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Endotoxin levels in homes and classrooms of Dutch school children and respiratory health

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Abstract

Several studies describe indoor pollutant exposure in homes and to a lesser extent in schools. Population studies that include both environments are sparse.

This study aims to assess endotoxin levels in primary schools and homes of children. Endotoxin was also studied in relation to asthma and sensitization.

Ten schools with (index) and without (reference) dampness were selected, based on reports and inspections. Cases and controls were selected from 169 homes, based on the presence or absence of asthma-like symptoms of children. Classrooms and bedrooms airborne settled dust was sampled with the Electrostatic Dust fall Collectors (EDC).

Average endotoxin levels in schools ranged from 2178 to 6914 EU/m^2 per week compared to 462 to 1285 EU/m^2 per week in homes. After mutual adjustment for home and school endotoxin, home endotoxin was inversely associated with asthma (OR=0.90 (0.82-1.00). School exposure was positively associated with non-atopic asthma (OR=1.13; 95%CI 1.03-1.25).

The high endotoxin levels in schools compared to homes indicate that exposure at school can contribute considerably to environmental endotoxin exposure of children and teachers. Our results also suggest that endotoxin in schools may be associated with non-atopic asthmatic symptoms in pupils, although the results require reproduction because of the modest sample size.

Keywords: asthma, children, endotoxin, homes, schools, sensitization

Introduction

School is, next to home, an important indoor environment for young children and may contribute to children's exposure. Several studies describe the variety and extent of indoor pollutant exposure in homes and to a lesser extent in schools. Using levels from a single microenvironment may however not adequately represent daily personal exposure in children and also other environments need to be taken into account. Studies that include both home and school environment at the same time are however sparse.

Poor indoor air quality in schools may affect the performance and attendance of students [1]. Home dampness and elevated microbial exposures have been associated with respiratory health effects. Specific causative agents have not been identified, but most observations are available for endotoxin[2,3,4]. In schools, allergen levels have been studied most extensively[5], but only few studies examined endotoxin or levels of other microbial markers[6-13]. Endotoxin is a cell wall component of the outer membrane of gram-negative bacteria and is present in indoor environments as constituents of house dust. In homes endotoxin have been linked to decreased rates of sensitization, asthma and hay fever in early childhood [14-16], but endotoxin is also associated with increased symptom prevalence and non-allergic asthma [14,17]. In most studies, endotoxin in schools was studied in a small number of measurements and endotoxin was mainly measured in floor dust[6,8-11], which is a poor proxy of inhalatory endotoxin exposure, as classroom floor dust consists for a major part of large and heavy particles (sand, breadcrumbs). Only one recent study measured floor endotoxin levels in homes and schools of asthmatics and found higher endotoxin levels in school compared to homes of asthmatics.

They however did not study associations between endotoxin levels and respiratory outcomes [13].

The present study is part of the European project: Health Effects of Indoor Pollutants: Integrating microbial, toxicological and epidemiological approaches (HITEA) [18], and is designed to identify the role of dampness-related indoor air quality in primary schools in relation to respiratory health in pupils and teachers across Europe. The aim of this study was to assess indoor endotoxin levels and compare levels in primary schools and homes of children in the Netherlands. In addition we explored associations with asthmatic symptoms and sensitization.

Methods

Selection of schools

This study is a Dutch extension of the HITEA project. A more detailed description of the school selection is given elsewhere [18]. The aim was to select schools with (index) and without (reference) moisture, mold and/or dampness problems, representing the strongest moisture damaged schools and control schools as previously described [19].

Briefly, schools were invited by a letter addressed to the school principals, to complete a questionnaire by regular mail, phone interview or internet, focusing on current and past dampness, moisture damage and mold problems and collecting extensive general information on the school buildings

Based on this questionnaire, schools were selected and building inspections were performed by centrally trained research personnel and included walkthroughs, utilizing pre-designed checklists, and simple indoor climate measurements. Based on severity, extent and location of the dampness observations during the school inspections schools were categorized as affected by moisture damage / dampness (index schools) or as non-affected (reference schools) as previously described [19].

In the Netherlands, five index schools and five reference schools were included for detailed exposure and health characterization.

Study population: Selection of cases and controls

A clarification of the study design is presented in figure 1. Participants were selected out of a population of 796 children who had already participated in a questionnaire survey and had complete lung function data. The protocol was based on the ISAAC study [20]. Lung function test were performed at school according to the European Respiratory Society guidelines [21]. All children were aged between 6 and 12. Parents from 84 children with asthma-like symptoms (cases) and 170 children without (controls) as reported in a questionnaire study, were invited by regular mail to participate with home exposure measurements. Children were considered asthmatic when a positive answer was given to at least one of following items:

1) Wheezing or whistling in the chest in the past 12 months;

2) Ever had asthma;

3) Using any medicines, pills, inhalers or other medication for wheezing or asthma in the past 12 months;

4) Dry cough at night, apart from a cough associated with a cold or chest infection in the past 12 months.

Children were also invited to donate venous blood samples for IgE analysis and 97 (57%) of the invited children participated. The study protocol was approved by the Medical Ethical Committee of the Utrecht Medical Center and complied with all requirements of international regulations.

IgE measurements

Blood sampling was performed at school. Aeroallergen-specific immunoglobulin E (IgE) serum concentrations were measured by an earlier developed enzyme immunoassay, which has been validated against commercially available immunoassays[22]. IgE levels were screened against house dust mites, cat epithelium, dog dander, birch, and grass pollen mixture. A child was considered sensitized to a specific allergen if their IgE test result exceeded 0.050 optical density after correction.

Dust sampling

School and home endotoxin was measured during March and April 2010. Airborne settled dust was passively sampled with Electrostatic Dustfall Collectors (EDC), a recently developed low cost sampling approach that consists of a folder with two electrostatic microfiber cloths, each with a surface area of $0.02083 m^2$ [23]. In all schools dust was collected simultaneously during an 8-week sampling period. Trained field workers placed EDCs at a minimum height of 150cm from the floor and away from windows, doors, ventilation ducts

and heating [23], targeting approximately 15 locations and covering all areas, location types and grades in a school. Relative humidity was measured for 1 week in one classroom per school. EDCs were closed after sampling and stored at room temperature for maximally two weeks. After removal from the EDC, cloths were stored at -20ºC until analysis.

EDCs were also applied for dust sampling in homes of the participating children according to previously used home protocols, over a two week period [23,24]. These EDC's were sent by regular mail, accompanied by an invitation letter, detailed sampling instructions and a short questionnaire that included questions about the measurement and home and household characteristics. EDCs were placed in the child's bedroom, at a minimum height of 150cm and away from windows, doors, ventilation ducts and heating. After sampling, parents returned the EDC and questionnaire by mail. Home EDCs were handled similarly as the school samples.

Sample extraction and analysis

Endotoxin was extracted from the EDC as described elsewhere [23,24]. Briefly, cloths were incubated for 60 minutes in 20ml pyrogen free water (B. Braun NPBI, Oss, The Netherlands) in an end-over-end roller. After centrifugation for 15 minutes at 1000g, the supernatant was stored in 200µl aliquots in pyrogen free glass tubes at -20ºC until analysis.

Extracts were tested for endotoxin with the Limulus Amoebocyte Lysate (LAL) assay (Lonza Group, Basel, Switzerland) according to the manufacturer's protocol. Resulting endotoxin units (EU) per ml values were converted into EU/m^2 per week. The limit of detection (LOD)

of the assay was assessed at 150 EU/m^2 per week. None of the 148 school samples and 3 out of 169 home samples were below the LOD and were assigned a value 100 EU/m^2 per week.

Statistical Methods

Endotoxin levels were log10-transformed because of the skewed distribution. Endotoxin was measured in the classroom of 75% of our study population. Missing levels were replaced by grade averages or, when unavailable, the average of classrooms in the same area within the school or the classroom school average was used. All classrooms were occupied by the same group of pupils during the week.

The MIXED procedure was used to explore the variability of endotoxin levels and to study potential determinants affecting endotoxin levels. Associations between exposure in classrooms and health outcomes were analyzed by the GENMOD procedure. Odds ratios were calculated for an interquartile range increase in log10-transformed endotoxin exposure, which corresponded with a ($10^{0.29}$) 2.0-fold exposure increase in school levels and a ($10^{0.57}$) 3.7-fold endotoxin exposure increase in home levels. ´School´ was included as random effect to adjust for correlation between exposure and health outcomes of children within the same school. Children from schools affected by moisture might be oversampled due to the study design, so all models were adjusted for the school's moisture status. Potential confounders were selected based on prior knowledge and whether they were significantly associated with the outcome of interest. Covariates were retained in the final model if they substantially (>10%) modified the crude odds ratio (OR).

The shape of the association between endotoxin exposure and health outcomes was further studied by generalized additive modeling (smoothing), with the degrees of freedom (df) set at 2. All statistical analyses were performed with SAS version 9.2.

Results

A total of 148 school samples, including 97 classroom samples, and 169 home samples (response rate homes: 67%) were included. The week-average relative humidity in classrooms was 37% (min-max: 30-49%). Responders and non-responders did not differ with regard to most home characteristics and reported parental allergies. Available questionnaire survey data indicated that non-responders had smoked more inside the home and reported mould odour more often (smoking: 20% vs 11%; mould odour: 33% vs. 20%). No significant differences were observed in characteristics between children who provide serum and who did not.

Endotoxin levels in schools were significantly higher in full-time classrooms and in lower grades (Table 1). Home endotoxin levels were higher in larger households and in older houses. Other characteristics, like floor type, the presence of pets in the home, and smoking inside the home, were not associated with endotoxin EDC levels.

Endotoxin levels in classrooms were on average 4.5 fold higher ($p<0.05$) than in bedrooms. Average school levels ranged from 2178 to 6914 EU/m^2 per week (geometric mean (GM)) compared to 462 to 1285 EU/m^2 per week in homes (GM per school). School and home endotoxin levels were significantly higher (respectively 30% and 20%) in the index compared

to the reference category. The correlation between home and school endotoxin levels was low (r=0.09; p=0.29). Home endotoxin levels did not clearly differed between different schools.

Of the children with asthma-like symptoms, 17% reported wheeze at present, 20% had asthma (ever or doctor diagnosed), 20% had used asthma medication last 12 months and 39% reported dry cough at night during the last 12 months. The FEV1 of those with asthmatic symptoms was 2% lower, adjusted for gender and age. Although differences were not statistically significant, children with asthmatic symptoms more often had asthmatic parents (34% vs 22%; p=0.10). Surprisingly, non-asthmatic children showed more often elevated serum IgE levels, although the difference was not significant (40% vs. 24%; p=0.11) (Table 2).

Home endotoxin levels were significantly lower in children with asthma-like symptoms compared to children with no asthmatic symptoms (GM=698 vs. 925 EU/m^2 per week, p=0.03), regardless the sensitization status of the child (Figure 2). In contrast, higher endotoxin loads were found in classrooms of children with asthma compared to non-asthmatic children, however differences were only statistically significant for non-sensitized asthmatics (GM=4145 vs. 3184 EU/m^2 per week, p=0.04). There was no difference in endotoxin home or school levels between sensitized and non-sensitized children.

Logistic regression modelling (Table 3) showed that home endotoxin remained inversely associated with asthma symptoms. Including sensitization status, which was available for part of the population, showed that associations were significant for both atopic and non-atopic asthmatic children, also after adjustment for potential confounders (adjusted OR=0.90 (0.82-

1.00)). School exposure was positively associated with non-atopic asthma symptoms (adjusted OR=1.13 (1.03-1.25)), but not with atopic asthma. Sensitization, without taking into account case (asthma) status, was not associated with endotoxin levels at home or at school (data not shown). Splines generally showed a decrease in prevalence of sensitization and asthma- symptoms with increasing home endotoxin levels (Figure 3). Similar trends were found for atopic and non-atopic asthma symptoms. Asthma symptoms generally increased with increasing classroom endotoxin levels (Figure 3). This seemed attributable to asthma symptoms in non-atopics.

Discussion

The present study shows that endotoxin levels were considerably higher in schools than in homes. Furthermore, endotoxin levels were associated with asthma status; levels were lower in homes of asthmatic children compared to homes of non-asthmatics. In contrast, school endotoxin levels tend to be higher for children with asthma-like symptoms. Associations were strongest for non-atopic asthma and remained significant after mutually adjustment of children bedroom and schools levels. Determinants associated with home levels were the number of people in the household and the construction year of the house, while in schools mainly grade and occupancy affected endotoxin levels.

Although this is an exploratory study, with a relatively limited number of home measurements, the major strength of this study is that we measured endotoxin levels in schools and homes in parallel among the same children. Our results confirm the findings from a recent study that endotoxin levels in schools were higher than in homes [13]. In our study, endotoxin levels were on average more than 4-fold higher in schools and also in schools without dampness levels were 4 times higher compared to homes. An earlier survey showed that prolonged exposure of the EDC did lead to underestimation of endotoxin[24]. In our survey, sampling time between homes and schools was different (2 vs. 8 weeks) and we converted results to a 1-week sampling time by dividing levels by respectively 2 and 8. Accounting for this underestimation would have led to higher school endotoxin levels. This means that difference in endotoxin levels between school and homes might even be larger.

Domestic endotoxin has been associated with home dampness, pet ownership, farm animal contact, environmental tobacco smoke exposure, family size, and social economic status [3,4]. Schools differ in function, building characteristics, maintenance level and occupant density from homes, so also other determinants might affect school endotoxin levels. Shedding from human skin or dirt brought in by shoes are probably important sources of endotoxin in classrooms, while in homes endotoxin is also derived from pets.

We did find a poor correlation between home and school endotoxin levels and home levels were lower. This makes it unlikely that children transport endotoxin by their clothing from home to school, as observed earlier for cat allergens[25]. No other studies have characterized in detail factors affecting endotoxin levels in schools. Some studies found however high dust

levels, which contain endotoxin, in schools. Average airborne PM_{10} levels in classrooms ranged from 65 to 157 $\mu g/m^3$ in different European countries [26,27] and were considerably higher in classrooms compared to homes [28]. High dust levels in schools might be caused by re-suspension of particles caused by activity of pupils and also cleaning frequency might affect dust levels[26,27]. These are also likely explanations for the high endotoxin levels in schools in our study.

Analysis of effects of home and school endotoxin exposure on asthma status showed some remarkable findings. We observed an inverse relationship between endotoxin exposure at home and asthma symptoms, in agreement with other studies in which similar observations were made for the risk of having asthma and other respiratory allergies [14-16]. Surprisingly, we generally found a positive association between asthma symptoms and classroom endotoxin levels. These apparent differences in exposure response relations are remarkable and possible explanations for these different associations might exist. First, the composition of endotoxin may be different in schools. Is has been well established that different micro-organisms produce different chain lengths of endotoxin and these might have a different biological potency[29]. A study in 10 Chinese schools found a negative association between asthmatic symptoms and short chain endotoxins (C10, C12 and C14 LPS), while positive associations were observed with longer chain lengths [6]. A two year follow-up study observed a protective effect of bacterial compounds (including LPS) on the development of mucosal and general symptoms [7]. These findings have not been yet replicated in other studies, but might not easily be generalized. For instance, studied classrooms were considerably more crowded (~50

students per classrooms) and probably other aspects differed as well (climate, building structure and maintenance) and may have influenced the microbial exposure qualitatively and quantitatively, although a detailed comparison is not possible because different agents have been measured or techniques differed.

Second, some studies have demonstrated that in addition to the protective effects of endotoxin on allergies, high endotoxin exposure may also cause non-atopic respiratory effects[14,17] what might explain our findings that the relatively high school levels were mainly associated with non-atopic asthma. The overlap in exposure distributions between schools and homes is however considerable and therefore seems a less likely explanation. On the other hand, this may in part also be the result of an adjustment for different sampling times, and the adjustment needs further verification. Third, timing and duration of endotoxin exposure might be different for schools and homes. Home levels more likely reflect longer-term exposure to microbial agents from birth onward, whereas endotoxin exposure at schools reflect more recent exposure that occurred during the past few years, since school going age. Fourth, in addition to endotoxin, also other microbial agents might affect occupants' health. Fungal exposure in schools has been adversely related to respiratory health effects in a few studies[7-9]. Because the schools in our study were selected on the basis of moisture problems, indoor pollution may differ between schools and homes, and endotoxin exposure at school may interact with other (dampness-related) agents [30]. On the other hand, endotoxins are still considered as one of the more potent microbial agents. Large and especially longitudinal

studies, with more refined clinical data, are necessary to explore the above mentioned potential explanations further.

Exposure has likely been estimated adequately over a relatively long period. Previous studies showed that endotoxin levels measured with the EDC were relatively constant over time [23]. Also, none of the participants moved the year before the home exposure measurements. Also avoidance behavior (reverse causation), i.e. measures to reduce exposure in persons with respiratory problems, might bias the results, especially in homes. Comparing asthmatic and non-asthmatic children regarding potentially avoidance-related characteristics at home, like smoking, having pets, cleaning frequency and the use of carpet in the bedroom, indicated that avoidance probably seems not a major concern, however can not be ruled out. Reverse causation in schools is unlikely, as parents probably do not select the school on hygiene status.

In conclusion, this study showed high endotoxin levels in schools compared to homes, what indicates that exposure during the school day may contribute substantially to the total endotoxin load in children and teachers. This makes endotoxin exposure in schools interesting to study in relation with respiratory health of pupils and teachers. Our results also suggest that endotoxin in schools may be associated with asthmatic symptoms in pupils, although the sample size of our study is relatively low. To confirm these findings and explore whether also other agents might affect respiratory health, additional exploration of these result are needed in a larger dataset, including more detailed (longitudinal) exposure and

health measurements in the other schools of the three countries of the HITEA study, but also in other school studies.

Funding

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TABLES

Table 1: Association between general characteristics and endotoxin levels in schools and homes from the study participants (univariate analysis)

	Geometric Mean ratio (95%CI)
Schools (n=148)	
Full-time classrooms (compared to other locations in school)	1.21 (1.13-1.3)
Higher grade (1 grade increase)	0.98 (0.97-1.00)
Occupancy school (5 persons increase)	1.04 (0.99-1.10)
Building year (10 years increase)	1.00 (0.97-1.03)
Moisture/mold observations	1.15 (0.95-1.39)
Type of floor	
- Smooth floor without carpet	Ref
- Smooth floor with carpet	-
- Carpet	0.96 (0.67-1.39)
Homes (n=169)	
Higher grade at school (1 grade increase)	1.03 (0.98-1.07)
Number occupants (1 person increase)	1.14 (1.06-1.22)
Building year (10 years increase)	0.96 (0.94-0.99)
Moisture/mold observations	1.02 (0.90-1.16)
Type of floor	
- Smooth floor without carpet	Ref
- Smooth floor with carpet	1.03 (0.90-1.18)
- Carpet	0.99 (0.85-1.15)
Smoke inside	1.00 (0.91-1.09)
Pets in home	1.04 (0.93-1.17)

Pets in bedroom	0.90 (0.78-1.04)
Education level parents	
- High	Ref
- Intermediate	0.89 (0.79-1.01)
- Low	1.04 (0.80-1.35)
Parental allergies	1.03 (0.89-1.18)

Table 2: General characteristics of children with symptoms indicative for asthma

	Asthma suggestive symptoms*	
	Controls	Cases
Subjects n	103	66
Basic characteristics		
Males (n, %)	52 (50%)	30 (45%)
Age (y, sd)	8.7 (1.5)	8.5 (1.4)
BMI (kg/m^2, sd)	16.3 (1.9)	16.6 (2.0)
Number older siblings (median, interquartile range) †	1 (1)	0 (1)
Parental education (n, %)		
- Low	8 (8%)	1 (2%)
- Intermediate	28 (28%)	29 (44%)
- High	65 (64%)	36 (55%)
Parental smoking (n, %)		
Current	9 (9%)	8 (13%)
During 1st yr of child's life	9 (9%)	7 (11%)
During pregnancy	6 (6%)	6 (9%)
Birth weight (n, %)		
< than 2.5 kg	4 (4%)	5 (8%)
2.5-4 kg	80 (78%)	53 (82%)
>4 kg	18 (17%)	7 (11%)
Daycare attendance (n, %)	41 (40%)	25 (38%)
Parental asthma (n, %)	23 (22%)	22(34%) #
Farm animal contact 1st yr (n, %)	12 (12%)	8 (12%)
Pets present in home (n, %)		
Current	53 (51%)	28 (42%)
During 1st yr	45 (44%)	33 (50%)
Mould spots/odour at home (n, %)		

During last 12 mths	20 (20%)	18 (27%)
Ever	31 (30%)	24 (36%)
Water damage at home (n, %)		
During last 12 mths	27 (26%)	16 (25%)
Ever	38 (37%)	31 (48%)
Sensitisation >1 common allergen[§]	24 (40%)	9 (24%)

* Not all categories add to the total sample size due to missing information.

[†]Because the distribution is skewed to the right, the median is reported instead of the mean.

[§] Serum information was available from 97 children

[#] $p \leq 0.05$ different from controls

Table 3: Association between endotoxin levels at home and school and asthma suggestive symptoms

Endotoxin exposure	**Asthma symptoms**[#]	**Asthma symptoms including sensitization status**[#]	
		Atopic asthma	*Non-atopic asthma*
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*
Number cases/controls	66/103	9/36	28/36
In classroom (of participants with home endotoxin measurements)	1.08 (0.98-1.19)	1.03 (0.89-1.19)	**1.16 (1.03-1.3)**
At home	**0.90 (0.81-0.99)**	**0.79 (0.63-1.00)**	**0.74 (0.62-0.87)**
Endotoxin exposure mutually adjusted:			
School	1.08 (0.98-1.20)	1.09 (0.90-1.31)	**1.17 (1.02-1.35)**
Home	**0.89 (0.80-0.98)**	**0.77 (0.59-1.00)**	**0.73 (0.64-0.84)**
Endotoxin exposure mutually adjusted and adjusted for additional confounders*			
School	**1.06 (1.00-1.12)**	1.08 (0.96-1.20)	**1.13 (1.03-1.25)**
Home	**0.90 (0.82-1.00)**	**0.77 (0.61-0.98)**	**0.73 (0.61-0.86)**

Reference categories (controls) were no asthma symptoms, and not sensitized *and* no asthma symptoms respectively. Data are presented as the odds ratio and 95% confidence interval (OR (95% CI)) for an interquartile range increase, which corresponds with a factor 2.0 endotoxin increase in schools and a factor 3.7 increase in homes

*Adjusted for age, gender, building year home, number people in household, smoking inside home, pets inside bedroom, parental allergies, parental education, school grade and dampness status of the school

FIGURES

Figure 1: Flow chart of the study design

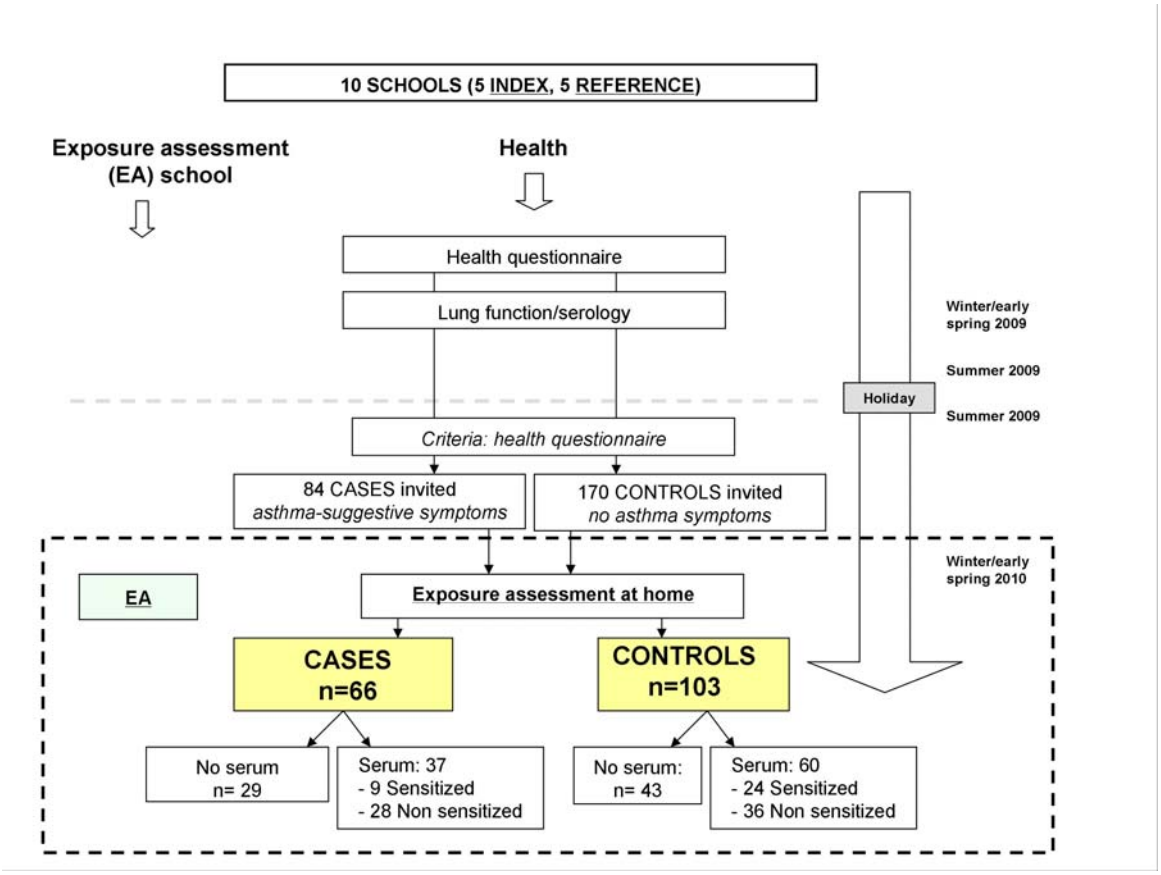


Figure 2: Airborne endotoxine levels in homes and schools by health status

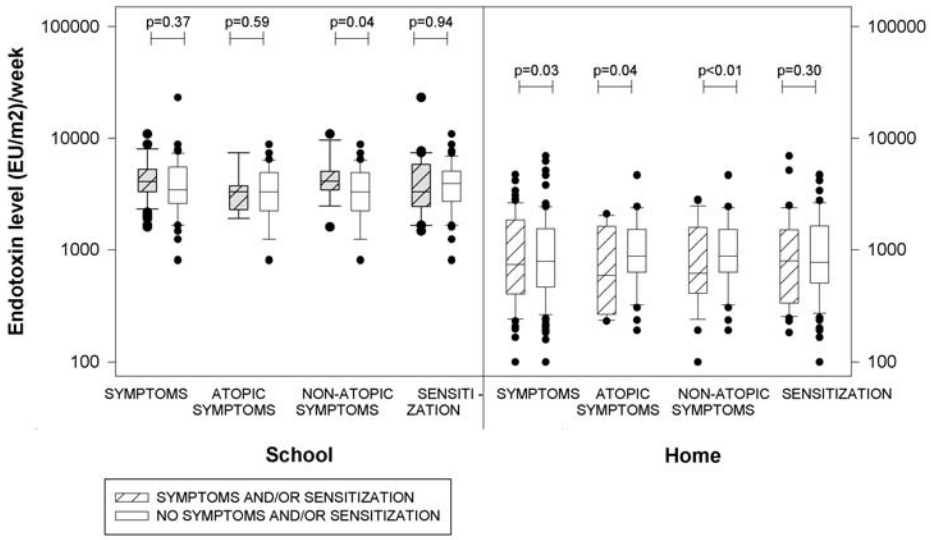
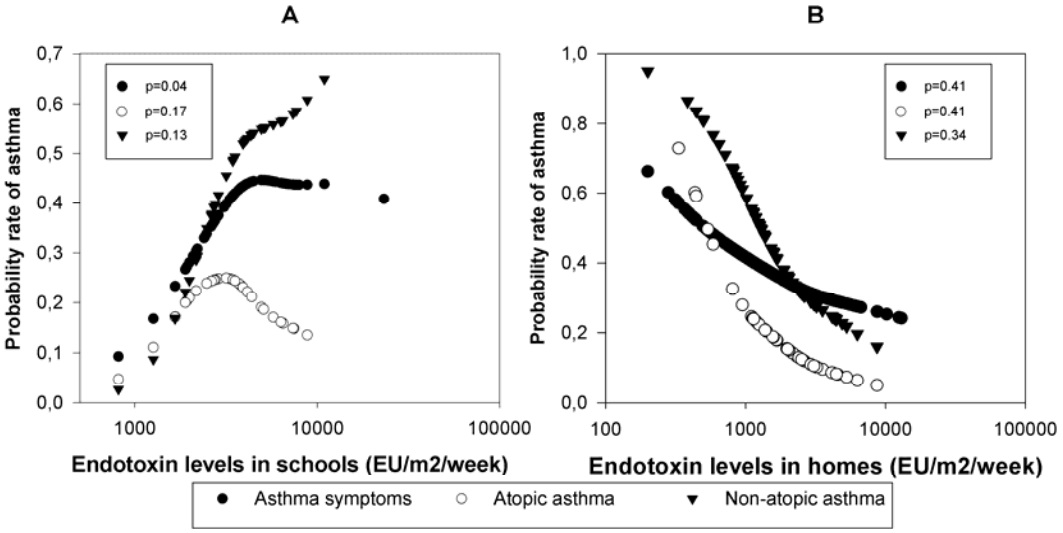


Figure 3: Non-linear associations (df=2) between asthmatic symptoms (●), atopic asthma (○) and non-atopic asthma (▼) in children and: (A) endotoxin exposure in schools and (B) in homes. Non-linear associations were statistically significant ($p<0.05$) for endotoxin levels at school and asthma (●; model A)


A
B
Probability rate of asthma
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
p=0.04
p=0.17
p=0.13
1000
10000
100000
Endotoxin levels in schools (EU/m2/week)
1,0
0,8
0,6
0,4
0,2
0,0
p=0.41
p=0.41
p=0.34
100
1000
10000
100000
Endotoxin levels in homes (EU/m2/week)
Asthma symptoms
Atopic asthma
Non-atopic asthma

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