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Airborne Allergens, Endotoxins, and Particulate Matter in Elementary Schools, Results from Germany (LUPE 2)

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Allergic disorders are the most common childhood-related chronic diseases in developed countries. It is essential to assess the exposure, especially in schools, where children spend a large portion of their time. We aimed to investigate allergen and endotoxin levels in the air of schools and to observe seasonal variations of these factors. We evaluated airborne concentrations of house dust mites allergens (Der p 1, Der f 1), cat allergen (Fel d 1), and endotoxin in PM₁₀ in 14 classrooms during the school days in the region of Munich, each over 20 consecutive days and in 1 classroom over the course of a year (at 83 days); we also tested outdoor air close to the schools. Endotoxin levels were quantified using two different analytical methods. In addition, indoor air climate parameters were measured. The median daily indoor CO₂ and PM₁₀ concentrations in the classrooms ranged from 423 to 3,135 ppm (median: 1,211 ppm) and 9 to 390 µg/m³ (median: 127 µg/m³), respectively.

Fel d 1 in the PM₁₀ samples was the most frequently detected allergen, with levels from 0.02 to 1.15 ng/m³ in a total of 301 samples (median: 0.19 ng/m³, 95th percentile: 0.57 ng/m³). Der p 1 and Der f 1 were detected in only 51% and 19% of the samples, with 95th percentiles at 0.5 and 0.3 ng/m³. Endotoxin levels in the PM₁₀ and inhalable dust samples ranged from 0.5 to 84.1 EU/m³ (median: 15.3 EU/m³; 95th percentile: 58.2 EU/m³) and from 0.03 to 115 EU/m³ (median: 8.4 EU/m³; 95th percentile: 27.9 EU/m³). Fel d 1 and endotoxin were found in higher levels in the winter months. The results of the two different indoor sampling techniques for endotoxin were statistically significantly correlated. The results of airborne allergens indicate a generally low exposure level in classrooms. With regard to endotoxin, our study showed higher levels in schools compared with residences.

Keywords indoor, schools, endotoxin, cat allergen, PM₁₀

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INTRODUCTION

Asthma and related allergic disorders are the most common chronic diseases in childhood in the Western world, with increasing prevalence during recent decades.⁽¹⁾ Although there is a known and generally accepted genetic component to allergy susceptibility, various environmental factors also contribute to the development and severity of these diseases. One of these additional factors is the indoor environment. There is evidence that exposure to allergens, such as mite dust and pet dander, increases the risk of an IgE response, sensitization rates, and the prevalence of symptoms.^(2–6) Nevertheless, there are still conflicting reports regarding a relationship between early allergen exposure and the development of asthma.^(7–11)

Potential health problems arising from indoor allergen exposure are often related to the modern lifestyle, e.g., living in warm houses with extensive furnishings and poor ventilation and the fact that people spend more than 90% of their time inside and a considerable portion of their time in school spaces (approximately 30%). Even though pets are not allowed in schools, various studies have found pet allergens in these locations and have suggested that the number of pupils keeping a pet at home is correlated with the amount of allergens found

in classroom dust.^(6,12–14) The transport of Fel d 1 on clothing from domestic to school environments is a major source of classroom cat allergens.^(13,15)

Better knowledge of the characteristics of indoor allergens in school environments is critical for understanding the risks of exposure and developing adequate prevention measures. Generally, sampled levels of allergens have been found to be highly variable over time and space, and whether a single sample is representative of the exposure risk is still a matter of debate.⁽¹⁶⁾ Some studies have described the aerodynamic properties of allergen-containing particles and their distribution between settled and airborne dust in more detail. A correlation was observed between air and dust concentrations of Fel d 1, but not Der p 1.^(17–22) The different particle-binding characteristics of these allergens could explain this observation. Der p 1 is strongly bound to coarse particles and therefore settles rapidly after resuspension from surfaces, while Fel d 1 is bound to fine particles and thus remains airborne for longer periods.^(23,24)

Endotoxin is a well-known component of the outer layer of gram-negative bacteria and consists mainly of lipopolysaccharides (LPS). LPS is known to initiate Th1 (or innate immune) responses, with the production of IFN- γ (interferon- γ) causing neutrophilic inflammation.⁽²⁵⁾ Endotoxin shows dichotomous effects in relation to allergic diseases. During a brief time window in early childhood, exposure to high levels of endotoxin is thought to be protective against the development of atopy and asthma in children.^(26,27) On the other hand, the presence of allergens could also exacerbate asthma symptoms.⁽²⁸⁾ Nevertheless, inhaled endotoxin can induce asthma-like bronchial reactivity and airway inflammation in healthy subjects and asthmatics, and co-exposure with al-

lergen markedly augments inflammatory response compared with those observed with either stimulus alone.⁽²⁹⁾

We have previously investigated the prevalence of volatile organic compounds, allergens in settled dust, and particle fractions in the air of schools in southern Germany.^(30,31) The main objective of the current study was to generate exploratory results of allergens from cats (*Felis domesticus*, Fel d 1) and house dust mites (*Dermatophagoides pteronyssinus* and *farinae*, Der p 1 and Der f 1), as well as endotoxin (LPS) in the air of German classrooms. In addition, we present a comparison of the results from the two different sampling techniques and analytical methods used to detect endotoxin. The investigation (LUPE 2) is part of the LUPE (Länderuntersuchungsprogramm) projects.

MATERIALS AND METHODS

Sampling Location

Schools in Munich and the surrounding areas and in the city of Berlin were identified and contacted by phone to request their participation in the study. As can be seen from Table I, seven schools in the region of Munich (school A–G) and one school in Berlin (H) were included after the school managers agreed to participate. All buildings were constructed between 1901 and 1999 and hosted elementary schools. Each of the 15 classrooms (second to third grade) were covered with smooth floorings and had sizes and volumes from 59 to 79 m² and 183 to 313 m³, respectively. All classrooms were naturally ventilated. No special recommendations for ventilation conditions were given to the room users. As is not uncommon in Germany, all classrooms were fitted with curtains in front of the windows,

TABLE I. Basic Characteristics of Schools and Classrooms

School	Room	Construction [year]	Flooring	Room size [m ²]	Room volume [m ³]	Curtains	Cozy corner	Sampling period
Munich								
A	1	1976	linoleum	59	187	Yes	Yes	November
	2			59	187	Yes	Yes	December
B	1	1972	linoleum	72	237	Yes	Yes	June
	2			72	236	Yes	Yes	July
C	1	1963	linoleum	71	228	Yes	No	October
	2			72	243	Yes	No	November
D	1	1991	linoleum	59	183	Yes	No	November
	2			62	199	Yes	No	December
E	1	1901	linoleum	79	311	Yes	Yes	January
	2			79	313	Yes	Yes	February
F	1	1964	linoleum	69	218	Yes	Yes	February
	2			69	218	Yes	Yes	March
G	1	1965	linoleum	72	227	Yes	Yes	June
	2			72	226	Yes	Yes	July
Berlin								
H		1999	linoleum	69	206	Yes	No	Whole year

and in all except five cases, the rooms contained a cozy corner with a sofa and various types of toys and books. Nevertheless, there were some differences between the schools, especially in terms of the current cleaning arrangements. Normally, the rooms were cleaned two to five times a week.

Sampling and Analysis

Indoor air samples were collected in 14 classrooms in the Munich area (two in each school), during the school day over a period of 20 consecutive days. Due to malfunction of the sampling pumps or electrical problems some parameters could not be measured every day. Measurements were not taken on the weekends. Outdoor air samples were available from 5 of the 7 schools and were collected directly in front of the classroom windows. In Berlin, one classroom was investigated on 83 school days over the course of a year. The sampling and measurement position in each classroom was located opposite the black board, approximately one meter above floor level, i.e., the level at which the pupils would normally inhale. This was chosen as a typical location inside the room because it was away from the door, thus avoiding disturbances resulting from air currents. Teachers were asked to document the number of pupils in the room.

Indoor carbon dioxide (CO₂) concentrations as indicator of indoor air quality were analyzed using a continuously monitoring infrared sensor (Testo 445, Lenzkirch, Germany). The instrument was programmed for a one-minute data logging interval. Calibration of the CO₂ sensors involved linearity checks at four concentrations (0, 350, 1000, and 2000 ppm) during the weeks prior to sampling. Indoor humidity and temperature were measured simultaneously with a separate sensor connected to the Testo instrument.

Endotoxin and Allergens in PM₁₀

Over the teaching hours (5.5 h/day) filter-based measurements of PM₁₀ in the classrooms were conducted using a medium-volume sampler with a flow-controlled pump (Derenda, Stahnsdorf, Germany) working at a constant flow of 2.3 m³/hour. The sample inlet was a PM₁₀ sampler with a 50% collection efficiency for particles with a 10 μm aerodynamic diameter. Sample volumes were calculated from elapsed time indicators and flow measurements before sampling with calibrated rotameters. A 47-mm Teflon filter (Berlin: binder-free glass fiber filter [MN 85/90BF]) with a pore size of 2 μm was purchased from Pall Corporation. For the outdoor PM₁₀ measurements, a high-volume sampler (Derenda, Stahnsdorf, Germany) with a flow of 30 m³/h was used and was equipped with a 150-mm Teflon TE38 filter (Watman GmbH, Dassel, Germany) with a pore size of 5 μm. For the Berlin study the same filters as indoors were used.

The filters were stored and conditioned for 48 hours in a room with controlled temperature and humidity before they were weighed (according to the recommendation of DIN EN 12341) to prevent biases. Each filter was weighed four times

prior to sampling and four times afterwards using a microbalance (Mettler-Toledo AX26DR, Greifensee, Switzerland) with a reading accuracy of 10 μg.

PM₁₀ samples were stored at -20°C until extraction to prevent bacteria growth and to kill mites. A method described by Fahlbusch et al.⁽³²⁾ was used to measure the levels of indoor airborne allergens. In brief, the PM₁₀ samples plus filter matrices were extracted with 0.125 M NH₄HCO₃ plus 0.05% Tween20 for 2 h at room temperature under constant shaking, with an extraction ratio of 1:10 to 1:100 (w/v), depending on the amount of dust sampled. Endotoxin levels were quantified using the kinetic Limulus Amebocyte Lysate assay (Bio Whittaker, Walkerville, Md.) with a detection limit of 50 EU/g dust. Major mite allergens, Der p 1 and Der f 1, and cat allergen Fel d 1, were measured using a two-site monoclonal enzyme-linked immunosorbent assay (Indoor Biotechnologies, Clwyd, U.K.). The lower limit of detection (LOD) was 0.5 ng/ml for Der p 1 and Der f 1 and 0.2 ng/ml for Fel d 1.

Endotoxin in Inhalable Dust

For comparison of a conventional environmental monitoring method (PM₁₀ sampling) and a conventional occupational monitoring method (inhalable dust sampling) in an environmental setting such as classrooms during teaching hours, endotoxin concentrations in the inhalable dust were collected simultaneously with endotoxin levels in PM₁₀ samples and involved a different sampling technique often used for the workplace atmosphere. Inhalable dust comprises the fraction of airborne particles that enter the nose and mouth during normal breathing. For cost-saving reasons only endotoxin (and not the allergens) in inhalable dust could be measured. In this case, according to EN 14031 an air sampler with a constant flow of 3.5 l/min and having a 50% cut-point of 100 μm was used and placed close to the PM₁₀ sampler.

The inhalable dust samples were collected exclusively during teaching hours (5.5 h/day). In brief, filters were extracted with 7 ml endotoxin-free water, under constant shaking, for 1.5 h. The inhalable endotoxin fraction was determined in strict adherence to the German DIN BIA 9450 (DIN CEN 14031)⁽³³⁾ for endotoxin concentrations at the workplace which recommends endotoxin-free water for filter extraction. After centrifugation for 3 min with 3000 U/min and dilution (1:1, 1:10), endotoxin concentrations were quantified using the kinetic Limulus Amebocyte Lysate assay (Lonza, Kit LOT-Nr: 76367; potency to E.coli 055:B5: 13EU/ng) with a new calibration curve (50/5/0.5/0.05/0.005 EU/ml) for each day. The detection limit was 0.2 EU/m³ with a sampling volume of 1.1 m³. Airborne endotoxin concentrations (EU/m³) were calculated from sampled air volume (m³). For quality assurance control a standard was added to each sample requiring recovery rates from 50 to 200%. Samples with outlying concentrations were further diluted. Additionally, an internal standard was quantified for each series (15 measurements: 71.2 ± 23.8 EU/mg).

Statistical Analysis

The data were analyzed using the statistical software package IBM-SPSS (IBM Deutschland GmbH, Ehningen, Germany). The Mann-Whitney U test and the Kruskal-Wallis test were used to compare various sampling locations and seasons. Samples collected in June and July were designated "summer" and those collected in December, January, and February "winter." Correlations were assessed using the Spearman correlation coefficient test. If not stated otherwise, values below the LOD were assigned a value of half of the LOD. A single indoor PM₁₀-value of 1,175 $\mu\text{g}/\text{m}^3$ was excluded as an outlier because this value was 14 times higher than the median value in this classroom. Moreover, parallel measurements with an optical laser aerosol spectrometer showed concentrations of 174 $\mu\text{g}/\text{m}^3$ on that day.

RESULTS

During the period of the study, the classrooms were occupied on an average daily basis by 9–46 subjects (mean: 22 ± 5 pupils). Some basic characteristics are given in Table I. The daily temperature inside the classrooms fluctuated between 12°C and 30°C, with median values from 18°C to 30°C. The daily relative humidity was between 19% and 92%, with medians from 26% to 74%.

Carbon Dioxide (CO₂)

The CO₂ single levels in the classrooms ranged between 276 ppm and 4,247 ppm with daily medians in the schools from 423 ppm to 3,135 ppm. The results for each classroom are given in Table II. Significantly higher CO₂ concentrations were detected in winter compared with summer. The corresponding median value of the daily outdoor air CO₂ concentrations in front of five schools over the measuring period was 440 ppm (results were only available for Munich).

Particulate Matter (PM₁₀)

The results of the PM measurements at the different sampling sites are given in Table II. Overall, the concentrations of indoor PM₁₀ ranged from 9 to 390 $\mu\text{g}/\text{m}^3$ (median: 127 $\mu\text{g}/\text{m}^3$). Compared with summer (months 6 and 7), the winter (months 12, 1, and 2) readings were significantly higher (median values: 78 $\mu\text{g}/\text{m}^3$ vs. 162 $\mu\text{g}/\text{m}^3$). The single outdoor values in front of five schools ranged from 3 to 118 $\mu\text{g}/\text{m}^3$ (median: 21 $\mu\text{g}/\text{m}^3$). Looking at all observations, we found a statistically significant correlation between indoor PM₁₀ and the median CO₂ concentrations ($r = 0.33$, $p < 0.001$), but not with the mean number of pupils in the classroom. Considering the season, it was obvious that the correlation with CO₂ was only significant for winter months and did not apply in summer. No correlation was observed between PM values indoors and outdoors. Furthermore, the PM₁₀ concentrations in Berlin were significantly higher than in the Munich classrooms.

Allergens

For each sampling site, the concentrations of the tested allergens are given in Table III. Overall, we were able to detect Fel d 1, Der p 1, and Der f 1 in 93%, 51%, and 19% of the indoor PM₁₀ samples, respectively. We found Fel d 1 concentrations between 0.02 ng/m³ and 1.15 ng/m³ (median: 0.19 ng/m³; 95th percentile: 0.57 ng/m³). For the dust mite allergens, the 95th percentiles and maximum values were 0.50 ng/m³ and 1.10 ng/m³ for Der p 1 and 0.30 ng/m³ and 1.28 ng/m³ for Der f 1, respectively.

A significant correlation was found between airborne cat allergen levels and the mean number of pupils in the room ($r = 0.24$, $p < 0.001$). Significantly higher concentrations were observed in winter months compared with summer, while no significant differences were observed between the levels in rooms with and without a cozy corner.

Endotoxin

The results of the endotoxin measurements in PM₁₀ at the different sampling sites are given in Table IV. Endotoxin in PM₁₀ could be quantified in 301 samples, with concentrations ranging from 0.5 to 84.1 EU/m³, and the 95th percentile at 58.2 EU/m³. As could be deduced from Table IV, the levels of endotoxin in Munich classrooms were higher than in Berlin. But observations depend on only one classroom in Berlin, thus the significance is limited. In a total of 127 samples, we quantified outdoor endotoxin levels in the PM₁₀ in front of five schools with median and 95th percentile concentrations of 0.15 EU/m³ and 0.76 EU/m³ (range: 0.02 to 1.73 EU/m³), respectively. The indoor endotoxin levels detected were significantly correlated with the number of pupils ($r = 0.20$; $p = 0.001$), the PM₁₀ concentration ($r = 0.54$; $p < 0.001$), and the median CO₂ level ($r = 0.24$; $p < 0.001$). No significant differences were found between the levels in rooms with and without a cozy corner. For the outdoor samples, no correlation was observed with temperature and humidity, but only 48 data pairs were available. Nevertheless, we found significant differences between winter and summer samples, with higher concentrations in winter. A time course over a year, depicting daily PM₁₀ and endotoxin levels in PM₁₀, is shown in Figure 1.

The results of the measurements of endotoxin in inhalable airborne dust in Munich are additionally given in Table IV. In 235 samples endotoxin could be quantified and levels ranged from 0.03 to 115.2 EU/m³ (median: 8.4 EU/m³).

In a total of 174 indoor air samples, we simultaneously quantified endotoxin both in the PM₁₀ fraction and in the inhalable airborne dust. We found a significant correlation between the two sampling techniques ($r = 0.67$; $p < 0.001$), as can be seen in Figure 2.

DISCUSSION

In this study, measurements of particulate matter (PM₁₀), airborne allergens and endotoxin are presented for a number

TABLE II. Summary Statistics of the Measured Daily Median CO₂ and PM₁₀ Concentrations Inside Classrooms

Room	N	mean	median	Min	Max	Outdoor Median (Min-Max)
CO₂ [ppm]						
Munich						
A1	19	1899	1930	1570	2297	394
A2	19	1382	1321	968	2031	—
B1	20	837	822	443	1396	—
B2	20	1180	1073	647	1953	—
C1	17	952	893	695	1397	—
C2	17	993	973	671	1418	—
D1	19	1611	1588	1016	2214	345 (322–412)
D2	19	1185	1139	882	1642	—
E1	15	1952	1749	1195	2790	348 (268–426)
E2	15	1574	1502	855	2326	—
F1	15	1046	1045	821	1402	515 (454–615)
F2	15	1152	1113	707	1809	—
G1	18	1668	1592	837	3135	440 (394–511)
G2	17	1210	967	557	2605	—
Overall	242	1329	1241	443	3135	
Berlin						
H	82	1143	1178	423	1963	—
PM₁₀ [$\mu\text{g}/\text{m}^3$]						
Munich						
A1	19	125	123	79	161	27
A2	19	125	127	84	173	—
B1	20	79	58	42	210	—
B2	20	88	79	41	147	—
C1	20	76	78	26	127	—
C2	20	80	78	26	142	—
D1	20	194	191	31	335	27 (10–73)
D2	20	145	140	85	219	—
E1	14	184	174	100	265	30 (15–77)
E2	15	174	168	86	291	—
F1	15	157	161	51	247	20 (3–41)
F2	15	150	154	44	277	—
G1	20	83	84	19	183	18 (5–40)
G2	18	71	63	9	181	—
Overall	253	121	115	9	335	
Berlin						
H	83	171	168	55	390	15 (6–61)

of classrooms in German schools and in one classroom over the course of a year.

Carbon Dioxide (CO₂)

in the classrooms we partly found high concentrations of CO₂ (median: 1211 ppm), a substance commonly used as an indicator of indoor air quality. According to the American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) Standard 62, indoor CO₂ levels should not exceed 1,000 ppm to ensure satisfactory comfort. Values exceeding this threshold indicate insufficient fresh air and are associated with a higher frequency of health complaints.⁽³⁴⁾ In line with other recommendations, the German

Working Group on Indoor Guidelines of the Federal Environment Agency and the States' Health Authorities stated that air quality can be regarded as harmless if the CO₂ levels are below 1000 ppm, elevated if between 1,000 and 2,000 ppm, and unacceptable if above 2,000 ppm.⁽³⁵⁾ Comparable to our results, however, particularly in wintertime, increased CO₂ levels above the mentioned values have been observed in classrooms in numerous countries (e.g. ^(36–39)). For example in 91 German classrooms, median CO₂ levels ranged between 598 and 4,172 ppm in winter and between 480 and 1,875 ppm in summer.⁽³⁰⁾ These high levels of CO₂ are a clear indication of an insufficient ventilation routine in naturally ventilated schools.

TABLE III. Summary Statistics for Allergen Concentrations Inside Classrooms in ng/m³

	Fel d 1	Der p 1	Der f 1
Munich			
Number (n>LOD)	218 (213)	218 (96)	218 (34)
Mean	0.24	0.08	0.07
Median	0.21	0.04	0.04
95th percentile	0.58	0.22	0.16
Minimum	0.02	0.02	0.02
Maximum	1.15	0.36	1.28
Berlin			
Number (n>LOD)	83 (68)	83 (57)	83 (23)
Mean	0.15	0.29	0.12
Median	0.12	0.25	0.08
95th percentile	0.39	0.73	0.32
Minimum	0.02	0.06	0.04
Maximum	0.67	1.10	0.70
All			
Number (n>LOD)	301 (281)	301 (153)	301 (57)
Mean	0.22	0.14	0.08
Median	0.19	0.07	0.04
95th percentile	0.57	0.50	0.30
Minimum	0.02	0.02	0.02
Maximum	1.15	1.10	1.28

Particulate Matter (PM₁₀)

Our results are in line with previously published data from other researchers who observed high levels of particulate mat-

ter in naturally ventilated schools compared with other indoor spaces such as residences.^(30,40,41) The indoor PM concentration was especially high in winter. Mechanisms that could explain these results need to be identified. A high number of active pupils combined with limited space might resuspend more PM from surfaces; this, together with reduced ventilation rates, is one possible explanation for these findings. With an indoor-outdoor ratio of 4.7 (mean concentrations of 135.7 $\mu\text{g}/\text{m}^3$ vs. 29.1 $\mu\text{g}/\text{m}^3$) indoor generated particulate matter dominated the dust levels indoors.

Allergens

Exposure to indoor allergens has been examined by numerous studies, but most studies focused on residences or used settled dust as an exposure matrix. Different studies have been published, mainly from Scandinavia and the United States, in which the amounts of allergens in dust were quantified.^(6,14,15,21,31,42–48) Summarizing these studies, Fel d 1 was the most common allergen, with geometric means ranging from the detection limit up to 11.3 $\mu\text{g}/\text{g}$, while Der p 1 levels were similar or slightly lower.

In addition to the measurement of allergens in settled dust, which acts as a reservoir, the collection of individual samples using breathing zone and stationary samplers would improve our knowledge of exposure. Only some studies analyzed indoor concentrations of airborne allergens in schools. The studies in the U.K.,⁽⁴⁹⁾ Scandinavia,^(13,21,50) and the United States,^(22,44,51) are summarized in Table V. Overall, it must be taken into account that the number of observations is often very low and that different sampling techniques and analytical

TABLE IV. Summary Statistics of Endotoxin Concentrations in PM₁₀ (Inhalable Dust) Inside Classrooms in EU/m³

Room	N	Mean	Median	Min	Max
Munich					
A1	19 (—)	6.8 (—)	6.3 (—)	3.1 (—)	11.1 (—)
A2	19 (—)	8.9 (—)	9.1 (—)	5.1 (—)	12.8 (—)
B1	20 (18)	10.1 (8.8)	5.8 (7.6)	1.3 (2.3)	33.4 (17.8)
B2	20 (18)	13.5 (13.9)	9.3 (11.2)	0.8 (2.9)	45.9 (37.7)
C1	20 (20)	20.3 (6.1)	17.3 (5.4)	0.8 (1.0)	47.7 (15.1)
C2	20 (20)	7.8 (3.6)	7.2 (3.3)	0.5 (0.7)	16.0 (7.4)
D1	20 (20)	34.9 (16.9)	34.3 (11.1)	1.4 (3.2)	67.5 (115)
D2	20 (19)	21.1 (6.7)	20.3 (5.1)	4.6 (1.0)	50.6 (25.3)
E1	15 (20)	23.9 (13.9)	20.7 (14.1)	10.5 (7.9)	43.2 (22.8)
E2	15 (20)	44.0 (21.4)	36.0 (18.0)	14.6 (8.2)	80.4 (42.4)
F1	15 (20)	48.3 (17.1)	46.3 (12.1)	15.3 (0.04)	84.1 (49.9)
F2	15 (20)	50.8 (15.0)	54.3 (14.0)	22.1 (0.03)	64.4 (42.9)
G1	— (20)	— (6.3)	— (6.1)	— (0.4)	— (13.5)
G2	— (20)	— (5.2)	— (4.5)	— (0.7)	— (13.9)
Whole study sites in Munich					
	218 (235)	22.7 (11.2)	15.3 (8.4)	0.5 (0.03)	84.1 (115.2)
Berlin					
H	83 (—)	15.1 (—)	11.3 (—)	0.6 (—)	56.4 (—)

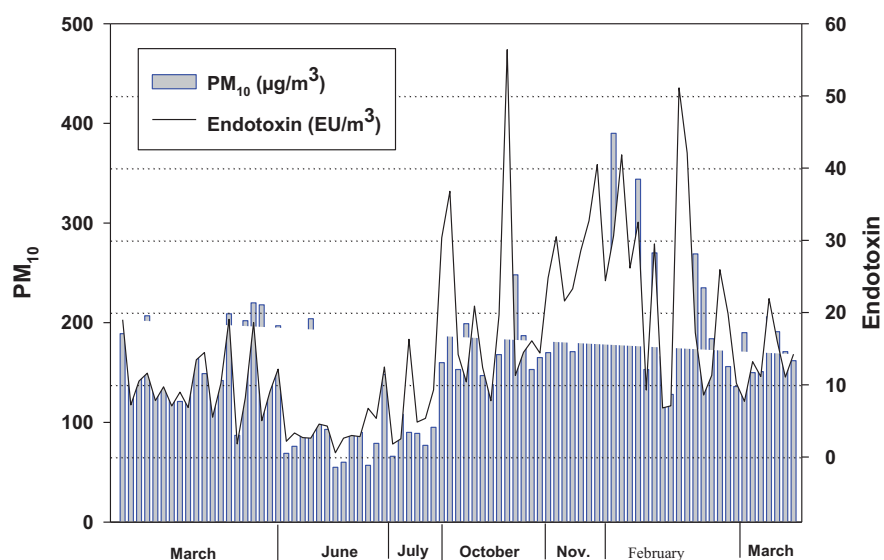


FIGURE 1. Time course of daily PM_{10} and endotoxin concentrations in a classroom in Berlin. (color figure available online)

methods, some of which had higher detection limits, were used. In the U.K. study, nine pupils attending classrooms were studied for 8 hours using personal air samplers.⁽⁴⁹⁾ Eight of 9 Fel d 1 samples were below the detection limit (0.5 ng/m^3), and one was positive at 0.79 ng/m^3 (corresponding to a child with

a cat), while Der p 1 levels were always under the detection limit.

Almqvist et al.⁽¹³⁾ collected cat allergens with personal samplers for 5 children attending 12 classrooms. They found median levels of Fel d 1 in classes with a high percentage

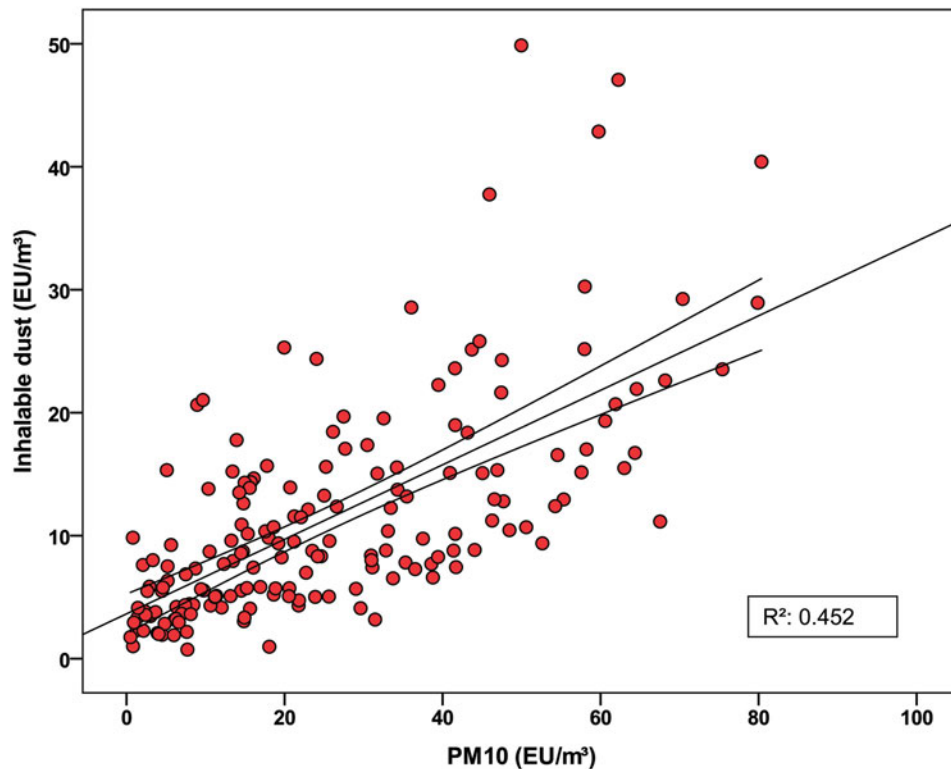


FIGURE 2. Correlation between endotoxin levels measured in PM_{10} and in inhalable dust (lines represent the regression line and 95% confidence interval). (color figure available online)

TABLE V. Airborne Levels of Cat Allergen in Schools in ng/m³ (Median and Range)

Reference	Fel d 1 levels	Sampling	Comments
Custovic et al. 1996 ⁽⁴⁹⁾	<0.5	8-hours, personal air sampling	9 classrooms
Almqvist et al. 1999 ⁽¹³⁾	0.59/2.94 ^B	3-hours, personal air sampling	5 children in each of 12 classrooms on 2 different occasions
Munir et al. 2003 ⁽²¹⁾	0.014 (0.0003–0.0004)	24-hours, high-volume electrostatic air-cleaning device	7 classrooms
Karlsson et al. 2004 ⁽⁵⁰⁾	0.8–1.5	morning lessons, personal air sampling	14 control schools, every five to six weeks, over six occasions
Forade and Berry 2004 ⁽⁴⁴⁾	63.5/96.6 ^{A,C}	24-hours, stationary, PM _{2.5}	2 schools, five times throughout the school year, five sampling sites in common areas
Raja et al. 2010 ⁽²²⁾	0.25 (0–0.76)	24-hours, stationary, PM ₁₀	6 classrooms
Hopke 2011 ⁽⁵⁴⁾			
Permaul et al. 2012 ⁽⁵¹⁾	1.82 (0.47–3.3) ^A	1 week, stationary, charged plate air sampler	12 schools; classroom, cafeterias, and gymnasiums
This study	0.19 (0.02–1.15)	4 hours, during school day, stationary, PM ₁₀	15 classrooms, altogether at 301 days

^Ageometric mean.^Bmeans in rooms with few and many cat owners.^Ccarpeted and tiled floorings.

(>25%) of cat owners are approximately 2.94 ng/m³ (25–75th percentile: 2.37–4.77 ng/m³), which is five-fold higher than the levels found in classrooms with few (<10%) cat owners. Allergens are transported on clothing from homes with cats, and levels in non-cat owners' clothes increased significantly after a school day. This finding was supported by other researchers.^(15,52) In another Swedish investigation, 96 air samples were collected from 7 classrooms using a high-volume electrostatic air-cleaning device.⁽²¹⁾ The concentration of airborne Fel d 1 varied from 0.00032 to 0.042 ng/m³, and an included variability study showed high levels of variation both within and between days. Karlsson et al.⁽⁵⁰⁾ reported the results from 25 classrooms in 2 schools in an allergy avoidance and intervention study that involved conducting personal air sampling every 5–6 weeks over a time period of 19 weeks. They found mean weekly levels in 14 control classrooms of 0.8 to 1.5 ng/m³.

In the United States, Raja et al.⁽²²⁾ sampled PM₁₀ with a personal sampler twice in three schools. They did not report their observed PM₁₀ and Fel 1 levels, but based on a personal communication, Fel d 1 in the classrooms was up to 0.76 ng/m³.⁽⁵³⁾ The median concentration is in line with our findings. The concentrations of Der p 1, however, varied from 0.05 to 0.18 ng/m³. In another study from the United States sampled PM_{2.5} over 2 hours in two schools with carpeted and hard surface floorings.⁽³⁷⁾ The concentrations of Fel d 1 and Der p 1 were 63.5 ng/m³ and < 32.3 ng/m³ in carpeted rooms and 96.6 ng/m³ and 73.7 ng/m³ in rooms with tiled surfaces, respectively. In the third study different rooms in 12 schools were investigated using a charged plate air sampler.⁽⁵¹⁾ They

found Fel d 1 levels from 0.47 to 3.3 ng/m³ with a geometric mean of 1.8 ng/m³, obviously higher than in our study.

As described previously, the results are only partially comparable to ours because of methodological differences. Nevertheless, our results are in a range similar to those described in some of these studies. In contrast, Munir et al.⁽²¹⁾ found very low levels using an electrostatic sampling system, while Foarde and Berry⁽⁴⁴⁾ reported very high levels in two schools.

Endotoxin

Few studies have been published describing endotoxin levels in classroom air, but there are two from the United States.^(22,44) The first sampled PM_{2.5} in two schools, five times per school year, on polytetrafluoroethylene filters over 2 hours (16.7 L/min). The geometric means of endotoxin levels in carpeted and tiled rooms were 0.3 EU/m³ and 0.9 EU/m³, respectively, while in outdoor air, the levels were 0.2 and 0.3 EU/m³. Raja et al.⁽²²⁾ collected PM₁₀ in six school rooms, but there was no publication of the results in this publication. Compared to our median value of 14.4 EU/m³ in PM₁₀ and 8.4 EU/m³ in inhalable dust, the aforementioned working group reported considerably lower mean levels of 0.3 EU/m³ in a personal communication.⁽⁵³⁾

On the other hand, PM₁₀ concentrations were approximately 100 µg/m³, only slightly lower than those observed in our study. Overall, the concentrations of endotoxin in classroom air samples, ranging from 0.5 to 84.1 EU/m³ in PM₁₀, were higher than the levels previously reported. One possible explanation for the diverging indoor levels might be the different sampling techniques and extraction methods, as well

as differences in the ELISAs that were used for the analysis. It is well known that endotoxin levels are strongly related to the sampling techniques and analytical methods.⁽⁵⁴⁾ This fact could also explain the diverging results between the endotoxin levels in PM₁₀ and in inhalable dust sampled simultaneously in our study. Our data support the findings of Spaan et al.⁽⁵⁴⁾ that extraction with Tween20 results in higher concentrations of airborne endotoxin. Nevertheless, endotoxin concentrations from both techniques were well correlated (Figure 2).

With respect to outdoor airborne endotoxin, our results were higher (medians: 0.15 vs. 0.017 EU/m³) than previously described in Munich.⁽⁵⁵⁾ Unlike our study, they measured a different dust fraction (PM_{2.5}), each filter was used over approximately 14 days, and the samples were stored for some years before the analysis. Nevertheless, we observed the same seasonal variability of endotoxin levels. In the United States, geometric means of 0.74 EU/m³ (winter) and 2.78 EU/m³ (summer) were observed in front of six homes, using open filter cassettes with a flow rate of 12.5 L/min.⁽⁵⁶⁾

Until now, no health effect threshold or exposure limit for endotoxins for the general population has been estimated. For occupational settings, an exposure level of 90 EU/m³ was assessed as a “no observed effect level” (NOEL)() on the basis of a study of the effects of 6 hr exposure to endotoxins in volunteers selected for their sensitivity to endotoxins.⁽⁵⁷⁾ The Dutch Expert Committee on Occupational Safety recommended that a health-based exposure limit of 90 EU/m³ (8 hr time-weighted average [TWA]) affords adequate protection against the effects of both acute and chronic exposure to endotoxins. The same committee recommended a maximum level of 135 EU/m³ when a kinetic LAL assay was used. Rylander⁽⁵⁸⁾ suggested a NOEL for airway inflammation of 100 EU/m³ and 1,000 EU/m³ for systemic effects. Nevertheless, the use of these exposure limits for work environments is still a matter of debate. Compared with the aforementioned occupational exposure limit our data showed lower levels of endotoxins in schools. On the other hand, these limits could not be used directly for the whole population and especially not for children.

CONCLUSION

We were able to collect indoor air samples in 14 classrooms during the entire school day (two in each school) over 20 consecutive days and in one classroom over the course of a year. Cat allergen Fel d 1, major mite allergens Der p 1 and Der f 1, and endotoxin in airborne dust were the target analytes. In line with previous studies, our results show that exposure to CO₂ and PM₁₀ in school classrooms is high, which might be explained by the reduced airing of the rooms and the high density of room users who swirl up sedimented dust from the floors. Moreover, our study shows higher endotoxin levels in schools compared to the results found in residences in previous studies. This indicates that exposure during the school day may contribute substantially to the total exposure of children in Germany and has to be considered for risk assessment. In

addition, levels of airborne allergens in classrooms were low and of the same magnitude reported for indoor environments without pets.

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