

Endotoxin Exposure Is a Risk Factor for Asthma

The National Survey of Endotoxin in United States Housing

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Background: Although research has shown that early life exposure to household endotoxin protects against development of allergies, studies are less clear on the relationship between household endotoxin exposure and prevalence of wheezing and asthma. We assayed 2,552 house dust samples in a representative nationwide sample to explore relationships between endotoxin exposures and risk factors for asthma, asthma symptoms, and medication use.

Methods: House dust was vacuum-sampled from five locations within homes and assayed for endotoxin. Health, demographic, and housing information was assessed through questionnaire and on-site evaluation of 2,456 residents of 831 homes selected to represent the demographics of the United States.

Results: Endotoxin concentration (EU/mg) and load (EU/m²) were highly correlated ($r = 0.73$ – 0.79). Geometric mean endotoxin concentrations were as follows (in EU/mg): bedroom floors, 35.3 (5th–95th percentile, 5.0–260); bedding, 18.7 (2.0–142); family room floors, 63.9 (11.5–331); sofas, 44.8 (6.4–240); and kitchen floors, 80.5 (9.8–512). Multivariate analysis demonstrated significant relationships between increasing endotoxin levels and diagnosed asthma, asthma symptoms in the past year, current use of asthma medications, and wheezing among residents of the homes. These relationships were strongest for bedroom floor and bedding dust and were observed in adults only. Modeling the joint effect of bedding and bedroom floor endotoxin on recent asthma symptoms yielded an adjusted odds ratio of 2.83 (95% confidence interval, 1.01–7.87). When stratified by allergy status, allergic subjects with higher endotoxin exposure were no more likely to have diagnosed asthma or asthma symptoms than nonallergic subjects.

Conclusion: This study demonstrates that household endotoxin exposure is a significant risk factor for increased asthma prevalence.

Keywords: airway inflammation; house dust; lipopolysaccharide; wheeze

Endotoxins are inflammatory lipopolysaccharide (LPS) molecules from gram-negative bacteria that are ubiquitous in the indoor environment (1). Endotoxins are amphipathic molecules bearing both polysaccharide and lipid moieties. The lipid A component of the molecule is conserved across bacterial species and carries the toxic potency. Inhalation exposure to endotoxins is common in occupational environments (2) and in homes (3, 4). Recognized determinants of endotoxin in homes include indoor

sources, such as pets, pests, humidifiers, kitchen compost bins, and outdoor air (5–8). The hazards of inhaled endotoxin first became apparent from studies of lung disease among workers exposed to vegetable and cotton dust (9, 10), which were later refined with measured endotoxin exposures (11–13). Studies in the 1990s characterized pulmonary responses, including lung inflammation and cytokine upregulation, on inhalation of endotoxin and organic dusts containing endotoxin in humans (14–19) and mice (17, 20–22). Inhaled endotoxins trigger cellular activation and cytokine release from macrophages and other myeloid cells through LPS binding protein and CD14-dependent delivery of monomeric endotoxin to cells expressing MD-2 and Toll-like receptor 4 on their surface (23, 24). These responses to endotoxin inhalation may differ between individuals by genetics (19) or by degree of developed tolerance (25).

A number of epidemiologic studies have drawn attention to endotoxin exposure in domiciles and its role in asthma. Michel and colleagues (3) studied endotoxin in settled dust in the homes of patients with stable chronic rhinitis or asthma and noted a significant association of mattress and floor dust endotoxin concentration, but not mite allergen concentration, with the severity of asthma and asthma medication use. Studies from Bavaria (26), Canada (27), and Sweden (28) have provided evidence that growing up on a family farm leaves one less likely to develop allergies and allergic asthma. This protective effect (the hygiene hypothesis) is supported by studies demonstrating a shift away from an allergic phenotype with early life exposure to endotoxin (29). However, studies from Australia (30), Europe (31), New Zealand (32), and the United States (33, 34) have found the same or higher asthma rates (i.e., no protective effect) between farm and nonfarm children in one or more study groups. The role of endotoxin as a risk factor for asthma and wheezing has been investigated in urban cohort studies (35, 36), with the finding that higher endotoxin in house dust was associated with higher prevalence of wheeze. To date, studies relating endotoxin exposure to health outcomes have been limited to one or two locales and may not be representative of a wider geographic area with its associated diversity.

The first national survey of endotoxin in U.S. housing provides a unique opportunity to explore hypotheses regarding the geographic distribution of endotoxin, socioeconomic and housing characteristics related to endotoxin exposure, and the association of endotoxin exposure with asthma and allergy in a representative sample of the United States. The latter is the subject of this report. Some of the results of these studies have been previously reported in the form of an abstract (7).

METHODS

Study Design

This study was conducted using samples collected for the National Survey of Lead and Allergens in Housing. The study design, sampling, and endotoxin analysis methods have been published (37). The study was performed in 831 housing units representative of the nation's 96 million homes.

(Received in original form May 13, 2005; accepted in final form September 1, 2005)

Supported by National Institute of Environmental Health Sciences (NIEHS) P30 ES05605, the U.S. Department of Housing and Urban Development (HUD/OHHLHC), and the NIEHS Division of Intramural Research.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 172, pp 1371–1377, 2005

Originally Published in Press as DOI: 10.1164/rccm.200505-758OC on September 1, 2005
Internet address: www.atsjournals.org

Assessment of Health Outcomes

Analyses included seven health outcome variables assessed at the individual level. These were ascertained as described previously (37) and in the online supplement as follows: diagnosed hay fever; diagnosed asthma; asthma symptoms past year; current asthma medication use; and wheezing ever, in the past month, and in the past year. Because exposure data were available by household, health outcomes were aggregated to the household level for primary analysis. Verification analyses were performed at the individual level for adults and children.

Exposure Assessment

Each household was visited by two field workers who administered a detailed questionnaire, conducted a home inspection, and collected samples. The questionnaire included information on demographics and health of the residents plus conditions of the home (37). Dust was sampled by vacuum collection into an in-line filter using a standardized protocol. Dust was sieved (425 μ m), aliquoted into 100-mg lots, and then frozen at -80°C before indoor allergen and endotoxin assays.

Endotoxin and allergen analysis methods have been described previously (37). Briefly, endotoxin was analyzed in sieved dust extracted at a 50 mg/ml concentration in 1.0 ml pyrogen-free water containing 0.05% Tween-20 using the kinetic chromogenic *Limulus* amoebocyte lysate assay (38). After linkage with housing unit and allergen data, 2,512 endotoxin determinations were available for statistical analysis.

Statistical Analysis

Logistic regression analyses were performed to assess the effect of the level of endotoxin concentration (EU/mg) on the disease outcomes. Endotoxin was evaluated as a continuous variable in unadjusted logistic models and was dichotomized in adjusted models. Full logistic models were adjusted for census region, season, frequency of indoor cigarette smoking, education, poverty, ethnicity, race, presence of a child younger than 6 yr living in the home, as well as exposure to house dust mite, cat, and dog allergens. Cutoffs for dichotomization were selected at the first quartile for bedroom floor and family room floor and at the second quartile for bedding endotoxin, on the basis of smoothing plots of health outcomes versus endotoxin concentration to maximize the strength of association. Models were tested for significant interactions between categoric endotoxin exposure and allergens and none were found. Sample weights were applied to all household level estimates to account for housing unit selection probabilities, nonresponse, and post-stratification. Taylor series linearization methods were used to obtain variance estimates adjusted for clustering associated with the multistage, complex survey design. The analyses were conducted in SUDAAN (version 8.0; RTI, Research Triangle Park, NC).

The relationship between endotoxin and disease outcomes was illustrated graphically, using smoothed plots to exhibit trends in outcome prevalence across a range of concentrations. The relationship was first modeled through nonparametric regression analyses, conducted with-

out weighting or incorporation of survey design information using S-Plus (version 6.2; Insightful Corp., Seattle, WA). The logit of rate of disease outcome was expressed as a continuous function of the log-transformed endotoxin level, obtained using a locally weighted regression smoother while controlling for the covariates mentioned above. The smoothing parameter for each model was selected based on Akaike's information criterion.

Correlations between endotoxin and eight allergens were calculated as Pearson correlation coefficients using log-transformed endotoxin concentration.

RESULTS

Weighted estimates for endotoxin recovered from the five sampling locations were expressed as both endotoxin concentration in the sieved dust (EU/mg) and endotoxin load (EU/m² of vacuumed area). For samples recovered from the kitchen floor and family room sofa, the area sampled was variable and so total load (total EU in the sample) was specified. The geometric mean endotoxin values, geometric standard errors, 95% confidence intervals (CIs), and 5th and 95th percentiles are reported in Table 1 (unweighted values are provided in the online supplement). These data show that concentrations were highest for kitchen floors and living room floors and lowest for the bedding, which includes the mattress and pillow. Although there was a high degree of variability for kitchen floors, family room floors, and sofas, the variability was considerably less for bedding and bedroom floors. Still, the 5th to 95th percentile values displayed a 70-fold range for bedding and a 50-fold range for bedroom floor endotoxin concentrations. The geometric mean concentration of endotoxin in the kitchen floor dust was 2.3-fold higher than bedroom floor dust and 4.3-fold higher than bedding dust. However, because the degree of contact with bedroom floor/bedding dust and the contact time are high, especially for children, exposure to dust from these sites is of great importance.

To enhance our understanding of the relationship of endotoxin concentration to endotoxin load, we plotted one against the other separately for the sampling locations (Figure E1 in the online supplement). There was a linear relationship between the logarithms of the two variables, with correlation coefficients of 0.73 for the bedroom floor samples and 0.79 for the bedding samples. The coefficient for family room floor samples was also 0.73. These data showed that either concentration or load could be used as measures of household endotoxin. Thus, subsequent analyses focused on concentration because the mass of dust collected is known with greater accuracy than the area sampled, particularly for sofa and kitchen floor.

TABLE 1. GEOMETRIC MEAN AND DISTRIBUTION OF ENDOTOXIN CONCENTRATION AND LOADING FOR THE FIVE SURFACES SAMPLED

Sampled Surface	Number	GM (GSE), 95% CI	5th Percentile	95th Percentile
Endotoxin concentration, EU/mg				
Bedroom floor	588	35.3 (1.05), 31.9–39.1	5.0	260
Bedding	470	18.7 (1.06), 16.5–21.1	2.0	142
Family room floor	489	63.9 (1.07), 56.4–72.4	11.5	331
Family room sofa	468	44.8 (1.08), 38.8–51.8	6.4	240
Kitchen floor	454	80.5 (1.07), 70.2–92.4	9.8	512
Endotoxin load, EU/m ² or EU/sample*				
Bedroom floor	585	10,500 (1.09), 8,830–12,400	1,550	113,000
Bedding	463	4,160 (1.06), 3,720–4,660	500	36,200
Family room floor	488	17,600 (1.08), 15,000–20,600	2,080	152,000
Family room sofa*	468	19,500 (1.11), 15,900–23,900	2,700	213,000
Kitchen floor*	454	18,700 (1.13), 14,800–23,500	1,170	266,000

Definition of abbreviations: CI = confidence interval; GM = geometric mean; GSE = geometric standard error. Values were derived using survey design information and sample weighting.

* Family room sofa and kitchen floor endotoxin load have the units EU/sample.

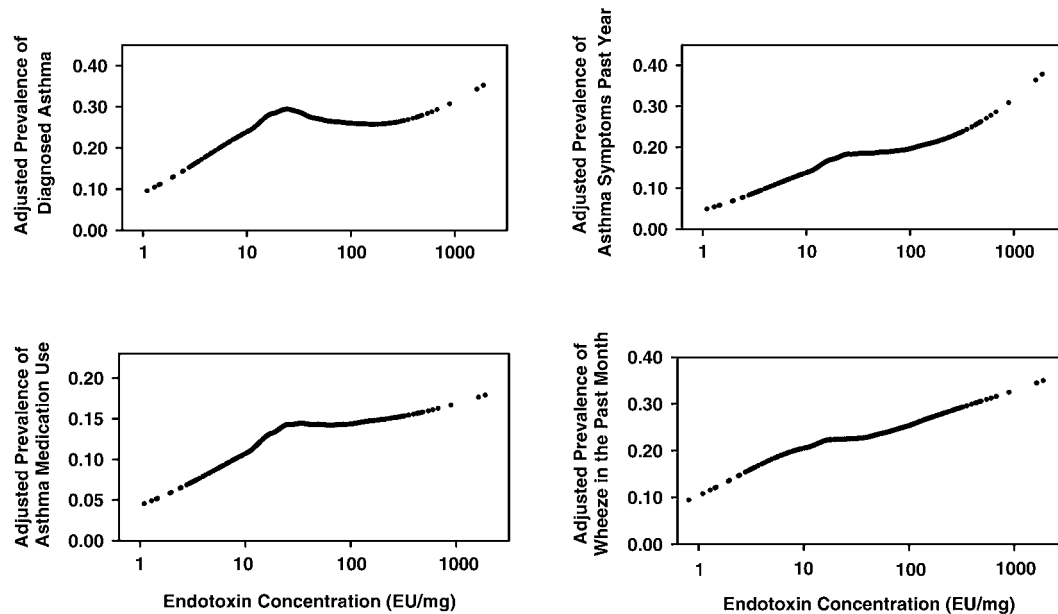


Figure 1. Smoothed plots showing adjusted prevalence of various health outcomes by endotoxin concentration for bedroom floor. *Top left:* adjusted prevalence of doctor-diagnosed asthma; *bottom left:* adjusted prevalence of asthma medication use in the past 12 mo; *top right:* adjusted prevalence of asthma symptoms in the past year; and *bottom right:* adjusted prevalence of wheeze in the past month. The span parameter for each model was 0.8.

It was of interest to explore the relationship of endotoxin concentrations to those of household allergens measured from the same samples to assess the potential for confounding. Correlation coefficients between endotoxin and eight animal, arthropod, and fungal allergens in the five household sites were low, ranging from -0.03 to 0.43 (*see* Table 2). Only 5 of the 40 coefficients exceeded 0.30, and four of those were for *Alternaria alternata*, a common species of fungus. We also determined overall Pearson correlation coefficients for endotoxin pairs within the same households (Table 3). Endotoxin concentrations were not highly correlated between rooms of the same households, and coefficients ranged from 0.44 to 0.12.

This study yielded a prevalence rate of 11.3% for diagnosed asthma among persons living in noninstitutional U.S. housing that is permanently occupied and allows resident children. This compared favorably with the 11.1% prevalence of diagnosed asthma determined from the 2002 National Health Interview Survey (39). This study also compared favorably for diagnosed asthma when separated by sex and age categories (*see* the online supplement). Logistic regression analyses were performed without adjustment for health outcomes using $\log_{10}$ endotoxin as a continuous variable to determine if there is a relationship be-

tween household endotoxin and health outcomes (Table 4). Although there was no relationship of endotoxin exposure with hay fever, the relationships between endotoxin and other health outcomes were highly significant for bedroom floor endotoxin. Endotoxin from other sampling locations yielded p values significant or suggestive of an effect for asthma outcomes ($p = 0.02$ – 0.10).

To further explore the role of bedroom and family room endotoxin in asthma, we developed logistic regression models using dichotomized endotoxin levels (Table 5). Significantly elevated crude odds ratios (ORs) were identified with exposure to bedroom floor endotoxin for the following outcomes: asthma symptoms in the past year (OR, 2.82), current asthma medication use (OR, 2.42), wheezing ever (OR, 2.13), wheezing in the past month (OR, 1.98), and wheezing in the past year (OR, 2.23). After adjustment, ORs were still significantly elevated for most of these outcomes (Table 5). Similar results (but with lower ORs) were observed when the prevalence of disease outcomes was evaluated for association with bedding endotoxin concentration (Table 5). In both the unadjusted and fully adjusted models for bedding, increased endotoxin concentration was most strongly associated with wheezing ever, in the past month, and in the past year. ORs for the effect of family room floor endotoxin on

TABLE 2. WEIGHTED PEARSON CORRELATION AT EACH HOUSEHOLD SITE FOR LOG-TRANSFORMED ENDOTOXIN AND LOG-TRANSFORMED ALLERGEN CONCENTRATIONS

Endotoxin vs. Allergen	Bedroom Floor	Bedding	Family Room Floor	Family Room Sofa	Kitchen Floor
Endo vs. Bla g	0.23	0.18	0.13	0.11	0.37
Endo vs. Can f	0.26	0.18	0.13	0.21	0.14
Endo vs. Fel d	0.18	0.27	0.03	0.17	0.03
Endo vs. Mus m	0.20	0.17	0.22	0.20	0.29
Endo vs. Alt a	0.43	0.29	0.34	0.42	0.34
Endo vs. Der f	0.05	0.15	-0.02	-0.03	0.06
Endo vs. Der p	0.19	0.22	0.13	0.10	0.18
Endo vs. Der f + Der p	0.12	0.20	0.08	0.03	0.15

Definition of abbreviations: Bla g = cockroach allergen; Can f = dog allergen; Fel d = cat allergen; Mus m = mouse allergen; Alt a = *Alternaria alternata* mold; Der f and Der p = house dust mite allergens.

Values over 0.30 are shown in bold type.

TABLE 3. OVERALL WEIGHTED PEARSON CORRELATION BETWEEN PAIRS OF HOUSEHOLD SITES FOR LOG ENDOTOXIN CONCENTRATION

Endotoxin Concentration	Bedroom Floor	Bedding	Family Room Floor	Family Room Sofa	Kitchen Floor
Bedroom floor	1.00				
Bedding	0.44	1.00			
Family room floor	0.33	0.24	1.00		
Family room sofa	0.30	0.27	0.28	1.00	
Kitchen floor	0.28	0.16	0.22	0.12	1.00

health outcomes were not significant, although diagnosed asthma, symptomatic asthma, and taking asthma medication had adjusted ORs of 2.0 or more. It is noteworthy that bedroom floor, family room floor, or bedding endotoxin were not protective factors for self-reported doctor-diagnosed hay fever, an indicator of atopy.

To understand the effect of aggregation to the household level, we also performed individual level analyses for bedroom floor endotoxin separately for adults (≥ 18 yr, 65% of subjects) and children (< 18 yr). This allowed for adjustment for potential confounders at the individual level. This analysis yielded findings consistent with the household level analysis for adults with significantly elevated adjusted ORs for symptomatic asthma and medication use. None of the ORs for children were significant and point estimates were generally close to 1.0, demonstrating that the effect of endotoxin was in adults. These results are provided in the online supplement.

Relationships between endotoxin concentration and disease outcomes were assessed using nonparametric regression analysis while controlling for the covariates mentioned above. These smoothed plots are shown in Figure 1 for bedroom floor endotoxin and four health outcomes. There was a marked increase in prevalence with increasing endotoxin for each health outcome. None of the outcome variables demonstrated a threshold below which there was no increase in prevalence with increasing endotoxin. Thus, no level of endotoxin observed in this study could be clearly identified as a no-effect level.

To further evaluate the role of bedroom floor and bedding endotoxin on prevalence of asthma symptoms in the past year, we modeled the joint effect above and below 19.6 EU/mg for 373 homes that had bedroom floor and bedding endotoxin measurements and data on asthma symptoms. The OR for households with both bedroom floor and bedding endotoxin over 19.6 EU/mg compared with those with both values below this mark was 3.18 (95% CI, 1.31–7.72; $n = 373$). Adjustment for census region,

season, smoking inside, education, poverty, ethnicity, and race resulted in an OR of 2.83 (95% CI, 1.01–7.87; $n = 338$).

Several recent studies have suggested that the role of endotoxin in asthma may differ for those with allergic asthma and those with nonallergic asthma. We compared diagnosed asthma, asthma symptoms, and wheeze in the past year, stratified by allergy status at the household level (self-reported doctor-diagnosed allergy). The adjusted OR for wheezing was higher for households with allergic residents that had higher bedding endotoxin (OR, 2.16; 95% CI, 1.12–4.15; referent is allergic with low endotoxin) as compared with nonallergic wheezing subjects with higher endotoxin exposure (OR, 0.80; 95% CI, 0.33–1.92; referent is nonallergic with low endotoxin). However, this analysis demonstrated no significant interaction between health outcomes and allergy status (interaction p value = 0.11; see Table E5 in the online supplement). This suggests that, in this cohort, atopic wheeze increased with higher bedding endotoxin exposure. For bedroom floor endotoxin, asthma symptoms and wheezing both yielded elevated adjusted ORs (OR > 2.20) in allergic subjects, again with no significant interaction terms. No other health outcomes or sampling sites showed differences with stratification by allergy status.

DISCUSSION

Despite recent interest in the role of endotoxin in asthma, there has not been a prior study of national scope to examine relationships between asthma outcomes and indoor exposures. This study clearly demonstrates significant relationships between household endotoxin and diagnosed asthma, recent asthma symptoms, current use of asthma medications, and wheezing. No effect was observed of allergy status on the relationship between endotoxin and asthma outcomes. This suggests that current endotoxin exposure may have little impact on allergy status and that airway inflammation is the most significant effect of endotoxin exposure

TABLE 4. p VALUES AND β COEFFICIENTS FOR UNADJUSTED LOGISTIC REGRESSION MODELS FOR PRESENCE OF HEALTH OUTCOMES AMONG ANYONE IN THE HOUSEHOLD USING LOG ENDOTOXIN CONCENTRATION AS A CONTINUOUS VARIABLE

Disease Outcomes	Bedroom Floor		Bedding		Family Room Floor		Family Room Sofa		Kitchen Floor	
	p	β	p	β	p	β	p	β	p	β
Diagnosed hay fever	0.93	0.02	0.35	-0.18	0.33	0.25	0.83	0.05	1.00	0.00
Diagnosed asthma	0.01	0.41	0.23	0.28	0.18	0.33	0.07	0.46	0.84	-0.05
Asthma symptoms past yr	< 0.001	0.75	0.14	0.36	0.03	0.52	0.06	0.57	0.30	0.25
Current asthma medication use	0.001	0.72	0.05	0.46	0.04	0.55	0.37	0.34	0.06	0.48
Wheezing, ever	0.005	0.58	0.16	0.27	0.20	0.34	0.36	0.20	0.08	0.37
Wheezing, past mo	0.009	0.58	0.02	0.47	0.10	0.35	0.15	0.38	0.10	0.40
Wheezing, past yr	0.02	0.44	0.07	0.35	0.12	0.30	0.85	-0.05	0.20	0.27

Values shown in bold are significant at $p \leq 0.05$. Household numbers for the seven health outcomes are as follows: bedroom floor, $n = 486$ –583; bedding, $n = 397$ –466; family room floor, $n = 409$ –485; family room sofa, $n = 407$ –463; kitchen floor, $n = 380$ –450.

TABLE 5. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FROM LOGISTIC REGRESSION MODELS FOR THE PRESENCE OF HEALTH OUTCOMES USING ENDOTOXIN EXPOSURE LEVELS ABOVE AND BELOW THE FIRST QUARTILE FOR BEDROOM FLOOR AND FAMILY ROOM FLOOR ENDOTOXIN AND THE SECOND QUARTILE FOR THE BEDROOM BEDDING ENDOTOXIN

Disease Outcomes	Bedroom Floor Endotoxin					Bedroom Bedding Endotoxin					Family Room Floor Endotoxin				
	Endotoxin Category (EU/mg)	Model 1*		Model 2†		Endotoxin Category (EU/mg)	Model 1*		Model 2†		Endotoxin Category (EU/mg)	Model 1*		Model 2†	
		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI		OR	95% CI	OR	95% CI
Diagnosed hay fever	≤ 16.6	1.00	0.82, 2.08	1.00	0.81, 2.76	≤ 19.6	1.00	0.59, 1.31	1.00	0.59, 1.75	≤ 33.9	1.00	0.68, 2.45	1.00	0.61, 2.22
	> 16.6	1.31		1.50		> 19.6	0.88		1.02		> 33.9	1.29		1.16	
Diagnosed asthma	≤ 16.6	1.00	0.91, 2.64	1.00	0.76, 3.22	≤ 19.6	1.00	1.00, 2.76	1.00	0.90, 3.93	≤ 33.9	1.00	0.94, 3.02	1.00	0.99, 3.95
	> 16.6	1.55		1.57		> 19.6	1.66		1.88		> 33.9	1.69		1.98	
Asthma symptoms past yr	≤ 16.6	1.00	1.47, 5.41	1.00	1.21, 6.38	≤ 19.6	1.00	0.91, 2.81	1.00	0.63, 3.38	≤ 33.9	1.00	0.84, 4.28	1.00	0.86, 5.18
	> 16.6	2.82		2.78		> 19.6	1.60		1.46		> 33.9	1.90		2.12	
Current asthma medication use	≤ 16.6	1.00	1.20, 4.88	1.00	0.91, 5.71	≤ 19.6	1.00	1.00, 2.95	1.00	0.79, 4.23	≤ 33.9	1.00	0.86, 4.25	1.00	0.85, 5.21
	> 16.6	2.42		2.28		> 19.6	1.72		1.83		> 33.9	1.91		2.11	
Wheezing, ever	≤ 16.6	1.00	1.32, 3.43	1.00	1.28, 4.00	≤ 19.6	1.00	1.09, 2.98	1.00	1.23, 3.30	≤ 33.9	1.00	0.64, 2.75	1.00	0.69, 2.66
	> 16.6	2.13		2.27		> 19.6	1.80		2.01		> 33.9	1.20		1.35	
Wheezing, past mo	≤ 16.6	1.00	1.27, 3.11	1.00	1.05, 3.61	≤ 19.6	1.00	1.33, 2.98	1.00	1.30, 3.23	≤ 33.9	1.00	0.54, 2.62	1.00	0.46, 2.55
	> 16.6	1.98		1.95		> 19.6	2.00		2.05		> 33.9	1.19		1.09	
Wheezing, past yr	≤ 16.6	1.00	1.34, 3.73	1.00	0.95, 3.01	≤ 19.6	1.00	1.24, 3.27	1.00	1.20, 3.43	≤ 33.9	1.00	0.74, 2.14	1.00	0.44, 1.55
	> 16.6	2.23		1.70		> 19.6	2.01		2.03		> 33.9	1.26		0.83	

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

* No adjustments are made for Model 1.

† Model 2 is adjusted for census region, season when sampled, frequency of indoor cigarette smoking, education, poverty, ethnicity, race, presence of a resident child < 6 yr, log(Der p + Der f), log(Can f), log(Fel d).

in a cross-section of the population. Secondary analyses showed the effect of endotoxin on asthma and wheeze was driven by adults.

Recognition that house dust contains endotoxin dates to a 1964 study (40); however, detailed investigations of the role of endotoxin in household dust came more than 30 yr later. Michel and colleagues (3) demonstrated that the severity of asthma and asthma medication use in a series of Belgian clinic patients was significantly associated with mattress and floor dust endotoxin concentration. Several European studies reported endotoxin levels in house dust. Wouters and coworkers (6) analyzed dust from 100 Dutch households and demonstrated that living room floor endotoxin was threefold higher for households that had a biopail for compostable waste in their kitchen versus those who did not. Endotoxin loads were considerably lower than those in the current study. The Lifestyle–Immune System–Allergy (LISA) study is a birth cohort study of allergy in Munich and Leipzig, Germany, in which mattress dust was collected from mothers of newborns (35, 41). This study yielded a very low geometric mean endotoxin concentration (2.9 EU/mg; $n = 1884$; range, 0.057–1290 EU/mg). Exposure to bedding endotoxin in the first few months of life protected against atopic eczema but was a risk factor for respiratory infection, cough with infection, and wheezing at 6 mo of age (41). By 1 yr of age, the protective effect was no longer apparent, yet the risk of wheezing was still evident in the highest exposure group (> 10.2 EU/mg; OR, 1.60). The highest quintile group in the LISA study was exposed at the median level found in the current study.

A second large European study, the Allergen and Endotoxin (ALEX) study, investigated the role of endotoxin exposure early in life in farm children born in Bavaria, Austria, and Switzerland (28) and found that endotoxin concentration in the child's bedding was associated with reduced risk of hay fever, sneezing, and itchy eyes. Endotoxin load was further associated with a reduction in atopic sensitization and atopic wheeze. Asthma and wheeze among nonallergic subjects were nonsignificantly related to endotoxin exposure. Smoothing plots showing adjusted prevalence of wheeze for bed endotoxin stratified by atopic status

demonstrated a protective effect of endotoxin for those with atopy and increased risk of wheeze for nonatopic subjects exposed at the highest endotoxin loads. Although not focused on children, data from the current study present a different picture. In the National Survey, both allergic and nonallergic subjects had higher adjusted ORs for diagnosed asthma, asthma symptoms, and wheeze in the past year, with increasing endotoxin exposure for bedroom floor endotoxin. For bed endotoxin, allergic subjects were more likely than nonallergic subjects to wheeze with higher endotoxin concentration. For this analysis, none of the interaction terms were significant. It should be pointed out that this study was somewhat underpowered for analysis of health outcomes stratified by both endotoxin exposure and allergy status.

This study had several weaknesses. One limitation of this study is that exposures and health outcomes were assessed cross-sectionally. Health outcome variables were assessed at the same time as dust samples were collected; however, the age of onset of asthma and symptoms was not ascertained. Thus, we were not able to establish what endotoxin exposures were before the development of asthma. The fact that wheezing in the past month and year and current asthma medication use yielded similar results to diagnosed asthma supports the study findings. In addition, the use of self-reporting of doctor-diagnosed asthma and hay fever could have led to misclassification due to recall bias.

Multivariate analyses reported above were performed at the household level because the study was designed as a household survey. This analysis makes the assumption that endotoxin levels in bedrooms within a home are comparable. To test the effect of aggregation at the household level, we performed two additional analyses. First, we analyzed exposure-outcomes data at the individual level for all subjects. Next, we recognized that we could include more households and individuals in the analysis by creating a house index and imputing missing bedroom endotoxin values from available data from other rooms in the house. For instance, for the bedroom floor, this led to inclusion of an additional 202 endotoxin concentrations to yield a total of 790 values. Both these individual-level analyses yielded similar OR point

estimates to those from the household-level results, suggesting minimal bias in our data.

Individual-level analyses were then performed separately for adults and children. This revealed that the higher prevalence of diagnosed asthma and asthma symptoms was driven mostly by an effect of endotoxin exposure among adults. Because this study was designed as a representative survey for the demographics of the United States, it was not designed or powered to explore specific hypotheses regarding endotoxin and asthma exacerbation in young children, as was done in the LISA and ALEX studies.

The National Survey demonstrates on a national scale that U.S. household endotoxin exposures are high relative to those in Europe and are associated with asthma symptoms, current asthma medication use, and wheezing, but not allergy. Bedroom floor and bedding endotoxin imparted the greatest risk and no no-effect thresholds could be identified. Stratification by allergy status did not reveal a significant protective effect of endotoxin exposure for atopic asthma or asthma symptoms.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank individuals who contributed to conducting the National Survey, including Warren Friedman, Patrick Vojta, and the staff at Westat, Inc. They thank Renee Jaramillo for her assistance with data analysis.

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