

DR. INGRID SANDER (Orcid ID : 0000-0002-5014-4112)

Article type : Original Article: Experimental Allergy and Immunology

Editor : Reto Crameri

Indoor allergen levels in settled airborne dust are higher in day-care centres than at home

Sander I¹, Lotz A¹, Neumann HD², Czibor C¹, Flagge A¹, Zahradnik E¹, Raulf M¹

¹Institute for Prevention and Occupational Medicine, German Social Accident Insurance, Institute of the Ruhr-Universität Bochum (IPA), Bochum, Germany

²German Social Accident Insurance Institution for the public sector in North Rhine-Westphalia, Düsseldorf, Germany

Short title

Indoor allergens in day-care centres and at home

Corresponding author:

Ingrid Sander

Forschungsinstitut für Prävention und Arbeitsmedizin der Deutschen Gesetzlichen Unfallversicherung, Institut der Ruhr-Universität Bochum (IPA)

Department of Allergology / Immunology

Bürkle-de-la-Camp-Platz 1

D-44789 Bochum

Germany

Phone: +0049 234 302 4606

Fax: +0049 234 302 4610

email: Sander@ipa-dguv.de

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/all.13371

This article is protected by copyright. All rights reserved.

Heinz-Dieter Neumann,

Phone: +0049 151 14828842, email: h.neumann@unfallkasse-nrw.de

Anne Lotz,

Phone: +0049 234 302 4533, Fax: +0049 234 302 4510, email: lotz@ipa-dguv.de

Christina Czibor,

Phone: +0049 234 302 4702, Fax: +0049 234 302 4610, email: czibor@ipa-dguv.de

Angelika Flagge,

Phone: +0049 234 302 4618, Fax: +0049 234 302 4610, email: flagge@ipa-dguv.de

Eva Zahradnik,

Phone: +0049 234 302 4304, Fax: +0049 234 302 4610, email: zahradnik@ipa-dguv.de

Monika Raulf,

Phone: +0049 234 302 4582, Fax: +0049 234 302 4610, email: raulf@ipa-dguv.de

Background

Early life sensitization to indoor allergens predicts asthma development. Aim of study was to compare allergen concentrations in day-care centres (DCC) to those in private homes.

Methods

Settled airborne dust was collected four times a year from 20 German DCC (620 samples), and from the homes of children and day-care workers (602 samples) using electrostatic dust collectors (EDC).

This article is protected by copyright. All rights reserved.

The samples were analysed with fluorescence-enzyme-immunoassays recognising domestic mite allergens (DM), Fel d 1, Can f 1 and Mus m 1. Pet allergen thresholds that discriminate samples from homes with cats or dogs to those without were calculated using receiver-operator characteristics. Influences on allergen levels were analysed using multilevel models.

Results

Allergen loads were on average higher in DCC than in homes. In DCC, 96% of the samples were positive for DM, 95% for Can f 1, 90% for Fel d 1, and 83% for Mus m 1. In homes, 84% contained DM, 48.5% Can f 1, 33% Fel d 1, and 43% Mus m 1. The threshold level for homes with dogs was 75 ng/m² Can f 1 (96.8% sensitivity, 96% specificity), and 46 ng/m² Fel d 1 for homes with cats (92% sensitivity, 94.9% specificity). In DCC, Can f 1 and Fel d 1 loads were higher than these thresholds in 37% and 54% of the samples, respectively. Allergen levels were significantly influenced by the season and room type; however, carpets on floors had no influence.

Conclusions

Mite, mouse, cat and dog allergens were mostly higher in DCC than in homes. Exposure to dog and cat allergens in DCC often reached levels of households with pets.

Keywords

Allergen exposure, Can f 1, domestic mites, Fel d 1, Mus m 1

Introduction

Early life sensitization to indoor allergens is predictive of asthma development later in life (1).

Identification of allergen molecules and the development of antibody-based detection methods enabled allergen quantification in dust. Several studies on the prevalence of sensitization or symptoms and allergen quantification after vacuuming mattresses or floors in homes implied a dose-response relationship (2–4). Hence preventative measures, such as the use of mite-impermeable mattresses covers or high efficiency particulate air filter in vacuums, increased frequency of washing and cleaning, or even the removal of dogs and cats from families with atopic history were recommended. However, contradictory results were reported regarding the success of allergen avoidance. Morgan et al. (5) found that one year after intervention, asthma-associated morbidity decreased in asthmatic children belonging to the intervention group with reduced allergen concentrations in beds and on the bedroom floor. Recent analyses of birth cohorts and intervention studies, however, challenge the longtime benefit of allergen avoidance, as no association between early allergen exposure and risk of sensitization or allergic symptoms could be found (6–11).

However, allergen exposure was not always proven by measurements in these studies, and even if allergens were quantified, reservoir dust from beds or floors in homes was used. Tovey et al. (12, 13) have questioned whether current methods are effective to reduce chronic personal exposure.

Quantification of mite allergens after sampling with time-based air samplers prove that there was higher individual exposure during the day than at night, and that there was no correlation of aeroallergen exposure with the levels of allergen quantified in bed surface dust (14). In addition, about 40% of mite allergen exposure in adults occurred outside the home (15). However, measurements with personal air-samplers are work-intensive and expensive, and therefore are not practical for huge studies. As a compromise between personal airborne dust and reservoir dust, electrostatic dust collectors (EDC) were invented for endotoxin quantification (16), and were recently used with success for allergens (17–21). The EDC is placed at a height of at least 1.5 m and

captures settled dust which has been previously airborne, and therefore consist primarily of the inhalable dust fraction which is most important for allergen quantification (22).

Young children in Germany spend a large proportion of their time in day-care. In 2015, 35.8% of the children between the ages of 1 and 2 years, 61.3% of the children between 2 and 3 years, and 94.9% of the children between the ages of 3 and 6 years were under regular child care. Furthermore, 43.9% of the latter group spent more than 7 hours a day under such care (23). Thus, children's allergen exposure outside the home environment may play an underrated role in German birth cohort studies.

The aim of our study was to estimate allergen exposure in DCC compared to the home environment. Therefore, in addition to allergen quantification in reservoir dust from floors and furniture surfaces (24), settled airborne dust on EDC was sampled in group rooms, adjoining side rooms, staff rooms, bedrooms, and corridors in 20 DCC in North Rhine-Westphalia, and simultaneously in the children's and day-care workers' homes. Main allergens of dog, cat and mouse were quantified with antibodies from Indoor Biotechnologies Ltd. (Charlottesville, VA) using their universal allergen standard based on amino-acid analysis as the reference (25). Since previous studies have shown that single house dust mite allergens are usually below the detection limit in airborne dust or on EDC, domestic mite allergens (DM) were quantified using polyclonal antibodies directed to *Dermatophagoides farinae* (19, 20, 26). This species seems to be the most frequently detected house dust mite according to previous studies in Germany (21, 26, 27). In an initial methodological study, EDC deployment time, extraction buffer and storage conditions were evaluated to determine the influence of each factor on the results (21). The allergens could be reproducibly quantified and increased proportionally with deployment time over a period of 4 weeks. For further validation of the DM method, we tested whether the polyclonal antibodies detected the same proteins and epitopes as human IgE of mite-sensitized patients by inhibition experiments.

This is the first study using EDC to quantify allergens in DCC, and also the first study in DCC with parallel measurements in participants' homes. Furthermore, samples were taken four times a year to account for seasonal variation.

The following questions were addressed by analyzing the EDC data from DCC and homes:

- 1) Is exposure to indoor allergens higher in DCC than at home?
- 2) Which threshold levels discriminate samples from homes with cat or dog from the ones without these pets?
- 3) Which parameters influence allergen levels in DCC and homes?

Methods

Sampling of settled airborne dust

Twenty DCC across North Rhine-Westphalia in Germany were visited four times during the different seasons from 2010 until 2012 (24). EDC with polyester cloths from Zeeman (Alphen aan de Rijn, The Netherlands) were placed for 14 days on a flat surface of shelves or styrofoam devices hanging on the ceiling at a median height of 1.8 m above the ground in 61 group rooms, 56 adjoining side rooms, 20 staff rooms, 12 bedrooms, and 19 corridors . Out of 1077 children (or their parents) who answered questions on pet ownership, 178 (16.5%) had a cat and 140 (13%) a dog at home. EDC were simultaneously placed in the homes of parents (n=141) and day-care workers (n=85), which included 128 children's rooms, 55 living rooms, 31 bedrooms and 12 other rooms in different seasons on shelves at a median height of 1.8 m for 14 days. Out of 226 households, 24 (10.6%) had a cat, 34 (15.5%) a dog, and 2 (0.9%) had mice. The final dataset included 620 samples from DCC and 602 samples from homes.

The standard procedure for EDC sampling, extraction and storage was previously described in detail (21). EDC cloths were placed in the freezer at -20°C for 24 hours to destroy mites, after which they were stored at room temperature (median 24 days, range 1-80 days) until extraction in 15 ml phosphate-buffered saline with 0.05% Tween 20 by rotation for 1 hour. The extracts were then stored at -80°C (median 27 days, range 1 – 171 days) before measurement in the DM assay. Long time storage of the cloths at room temperature had decreased Can f 1 levels (-4.8% loss for every 30 days); whereas long time storage of the extracts had decreased DM levels (-1.2% loss for every 30 days) (21). However, since only minor losses of activity were expected after storage (<15% for Can f 1 and <7% DM), these effects were not taken into account for data analysis.

In many cases, deviations from the sampling time of 14 days occurred because parents or day-care workers did not stick to the study protocol. In these cases, the measured allergen levels were adjusted by linear extrapolation to the expected 14 days level since a more or less proportional increase with deployment time had been observed (21). The sampling area on an EDC cloth was 209 cm². Allergen levels were calculated as ng/m² for two weeks sampling.

Allergen quantification with fluorescence enzyme immunoassays (FEIA)

The cat, dog, and mouse antibodies and the universal allergen standard were purchased from Indoor Biotechnologies. The DM immunoassay is based on rabbit antibodies to *Dermatophagoides farinae* and detects antigens from several DM species (26). The details on FEIA conjugate, substrate, and measurement were previously described (21). The detection limits (LOD) for EDC cloths were 35.9 ng/m² for DM and 7.2 ng/m² for Can f 1, Fel d 1, and Mus m 1.

Inhibition of IgE-binding to house dust mite allergens by DM antibodies

The rabbit antibodies of the DM immunoassay were used for competitive inhibition of IgE-binding to house dust mite ImmunoCAPs (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). As a control, serum from a non-immunized rabbit was used. Anti-DM antibodies in 30 µl rabbit serum were added to 30 µl of patients' serum with specific IgE to *Dermatophagoides pteronyssinus* (d1) and *D. farinae* (d2). Five sera with different IgE-antibody binding capacities were selected from house dust mite-sensitized patients. The sera had the following specific IgE (sIgE) values measured using CAP-FEIA (Thermo Fisher): Serum 1: 77.10 kU/L d1, >100 kU/L d2; Serum 2: 47.40 kU/L d1, 63.40 kU/L d2; Serum 3: 39.10 kU/L d1, 36.00 kU/L d2; Serum 4: 6.13 kU/L d1, 12.50 kU/L d2; Serum 5: 5.88 kU/L d1, 6.63 kU/L d2. Binding of IgE to d1 and d2 ImmunoCAPs in the presence of increasing amounts of anti-DM antibodies was measured using CAP-FEIA. The percentage of inhibition was calculated in relation to the sIgE-value obtained in the presence of the rabbit control serum.

Statistical analysis

Pet allergen thresholds that discriminate samples from homes with cat or dog to those without were calculated using receiver-operator characteristics (ROC) and Youden indices with GraphPad Prism 5.04 (GraphPad Software, Inc., La Jolla, CA) and Microsoft Excel 2010. For graphs and calculation of the geometric mean (GM), values below LOD were replaced by 2/3 LOD.

To analyze influences on allergen concentrations, multilevel models considering values below the detection limit were used as described before (24, 28) using the software R (29). The multilevel models for allergens in DCC, or for allergens in DCC and homes consisted of three levels (level 1: collected sample, level 2: room of collection, level 3: DCC or home). Models for allergens in the children's or day-care workers' homes consisted of two levels (level 1: collected sample, level 2: room of collection) because each home collected samples only in one room. Each allergen was

analyzed in a separate model. The dependent variables in the models were the log-transformed allergen concentrations on EDC. The tables 1-3 show the transformed coefficient estimates $\exp(\hat{\beta})$ and 95% confidence intervals. These estimates can be interpreted as factors of the change of allergen concentrations on EDC. To account for multiple comparisons, significance levels were corrected by the number of variables in each model using the method of Bonferroni.

Results

Inhibition of IgE-binding to house dust mite allergens by DM antibodies

In order to characterize the antibodies from the DM immunoassay, inhibition experiments were performed with house dust mite allergic patients' sera. The anti-DM antibodies were able to inhibit IgE-binding to *D. farinae* in a dose-dependent manner (Fig. 1). The inhibitions obtained with the highest dose reached 95% (Serum 1), 84% (Serum 2), 87% (Serum 3), 93% (Serum 4), and 98% (Serum 5). A corresponding experiment with high anti-DM antibody concentrations was performed with *D. pteronyssinus* ImmunoCAPs. The maximal inhibition of IgE-binding to *D. pteronyssinus* was 64% (Serum 1), 74% (Serum 2), 85% (Serum 3), and 84% (Serum 4, Serum 5). The competitive inhibition proves that anti-DM antibodies detect, at least partially, the same proteins and epitopes as patients' IgE.

Allergen levels in DCC and homes

Nearly all EDC samples from DCC contained allergen levels above the LOD. DM was measurable in 96% of all DCC samples (GM 367 ng/m²), Can f 1 in 95% (GM 50 ng/m²), Fel d 1 in 90% (GM 48 ng/m²) and Mus m 1 in 83% (GM 16.2 ng/m²). In homes, 84% of samples contained DM (GM 248 ng/m²), 48.5% contained Can f 1 (GM 16.3 ng/m²; prevalence of samples with dogs at home 15.8%),

33% contained Fel d 1 (GM 10.3 ng/m²; prevalence of samples with cats at home 8.3%) and 43% contained Mus m 1 (GM 7.7 ng/m²). As expected, EDC sampled high levels of Fel d 1 (GM 403 ng/m²) in homes with cats and high levels of Can f 1 (GM 614 ng/m²) in homes with dogs. ROC analysis was performed in order to define the pet allergen concentrations that discriminate best between households with pets versus households without pets. The Can f 1 levels predicted the presence of dogs (AUC: 98.53%) with a high sensitivity and specificity. The highest Youden index was obtained with a Can f 1 level of 75 ng/m² (96.8% sensitivity, 96% specificity). The Fel d 1 level predicted the presence of cats in homes with an AUC of 96.45%, and the maximal Youden index was obtained with a Fel d 1 level of 46 ng/m² (92% sensitivity, 94.9% specificity).

All allergen levels measured with EDC in DCC and homes are shown as box plots in figure 2. DM levels were similar in homes with or without pets, but these levels were lower than those measured in DCC. Mus m 1 levels were higher in homes with pets than in homes without pets, with highest levels measured in DCC. Fel d 1 levels were higher in DCC than in homes without cats, but lower than in homes with cats. Fifty-four percent of the samples in the DCC had Fel d 1 higher levels than 46 ng/m², which predicts the presence of cats in homes. Can f 1 levels were highest in homes with dogs, followed by DCC, and lowest in homes without dogs. Thirty-seven percent of samples in DCC had Can f 1 loads higher than 75 ng/m², which is the level that predicts the presence of dogs in homes.

In the multilevel models (Table 1), the location of sampling was a dominant determinant for DM, Fel d 1 and Can f 1, but not for Mus m 1 (ICC 54.47%, 48%, 44.4%, and 2.82%, respectively).

Group rooms, side rooms and bedrooms in the DCC had significantly higher DM levels compared to the staff rooms in the DCCs, as well as living and other rooms in homes. DM levels in autumn and summer were about twice as high as those measured in winter and spring. Dog, cat and mouse allergen levels were significantly higher in group rooms at the DCC compared to the bedrooms and staff rooms, as well as all rooms in homes. However, the presence of a dog or cat at home was included as a fixed effect variable in the models for dog and cat allergens, respectively, and

drastically increased allergen levels in homes. Significantly higher levels of Fel d 1 were found in autumn compared to all other seasons; whereas, Can f 1 levels were highest in winter, although not significantly different from autumn. The lowest Can f 1 levels were found in summer.

Influences on allergen levels in DCC

The estimates for all allergens in the multilevel models were predominantly affected by room type and season (Table 2). For all allergens, significantly lower allergen loads were found in staff rooms. Cat, dog and mouse allergen levels, but not DM levels, were also significantly lower in bedrooms compared to the group rooms. DM and Mus m 1 allergen levels were significantly lower in winter and spring than in autumn and summer; Can f 1 levels were significantly lower in spring and summer than in autumn and winter. Higher air humidity increased DM levels, while significantly decreasing mouse allergens. Relative air humidity in DCC during the study ranged from 22% - 85%. This 63% humidity difference influenced DM and Mus m 1 allergen levels by estimated factors of 2.55 (1.015⁶³) and 0.53 (0.99⁶³), respectively. Dog allergen levels were significantly lower in rooms that were used less than 70% during the sampling time.

Influences on allergen levels in homes

Mouse allergen levels were significantly higher in homes with more than 4 residents compared to those with only one or two residents (Table 3). The presence of cats in the homes increased Fel d 1 levels by a factor of 125; the presence of dogs increased Can f 1 levels by a factor of 108. Mus m 1 levels were significantly higher in dwellings with cats and/or dogs; whereas, the DM levels were not significantly influenced. DM loads were significantly lower in living rooms compared to children's rooms and bedrooms; Mus m 1 loads were significantly lower in 'other rooms' (kitchen, bathroom, office) than in children's rooms. In summer, cat and dog allergen loads were less than those

This article is protected by copyright. All rights reserved.

measured in autumn. DM levels were significantly lower in spring and winter than in autumn and summer. Finally, Mus m 1 levels were highest in spring, followed by summer and autumn, with lowest levels measured in winter. The interior, including carpets, plants and soft toys did not significantly influence allergen loads with the exception of upholstered furniture, such as beds and furniture with textile surfaces. The higher the density of such furniture in a room resulted in a significant increase in DM loads.

Discussion

Pet allergen exposure

It is well-known that dwellings with cats or dogs contain sufficient levels of the respective allergens to provoke symptoms in sensitized allergic individuals. Since the identification of major allergens and the development of specific immunoassays to quantify these allergens, Fel d 1 and Can f 1 concentrations in surface dust from houses with these pets have been determined repeatedly. More than 95% of houses with cats had Fel d 1 concentrations of 8 µg/g or greater, and more than 95% of houses with dogs had Can f 1 concentrations of at least 10 µg/g (30). Eight µg/g Fel d 1 has been shown to induce symptoms in almost all patients allergic to cats, and was therefore proposed as the threshold level (31, 32); whereas, 10 µg/g Can f 1 was used as the threshold level eliciting symptoms to dog allergens (33). No such threshold levels existed for sampling allergens with the new EDC method. In addition, standards for allergen quantification using these immunoassays have changed since the definition of former thresholds (34). Therefore, a first step in our data analysis of 602 EDC samples from homes was to define exposure levels typical for households with pets. Can f 1 levels of ≥ 75 ng/m² predicted the presence of dogs with 96.8% sensitivity and 96% specificity; whereas Fel d 1 levels of ≥ 46 ng/m² predicted the presence of cats in homes with 92% sensitivity and 94.9% specificity. It could therefore be assumed that such levels are sufficient to elicit symptoms in allergic patients.

In DCC, exposure to cat and dog allergens often reached such high levels: 54% of samples had levels higher than 46 ng/m² Fel d 1, and 37% of samples had Can f 1 loads higher than 75 ng/m². The higher percentage of samples with elevated Fel d 1 levels compared to Can f 1 levels may be due to the higher frequency of children with cats than dogs at home (16.5% vs 13%, respectively, which may not be altogether accurate due to the incomplete participation in the question on pet ownership). Importantly, volunteers who sampled in their homes more often were dog rather than cat owners (15.5% vs. 10.6%), which was partially due to the participation of day-care workers who more often had dogs than cats (17.6% vs. 7.1%). Frequency of households with pets in our study was rather low compared to an estimated average of dog ownership of 33% worldwide and 21% in Germany, and cat ownership of 23% worldwide and 29% in Germany (35). Since the number of pet owners at schools or DCC is one of the strongest predictors of elevated cat and dog allergen levels in these settings (36), cat and dog allergen exposure in DCC might be even higher in other regions than in our study and highly relevant for the question about how children without animals at home become sensitized. According to the population-based KiGGS study (37), 9.7% or 8.1% of German children and adolescents were sensitized to dogs or cat dander, respectively.

Similar high exposure to pet allergens was found in schools in the Netherlands using EDC and Indoor multiplex assays for Fel d 1 and Can f 1 quantification (19). In addition, pet allergen exposure in schools was much higher when compared to homes without pets, which concurs with our comparison between pet exposure levels in DCC and homes. The study results are comparable since the multiplex assay results were similar to the amplified FEIA results obtained when 132 of the samples from the study performed in the Netherlands were reanalysed using the FEIA assays (38). Overall, with the exception of households with pets, pet allergen levels are generally higher in DCC compared to those in households. Major factors that influence exposure include the room type, frequency of usage, and the season.

Mouse allergen exposure

In Europe, mouse allergens have been rarely studied outside laboratory animal facilities. In the school study from the Netherlands, mouse allergen levels were low, but still two times higher in schools compared to homes (19). In addition, mouse allergen levels were significantly higher in homes with pets than without pets. Our study shows similar results. Overall mouse allergen levels were low, but about two times higher in DCC than in homes. Furthermore, levels were significantly higher in homes with pets than in homes without pets. However, the location of sampling explained less than 3 % of the total variation in the multilevel model of all samples.

In 1995, Jones et al. (39) observed a decrease in airborne mouse allergen due to increased air humidity in an environmentally controlled mouse room (3 ng/m³ at 15% humidity and 0.5 ng/m³ at 65% humidity). We also observed a significant decrease in Mus m 1 levels with increased air humidity, although the estimated loss due to the 50% elevation in humidity is 40% in the multilevel model in DCC. Households with more than four residents had twofold higher mouse allergen levels. However, mouse allergen concentrations on EDC in laboratory animal facilities (18) were more than 100fold higher than Mus m 1 levels in DCC and households. In Europe, primary sensitization and allergy to mice outside occupational settings is generally rare (40); therefore, the low Mus m 1 levels found in our study may not be of relevance to human health.

Domestic mite allergen exposure

For quantification of mite allergens, the polyclonal antibody-based DM FEIA was used since previous studies showed that single house dust mite allergens were usually below the detection limit in airborne dust or on EDC (19). This assay detects domestic mite antigens, not only from *Dermatophagoides* but also from several storage mites (26). Anti-DM-antibodies inhibited human IgE-binding to *D. farinae* and *D. pteronyssinus* ImmunoCAPs in a dose dependent manner by 84-98%

and 64-85%, respectively. This indicates that the DM-immunoassay is able to detect the majority of *Dermatophagoides* allergens. To date, 31 *D. farinae* allergens and 20 *D. pteronnyssinus* are listed in the allergen nomenclature database of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) (41). Whereas monoclonal antibody-based mite immunoassays detect only single major allergens, e.g. Der f 1 or Der p 1, the DM-immunoassay detects several allergens, as well as antigens that might not be allergenic. Both strategies for allergen quantification have their advantages and disadvantages. Most important is that the measurement is a good proxy for the exposure that is relevant for sensitization and allergic symptoms. Although absolute values differ between assays (DM at mean 144 times higher than Der f 1), there was an excellent correlation between the results when surface samples were analyzed (26), and a moderate correlation in EDC samples that were, however, rarely above the detection limit for Der f 1 (38).

DM was measurable in 96% of DCC samples (GM 367 ng/m²) and 84% of the samples collected from homes (GM 248 ng/m²). DM levels were higher in group, side and bedrooms in DCC compared to all rooms in homes. Significantly higher levels were obtained in group and side rooms in comparison to living rooms and other rooms in homes. Thus, on average DM levels were higher in DCC than in homes. These results are in contrast to data obtained by surface measurements, which imply much higher domestic mite allergen levels in dwellings than in DCC (24, 42). The different outcome may be explained by higher activities and air movements in rooms with many children, which increases the allergen levels on EDC collected from DCC. Conversely, less frequent surface cleanings may increase the allergen concentrations in surface dust at home. Although stationary sampling using EDC that were placed at an average height of 1.8 m above the floor may produce results that differ considerably from personal air sampling, we consider EDC results to be a better proxy for exposure than surface allergen levels or concentrations. DM levels measured in schools and homes in the Netherlands using EDC (19), revealed that homes in the Netherlands had higher geometric mean values than homes in Germany; whereas, the values in the schools in the Netherlands were lower than those measured in German DCC.

This article is protected by copyright. All rights reserved.

More than 50% of total variation in the multilevel model of all samples depended on the location of sampling. Major factors that influenced mite allergen exposure were the room type, season and air humidity, and the number of upholstered furniture, including beds. Carpets on the floor had no effect, which contradicts the findings of previous studies (42–44) reporting higher mite allergen levels on carpets than on smooth floors, including our own observations in DCC (24). However, although carpets are a reservoir for indoor allergens, they are able to trap particulates and may thus prevent allergens from becoming airborne.

Conclusion

Overall, indoor allergen levels on EDC were higher in DCC than in homes. These findings are new and contradict previous studies in DCC where allergens were quantified in surface dust (42, 44). Dog and cat allergen levels predicted the presence of a dog or cat at home, with an AUC of 98.5 and 96.5%, respectively. The threshold level for homes with dogs was 75 ng/m² Can f 1, and 46 ng/m² Fel d 1 for homes with cats. Indoor allergen levels were significantly influenced by the room type and upholstered furniture per room, season and humidity; whereas, carpets on the floor had no influence.

Acknowledgments

We express our gratitude to the day-care workers and the children's parents for their support and participation in our study. We acknowledge the efforts of Martin Buxtrup, Michael Stolle and Wolfgang Swoboda on data acquisition during repeated visits to the day-care centres. In addition, we would like to thank Rosemarie Marchan for improving the language and Laura Hoyden for initial statistical analysis of data in her Master's thesis.

Author contributions

HN, MR and IS were responsible for the conception and design of the study. IS, AL, CC, AF and EZ were responsible for acquisition of data and the statistical analysis. IS and AL wrote the first draft of the manuscript. All coauthors contributed substantially to the analyses and interpretation of the data, writing and approval of the final version of the manuscript.

Funding

The study was financially supported by German Social Accident Insurance as part of the project IPA-47. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Sheehan WJ, Phipatanakul W. Indoor allergen exposure and asthma outcomes. *Curr. Opin. Pediatr.* 2016;**28**:772-777.
2. Lau S, Falkenhorst G, Weber A, Werthmann I, Lind P, Buettner-Goetz P et al. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J. Allergy Clin. Immunol.* 1989;**84**:718-725.
3. Torjusen EN, Diette GB, Breyse PN, Curtin-Brosnan J, Aloe C, Matsui EC. Dose-response relationships between mouse allergen exposure and asthma morbidity among urban children and adolescents. *Indoor air* 2013;**23**:268-274.
4. Brussee JE, Smit HA, van Strien RT, Corver K, Kerkhof M, Wijga AH et al. Allergen exposure in infancy and the development of sensitization, wheeze, and asthma at 4 years. *J. Allergy Clin. Immunol.* 2005;**115**:946-952.

5. Morgan WJ, Crain EF, Gruchalla RS, O'Connor GT, Kattan M, Evans R3 et al. Results of a home-based environmental intervention among urban children with asthma. *N. Engl. J. Med.* 2004;**351**:1068-1080.
6. Lødrup Carlsen KC, Roll S, Carlsen K-H, Mowinckel P, Wijga AH, Brunekreef B et al. Does pet ownership in infancy lead to asthma or allergy at school age? Pooled analysis of individual participant data from 11 European birth cohorts. *PLoS ONE* 2012;**7**:e43214.
7. Bremner SF, Simpson EL. Dust mite avoidance for the primary prevention of atopic dermatitis: A systematic review and meta-analysis. *Pediatr. Allergy. Immunol.* 2015;**26**:646-654.
8. Gøtzsche PC, Johansen HK. House dust mite control measures for asthma: systematic review. *Allergy* 2008;**63**:646-659.
9. Gehring U, Jongste JC de, Kerkhof M, Oldewening M, Postma D, van Strien RT et al. The 8-year follow-up of the PIAMA intervention study assessing the effect of mite-impermeable mattress covers. *Allergy* 2012;**67**:248-256.
10. Toelle BG, Garden FL, Ng KKW, Belousova EG, Almqvist C, Cowell CT et al. Outcomes of the childhood asthma prevention study at 11.5 years. *J. Allergy Clin. Immunol.* 2013;**132**:1220.
11. Schoos A-MM, Chawes BL, Jelding-Dannemand E, Elfman LB, Bisgaard H. Early indoor aeroallergen exposure is not associated with development of sensitization or allergic rhinitis in high-risk children. *Allergy* 2016;**71**:684-691.
12. Tovey ER, Marks GB. It's time to rethink mite allergen avoidance. *J. Allergy Clin. Immunol.* 2011;**128**:723.
13. Tovey E, Ferro A. Time for new methods for avoidance of house dust mite and other allergens. *Curr. Allergy. Asthma Rep.* 2012;**12**:465-477.
14. Tovey ER, Willenborg CM, Crisafulli DA, Rimmer J, Marks GB. Most personal exposure to house dust mite aeroallergen occurs during the day. *PLoS ONE* 2013;**8**:e69900.
15. Tovey ER, Liu-Brennan D, Garden FL, Oliver BG, Perzanowski MS, Marks GB. Time-Based Measurement of Personal Mite Allergen Bioaerosol Exposure over 24 Hour Periods. *PLoS ONE* 2016;**11**:e0153414.
16. Noss I, Wouters IM, Visser M, Heederik DJ, Thorne PS, Brunekreef B et al. Evaluation of a low-cost electrostatic dust fall collector for indoor air endotoxin exposure assessment. *Appl. Environ. Microbiol.* 2008;**74**:5621-5627.
17. Samadi S, Heederik DJJ, Krop EJM, Jamshidifard A-R, Willemse T, Wouters IM. Allergen and endotoxin exposure in a companion animal hospital. *Occup. Environ. Med.* 2010;**67**:486-492.
18. Feistenauer S, Sander I, Schmidt J, Zahradnik E, Raulf M, Brielmeier M. Influence of 5 Different Caging Types and the Use of Cage-Changing Stations on Mouse Allergen Exposure. *J. Am. Assoc. Lab. Anim. Sci.* 2014;**53**:356-363.

- Accepted Article
19. Krop EJM, Jacobs JH, Sander I, Raulf-Heimsoth M, Heederik DJJ. Allergens and β -Glucans in dutch homes and schools: characterizing airborne levels. *PLoS ONE* 2014;**9**:e88871.
 20. Kilburg-Basnyat B, Metwali N, Thorne PS. Effect of deployment time on endotoxin and allergen exposure assessment using electrostatic dust collectors. *Ann. Occup. Hyg.* 2015;**59**:104-115.
 21. Sander I, Lotz A, Zahradnik E, Raulf M. Allergen Quantification by Use of Electrostatic Dust Collectors (EDCs): Influence of Deployment Time, Extraction Buffer, and Storage Conditions on the Results. *Ann. Occup. Hyg.* 2016;**60**:845-859.
 22. Raulf M, Bergmann KC, Kull S, Sander I, Hilger C, Brüning T et al. Mites and other indoor allergens - from exposure to sensitization and treatment. *Allergo Journal International* 2015;**24**:68-80.
 23. Statistische Ämter des Bundes und der Länder. Kindertagesbetreuung regional 2014. Ein Vergleich aller 402 Kreise in Deutschland, 2015. Wiesbaden.
https://www.destatis.de/DE/Publikationen/Thematisch/Soziales/KinderJugendhilfe/KindertagesbetreuungRegional5225405147004.pdf?__blob=publicationFile.
 24. Sander I, Neumann H-D, Lotz A, Czibor C, Zahradnik E, Flagge A et al. Allergen quantification in surface dust samples from German day care centers. *J. Toxicol. Environ. Health A* 2016;**79**:1094-1105.
 25. Filep S, Tsay A, Vailes LD, Gadermaier G, Ferreira F, Matsui E et al. Specific allergen concentration of WHO and FDA reference preparations measured using a multiple allergen standard. *J. Allergy Clin. Immunol.* 2012;**129**:1408-1410.
 26. Sander I, Zahradnik E, Kraus G, Mayer S, Neumann HD, Fleischer C et al. Domestic mite antigens in floor and airborne dust at workplaces in comparison to living areas: a new immunoassay to assess personal airborne allergen exposure. *PLoS ONE* 2012;**7**:e52981.
 27. Zock JP, Heinrich J, Jarvis D, Verlato G, Norbäck D, Plana E et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;**118**:682-690.
 28. Vaida F, Liu L. Fast Implementation for Normal Mixed Effects Models With Censored Response. *J. Comput. Graph. Stat.* 2009;**18**:797-817.
 29. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria, 2015. <http://www.R-project.org>.
 30. Ingram JM, Sporik R, Rose G, Honsinger R, Chapman MD, Platts-Mills TA. Quantitative assessment of exposure to dog (Can f 1) and cat (Fel d 1) allergens: relation to sensitization and asthma among children living in Los Alamos, New Mexico. *J. Allergy Clin. Immunol.* 1995;**96**:449-456.
 31. Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TA. Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. *Am. Rev. Respir. Dis.* 1993;**147**:573-578.

- Accepted Article
32. Woodfolk JA, Luczynska CM, Blay F de, Chapman MD, Platts-Mills TA. Cat allergy. *Ann. Allergy* 1992;**69**:273-275.
 33. Tranter DC, Wobbema AT, Norlien K, Dorschner DF. Indoor allergens in Minnesota schools and child care centers. *J. Occup. Environ. Hyg.* 2009;**6**:582-591.
 34. Filep S, Tsay A, Vailes L, Gadermaier G, Ferreira F, Matsui E et al. A multi-allergen standard for the calibration of immunoassays: CREATE principles applied to eight purified allergens. *Allergy* 2012;**67**:235-241.
 35. Zahradnik E, Raulf M. Respiratory Allergens from Furred Mammals. *Environmental and Occupational Exposure. Veterinary Sciences* 2017;**4**:38.
 36. Salo PM, Sever ML, Zeldin DC. Indoor allergens in school and day care environments. *J. Allergy Clin. Immunol.* 2009;**124**:185-92, 192.e1-9; quiz 193-4.
 37. Schmitz R, Ellert U, Kalcklösch M, Dahm S, Thamm M. Patterns of sensitization to inhalant and food allergens - findings from the German Health Interview and Examination Survey for Children and Adolescents. *International archives of allergy and immunology* 2013;**162**:263-270.
 38. Sander I, Zahradnik E, Krop E, Fleischer C, Heederik DJJ, Brüning T et al. Indoor allergen exposure assessment comparison of amplified fluorescence enzyme immunoassays for quantification of Fel d 1, Can f 1 and domestic mite antigens with a multiplex array. *Allergy* 2013;**68**:171.
 39. Jones RB, Kacergis JB, MacDonald MR, McKnight FT, Turner WA, Ohman JL et al. The effect of relative humidity on mouse allergen levels in an environmentally controlled mouse room. *American Industrial Hygiene Association journal* 1995;**56**:398-401.
 40. Liccardi G, Salzillo A, Sofia M, Piccolo A, Dente B, Russo M et al. Sensitization to rodents (mouse/rat) in an urban atopic population without occupational exposure living in Naples, Italy. *European annals of allergy and clinical immunology* 2012;**44**:200-204.
 41. Allergen Nomenclature Sub-committee. WHO/IUIS Allergen Nomenclature Home Page. <http://www.allergen.org/index.php> (Mar 2, 2017).
 42. Engelhart S, Bieber T, Exner M. House dust mite allergen levels in German day-care centers. *Int. J. Hyg. Environ. Health* 2002;**205**:453-457.
 43. Causer S, Shorter C, Sercombe J. Effect of floorcovering construction on content and vertical distribution of house dust mite allergen, Der p I. *J. Occup. Environ. Hyg.* 2006;**3**:161-8; quiz D45.
 44. Arbes SJ, Sever M, Mehta J, Collette N, Thomas B, Zeldin DC. Exposure to indoor allergens in day-care facilities: results from 2 North Carolina counties. *J. Allergy Clin. Immunol.* 2005;**116**:133-139.

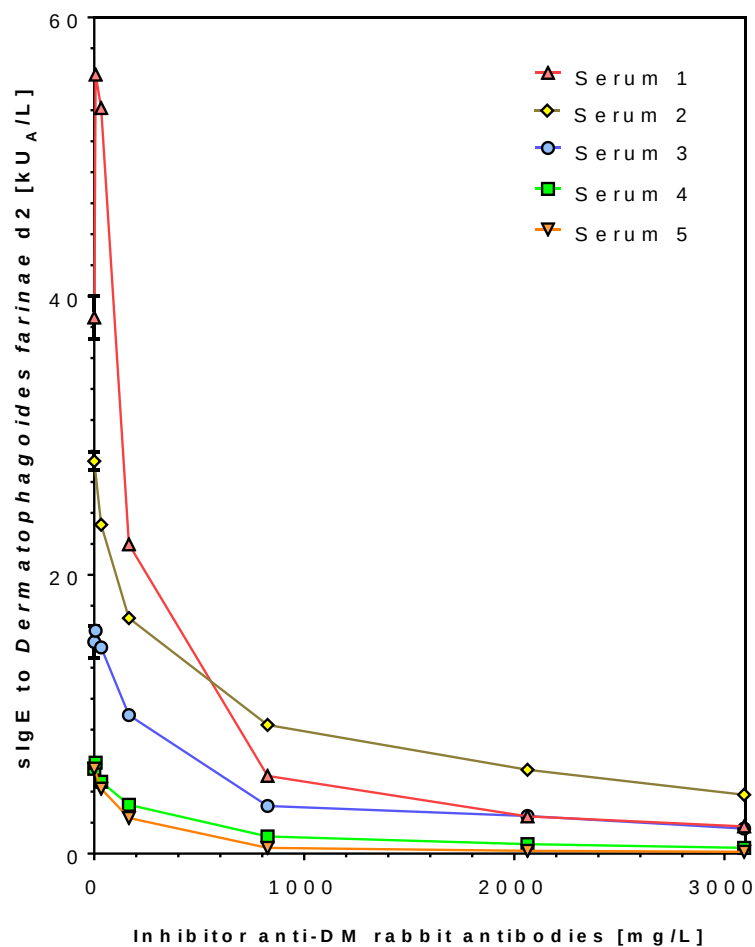


Fig. 1: Competitive inhibition of IgE-binding to *D. farinae* ImmunoCAPs by increasing concentrations of anti-DM rabbit antibodies. Sera from five patients (Serum 1 - Serum 5) with different IgE-binding capacity to *D. farinae* were used.

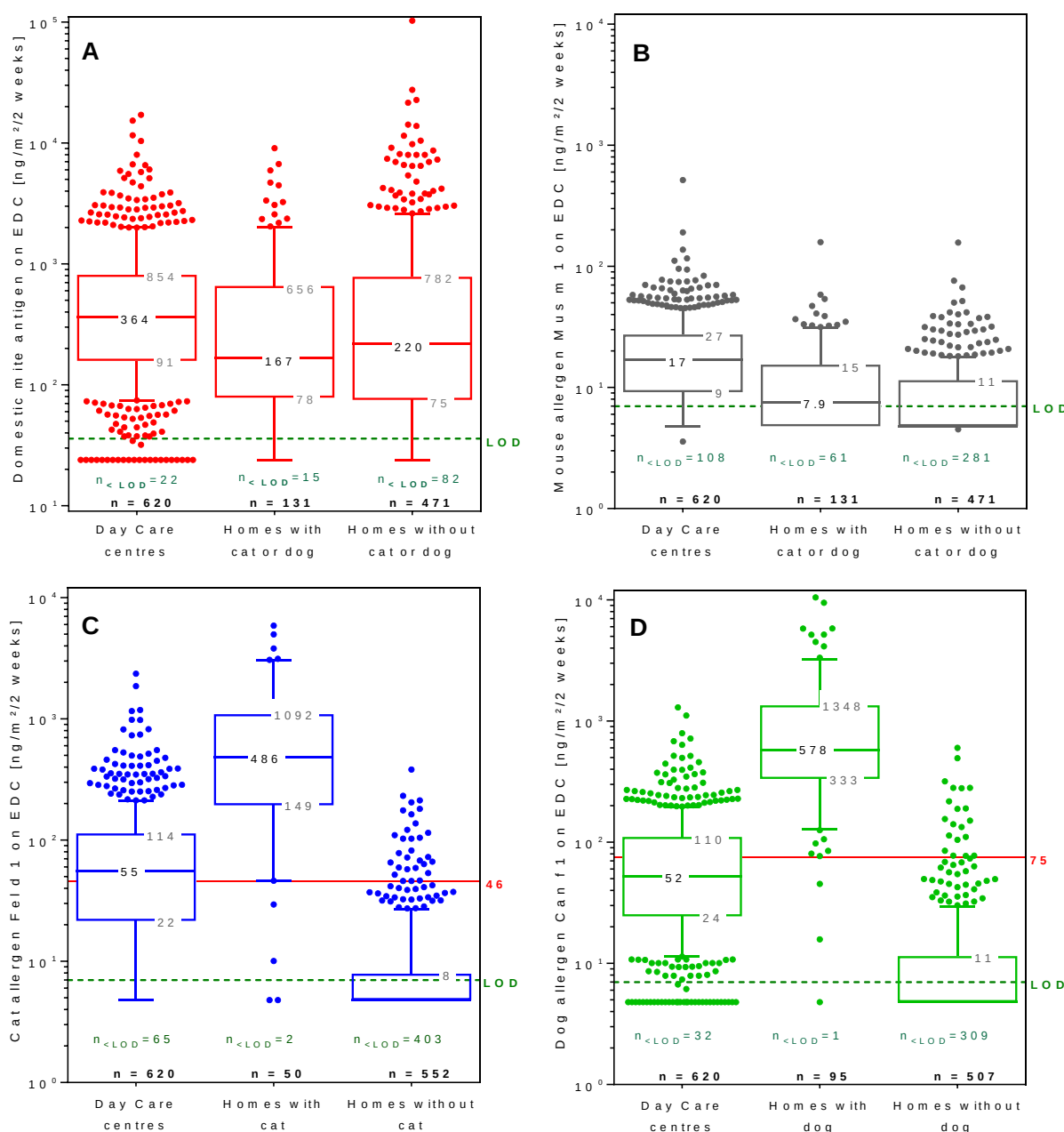


Fig. 2: DM (a), Mus m 1 (b), Can f 1 (c), and Fel d 1 (d) levels on EDC in DCC and homes. Boxes and numbers comprise the 25th and 75th percentile and the median values. Vertical bars show the 10th and 90th percentiles and circles represent values outside of the given percentiles. Sample numbers and the numbers of values below LOD are indicated. In addition, the cat and dog allergen levels that discriminate best between samples from homes with or without pet are marked with a solid line.

Table 1. Multilevel models of allergen levels on all EDC in DCC and homes.

Fixed effects			Domestic mite allergen			Cat allergen Fel d 1			Dog allergen Can f 1			Mouse allergen Mus m 1		
Variable	Category	Reference	estimate	CI	p-value	estimate	CI	p-value	estimate	CI	p-value	estimate	CI	p-value
Pets	Intercept (ng/m ²)		577.99	(349.8–955.05)		77.09	(49.13–120.94)		91.64	(61.63–136.25)		22.66	(19.92–25.78)	
	Cat / Dog yes* (n=50, 95, 131)	Cat / Dog no* (n=1172, 1127, 1091)				67.76	(39.82–115.31)	<0.0001	91.56	(62.87–133.34)	<0.0001	1.20	(1.04–1.39)	0.014
Type of room	Bedroom DCC (n=46)	Group room DCC (n=227)	0.97	(0.63–1.50)	0.885	0.30	(0.19–0.49)	<0.0001	0.55	(0.38–0.81)	0.002	0.60	(0.47–0.76)	<0.0001
	corridor DCC (n=71)	Group room DCC (n=227)	0.67	(0.47–0.97)	0.033	0.94	(0.63–1.39)	0.748	0.83	(0.61–1.14)	0.261	0.98	(0.80–1.20)	0.845
	Side room DCC (n=203)	Group room DCC (n=227)	1.22	(0.95–1.59)	0.125	0.91	(0.69–1.20)	0.506	0.85	(0.68–1.06)	0.156	0.95	(0.82–1.09)	0.431
	Staff room DCC (n=73)	Group room DCC (n=227)	0.26	(0.18–0.37)	<0.0001	0.21	(0.14–0.31)	<0.0001	0.28	(0.20–0.38)	<0.0001	0.42	(0.35–0.52)	<0.0001
	Children's room home (n=343)	Group room DCC (n=227)	0.77	(0.45–1.33)	0.347	0.07	(0.04–0.12)	<0.0001	0.09	(0.06–0.14)	<0.0001	0.47	(0.40–0.54)	<0.0001

Season	Living room home (n=142)	Group room DCC (n=227)	0.28	(0.15–0.51)	<0.0001	0.06	(0.03-0.19)	<0.0001	0.08	(0.05–0.13)	<0.0001	0.39	(0.33–0.47)	<0.0001
	bedroom home (n=85)	Group room DCC (n=227)	0.90	(0.46–1.78)	0.761	0.07	(0.04-0.14)	<0.0001	0.06	(0.03–0.11)	<0.0001	0.42	(0.34–0.51)	<0.0001
	Other rooms home (n=32)	Group room DCC (n=227)	0.21	(0.09–0.53)	0.001	0.04	(0.02–0.11)	<0.0001	0.05	(0.02–0.12)	<0.0001	0.36	(0.27–0.47)	<0.0001
	Winter (n=327)	Autumn (n=335)	0.52	(0.45–0.62)	<0.0001	0.72	(0.62–0.85)	0.0001	1.11	(0.98–1.27)	0.103	0.75	(0.69–0.81)	<0.0001
	Spring (n=291)	Autumn (n=335)	0.49	(0.41–0.58)	<0.0001	0.65	(0.55–0.76)	<0.0001	0.61	(0.53–0.70)	<0.0001	0.81	(0.75–0.88)	<0.0001
	Summer (n=269)	Autumn (n=335)	0.96	(0.81–1.14)	0.629	0.65	(0.55–0.77)	<0.0001	0.38	(0.33–0.44)	<0.0001	0.98	(0.90–1.07)	0.686
ICC Location (Room, Building)			54.47%			48.00%			44.40%			2.82%		

Influences with p-values <0.00417 (Bonferroni correction of a significance level of .05 by the number of variables in each model) are printed in bold.
Estimate: This is the transformed coefficient estimate exp (β) of the multilevel model. CI: confidence interval. Intercept: This is the calculated allergen level for the reference classes. *The presence of dogs is included in the model for Can f 1, the presence of cats is included in the model for Fel d 1, and the presence of cats or dogs is included in the model for Mus m 1. ICC: Intraclass correlation coefficient of the model.

Table 2. Multilevel models of allergen levels on EDC in DCC.

			Domestic mite allergen				Cat allergen Fel d 1				Dog allergen Can f 1				Mouse allergen Mus m 1			
	Category	Reference	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value
Intercept (ng/m²)			218.357	76.292	624.961		23.342	6.096	89.374		113.240	44.995	283.994		37.937	19.875	72.411	
Number of Children	(per 1 child increase)		1.002	0.993	1.012	0.677	1.009	0.995	1.022	0.227	0.999	0.99	1.007	0.752	1	0.994	1.005	0.876
Animals (non-fur)	Yes (n=142)	No (n=478)	1.326	0.6	2.932	0.485	1.232	0.392	3.88	0.721	1.725	0.826	3.604	0.147	1.271	0.811	1.99	0.295
Location	Rural (n=290)	Urbanized (n=330)	1.01	0.622	1.639	0.97	0.908	0.452	1.825	0.787	1.306	0.833	2.048	0.244	0.989	0.751	1.302	0.936
Renovation	Yes (n=439)	No (n=181)	0.711	0.436	1.157	0.17	1.04	0.518	2.086	0.913	1.359	0.865	2.135	0.183	1.131	0.857	1.494	0.385
Type of room	Bedroom (n=46)	Group room (n=227)	0.925	0.586	1.46	0.737	0.284	0.174	0.462	<0.0001	0.495	0.334	0.733	0.0004	0.463	0.341	0.63	<0.0001
	Side-room (n=203)	Group room (n=227)	1.145	0.839	1.563	0.392	0.879	0.633	1.222	0.443	0.791	0.606	1.032	0.084	0.89	0.727	1.091	0.263
	Staff room (n=73)	Group room (n=227)	0.259	0.171	0.392	<0.0001	0.199	0.128	0.31	<0.0001	0.241	0.169	0.344	<0.0001	0.311	0.235	0.412	<0.0001
	Corridor (n=71)	Group room (n=227)	0.692	0.468	1.022	0.064	0.874	0.58	1.319	0.522	0.75	0.538	1.046	0.09	0.89	0.689	1.15	0.374
Area of the room	(per 1 m² increase)		0.997	0.992	1.002	0.209	0.998	0.993	1.003	0.376	0.998	0.995	1.002	0.445	0.998	0.995	1.001	0.191
Height of room	>2.4 m	≤2.40 m	1.462	1.023	2.089	0.037	1.614	1.094	2.381	0.016	0.935	0.688	1.271	0.667	1.242	0.979	1.576	0.074

	(n=540)	(n=80)																
Season	Winter (n=165)	Autumn (n=165)	0.612	0.5	0.75	<0.0001	0.772	0.641	0.93	0.007	1.157	0.997	1.343	0.055	0.587	0.51	0.676	<0.0001
	Spring (n=146)	Autumn (n=165)	0.692	0.545	0.877	0.002	0.769	0.616	0.959	0.02	0.599	0.502	0.714	<0.0001	0.618	0.524	0.729	<0.0001
	Summer (n=144)	Autumn (n=165)	0.929	0.747	1.155	0.507	0.807	0.659	0.987	0.037	0.368	0.313	0.433	<0.0001	1.029	0.886	1.194	0.71
Air humidity	(per 1% increase)		1.015	1.006	1.024	0.001	1.006	0.998	1.015	0.15	0.996	0.99	1.003	0.278	0.99	0.984	0.996	0.002
Occupancy	≤ 70% (n=157)	70–≤75% (n=383)	0.892	0.726	1.096	0.278	0.753	0.623	0.911	0.003	0.651	0.558	0.759	<0.0001	0.841	0.729	0.971	0.018
	> 75% (n=80)	70–≤75% (n=383)	1.026	0.8	1.316	0.838	1.106	0.878	1.393	0.391	0.984	0.817	1.185	0.861	1.095	0.924	1.298	0.293
Underfloor heating	Yes (n=95)	No (n=525)	1.343	1.003	1.798	0.047	1.072	0.814	1.412	0.62	0.901	0.723	1.122	0.352	1.164	0.955	1.419	0.133
Carpet (%)	10–≤50 % (n=270)	≤10 % (n=247)	0.98	0.75	1.28	0.883	0.845	0.641	1.114	0.232	0.846	0.677	1.057	0.141	0.827	0.692	0.987	0.036
	>50 % (n=103)	≤10 % (n=247)	0.992	0.717	1.372	0.96	1.033	0.735	1.452	0.852	0.948	0.723	1.243	0.701	0.943	0.757	1.173	0.597

Influences with p-values <0.00263 (Bonferroni correction of the significance level of .05 by the number of variables in each model) are printed in bold.

Estimate: This is the transformed coefficient estimate $\exp(\beta)$ of the multilevel model. CI_l: lower confidence interval. CI_u: upper confidence interval.

Intercept: This is the calculated allergen level for the reference class.

Table 3. Multilevel models of allergen levels on EDC in dwellings.

			Domestic mite allergen				Cat allergen Fel d 1				Dog allergen Can f 1				Mouse allergen Mus m 1			
	Category	Reference	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value	estimate	CI_l	CI_u	p-value
Intercept (ng/m ²)			144.90 0	53.45 4	392.78 7		11.086	3.205	38.350		10.291	4.056	26.11 4		9.134	5.168	16.142	
Number of residents	3 (n=129)	1-2 (n=153)	1.036	0.598	1.792	0.9	1.136	0.599	2.153	0.697	0.868	0.519	1.453	0.59	1.036	0.759	1.415	0.824
	4 (n=254)	1-2 (n=153)	1.993	1.187	3.345	0.009	0.875	0.472	1.623	0.673	0.832	0.513	1.35	0.456	1.184	0.885	1.585	0.256
	>4 (n=66)	1-2 (n=153)	2.345	1.172	4.695	0.016	0.905	0.389	2.102	0.816	0.661	0.343	1.272	0.215	2.271	1.559	3.309	<0.0001
Mite allergic resident	Yes (n=166)	No (n=436)	1.499	0.946	2.373	0.085	1.357	0.78	2.361	0.28	1.628	1.056	2.509	0.027	0.988	0.759	1.285	0.927
Mite avoidance	Yes (n=149)	No (n=453)	0.639	0.383	1.066	0.087	0.83	0.453	1.521	0.547	0.762	0.473	1.23	0.266	0.665	0.5	0.886	0.005
Cat* Dog* Cat/Dog	Yes* (n=50, 95, 131)	No* (n=552, 507, 471)	1.111	0.717	1.721	0.638	124.648	66.46	233.77	<0.0001	108.163	69.633	168.01	<0.0001	1.451	1.142	1.843	0.002
Type of room	Living room (n=142)	Children's room (n=343)	0.425	0.248	0.73	0.002	1.076	0.509	2.272	0.849	0.697	0.383	1.271	0.239	0.636	0.445	0.91	0.013
	Bedroom (n=85)	Children's room (n=343)	1.94	1.02	3.69	0.044	1.203	0.561	2.58	0.635	0.638	0.343	1.188	0.157	0.709	0.49	1.027	0.069
	Other rooms (n=32)	Children's room (n=343)	0.464	0.182	1.18	0.107	0.434	0.134	1.402	0.163	0.514	0.203	1.299	0.159	0.418	0.242	0.721	0.002
Area of the room	(per 1 m ² increase)		0.988	0.967	1.009	0.253	0.959	0.932	0.987	0.004	1.002	0.983	1.022	0.819	0.994	0.982	1.006	0.315
Height of room	>2.4 m (n=363)	≤2.40 m (n=239)	1.459	1.012	2.103	0.043	1.332	0.863	2.058	0.196	0.749	0.532	1.055	0.098	1.027	0.84	1.257	0.794
Season	Winter (n=162)	Autumn (n=170)	0.497	0.378	0.653	<0.0001	0.699	0.481	1.016	0.06	1.178	0.908	1.528	0.218	0.692	0.562	0.852	0.001
	Spring	Autumn	0.461	0.347	0.612	<0.0001	0.598	0.405	0.885	0.01	0.68	0.514	0.898	0.007	1.468	1.212	1.777	0.0001

	(n=145)	(n=170)																
	Summer (n=125)	Autumn (n=170)	1.084	0.807	1.455	0.594	0.408	0.265	0.628	<0.0001	0.47	0.345	0.64	<0.0001	1.277	1.042	1.564	0.018
Location of room	2. floor (n=250)	≤ ground floor (n=170)	1.292	0.82	2.036	0.27	0.715	0.417	1.229	0.225	0.886	0.577	1.36	0.579	0.822	0.639	1.058	0.129
	≥ 3. Floor (n=82)	≤ ground floor (n=170)	0.883	0.55	1.419	0.608	0.604	0.348	1.049	0.074	0.834	0.534	1.304	0.426	0.893	0.689	1.158	0.395
Plants	Yes (n=273)	No (n=329)	0.811	0.553	1.189	0.283	1.254	0.782	2.011	0.347	0.819	0.568	1.179	0.283	1.078	0.859	1.353	0.519
Soft toys	Yes (n=379)	No (n=223)	0.702	0.468	1.054	0.088	1.102	0.67	1.812	0.703	0.749	0.508	1.104	0.144	0.745	0.584	0.952	0.019
Smooth furniture	(per 1 increase /10 m²)		1.114	0.995	1.248	0.06	0.995	0.867	1.142	0.944	1.09	0.977	1.216	0.121	1.019	0.955	1.087	0.572
Upholstered furniture	(per 1 increase /10 m²)		1.526	1.182	1.969	0.001	0.908	0.667	1.237	0.541	1.132	0.887	1.445	0.318	1.001	0.865	1.158	0.993
Occupancy	<100% (n=84)	100% (n=518)	0.703	0.496	0.996	0.048	1.011	0.625	1.635	0.965	0.831	0.586	1.177	0.297	0.763	0.601	0.968	0.026
Underfloor heating	Yes (n=81)	No (n=521)	0.984	0.576	1.681	0.952	0.662	0.343	1.277	0.218	0.899	0.543	1.49	0.68	1.004	0.753	1.339	0.978
Carpet (%)	10–≤50 % (n=143)	≤10 % (n=339)	1.167	0.803	1.696	0.417	1.127	0.71	1.79	0.612	1.072	0.755	1.523	0.697	1.076	0.864	1.341	0.511
	>50 % (n=120)	≤10 % (n=339)	0.934	0.585	1.491	0.774	0.664	0.37	1.189	0.168	0.557	0.352	0.88	0.012	0.711	0.542	0.932	0.014

Influences with p-values <0.00208 (Bonferroni correction of the significance level of .05 by the number of variables in each model) are printed in bold. Estimate: This is the transformed coefficient estimate exp (β) of the multilevel model. CI_l: lower confidence interval. CI_u: upper confidence interval. Intercept: This is the calculated allergen level for the reference class.*The presence of cats is included in the model for Fel d 1, the presence of dogs is included in the model for Can f 1, and the presence of cats or dogs is included in the model for domestic mites and Mus m 1.