geometric mean of 5 334.4 ng/m^2. All detailed data are showed in Table 2.

Table 1. The values of the microclimate parameters in the children's homes under the study

Microclimate parameter	Homes – indoor air (N = 50)				
	samples (n)	AM	SD	min.	max
Temperature (°C)	89	22.40	1.77	17.00	27.70
Relative humidity (%)	89	45.10	9.92	28.20	67.00
Concentration of CO_2 (ppm)	89	1 223.38	483.37	522.00	2 570.00
Airflow velocity (m/s)	89	0.001	0.004	0.000	0.020

AM – arithmetic mean; SD – standard deviation.
min. – minimal value of the range; max – maximal value of the range.

Table 2. The concentrations of (1→3)-β-D-glucans and endotoxins present in the settled dust from the floor in the children's homes under the study

Factor	Homes – floor (N = 50)						
	samples (n)	AM	SD	GM	GSD	min.	max
(1→3)-β-D-glucans	89						
ng/g of dust		32 853.88	53 443.13	20 478.41	2.28	5044.97	403 915.88
ng/m^2 of floor		7 999.46	6 898.04	5 334.42	2.69	382.77	33 138.96
Endotoxins	89						
ng/g of dust		8 990.91	19 550.74	3 833.27	3.22	409.38	150 649.82
EU/g of dust		98 900.05	215 058.15	42 166.00	3.22	4503.13	1 657 147.97
ng/m^2 of floor		2 218.14	2 996.63	1 001.06	3.98	21.39	13 829.65
EU/m^2 of floor		24 399.56	32 962.93	11 011.71	3.98	235.28	152 126.18

GM – geometric mean.
GSD – geometric standard deviation.
Other abbreviations as in Table 1.

The average concentrations of allergens determined in the settled dust collected from the floors in investigated premises per 1 m^2 of the floor, expressed by geometric mean (GM) and the range, reached the following values: Der p1 – 24.5 ng/m^2 (5.0–21 856.3 ng/m^2); Fel d1 – 31.3 ng/m^2 (2.0–3361.3 ng/m^2); Can f1 – 75.4 ng/m^2 (5–22 403.7 ng/m^2) and Bla g1 – 0.19 U/m^2 (0.02–2.5 U/m^2).

The average concentrations of allergens determined in the dust collected from the children'a, per 1 g of dust, expressed by geometric mean (GM) and the range reached the following values: Der p1 – 597.8 ng/g (12.5–54 0154.2 ng/g); Fel d1 – 54.1 ng/g (4.8–13 151.5 ng/g); Can f1 – 158.6 ng/g (20.5–2516.5 ng/g) and Bla g1 – 0.6 U/g (0.04–6.2 U/g).

Detailed values of the investigated allergens determined in the settled dust collected from the floor and from the children's beds are presented in Table 3.

The analysis of the investigated agents present in the square meter of the floor demonstrated that the concentrations of endotoxins correlated significantly positively with the concentrations of settled dust and (1→3)-β-D-glucans ($p < 0.05$). Besides, both the concentrations of endotoxins and (1→3)-β-D-glucans in the rooms where the floor was completely or partly covered with a carpet were higher in comparison to those in the premises with smooth floors ($p < 0.05$).

All the analyzed allergens in the concentration exceeding the sensitivity level of ELISA tests were found in 18% of

Table 3. The concentrations of selected allergens present in the settled dust from the floor per m^2 and per g and from the beds per g in the children's homes under the study

Dust sample	Factor (unit)	Homes – floor (N = 50)						
		sample (n)	AM	SD	GM	GSD	min.	max
Floor	Der p1 – mite	89						
	(ng/g)		948.40	3 864.42	93.88	6.58	10.12	33 554.89
	(ng/$m^{2)}$		677.15	2 914.98	24.52	7.79	5.00	21 856.31
	Fel d1 – cat	89						
	(ng/g)		3 579.09	21 653.90	119.82	12.98	4.13	201 992.54
	(ng/m^2)		258.46	494.04	31.29	11.82	2.00	3361.33
	Can f1 – dog	89						
	(ng/g)		1 115.63	4 073.05	288.88	3.39	19.49	30 018.91
	(ng/m^2)		594.23	2 755.41	75.44	5.73	5.00	22 403.71
	Bla g1 – cockroach	89						
	(U/g)		3.51	10.55	0.72	4.36	0.08	71.34
	(U/m^2)		0.30	0.38	0.19	2.57	0.02	2.52
Bed	Der p1 – mite (ng/g)	51	19 841.50	83 727.97	597.80	14.25	12.46	540 154.20
	Fel d1 – cat (ng/g)	51	436.10	1 971.11	54.15	4.37	4.77	13 151.49
	Can f1 – dog (ng/g)	51	301.73	424.16	158.63	3.14	20.49	2 516.49
	Bla g1 – cockroach (U/g)	51	0.96	1.08	0.60	2.92	0.04	6.16

Abbreviations as in Table 1 and 2.

all samples of settled dust from the floors in investigated homes. In the case of the dust coming from the children's beds this percentage reached 41%. In all samples of dust analyzed concerning the four selected allergens, both from the floor and from the bed, at least one of these proteins was found, in the amount above the detectability level.

The study showed that in homes where carpet was laid on the greater part or on the whole floor, the concentrations of all analyzed allergens in the dust from the floor were significantly higher than in homes with smooth floors ($p < 0.05$). However, in case of Der p1, Fel d1 and Bla g1 present in the children's beds, their significantly higher concentrations were found in homes with smooth floors ($p < 0.05$).

Furthermore, significantly higher concentrations of Can f1 were found in premises where there was a dog or whose inhabitants were in contact with this animal outside their homes. No such correlation was indicated in the case of Fel d1.

The analysis of the correlations between the investigated factors showed a significant ($p < 0.05$) positive correlation of the concentrations of all allergens determined in the settled dust (Der p1, Fel d1, Can f1 and Bla g1) expressed in ng or U per 1 m^2 of the floor with the concentrations of this dust. Besides, significant positive correlations were shown between the concentrations of Der p1, Fel d1 and Can f1 (also expressed per 1 m^2 of the floor) with the concentration of (1→3)-β-D-glucans, and between the concentrations of Der p1 and Can f1 allergens with the concentrations of endotoxins. The analysis of the correlation of the concentrations of investigated allergens expressed per 1 g of dust with the other factors showed much lower levels than in case of those expressed per m^2. It has been demonstrated that the concentrations of Bla g1 determined in the bed significantly negatively correlate with the dust concentration. Moreover, the concentrations of Fel d1 and Can f1, determined in the dust from the beds, significantly positively correlated with the concentrations of these proteins indicated in the settled dust collected from the floor. The concentration of mite (Der p1) and dog (Can f1) allergens analyzed in the dust from the beds correlated significantly positively with relative humidity, but the level of significance of this correlation was rather low.

Detailed results of the analysis of the correlations between the investigated variables are presented in Table 4 and Table 5.

The analysis of concentrations of determined factors in relation to the examined characteristic features shows a significant influence only for the type of floor and presence the of cats and dogs. Detailed results are presented in Table 6.

Table 4. Correlation analysis between the studied variables (Spearman's rank correlations coefficient) – settled dust (floor), (1→3)-β-D-glucans, endotoxins and selected allergens on the floor

Variable	Spearman's rank correlations coefficient									
	1	2	3	4	5	6	7	8	9	10
1. SD_G										
2. $SD_{E/A}$	**0.81***									
3. G	**0.78***	–								
4. E		**0.57***	**0.37***							
5. AD	–	**0.57***	**0.31***	**0.33***						
6. AF	–	**0.33***	**0.27***	0.20	0.14					
7. AC	–	**0.80***	**0.51***	**0.45***	**0.35***	**0.28***				
8. AB	–	**0.41***	0.10	0.16	**0.24***	**0.36***	**0.31***			

Table 4. Correlation analysis between the studied variables (Spearman's rank correlations coefficient) – settled dust (floor), (1→3)-β-D-glucans, endotoxins and selected allergens on the floor – cont.

Variable	Spearman's rank correlations coefficient									
	1	2	3	4	5	6	7	8	9	10
9. T	–0.05	0.03	–0.06	–0.08	–0.08	–0.15	0.01	–0.16		
10. RH	0.05	0.09	0.05	0.03	0.17	–0.15	0.13	–0.20	**–0.36***	
11. CO_2	0.07	0.07	0.13	–0.13	–0.01	< 0.01	**0.22***	–0.02	–0.14	**0.56***

SD_G – settled dust for the analysis of (1→3)-β-D-glucans.
$SD_{E/A}$ – settled dust for the analysis of endotoxins and allergens.
G – (1→3)-β-D-glucans; E – endotoxins; AD – allergen Der p1 (mite); AF – allergen Fel d1 (cat); AC – allergen Can f1 (dog); AB – allergen Bla g1 (cockroach).
T – temperature; RH – relative humidity; CO_2 – concentration of CO_2.
* $p < 0.05$.
"–" – lack of justification for calculation.

Table 5. Correlation analysis between the studied variables (Spearman's rank correlations coefficient) – settled dust (bed) and selected allergens on the floor and in the bed

Variable	Spearman's rank correlations coefficient								
	1	2	3	4	5	6	7	8	9
1. $SD_{A, B}$									
2. AD_F	–								
3. AF_F	–	0.15							
4. AC_F	**–**	–0.07	0.09						
5. AB_F	–	**0.35***	**0.43***	0.15					
6. AD_B	–0.18	**0.52***	0.08	–0.13	0.02				
7. AF_B	–0.16	0.10	**0.37***	< 0.01	0.25	0.04			
8. AC_B	0.13	0.06	–0.04	**0.64***	0.06	–0.04	0.21		
9. AB_B	**–0.76***	–0.07	–0.26	**–0.38***	–0.01	0.19	0.04	–0.18	
10. T	–0.14	**–0.26***	–0.18	–0.01	–0.04	–0.15	0.10	**–0.35***	0.09
11. RH	0.09	0.14	–0.18	0.05	–0.19	**0.28***	–0.15	**0.28**	–0.05
12. CO_2	0.10	–0.03	0.01	**0.24***	–0.05	0.26	**–0.29***	0.13	–0.23

$SD_{A,B}$ – settled dust in the bed for the analysis of allergens.
AD_F – allergen Der p1 (mite) in the settled dust on the floor.
AF_F – allergen Fel d1 (cat) in the settled dust on the floor.
AC_F – allergen Can f1 (dog) in the settled dust on the floor.
AB_F – allergen Bla g1 (cockroach) in the settled dust on the floor.
AD_B – allergen Der p1 (mite) in the settled dust in the bed.
AF_B – allergen Fel d1 (cat) in the settled dust in the bed.
AC_B – allergen Can f1 (dog) in the settled dust in the bed.
AB_B – allergen Bla g1 (cockroach) the in settled dust in the bed.
Other abbreviations as in Table 4.

Table 6. Correlation between the characteristics of the homes under the study (N = 50) and concentrations of the analyzed factors

Characteristics	Settled dust		Endotoxins		(1→3)-β-D-glucans		Cat alergen (Fel d1)			Dog alergen (Can f1)			Mites alergen (Der p1)			Cockroach alergen (Bla g1)			
	floor		floor		floor		floor	bed		floor	bed		floor	bed		floor	bed		
	GM (GSD) (g/m^2)	p	GM (GSD) (EU/m^2)	p	GM (GSD) (ng/m^2)	p	GM (GSD) (ng/m^2)	GM (GSD) (ng/g)	p	GM (GSD) (ng/m^2)	GM (GSD) (ng/g)	p	GM (GSD) (ng/m^2)	GM (GSD) (ng/g)	p	GM (GSD) (U/m^2)	GM (GSD) (U/g)	p	
Type of building		**≤ 0.01**		0.44		0.18			0.18			0.06			0.92			0.12	
block of premises	0.27 (3.97)		789.2 (3.6)		6 932.4 (2.3)		54.0 (14.4)	253.4 (17.5)		86.5 (4.1)	354.3 (1.8)		9.9 (2.2)	39.3 (4.3)		0.19 (2.67)	0.75 (6.45)		
block with four floors	0.36 (2.75)		1292.9 (3.5)		6 012.3 (2.7)		43.9 (12.1)	134.1 (9.0)		81.1 (4.4)	209.4 (2.8)		36.6 (10.3)	104.8 (7.1)		0.20 (2.88)	0.53 (3.15)		
tenement house	0.11 (6.61)		621.0 (9.8)		3 482.9 (2.8)		31.1 (6.5)	288.3 (7.2)		57.7 (6.6)	386.0 (4.7)		31.2 (12.6)	227.9 (5.0)		0.31 (2.24)	3.08 (6.00)		
detached house	0.19 (3.62)		948.4 (4.0)		4 110.6 (2.9)		12.3 (9.5)	63.4 (16.1)		62.9 (11.8)	265.4 (5.7)		28.9 (8.7)	138.7 (6.5)		0.16 (2.09)	0.83 (3.43)		
Inhabitants (n)		0.87		0.68		0.90			0.97			0.80			0.99			0.78	
3	0.27 (4.08)		1 132.6 (3.3)		5 554.2 (2.8)		33.3 (9.9)	142.5 (12.4)		73.9 (4.5)	256.9 (3.0)		21.9 (6.8)	81.4 (6.7)		0.17 (2.60)	0.64 (4.13)		
4	0.26 (3.43)		866.7 (4.8)		5 179.5 (2.7)		29.8 (15.4)	113.6 (14.7)		76.3 (8.1)	277.6 (3.9)		32.6 (9.6)	120.3 (6.0)		0.22 (2.62)	0.84 (4.85)		
5	0.21 (1.90)		961.4 (4.7)		4 603.1 (2.1)		25.4 (11.2)	194.2 (4.8)		83.4 (2.5)	352.3 (3.3)		8.0 (2.9)	36.6 (3.4)		0.15 (1.91)	0.72 (3.06)		
		0.32		0.16		0.49			0.56										

Condensation of water vapor during the winter on the windows and / or other surfaces		0.07																			
yes	0.32 (3.83)		1058.5 (3.8)	0.74	6 325.3 (2.7)	0.15	43.9 (11.6)	0.25	152.5 (11.3)	0.51	103.8 (4.5)	0.13	294.5 (3.2)	0.42	35.5 (10.7)	0.13	102.9 (8.2)	0.57	0.19 (3.00)	0.34	0.59 (4.32)	0.29
no	0.22 (3.51)		958.4 (4.2)		4 670.6 (2.6)		24.0 (11.9)		116.3 (14.3)		58.8 (6.7)		252.8 (3.5)		18.4 (5.7)		87.9 (5.1)		0.19 (2.26)		0.85 (4.40)	
Visible signs of moisture		0.18																			
yes	0.15 (6.70)		1 352.2 (5.7)	0.66	9 201.9 (1.6)	0.18	67.3 (6.9)	0.44	413.6 (12.0)	0.15	61.2 (7.6)	0.73	207.7 (1.2)	0.51	69.7 (31.0)	0.21	229.1 (7.7)	0.18	0.23 (3.22)	0.47	1.13 (3.37)	0.61
no	0.28 (3.56)		1 046.0 (3.8)		5 230.2 (2.7)		27.3 (12.5)		110.2 (13.1)		80.9 (5.6)		278.9 (3.6)		23.9 (7.1)		91.7 (6.4)		0.19 (2.48)		0.70 (4.21)	
Visible signs of mold		**≤ 0.01**																			
yes	0.07 (2.64)		613.7 (7.3)	0.30	3 768.1 (2.6)	0.30	66.4 (5.5)	0.40	1 466.9 (6.3)	**≤0.01**	16.6 (12.1)	**≤0.01**	189.6 (3.6)	0.26	5.0 (1.0)	**0.02**	95.6 (2.7)	0.99	0.18 (2.46)	0.76	3.95 (4.47)	**≤0.01**
no	0.30 (3.52)		1 050.6 (3.7)		5 520.7 (2.7)		29.0 (12.5)		104.7 (12.2)		87.6 (5.0)		278.2 (3.4)		28.7 (8.1)		93.7 (6.7)		0.19 (2.59)		0.63 (4.00)	
The frequency of cleaning floors (per/ week)		0.46																			
1	0.24 (3.71)		689.9 (3.7)	0.22	4 794.1 (2.4)	0.77	81.9 (13.0)	**≤0.01**	426.1 (17.2)	**≤0.01**	70.8 (7.9)	0.95	329.9 (4.0)	0.13	11.3 (3.2)	**0.02**	53.7 (4.2)	**≤0.01**	0.19 (2.55)	0.69	0.89 (4.27)	0.48

Table 6. Correlation between the characteristics of the homes under the study (N = 50) and concentrations of the analyzed factors – cont.

Characteristics	Settled dust		Endotoxins		(1→3)-β-D-glucans		Cat alergen (Fel d1)				Dog alergen (Can f1)				Mites alergen (Der p1)				Cockroach alergen (Bla g1)			
	floor		floor		floor		floor		bed		floor		bed		floor		bed		floor		bed	
	GM (GSD) (g/m²)	p	GM (GSD) (EU/m²)	p	GM (GSD) (ng/m²)	p	GM (GSD) (ng/m²)	p	GM (GSD) (ng/g)	p	GM (GSD) (ng/m²)	p	GM (GSD) (ng/g)	p	GM (GSD) (ng/m²)	p	GM (GSD) (ng/g)	p	GM (GSD) (U/m²)	p	GM (GSD) (U/g)	p
2	0.33 (2.96)		1 047.1 (3.0)		5 563.4 (3.2)		10.9 (7.3)		36.9 (9.8)		66.4 (4.1)		193.9 (2.2)		27.6 (3.8)		85.7 (3.4)		0.18 (2.18)		0.56 (3.75)	
> 2	0.24 (4.13)		1 223.2 (4.6)		5 671.4 (2.8)		26.1 (11.2)		103.5 (6.8)		77.0 (5.3)		259.3 (3.5)		43.0 (14.4)		174.7 (9.2)		0.19 (2.92)		0.77 (4.87)	
Animals at home		**≤ 0.01**																				
yes	0.12 (3.98)		601.6 (5.3)	0.06	3 909.9 (2.5)	0.11	27.0 (16.5)	0.80	258.9 (20.8)	0.06	42.5 (5.2)	0.10	363.1 (2.1)	0.09	7.0 (1.9)	**≤0.01**	64.0 (3.8)	0.14	0.21 (2.79)	0.61	1.81 (6.94)	**≤0.01**
no	0.32 (3.32)		1 160.3 (3.5)		5 837.1 (2.7)		32.7 (10.9)		106.8 (10.8)		89.1 (5.7)		247.2 (3.7)		35.2 (8.8)		104.9 (7.0)		0.18 (2.52)		0.56 (3.34)	
Contact with a dog		0.64		0.74		0.82		0.69		0.48		**≤ 0.01**		**≤ 0.01**		0.44		0.11		0.82		0.83
yes	0.28 (4.29)		1 062.2 (4.1)		5 181.5 (2.9)		34.7 (15.8)		155.4 (14.9)		148.3 (6.0)		568.4 (4.1)		29.9 (10.6)		124.5 (8.9)		0.19 (2.57)		0.72 (4.10)	
no	0.25 (3.33)		961.5 (3.9)		5 440.9 (2.5)		29.2 (9.7)		117.1 (11.9)		47.7 (4.8)		171.0 (2.2)		21.4 (6.2)		79.0 (4.9)		0.19 (2.59)		0.73 (4.61)	
Contact with a cat		0.11		0.17		0.12		0.15		**≤0.01**		0.74		**0.04**		0.59		0.19		0.51		0.55
yes	0.21 (3.83)		1 324.3 (4.0)		4 253.1 (2.8)		52.3 (15.3)		286.5 (18.4)		81.4 (9.2)		352.7 (4.6)		28.5 (11.2)		122.2 (6.9)		0.18 (2.35)		0.86 (4.06)	

no	0.29 (3.57)	868.3 (3.9)	5 985.7 (2.6)	24.1 (10.1)	88.2 (9.8)	72.6 (4.3)	235.9 (2.8)	22.7 (6.4)	82.4 (6.0)	0.20 (2.69)	0.67 (4.56)
Contact with animals outside the home	0.92	0.97	0.42	0.15	**0.03**	**0.02**	**≤ 0.01**	0.18	**0.03**	0.84	0.61
yes	0.26 (4.32)	1 028.1 (3.8)	5 014.1 (2.9)	48.8 (14.6)	218.5 (13.7)	122.2 (6.4)	459.4 (4.0)	33.9 (10.7)	129.9 (8.2)	0.18 (2.50)	0.73 (3.82)
no	0.27 (3.23)	1 040.5 (4.2)	5 952.8 (2.4)	23.7 (9.2)	92.8 (11.9)	51.4 (4.9)	174.6 (2.4)	18.7 (5.8)	70.3 (4.9)	0.20 (2.65)	0.73 (5.08)
Type of floor	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	**≤ 0.01**	0.18	**≤ 0.01**	0.12	**≤ 0.01**	**0.04**	0.16	**≤ 0.01**
smooth	0.08 (3.47)	319.3 (4.3)	2 180.3 (2.2)	10.8 (9.7)	191.3 (14.5)	13.3 (3.2)	218.4 (3.1)	8.1 (2.1)	144.2 (3.0)	0.15 (2.58)	2.20 (5.60)
covering	0.45 (2.50)	1 656.4 (2.7)	7 807.1 (2.2)	48.2 (11.4)	106.4 (12.1)	162.9 (4.0)	299.2 (3.5)	41.0 (9.5)	77.5 (8.1)	0.21 (2.54)	0.43 (2.67)

GM – geometric mean.
GSD – geometric standard deviation.
Bold: $p < 0.05$.

DISCUSSION

The microclimate inside the examined children's homes was characterized by moderate average values of air temperature and humidity, similar to the values of these parameters obtained at children's homes by other authors [19]. On the other hand, the mean value of the CO_2 concentration in the indoor air of the examined children's homes exceeded 1000 ppm, above which the quality of air in the premises where people stay is considered as low [20]. Presumably, this could be associated with poor movement and exchange of air in premises, as the air flow velocity in the investigated premises did not exceed the level of 0.02 m/s.

The cleanness of residential premises may significantly affect the inhabitants' health, because the settled dust particles may be the carriers of various biological contaminants (e.g. endotoxins, (1→3)-β-D-glucans, allergens). Because of the inhabitants' activity, the exposure to these agents is usually associated with rising of settled dust particles into the air from where they may be breathed in by people [21]. The average content of settled dust expressed in 1 m^2 of the floor, found at the investigated homes, was slightly higher or comparable to the mean values indicated by other authors in other European countries [22]. The values obtained by Douwes et al. [23] and Gehring et al. [19] in earlier studies carried out in Germany were higher than those obtained by us.

The study revealed a very high positive correlation between the amount of the settled dust collected from the floor and the concentrations of (1→3)-β-D-glucans, dog allergens (Can f1), endotoxins and mite allergens (Der p1) expressed per 1 m^2 of the surface. Similar correlations for selected agents (mainly (1→3)-β-D-glucans and endotoxins) were also observed by other authors [22,23,19].

Furthermore, the study showed that the settled dust loading of floor expressed per m^2 was significantly modified by the type of floor and the floor covered with a carpet was more conducive for the accumulation of dust on its surface than the smooth floor.

In comparison with other studies, we found that the mean concentration of endotoxins in Polish houses was similar to those obtained in Germany [24] and Croatia [25]. The mean levels of endotoxins found in the USA and in Germany were slightly higher than ours [26–30]. Slightly lower concentrations were observed in several studies from Europe, New Zealand, and Canada [23,31–35]. Our results indicate that the concentration of endotoxins present in settled dust depends significantly on the type of floor. Higher levels of this agent were found in the dust from carpest than in the dust from smooth floors. The results presented by us correspond well with the results showed by others authors [26,36,37].

Considering the dual effect of endotoxin (protective and asthma-enhancing described in the Introduction), we should take into account the latest findings stating that endotoxins are likely to exacerbate the response and inflammation in already sensitized individuals [38]. Children under our study were too young to diagnose allergy from the upper airways. We could only compare the concentrations found in our study with other studies conducted in children's homes. Significant differences between urban and rural environment were noticed [39]. It therefore seems that endotoxins concentrations demonstrated by us are too low to protect children in the way defined in the "hygienic hypothesis".

Researchers from the United States who used the Glucatell test, just as we did, obtained slightly higher, compared to ours, concentrations of (1→3)-β-D-glucans expressed in nanograms per 1 g of dust. They also noted the concentration one order of value higher than the average concentration of this agent obtained by us, expressed in nanograms per 1 m^2 of the floor [30]. Also, the mean level of (1→3)-β-D-glucans presented by Canadian authors, who used the above-mentioned test for their analysis, was by one order of value higher than the one obtained in this study [35].

The geometric mean values of (1→3)-β-D-glucans in our study were by two orders of magnitude lower than

those reported by others authors for homes in Europe [19,22,23,32,33,40], but the concentrations of (1→3)-β-D-glucans in these studies were determined with a specific inhibition enzyme immunoassay (EIA).

Considering the correlations between the investigated agents, it was found – similarly to several other authors – that there is a significant correlation between the concentrations of endotoxins and (1→3)-β-D-glucans present in the settled dust [22,30,41]. Furthermore, Gehring et al. demonstrated that endotoxins strongly correlated with the levels of the settled dust collected from 1 m^2 of the surface, which confirms our results [22].

Up to date, there have been no legally recommended values or standards of exposure to bacterial endotoxins measured in the settled dust. So far, the proposed reference values refer only to the concentration of airborne endotoxins.

Significant correlations obtained by Gehring et al. between the concentrations of (1→3)-β-D-glucans and the levels of endotoxins and allergens of cats and mites present in the dust collected from 1 m^2 of the floor confirm the correlations obtained in our analysis [19]. Also, analyses made in several European countries confirm the high positive correlations which we presented between the concentrations of (1→3)-β-D-glucans and the concentrations of the settled dust, obtained after vacuuming a square metre of the floor in the investigated premises [22]. Douwes et al., Gehring et al. and Giovannangelo et al. reported that the concentrations of (1→3)-β-D-glucans were higher in the dust from carpets than in the dust from smooth floors, which confirms our results [19,37,42].

Similarly to the case of endotoxins, also for (1→3)-β-D-glucans, there are no standards, recommended levels of exposure concerning the settled dust or even reference values for the air.

The range indicated in the study regarding the allergens concentrations of mites (Der p1), cat (Fel d1), dog (Can f1) and cockroach (Bla g1) present in the settled dust collected from the floor in the examined rooms should be considered as quite wide. The geometric means and maximum values of the concentrations of the dog and cat allergens in our study were higher in the case of the dust collected from the floor, as compared to the dust from the beds. On the other hand, in the case of the allergens of mites (Der p1), it was indicated that both the geometric mean value and the maximum value were higher in the dust collected from the bed.

While analyzing the concentrations of allergens obtained by other researchers in similar studies it must be noted that the results vary depending on the country and the test sample. Comparing them to the allergen concentrations reported in our study, it should be noted that the average values donot correspond well with the results of similar works by other authors [19,43]. Topp et al., in the research conducted over a few years in Germany, indicated in the dust both from the floor and the bed lower concentration values, compared to ours, for Der p1, but higher for Fel d1 [31]. Stelmach et al. conducted assessment studies on the prevalence of allergy and exposure to cockroach allergen Bla g2 in children from Łódź. In this paper, it was demonstrated that among children from the Łódź, allergy to cockroaches is more common than in children from other European countries. Moreover, some authors found high concentrations of protein Bla g2 in children' homes (in kitchens and bedrooms) [44,45]. Majkowska-Wojciechowska et al. also conducted a survey of children from the city of Łódź and showed a high incidence of allergies, compared with children from rural areas [46]. Analyzing the impact of the level of exposure to allergens present in the home environment, Wardzyńska et al. showed no linear relationship between the detected levels of the allergens present in the settled dust and the incidence of allergies in children. Most probably, that fact should be associated with the simultaneous exposure to other factors that modulate the children's immune response, such as (1→3)-β-D-glucans and endotoxins [14].

Literature presents two reference values for each allergen, established in empirical studies. The first, lower value (defined as the moderate level) is the concentration of the allergen above which the sensitized people exhibit allergic symptoms, and the other, higher value (referred to as the high level) is the concentration above which allergic people exhibit aggravated asthma. Allergens concentrations are expressed per 1 g of dust and are showed in Table 7. The average concentrations of the allergens which we examined, expressed by the geometric mean both in the dust, from the floor and from the bed, were much lower and did not exceed any of these levels. However, the analysis of individual results obtained for the premises indicated that in several cases, these values were exceeded. In the case of the allergenic protein Der p1 (mites), these are the following values respectively: ≥ 2000 ng/g and ≥ 10 000 ng/g [47,48]. The moderate level for Der p1 was exceeded in 5 premises (10%) in the dust from the floor and in 17 premises (34%) in the dust from the children's beds. Exceeded high levels were noted in the case of 1 home (2%) for the dust from the floor and 11 premises (22%) in the dust from the children's beds.

According to the literature data, the moderate level and the high level of the cat allergen (Fel d1) reach respectively ≥ 1000 ng/g and ≥ 8000 ng/g [47,49,50]. However, in 11 premises (22%) the moderate level was exceeded in the dust from the floor and in 2 premises (4%) in the dust from the children's beds. The high level was exceeded in 3 premises (6%) in the dust from the floor and in 1 home (2%) in the dust from the bed.

In the case of the dog allergen (Can f1), the concentrations defined as the moderate level and the high level reach respectively: ≥ 2000 ng/g and ≥ 10 000 ng/g [47,49,50]. The moderate level was exceeded in 4 premises (8%) in the dust from the floor and in 1 home (2%) in the dust from the bed. The high level was exceeded only in the dust from the floor in 2 premises (4%).

The literature values of the moderate level and the high level for the cockroach allergen (Bla g1) reach respectively: ≥ 1 U/g and ≥ 4 U/g [47]. The moderate level was exceeded in 20 premises (40%) in the dust from the floor and in 14 premises (28%) in the dust from the children's beds. The high level was exceeded in the case of 8 premises (16%) in the dust from the floor and in 1 home (2%) in the dust from the bed.

Leaderer et al., in a similar study carried out in the United States, checking the dust from the floor, indicated a higher, as compared to ours, percentage of premises with exceeded reference levels, both the moderate and the high level, for the mite allergen (Der p1) [47]. In the case of Fel d1 (cat), the percentage of exceeded moderate level was similar to the one indicated in our study, but the high level was exceeded in more premises than in our results. The differences refer also to the percentage of exceeded values for the dog and cockroach allergens, and Leadere et al. pointed to more, compared to our findings, premises with exceeded levels for the dog allergen (Can f1) and fewer for the cockroach allergen (Bla g1).

Table 7. The reference values of selected allergens [40,41]

Allergen	ML	HL
Fel d1 (ng/g)	≥ 1 000	≥ 8 000
Can f1 (ng/g)	≥ 2 000	≥ 10 000
Der p1 (ng/g)	≥ 2 000	≥ 10 000
Bla g1 (U/g)	≥ 1	≥ 4

ML – moderate level of the allergen (the concentration of the allergen above which the sensitized people exhibit allergic symptoms).
HL – high level of the allergen (the concentration above which allergic people exhibit aggravated asthma).
Fel d1 – cat allergen; Can f1 – dog allergen; Der p1 – mite allergen; Bla g1 – cockroach allergen.

Taking into account the reference value one needs to recognize that, with the exception of a few individual cases of homes, the exposure of children to allergens Can f1, Fel d1 and Der p1 was low. Only in the case of the cockroach allergen (Bla g1), relatively high concentrations were found. Comparing the concentrations of all analyzed allergens in the settled dust from the floors and the beds we demonstrated that in homes with a carpet, there were higher levels of allergens on the floor and in homes without carpeting higher concentrations of allergens Fel d1, Der p1 and Bla g1 were found in the bed. Such relationships were not found in the case of the dog allergen (Can f1). Most probably, dog owners clean up the floor more often and therefore smooth floor does not contribute to increased levels of the dog allergen in the bed.

We also demonstrated the significant correlation between the concentrations of Can f1 and Fel d1 on the floor and in the bed (irrespective of the type of floor). Chew conducted a review of the literature on exposure to the cockroach allergen and found that in this case the carpeting plays a protective role because children wipe their feet before they go into bed [51]. It is possible that the presence of carpet influences the reduction of allergens levels in the bed. We demonstrated this relation also in case of the mite allergen (Der p1), but it is not consistent with the prior findings that carpeting increases the risk of high levels of mite allergens [52].

The study included 50 children aged 1–2 years, too young to undergo the asthma diagnosis process. Therefore, it was impossible to analyze the correlation between the concentrations of allergens, endotoxins, (1→3)-β-D-glucans and the prevalence of symptoms and allergic diseases in children. The follow-up of the cohort under study will ensure the possibility of conducting such analysis in the near future.

Based on the results of our study and literature [51,52] it is possible to recommend a few protective measures to reduce the risk of allergy. The first is to be careful while resigning from the carpeting and to use the vacuum cleaner with High Efficiency Particulate Air (HEPA) filter. The bedding should be frequently washed and the smooth floor should be frequently wet cleaned. Animals should not sleep in children bed. However, according to Custovic et al., no single primary prevention strategy will be applicable to all children, but only to those with particular genetic susceptibility [53].

CONCLUSIONS

Although the number of homes under the study was too limited to formulate general conclusions, the obtained results point to the following conclusions:

1. Among the analyzed factors characteristic for the children's homes, only the type of floor significantly modified the level of biological indicators such as endotoxins and (1→3)-β-D-glucans. In the rooms where the floor was completely or partly covered with a carpet, the concentrations of both of them were higher in comparison to those in the rooms with smooth floors.
2. Also, the type of floor significantly modified the level of allergens. The presence of carpets increased their concentrations in the settled dust on the floor. The smooth floor was associated with higher concentrations of allergens (except the dog one) in the bed.
3. On average, the concentrations of cat, dog and cockroach allergens in the settled dust collected from the floor were higher than from the bed, but in the case of the mite allergen, higher average concentrations were found in the dust from the beds.
4. The average concentrations of the analyzed allergens (except the cockroach one) did not exceed the reference values given in literature, but in many individual cases of children's homes the values exceeded the moderate reference level for at least one allergen (38% in the floor dust and 40% in the bed).

5. The average concentrations of the cockroach allergen on the floor (3.5 U/g) was close to the high reference value given in literature, which can aggravate asthma (4 U/g).
6. The results of this study could be the base for verifying the hypothesis that the carpeting may have a protective role against high levels of cockroach, dog and cat allergens.

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