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## Endotoxin in inner-city homes: Associations with wheeze and eczema in early childhood

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### Abstract

**Background**— An inverse association between domestic exposure to endotoxin and atopy in childhood has been observed. The relevance of this aspect of the “hygiene hypothesis” to U.S. inner-city communities that have disproportionately high asthma prevalence has not been determined.

**Objectives**— To measure endotoxin in the dust from inner-city homes, evaluate associations between endotoxin and housing/lifestyle characteristics, and determine whether endotoxin exposure predicted wheeze, allergic rhinitis and eczema over the first three years of life.

**Methods**— As part of an ongoing prospective birth cohort study, children of Dominican and African-American mothers living in New York City underwent repeated questionnaire measures. Dust samples collected from bedroom floors at age 12 or 36 months were assayed for endotoxin.

**Results**— Among the samples collected from 301 participant's homes, the geometric mean endotoxin concentration [95% C.I.] was 75.9 EU/mg [66–87] and load was 3,892 EU/m<sup>2</sup> [3,351–4,522]. Lower endotoxin concentrations were associated with wet mop cleaning and certain neighborhoods. Endotoxin concentration correlated weakly with cockroach (Blatella germanica;  $r=0.22, p<0.001$ ) and mouse (Mus musculus;  $r=0.28, p<0.001$ ) allergens in the dust. Children in homes with

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**Capsule summary:** Endotoxin concentrations in the house dust from an inner-city New York City cohort are similar to other non-farm homes and are associated with a higher prevalence of wheeze and lower prevalence of eczema in the first years of life.

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higher endotoxin concentration were less likely to have eczema at age 1 year (O.R. 0.70 [0.53–0.93]) and more likely to wheeze at age 2 years (O.R. 1.34 [1.01–1.78]). These associations were stronger among children with a maternal history of asthma.

**Conclusions**— Endotoxin levels in this inner-city community are similar to non-farm homes elsewhere. In this community, domestic endotoxin exposure was inversely associated with eczema at age 1, but positively associated with wheeze at age 2.

**Clinical Implications**—Endotoxin exposure in the inner-city community may be related to wheeze in the early life; however, given the inverse association seen with eczema, the long term development of allergic disease is still in question.

## Keywords

Endotoxin; asthma; allergy; hygiene hypothesis; wheeze; inner-city; eczema

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## Introduction

The “hygiene hypothesis” asserts that the increase in the prevalence of allergic disease documented in Western Society in the 20<sup>th</sup> century can be attributed to, at least in part, lifestyle changes that have led to cleaner living conditions and reduced exposure to viruses and bacteria in the first years of life (1,2). Inverse associations between bacterial endotoxin in house dust and atopic disease have been observed in cross-sectional studies (3,4). Endotoxin, a lipopolysaccharide found in the outer membrane of gram negative bacteria, can downregulate T help (Th) 2 cytokine production and class switching to immunoglobulin (Ig) E isotype in mouse models (5). Hence, several authors have postulated that ambient exposure to high amounts of endotoxin in the home or farm environment could modulate the risk for atopy by suppressing molecular pathways responsible for IgE-mediated atopic disease (6–8).

It has been suggested that the “hygiene hypothesis” may explain inter-community differences reported in the prevalence of atopic asthma (9). The prevalence of asthma generally is greater among industrialized countries, where living conditions appear to be cleaner and fewer bacterial infections seem to occur, than among developing nations (10,11). However, it is unclear where inner-city homes in the U.S., generally afflicted by a disproportionately high prevalence of asthma, fall in the spectrum of environmental endotoxin exposure (4,12). One unanswered question is whether a greater level of hygiene associated with an inner-city lifestyle can explain the higher asthma prevalence relative to that observed in rural and suburban areas (12,13). Another is the extent to which endotoxin exposure modulates the risk for developing atopic disease or wheeze in this community during childhood.

Our study examined endotoxin exposure and asthma-related health outcomes in the first three years of life in a birth cohort of children living in New York City (NYC) neighborhoods that have reported a disproportionately high asthma prevalence (12). We hypothesized that differences in endotoxin exposure would be associated with asthma-like symptoms, allergic rhinitis and eczema. Our strategy was to 1) measure endotoxin in the dust from homes, 2) determine whether allergen levels, housing and/or lifestyle characteristics were associated with endotoxin exposure in the inner city, and 3) determine whether endotoxin exposure in this inner-city birth cohort predicted wheeze, allergic rhinitis and eczema over the first three years of life.

## Methods

### Study cohort

Participants included 301 children recruited as part of an ongoing birth cohort, described previously, being conducted by Columbia's Center for Children's Environmental Health (14–17). Non-smoking mothers not selected on allergic/asthmatic status of African-American and Dominican ethnicity living in Northern Manhattan and the South Bronx were recruited during the second or third trimester of pregnancy from prenatal clinics. This study was approved by Columbia University's Institutional Review Board.

### Endotoxin measurement

As a proxy for early life domestic endotoxin exposure, endotoxin was measured in bedroom floor dust collected when the child was age 12 months. For 47 of the children, due to difficulties associated with scheduling home visits, samples were not available at age 12 months, but bedroom floor dust that had been collected at age 36 months was available and tested. Samples were collected in a 2-square-meter area for 5 minutes onto 70 mm cellulose filters (Whatman International, Maidstone, England) with a canister vacuum cleaner (Eureka Mighty Mite, Bloomington, IN) and a modified collection nozzle (ALK, Horshølm, Denmark). For endotoxin analysis, dust was extracted in pyrogen-free water with 0.05% Tween for 1 hour shaking, centrifuged and the supernatant was removed and assayed without freezing. Endotoxin was analyzed by the kinetic chromogenic *Limulus* Amebocyte Lysate assay, described previously (18,19). Results are reported both as the concentration of endotoxin per milligram of dust (EU/mg) and as the load of endotoxin collected per square meter (EU/m<sup>2</sup>).

Dust samples also were collected from various locations in the home and were analyzed for cockroach (Blatella germanica, bed, n=255), mouse (Mus musculus, bed, n=243), and dust mite (Dermatophagoides farinae, bed, n=209), cat (Felis domesticus, bedroom floor, n=292) and dog (Canis familiaris, bedroom floor, n=289) allergens. The procedure for dust collection, allergen extraction and ELISA have been described previously (15,20–22).

### Questionnaire

Subsequent to a prenatal questionnaire, a detailed questionnaire including queries about wheezing; runny nose, sneezing or itchy eyes without a cold (allergic rhinitis symptoms); and report of doctor's diagnosed eczema was administered to the parents about their offspring at age 12, 24, and 36 months with additional less detailed health follow-ups at 3, 6, 9, and 30 months. A child was considered to have wheezed if wheeze was reported during at least one of the interviews pertaining to that year of life. Other symptoms were recorded similarly.

### Statistics

Due to the non-normal distribution, geometric mean values [95% confidence interval] were calculated for endotoxin and means were compared using ANOVA. Pearson correlations using logarithmically transformed values were used to compare endotoxin and allergen concentrations, and regression analysis was used to evaluate the associations between endotoxin and multiple allergens. Allergen concentrations below limit of detection were assigned a value of half the limit of detection. Analyses for wheeze and other allergic symptoms were performed using logistic multiple regression adjusting for ethnicity, gender, maternal asthma and smoking in the home. Generalized estimating equations (GEE) were used to correct variance estimates for analyses of repeated measures. Data were analyzed using SPSS version 12.0 (Chicago, IL). GEE was performed using SAS version 9.0 (Cary, NC).

To test the variability between samples collected at different time points in this cohort, 128 children who had endotoxin measured in a 12 month sample also had endotoxin measured in

a 36 month sample. These latter 36 month samples are only included in 1) the correlation between 12 and 36 month samples from the same home, 2) the analysis for the last sentence of the first health outcome paragraph in the results section.

## Results

### Study population

Among the 301 mothers, 64% and 36% were of Dominican and African-American ethnicity, respectively, and 19% reported having a doctor-diagnosis of asthma. The homes sampled were in East Harlem (18%), the South Bronx (36%), Washington Heights (15%) and West Harlem (32%). Seventy-three percent of the mothers reported an annual household income of \$20,000 or less, and 75% reported that they currently received Medicaid at the time the dust sample was collected.

### Endotoxin levels

The geometric mean [95% C.I.] endotoxin concentration in the dust from the bedroom floor samples was 75.9 EU/mg [66–87]. Calculated as the endotoxin load per square meter, the geometric mean was 3,892 EU/m<sup>2</sup> [3,351–4,522]. There was a significant correlation between endotoxin concentration and load ( $r=0.60$ ,  $p<0.001$ ). There was a modest correlation between the endotoxin measured at both 12 and 36 months for the same children ( $r=0.18$ ,  $p=0.043$  and  $r=0.21$ ,  $p=0.019$ , for concentration and load, respectively). The associations were stronger when only those children that had not moved between time points ( $n=73$ ) were analyzed ( $r=0.26$ ,  $p=0.025$  and  $r=0.25$ ,  $p=0.035$ ).

### Relationship between endotoxin and allergen levels

We found a modest correlation between endotoxin concentration and mouse allergen (MUP) ( $r=0.28$ ,  $p<0.001$ ) and cockroach allergen (Bla g 2) ( $r=0.22$ ,  $p<0.001$ ) concentrations. When both allergens were included as independent variables in a regression model, both Bla g 2 ( $\beta=0.17$ ,  $p=0.008$ ) and MUP ( $\beta=0.24$ ,  $p<0.001$ ) were significantly associated with endotoxin ( $r^2=0.11$  for model). No association was found between the concentration of endotoxin and the concentration of cat allergen (Fel d 1), either when the samples below the limit of detection were included ( $r=-0.016$ ,  $p=0.79$ ), or when the analysis was restricted to samples with  $>1 \mu\text{g}$  Fel d 1/g (40/292) were included ( $r=-0.19$ ,  $p=0.24$ ). Similarly, we found no association between endotoxin concentration and Can f 1 ( $r=-0.013$ ,  $p=0.83$ ) or Der f 1 ( $r=0.089$ ,  $p=0.20$ ).

### Associations between cleaning habits, built environment and endotoxin levels

Higher endotoxin concentrations were associated with frequent sightings of cockroaches and mice, and the reported use of Medicaid (Table I). Lower endotoxin concentration was associated with wet mopping as a home cleaning method. Assuming that wet mopping would be limited to rooms other than bedroom if a carpet was present, we stratified the analysis by carpet. Endotoxin was only significantly lower in homes that reported wet mopping among those without a carpet in the bedroom ( $p=0.006$ ) and not in those that had a carpet ( $p=0.18$ ). Higher endotoxin load was associated with a carpet in the bedroom and frequent sightings of cockroaches.

We found no significant differences in either endotoxin concentration or load among cat and/or dog owners compared to non-owners. We also did not find significant associations between endotoxin concentration or load and any of the questions addressing mold or housing disrepair (Table I), or when analyzing using a previously described housing disrepair scale as an independent variable in linear regression ( $\beta=0.02$ ,  $p=0.75$  for concentration endotoxin;  $\beta=0.014$ ,  $p=0.82$  for load) (23).

Mean endotoxin concentrations were significantly lower ( $p=0.001$ ) in East Harlem (57 EU/mg [40–79]) and Washington Heights (53 EU/mg [38–75]) as compared to West Harlem (101 EU/mg [78–129]) and the South Bronx (80 EU/mg [65–100]). Endotoxin loads did not differ significantly between these neighborhoods ( $p=0.19$ ). Ethnicity is strongly associated with neighborhood; however, there was no difference in either concentration ( $p=0.48$ ) or load ( $p=0.36$ ) by ethnicity of the mother.

The significant housing characteristics from Table I were evaluated in a linear regression model. For endotoxin concentration there was an  $R = 0.34$  for the model and significant associations for wet mop ( $\beta=-0.18$ ,  $p=0.004$ ), East Harlem residence ( $\beta=-0.22$ ,  $p=0.002$ ), and Washington Heights residence ( $\beta=-0.19$ ,  $p=0.008$ ). For endotoxin load an  $R=0.29$  was found for the model with significant associations for carpet ( $\beta=0.22$ ,  $p<0.001$ ) and cockroach sightings ( $\beta=0.13$ ,  $p=0.035$ ).

### Endotoxin and wheeze, allergic rhinitis symptoms and eczema

With increasing endotoxin concentration, we found lower adjusted odds of eczema in the first year of life (Table II). In addition, with increasing endotoxin concentration, we found a trend of increasing odds of wheeze during the second year of life. Similar trends (although not significant due to the reduced sample size) were observed when only those children with an endotoxin sample collected at 12 months were examined. No significant associations were detected when the 36 month outcomes were evaluated relative to the 36 month samples only (including those for which there were also 12 month samples).

The interaction term endotoxin by maternal asthma was significant for wheeze in the 2<sup>nd</sup> year ( $p=0.029$ ) as well as the 1<sup>st</sup> year ( $p=0.038$ ). The interaction term was not statistically significant for eczema in the 1<sup>st</sup> year ( $p=0.094$ ). The prevalence of wheeze and eczema by quartile of endotoxin concentration is shown in Figure 1 stratified by maternal history of asthma.

We also examined the association of endotoxin and wheeze as a longitudinal variable in GEE analysis. Endotoxin concentration was not associated with the presence of wheeze over time ( $p=0.88$ ), nor was it associated with change in wheeze over follow up ( $p=0.91$ ). The corresponding  $p$ -values when endotoxin load was evaluated were 0.91 and 0.36.

## Discussion

Inverse associations between endotoxin in house dust and allergic sensitization, eczema and atopic asthma in children have been reported (3,4,24,25), but have not been evaluated extensively in the inner-city communities of the United States. We have measured endotoxin in the homes of 301 children living in NYC. The novelties of this cohort are that it is limited to an urban inner-city population that is known to have high asthma prevalence, and that it includes children with and without maternal asthma. From our findings we conclude that domestic endotoxin exposure in this community is similar to that in other non-farming communities, and may be relevant to the development of allergic disease in this population. These similar levels and modest association with health outcomes in turn challenge the notion that lower domestic endotoxin exposure explains the higher asthma prevalence observed in the inner-city relative to the suburban U.S. communities.

In comparisons with other studies, endotoxin levels were moderately higher, when expressed as a concentration (Table III), but moderately lower when expressed as endotoxin load per square meter (Table IV). We recognize that, with the exception of our comparison to results presented by the U.S. National Survey of Lead and Allergens in Housing that was conducted by the same investigator (PT) and laboratory, some caution must be taken when comparing these data to other studies. Variations in the collection and extraction techniques and the

*Limulus* assay itself (26,27) can occur. Also, different locations in the home and sampling times may yield different endotoxin levels (28). In general, we can conclude that endotoxin exposure in these inner-city homes is comparable to that from other studies where health effects have been associated with domestic endotoxin exposure, but that the load of endotoxin we found is much lower than that from farm homes. Also of interest, these concentrations in the inner-city are slightly higher than those reported by the national survey (same laboratory) and this difference is of a similar magnitude to that seen between metropolitan Denver and the greater Boston area (same collection and assay technique) (29).

We identified several housing characteristics associated with higher endotoxin, including reports of cockroach and mouse infestation and their associated measured allergen levels. These findings are in keeping with a German study (30) that found an association with mouse sightings and the U.S. National Home Survey that found an association with cockroach allergen (29). With regression analysis, mouse and cockroach allergens were found to be independently associated with endotoxin; however, these variables combined only explained 11% of the variation in endotoxin. The modest correlation between endotoxin and pests might be driven by pest infestation which is dependent upon the hygiene of neighboring apartments and other building-wide factors.

This study is the first to report an inverse association between floor dust endotoxin and wet mopping, raising the possibility that this method may be more effective for removing endotoxin-laden dust from its reservoirs in the urban setting. A probable source of endotoxin in inner-city apartments is in the dust tracked into the apartments on shoes. We suspect that those homes that report wet mopping in the apartment also mop the bedroom if there is not a carpet, and this explains the lower levels of endotoxin in the bedroom floor dust specifically among non-carpeted bedrooms. Alternatively, wet mopping could be a proxy for some other housing characteristic not measured in our cohort.

Domestic endotoxin exposure may not be uniform across one large urban environment, as demonstrated by the differences in endotoxin concentrations we measured among the neighborhoods in our study, as well as difference in outdoor airborne endotoxin found by a study from Southern California (31). In NYC, differences in endotoxin among the neighborhoods may reflect the differences in housing stock. Unlike a German study, we did not find any associations with the story of the apartment (30). The same German study also reported higher endotoxin in older buildings (before 1980). While the ages of the buildings were not recorded in this study, we did see a non-significant ( $p=0.07$ ) trend of increased endotoxin in homes with ceiling heights of 9 feet or higher (GM 80 EU/mg) as compared to those with lower ceilings (60 EU/mg). In NYC higher ceilings are typically found in older buildings (constructed pre-World War II).

From the 128 children for whom we had measured endotoxin at both 12 and 36 months, substantial variability in dust-borne endotoxin between time points in these homes is evident. Additionally, in this urban environment moving is common, and 20% and 37% of the mothers reported a different address at 24 and 36 months, respectively, than at 12 months. Therefore, the variability in endotoxin within homes and movement to new homes may result in some exposure misclassification, probably biasing the results toward the null and potentially leading to an underestimation of the effect of endotoxin exposure. However, we have used 12 month samples for most of the children (84%), and the two significant associations observed (wheeze in year 2, eczema in year 1) represent outcomes within 12 months of the collection.

Two prior studies examined the association between endotoxin and early life asthma/allergy outcomes in urban U.S. populations. Results from a relatively small study in metropolitan Denver found an inverse association with endotoxin concentration and atopy (3), while a

positive association between endotoxin concentration and wheeze was found in a mixed urban and suburban birth cohort in Boston (32,33). This latter Boston study also found an inverse association between endotoxin and eczema (25).

The novelty of our study comes from the demographics of our population (all urban) and the fact that it was not restricted to children with a family history of asthma or allergy. Similar to the Boston study, we found both a negative association between endotoxin exposure and eczema and positive association between endotoxin exposure and wheeze (25,32). The decreased risk for eczema and potential “protection” from early atopic disease is consistent with the “hygiene hypothesis.” Given the variety of etiologies (e.g. viral infections) that contribute to wheeze in such young children, the positive association between endotoxin and wheeze in the first two years does not contradict the hygiene hypothesis. In our study the association with wheeze was found to be strongest among the children with a maternal history of asthma, which was also reported in a European birth cohort (34). Some caution must be taken given the relatively small number of children with allergic mothers in our study. Children with a family history of asthma are more likely to develop asthma and wheeze in the first years of life and this may be an early sign of increased airway hyper-reactivity (35).

Associations with allergic disease (both positive and negative) have been reported with endotoxin expressed both as a concentration and as a load (3,4,24,32,34), and we have analyzed our results throughout using both measures for comparison. The majority of the home characteristics and the only health effects significantly associated with endotoxin were observed with concentration of endotoxin. The debate over the more valid method persists, with the major critique of concentration misestimation of the actual amount of endotoxin in the sample and the major critique of the load difficulties in standardization of methods between studies (27,36). Given our findings and those from the Boston cohort, it appears as though analyzing endotoxin expressed as a concentration may be a better method within an urban cohort, possibly due to a methodology less sensitive to collection variation.

In summary, the similarities in endotoxin levels we report (compared to homes elsewhere) and the modest protection from one atopic disease (i.e. eczema) and the modest positive association with another (i.e. wheeze) lead us to doubt that inner city homes differ from other non-farm communities in levels of cleanliness as they pertain to endotoxin exposure. From these findings we also doubt that low endotoxin exposure explains the higher asthma prevalence observed in inner-city as compared to suburban communities in the United States.

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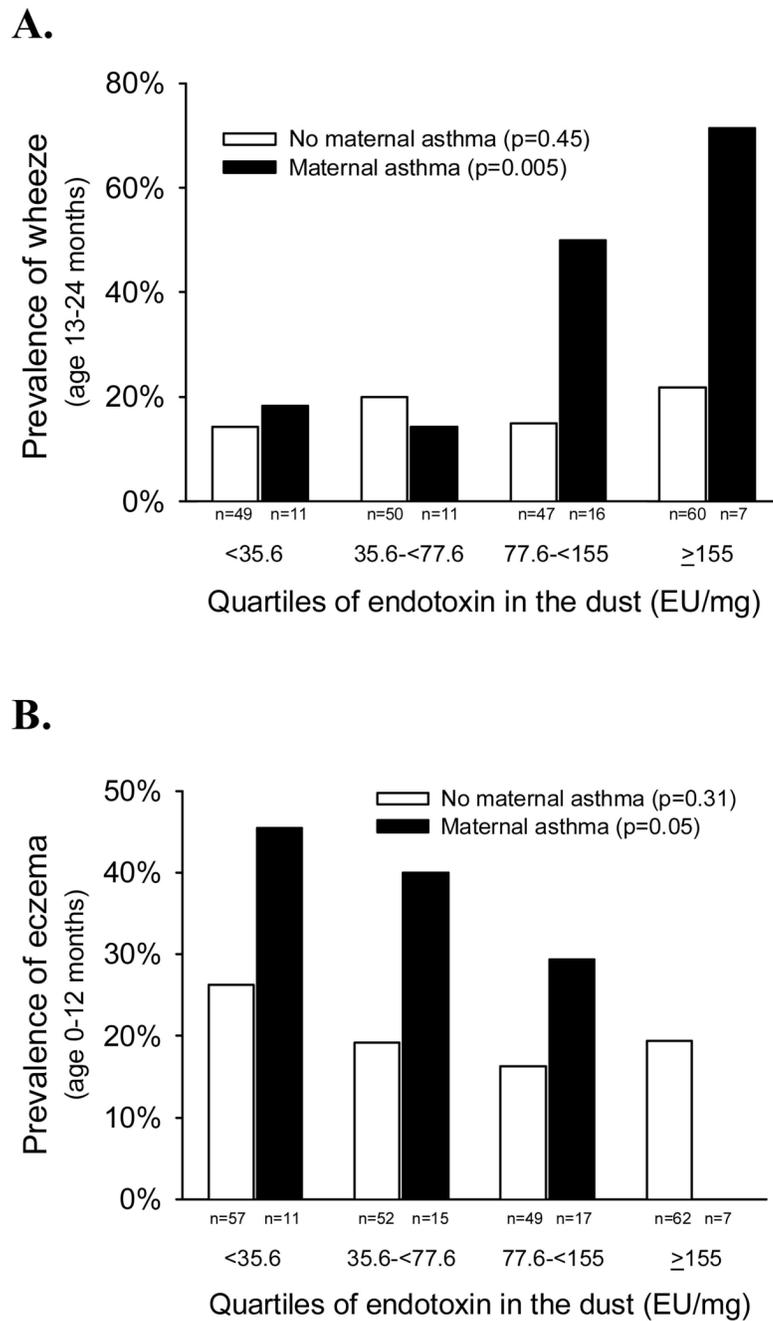
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## Abbreviations

<b>EU</b>	Endotoxin Units
<b>GEE</b>	Generalized Estimating Equations

<b>MUP</b>	Mouse Urinary Protein
<b>NYC</b>	New York City
<b>OR</b>	Odds Ratio



**Figure 1. Prevalence of (A.) wheeze in the 2<sup>nd</sup> year of life and (B.) report of physician diagnosed eczema in the 1<sup>st</sup> year of life by quartile of endotoxin in the bedroom floor dust stratified by maternal history of asthma**

P values calculated by chi square test for trend.

Geometric means of endotoxin concentration (per mg of dust) and load (per m<sup>2</sup>) by housing and other characteristics.

Table 1

	n for analysis	study prevalence		Concentration (EU/mg)		p <sup>†</sup>	Geometric mean [95% CI] of endotoxin Load (EU/m <sup>2</sup> )		P
		no	yes	no	yes				
Carpet in bedroom	295	79 [67-91]	66 [50-87]	0.37	3,528 [2,993-4,158]	6,757 [4,680-9,753]	0.002		
Cat or dog in home	270	77 [65-90]	66 [47-92]	0.50	4,061 [3,408-4,840]	3,835 [2,495-5,894]	0.82		
Leave waste uncovered*	267	76 [64-89]	74 [54-103]	0.93	3,981 [3,323-4,771]	4,326 [2,953-6,338]	0.73		
Eat in bedroom	270	72 [61-86]	83 [64-108]	0.39	3,969 [3,274-4,812]	4,181 [3,088-5,662]	0.78		
Report of mold	269	74 [63-86]	86 [59-127]	0.46	4,053 [3,393-4,841]	4,036 [2,703-6,026]	0.99		
Any housing disrepair <sup>‡</sup>	271	74 [61-91]	75 [61-93]	0.90	4,134 [3,278-5,213]	3,923 [3,141-4,899]	0.75		
Use wet mop for cleaning	268	93 [73-118]	62 [53-75]	0.008	4,517 [3,498-5,835]	3,661 [2,979-4,500]	0.21		
Medicaid	268	58 [45-73]	80 [68-96]	0.047	3,144 [2,237-4,419]	4,382 [3,651-5,259]	0.08		
Mom reports asthma	270	78 [66-92]	65 [49-86]	0.34	4,011 [3,335-4,825]	3,798 [2,741-5,261]	0.80		
Daily cockroach sightings	270	67 [57-80]	99 [77-127]	0.02	3,599 [2,992-4,329]	5,384 [3,891-7,449]	0.03		
Daily mouse sightings	269	69 [59-81]	114 [79-165]	0.013	3,772 [3,167-4,491]	5,653 [3,667-8,713]	0.07		

\* Report that they leave their waste uncovered more than once a week.

<sup>†</sup> ANOVA comparing log transformed endotoxin values.

<sup>‡</sup> Report of at least one of the following: holes in ceilings or walls, peeling or flaking paint, water damage, leaking pipes or lack of electricity in the last 6 months.

Geometric mean of endotoxin by wheeze, allergic rhinitis symptoms and eczema and the adjusted odds ratio of symptoms associated with endotoxin.

Table II

	Prevalence of outcome	n for analysis <sup>‡</sup>	Geometric mean of endotoxin concentration EU/ mg [95% CI]		Adjusted OR <sup>§</sup>
			no outcome	yes outcome	
Wheeze	0-12 months	265	76 [63-93]	73 [58-90]	0.96 [0.78-1.1]
	13-24 months	203	70 [58-86]	102 [76-138]	1.3 [1.01-1.8]
	25-36 months	163	68 [55-83]	70 [48-101]	1.04 [0.71-1.5]
Allergic rhinitis symptoms	0-12 months	265	69 [55-87]	81 [67-98]	1.1 [0.91-1.4]
	13-24 months	203	79 [64-97]	73 [56-97]	0.98 [0.77-1.3]
	25-36 months	163	66 [53-81]	73 [51-103]	1.08 [0.80-1.5]
Eczema	by age 12 months	265	83 [70-98]	51 [37-71]//	0.70 [0.53-0.93]
	by age 24 months	203	82 [68-100]	65 [46-90]	0.86 [0.64-1.2]
	by age 36 months	162	74 [60-91]	51 [35-75]	0.74 [0.52-1.05]
<b>Geometric mean of endotoxin load EU/m<sup>2</sup> [95% CI]</b>					
Wheeze	0-12 months	265	3.667 [2.943-4.568]	4.199 [3.292-5.356]	1.1 [0.90-1.3]
	13-24 months	203	4.093 [3.300-5.078]	4.483 [3.198-6.284]	1.02 [0.79-1.3]
	25-36 months	163	3.954 [3.096-5.050]	3.867 [2.987-5.006]	1.06 [0.78-1.4]
Allergic rhinitis symptoms	0-12 months	265	3.684 [2.856-4.751]	4.097 [3.344-5.020]	1.1 [0.89-1.3]
	13-24 months	203	4.026 [3.196-5.072]	4.502 [3.344-6.062]	1.03 [0.82-1.3]
	25-36 months	163	3.867 [2.987-5.006]	4.490 [3.021-6.672]	1.07 [0.84-1.4]
Eczema	by age 12 months	265	3.911 [3.268-4.680]	3.779 [2.576-5.546]	0.95 [0.76-1.2]
	by age 24 months	203	4.120 [3.339-5.082]	4.378 [3.007-6.376]	0.95 [0.73-1.2]
	by age 36 months	162	3.864 [3.019-4.944]	4.591 [2.923-7.210]	1.05 [0.80-1.4]

<sup>‡</sup> Means and OR are taken from the number of subjects used in the logistic regression.

<sup>§</sup> OR with 95% confidence interval from a logistic regression correcting for gender, maternal asthma, ethnicity and tobacco smoke exposure in the home.

// The only univariate significant difference ( $p < 0.05$ ) in means was observed in endotoxin concentration for eczema at age 0-12 months ( $p = 0.008$ ).

**Table III**

International comparisons of concentration of endotoxin in home dust.\*

Study Location	Dust sampling location	n	endotoxin (EU/mg)	associations with endotoxin
Rural New Zealand Wickens <i>et al.</i> 2002(37)	living room dust from farm homes	94	7.4 <i>geomean</i> #	endotoxin↓ on farms↑exposure to poultry ↑endotoxin no difference between with pet no association with allergic disease
	living room dust from non-farms	188	11.6 <i>geomean</i>	
Saxony-Anhalt, Germany Gehring <i>et al.</i> 2001(24) Bischof <i>et al.</i> 2002(30) Rural European Community Braun-Fahrlander <i>et al.</i> 2002 (4)	living room floor	405	22.7 [1–1,200] <i>geomean [max-min]</i>	↑endotoxin ↓sensitization.allergens association strengthened with increasing degree of sensitization
	bedding from farm homes	319	37.8 [14.4–88.9] <i>geomean [5%–95%]</i>	
	bedding from non-farm homes	493	22.8 [8.2–62.9] <i>geomean [5%–95%]</i>	
U.S. National Survey Thorne <i>et al.</i> 2005(29) Greater Boston area Park <i>et al.</i> 2001(32)	bedroom floor	588	35.3 [5.0–260] <i>geomean [5%–95%]</i>	↑endotoxin ↑asthma symptoms ↑endotoxin ↓wheezing
	bedroom floor	323	63 [2–761] <i>geomean [max-min]</i>	
Phipatanakul <i>et al.</i> 2004(25) Northern Manhattan, South Bronx, NYC	bedroom floor	301	75.9 [1.2–3,388] <i>geomean [max-min]</i>	↑endotoxin ↑wheezing, 2 <sup>nd</sup> year life ↑endotoxin ↓eczema, 1 <sup>st</sup> year life
Metropolitan Denver Gereda <i>et al.</i> 2001(38) Gereda <i>et al.</i> 2000(3)	multiple locations	86	178.2 <i>geomean</i>	↑endotoxin for allergen sensitized pets in home ↑endotoxin central air-conditioning,↓endotoxin

\*. Studies selected for comparison included those with endotoxin measurements and allergic or respiratory health outcomes.

**Table IV**

International comparisons of endotoxin load per square meter in the house dust.

Study Location	Dust sampling location	n	endotoxin (EU/m <sup>2</sup> )	sample collection method
Amsterdam Douwes <i>et al.</i> 2000(39)	bedroom floor non-symptomatic*	70	2,683 [6.7] <i>geomean [sd]</i>	One meter <sup>2</sup> for 2 minutes
	bedroom floor symptomatic	71	2,655 [5.3] <i>geomean [sd]</i>	
Northern Manhattan, South Bronx, NYC				
Rural New Zealand Wickens <i>et al.</i> 2002(37)	bedroom floor	301	3,892 [35–582,859] <i>geomean [max-min]</i>	Two meter <sup>2</sup> for 5 minutes
	living room dust from farm homes	94	3,216 <i>geomean</i>	One meter <sup>2</sup> for 1 minute
	living room dust from non-farms	188	4,507 <i>geomean</i>	
U.S. National Survey Thorne <i>et al.</i> 2005(29) Saxony-Anhalt, Germany Gehring <i>et al.</i> 2001(24) Rural European Community Braun-Fahrlander <i>et al.</i> 2002 (4)	bedroom floor	585	10,470 [1,550–113,100] <i>geomean [5%–95%]</i>	1.7 meter <sup>2</sup> for 5 minutes
	living room floor	444	24,221 [160–2,670,001] <i>geomean [max-min]</i>	One meter <sup>2</sup> for 2 minutes
	bed dust from farm homes	319	29,897 [5,452–157,208] <i>geomean [5%–95%]</i>	Two minutes per meter <sup>2</sup>
	bed dust from non-farm homes	493	14,456 [2,915–75,730] <i>geomean [5%–95%]</i>	