

Nonlinear relationship of mite allergen exposure to mite sensitization and asthma in a birth cohort

Euan R. Tovey, PhD,^{a,b} Catarina Almqvist, PhD,^{a,b,c,d} Qiang Li, BSc,^{a,e} Daniele Crisafulli, BSc,^{a,b,c} and Guy B. Marks, PhD^{a,b,c} Sydney, Australia, and Stockholm, Sweden

Background: There is uncertainty about the nature of the relationship between mite allergen exposure during infancy and the expression of allergic diseases in childhood.

Objective: We sought to explore the relationships between repeated measurements of mite allergen exposure during the first 5 years of life and clinical allergic disease outcomes at age 5 years.

Methods: In a birth cohort of 516 at-risk children, 13 bed dust samples were collected between birth and 5 years of age and analyzed for mite allergen. At age 5 years, the presence of mite atopy was assessed based on skin prick test results, and clinical assessments for asthma, eczema, and wheeze were conducted. The association of allergen exposure with each clinical outcome was examined by means of logistic regression, with adjustments for potential confounders.

Results: The lowest and highest mite exposure quintiles, expressed as mean allergen concentration averaged over 0 to 5 years, were associated with a lower prevalence of mite atopy and of asthma compared with intermediate levels of exposure. These relationships, when determined by using average allergen concentration over 0 to 18 months, were weaker.

Conclusions: This study demonstrates a nonlinear relationship between mite allergen exposure and clinical outcomes in this generally high mite allergen environment. (*J Allergy Clin Immunol* 2008;122:114-8.)

Key words: Allergens, *Der p 1*, house dust mite, asthma, sensitization, high-dose tolerance, childhood

The relationships between mite allergen exposure and the expression of sensitization, asthma, and eczema in young children are the topic of ongoing research and discussion. Although several earlier studies in children found a direct correlation between the intensity of house dust mite (HDM) exposure in infancy and the prevalence of mite sensitization,^{1,2} less

Abbreviations used

HDM: House dust mite

ICC: Intraclass correlation coefficient

IQR: Interquartile range

consistency has been observed for the relationship of mite exposure to asthma.³⁻⁵ Moreover, other studies of exposure to domestic pet allergens have shown that early and very high exposure could at times be associated with protection against sensitization, rhinitis, and asthma.⁶⁻⁸ However, the latter findings might also be confounded by selection for pet ownership. Recently, uncertainty about the shape of the dose-response relationship has been extended to mite allergen exposure. Although some recent studies show direct positive associations,^{9,10} others show a bell-shaped relationship¹¹ or no clear associations once a minimal threshold is exceeded.^{12,13} The causal role of exposure and sensitization in early childhood eczema is also uncertain.¹⁴⁻¹⁶

There are many sources of variation between different studies that might explain these apparent inconsistencies. These include the heterogeneous nature of the diseases and individual immune responses, the role of gene-environment interactions, the role of other nonallergen exposures related to lifestyle and geography,¹⁷⁻¹⁹ and changes in vulnerability to these exposures during infancy,^{1,20,21} particularly when exposure has been measured at different times. Additionally, there are many sources of variation in estimating "exposure." These include the changes in mite allergen concentrations that occur with time and bed type,²² coupled with the pragmatic limitation of using a single or a few dust samples as a summary measure for chronic aeroallergen exposure over this time. Typically, the measure of such exposure has been the allergen concentration or the quantity of allergen in samples of reservoir dust collected with a vacuum cleaner from the bed.²³

The aim of this analysis was to test for the presence of a relationship between mite allergen exposure during the first 5 years of life and the prevalence of atopy, wheeze, asthma, and eczema at age 5 years. We further wished to assess the shape of the dose-response relationship and relative predictive value of 3 measures of HDM allergen exposure.

METHODS

Subjects

The study population in the Childhood Asthma Prevention Study has been previously described.^{24,25} It comprised 616 children who were randomized at birth into active or control groups for both HDM avoidance and dietary intervention. All subjects were classified as being at risk of asthma on the basis of having at least 1 parent or sibling with symptoms of asthma, as assessed by means of a screening questionnaire. The active

From ^athe Woolcock Institute of Medical Research, Sydney; ^bthe Faculty of Medicine, University of Sydney; ^cthe Cooperative Research Centre for Asthma, Sydney; ^dthe Department of Medical Epidemiology and Biostatistics and the Department of Woman and Child Health, Karolinska Institutet, Stockholm; and ^ethe George Institute for International Health, Sydney.

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Reprint requests: Euan R. Tovey, PhD, Woolcock Institute of Medical Research, PO Box M77, Missenden Road Post Office, New South Wales 2050, Australia. E-mail: ert@med.usyd.edu.au.

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HDM intervention included the application of an allergen-impermeable barrier to the child's bed and pillow, provision of a washable rug to play on, and laundry of bedding at 3-month intervals in a solution containing the acaricide benzyl benzoate (final concentration, 0.03%). Both active and control groups were given advice about ensuring adequate ventilation, regular vacuuming, and avoidance of humidifiers or vaporizers. The data presented here relate to the 516 children who remained in the study at age 5 years. Families with a pet cat were excluded at enrollment, although 82 (15.9%) children acquired a cat during the study. Dog owners were not excluded. This study was approved by the Human Research Ethics Committees of the University of Sydney, Children's Hospital Westmead, and the South Western Sydney Area Health Services.

Exposure sample collection

Reservoir dust collection and analysis has been described previously.²² Briefly, children's bed dust was collected for all study subjects on 13 occasions at 1, 3, 6, 9, and 12 months and then at 6-month intervals up to the age of 5 years. Collection was by means of vacuuming with a standard protocol. Settling airborne dust was also collected by using Petri dishes²⁶ placed approximately 1 m from the child's bed and 1 m above the floor and left open for 5.65 ± 1.4 (SD) weeks at 1 month, 2.5 years, and 3.5 years. Mite allergen Der p 1 levels were measured by means of ELISA (Indoor Biotechnologies Ltd, Cardiff, United Kingdom).²² Allergen content of the reservoir dust was expressed as 2 different measures of exposure: as allergen concentration (micrograms of Der p 1 per gram of dust) and as quantity of allergen (micrograms of Der p 1 per sample), with the latter being the allergen concentration multiplied by the weight of the sample of fine dust. Settling dust was expressed as nanograms of Der p 1 per square meter per day. Only the results for allergen concentration are reported here; results for the quantity of allergen per sample and for settling airborne dust are reported in the [Results](#) section of the Online Repository (available at www.jacionline.org).

Measures of subject, social, and housing variables

Data on social, domestic, and lifestyle factors were collected either during 6-month home visits or by telephone. Household dampness was estimated as the difference between on-site indoor and outdoor absolute humidity averaged over 6 monthly assessments from ages 3 to 5 years and classified as greater than or less than the mean value.

Clinical outcomes

The measurement of clinical outcomes in the Childhood Asthma Prevention Study has been described elsewhere.^{24,25} Briefly, at age 5 years, parents were interviewed about symptoms and diagnoses relevant to asthma and allergic disease by using a *pro forma* questionnaire. Current eczema was defined as the presence of flexural eczema on inspection (by the assessment nurse) or by parental report of a history of itchy rash coming and going for a period of 3 months or more, together with a history of seeking medical care for eczema, using steroid or emollient creams in the last 12 months, or both. Atopy was measured by means of skin tests with 6 common aeroallergens and 4 food allergens. Children with any allergen wheal size of 2 mm or greater were classified as atopic, and HDM sensitization was defined as allergy to *Dermatophagoides pteronyssinus*. The definition of recent wheeze was any reported wheeze in the last 12 months. Current asthma was defined as parental report of any wheeze in the previous 12 months at age 5 years and either a parental report of diagnosed asthma at ages 18 months, 3 years, or 5 years or a greater than 12% increase in FEV₁ after bronchodilator use at age 5 years. FEV₁ was measured by means of spirometry.

Data analysis

The distribution of all allergen values was normalized by log₁₀ transformation. Time-weighted average allergen concentrations and time-weighted average quantities of allergen were calculated for periods from birth to 18 months and birth to 5 years.

The reliability of allergen measures within households was estimated as the intraclass correlation coefficient (ICC),²⁷ which was estimated by using a repeated-measures ANOVA (Stata 9; StataCorp LP, College Station, Tex). The relationship between each pair of the 3 different measures of allergen exposure was assessed by estimating pairwise correlation coefficients. The results for the ICCs for each measure and correlations between them are presented in the [Results](#) section of the Online Repository.

Quintiles of allergen exposure were defined for all 3 measures of allergen exposure. The 2 measures of reservoir dust exposure, allergen concentration and quantity of allergen, were analyzed for the time periods birth to 18 months and birth to 5 years.

The association of all 3 measures of allergen exposure with each of the 5 clinical outcomes was tested by using logistic regression separately for each clinical outcome and for each measure of allergen exposure. Nonlinear associations were tested by treating allergen exposure quintiles as a non-ordered categorical main effect, and linear associations were tested by using the log-transformed continuous measure of allergen exposure as a main effect. All models included both randomization groups (HDM and diet interventions) as covariates. The child's sex, the presence of a cat within the house at any visit during the first 5 years of life, maternal smoking during pregnancy, whether the child was breast-fed for 6 months or more, and the measure of indoor dampness were all included as covariates in all models.

Before fitting the main effect models described above, 2 interactions with the allergen exposure quintiles were tested separately for all models: the HDM intervention group allocation and the presence of a cat within the house at any time. Analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC), except where specified. The type I error level was set at 5%.

RESULTS

A wide range of values for allergen exposure were observed across the study population over time. A total of 6833 reservoir dust samples were analyzed; 3355 during the period from 0 to 18 months, 1534 from 2 to 3 years, and 1944 from 3.5 to 5 years. Mite allergen was detected in 97% of samples. Little difference was seen in the overall distribution of mean allergen concentration for the 3 periods of the study (see [Fig E1](#) in the Online Repository at www.jacionline.org). For birth to 18 months, the median was 10.0 µg/g (interquartile range [IQR], 3.3–20.9 µg/g); for 18 months to 3 years, the median was 13.2 µg/g (IQR, 5.1–25.0 µg/g), and for 3 to 5 years, the median was 10.0 µg/g (IQR, 4.0–21.2 µg/g). The cutoff points for the quintiles of allergen concentration over the 5-year period were 3.48, 7.78, 13.51, and 23.40 µg of Der p 1/g of dust.

As expected, HDM allergen levels were lower in the active HDM intervention group than in the control group. Furthermore, there were differences in the distribution of cat ownership ($P = .05$), the presence of any visible mold ($P = .03$), and household dampness ($P = .05$) among the quintiles of HDM allergen concentration. The distribution for the 9 housing and social factors is shown in [Table E1](#) (available in the Online Repository at www.jacionline.org).

The prevalence of the clinical outcomes at 5 years in this study population was as follows: any atopy at 5 years, 45.0%; HDM-induced atopy, 34.1%; current asthma, 22.4%; current eczema, 21.7%; and wheeze in the last 12 months, 31.9%.

There were significant differences in the prevalence of both HDM-induced atopy ($P = .029$) and asthma ($P = .0013$) at 5 years for the different quintiles of mite allergen exposure, expressed as time-weighted average allergen concentration, measured over the period of birth to 5 years ([Fig 1](#)). The prevalence of HDM-induced atopy was significantly higher in the second quintile (odds ratio, 2.37; 95% CI, 1.17–4.78) and the fourth quintile (odds ratio,

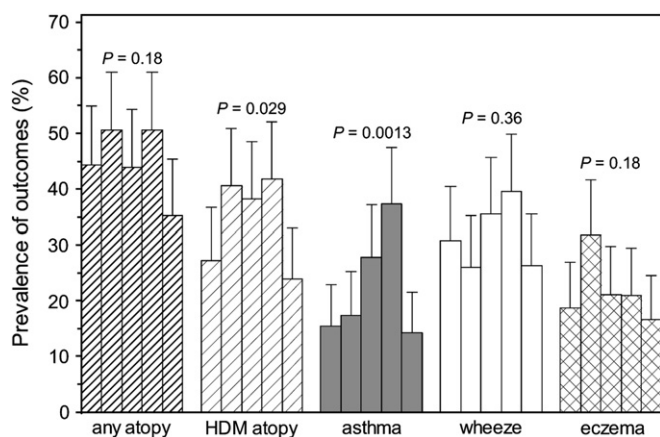


FIG 1. The prevalence of the 5 clinical outcomes at 5 years of age is shown for each quintile of time-weighted average mite allergen concentration over the period of birth to 5 years. The quintiles are 0 to 3.48, 3.48 to 7.78, 7.78 to 13.51, 13.51 to 23.40, and greater than 23.40 μg of Der p 1/g of dust. The bars show the 95% CIs.

2.51; 95% CI, 1.29-4.89) compared with that in the fifth quintile. The prevalence of asthma was also significantly higher in the fourth quintile compared with that in the fifth quintile (odds ratio, 3.99; 95% CI, 1.89-8.40) and the first quintile (odds ratio, 3.23; 95% CI, 1.44-7.25). There were no significant differences among the quintiles in the prevalence of the other outcomes: any atopy, eczema, and wheeze. There were no significant linear relationships between allergen concentration and any of the clinical outcomes.

The effect of allergen concentrations on any of the 5 clinical outcomes did not differ between active and control HDM intervention groups or between those with and without a cat in the house (all $P > .3$). For the exposure period of birth to 18 months, there were no significant overall or linear relationships between the quintiles of time-weighted average allergen concentration and any of the clinical outcomes at 5 years (all $P > .1$) (see Fig E2 in the Online Repository at www.jacionline.org). However, these relationships showed similar trends to those for the period of birth to 5 years. Additionally, there was a significant interaction between allergen concentration for 0 to 18 months and the HDM intervention group for the outcome of "any atopy" at 5 years ($P = .035$ for the interaction). In the nonintervention (control) group the prevalence of atopy tended to be higher in the second quintile compared with that in the highest (fifth) quintile, and in the active HDM intervention group, the prevalence of atopy tended to be lower in the third quintile compared with that in the fifth quintile. However, in neither of these subgroups was there a significant association between exposure quintiles and the prevalence of atopy ($P = .08$ in both cases). There were no other significant interactions (all $P > 0.06$), including with cat, and there were no significant overall associations between quintiles of allergen concentration, averaged from birth to 18 months, and outcomes at 5 years (all $P > 0.1$). Overall, there were no significant linear relationships between allergen concentration, averaged from birth to 18 months, and any of the outcomes at 5 years (all $P > 0.1$). There were no significant associations between exposure expressed as the time-weighted average quantity of allergen or when measured as settling airborne allergen exposure over the 5 years for any of the clinical outcomes (see the Results section in the Online Repository). There was no

significant relation between quintiles of allergen concentration, averaged from birth to age 5 years, and prebronchodilator FEV₁ at age 5 years after adjustment for HDM intervention group, sex, height, and HDM sensitization status ($P = .8$, data not shown).

DISCUSSION

The most striking feature of this cohort study is that children in the highest quintile of exposure were significantly less likely to have both mite sensitization and asthma compared with children in the fourth quintile of exposure, suggesting that very high mite exposure might be protective for these outcomes. Additionally, children in the lowest exposure quintile were also less likely to have asthma than those in the fourth exposure quintile. These associations were observed when the exposure metric was the time-weighted allergen concentration and the period for measurement was over the entire 5 years. When exposure was expressed as the average allergen concentration over the period from birth to 18 months, similar trends were observed, but they were not significant. Overall, this confirms the bell-shaped relationship between mite exposure and sensitization described recently by Schram-Bijkerk et al¹¹ and extends this finding to asthma.

It is noteworthy that a high prevalence of sensitization was observed across the second and fourth quintiles of exposure (ie, 3.48-7.78 $\mu\text{g}/\text{g}$ Der p 1 and 13.51-23.40 $\mu\text{g}/\text{g}$ Der p 1), whereas the highest prevalence of asthma was more tightly associated with the fourth quintile (ie, 13.51-23.40 $\mu\text{g}/\text{g}$ Der p 1). The prevalence of asthma, eczema, and HDM sensitization all decreased sharply for the highest allergen concentration quintile ($>23.4 \mu\text{g}/\text{g}$ Der p 1), and this effect was observed both when the timeframes of exposure were from birth to 5 years and from birth to 18 months, although only the former was significant.

We adjusted for confounders that might have affected our findings. These included HDM and diet intervention groups, presence of a cat, excess indoor humidity, sex, smoking during pregnancy, and breast-feeding for 6 months.

We also considered whether there might be other unmeasured factors associated with high mite allergen concentrations that might have affected these findings. The factors determining mite allergen concentrations in houses are not fully understood and are thought to include indoor humidity and possibly occupant density of houses, mattress age, pet ownership, and visible mold. However, as shown in Table E1, indoor humidity, mattress age, visible mold, and occupant number were approximately evenly distributed across exposure quintiles, indicating these domestic descriptors might not reflect the factors driving mite allergen concentrations in this climate. Although the proportion of cat owners was higher in households with higher concentrations of mite allergen, the clinical outcomes were independent of cat ownership. Furthermore, the association did not differ between households with and without cats, as shown by the nonsignificant interaction term in the regression model.

The occurrence of such nonallergen biologic factors remains of interest because high mite exposure might indicate high exposure to other biologically derived ligands that stimulate the innate immune system, which is consistent with the hygiene hypothesis. In this context high infant exposure to endotoxin²⁸ and β -glucans from fungi²⁹ have been shown to be protective. Endotoxin and β -glucans were not measured in this study, although we recently compared Der p 1 and endotoxin in bed dust of a different cohort

of Sydney children ($n = 112$), and these showed only a weak correlation ($R = 0.27$). In another study of adult beds ($n = 20$), we compared Der p 1 and β -glucans and found no correlation ($R = 0.14$, $P > .05$; unpublished data).

Another potential immunomodulator is chitin, which is derived from fungi, fragmented mite bodies, and the peritrophic membrane around mite feces. Chitin has been suggested to both stimulate^{30,31} and be protective^{32,33} for allergy in animal and human models. Chitin was not measured in this study.

Alternatively, nonlinearity of responses might be a direct function of high exposure to mite allergen itself. This might be analogous to the "high-dose tolerance" observed for cat allergen.³⁴ However, the extent to which this applies to other domestic and occupational aeroallergens is not clear, and the mechanisms and observations of high-dose tolerance are currently inconsistent.³⁵⁻³⁸

Several other studies have reported nonlinear responses to mite allergens, and there is a lack of consistency in findings between studies. Bell-shaped dose-response curves were recently described for the Prevention of Allergy Risk factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle study,¹¹ in which the middle-exposure tertile (range, 1.39-10.4 $\mu\text{g/g}$ Der p 1) had significantly higher rates of mite sensitization. In our study the prevalence of sensitization was high in the second, third, and fourth quintiles (7.78-23.6 $\mu\text{g/g}$ Der p 1) compared with that in the fifth and first quintiles. Although another recent multicenter prospective study² overall found that mite sensitization at age 4 years was unrelated to allergen exposure at 3 months, the plots show a trend toward sensitization decreasing at high allergen concentrations. A third study also found no significant relationships between mite allergen exposure at 2 months and either mite sensitization or atopic wheeze at age 5 years.³⁹ Here the exposure-response relationships for both outcomes increased steeply at low levels of exposure and were attenuated at high levels of exposure. The upper boundaries for the first and fourth quintiles were 0.5 and 7.9 $\mu\text{g/g}$, respectively, which again is lower than in our study. These patterns were modified by paternal atopy and birth order. Another study also observed the effects of parental atopy modifying clinical outcomes.¹³ Here dust mite exposure of greater than 10 $\mu\text{g/g}$ was associated with a decreased risk of atopic asthma in children with a parental history of atopy but was associated with an increased risk if there was no parental history of atopy. Conversely, the risk of mite sensitization increased at levels of greater than 10 $\mu\text{g/g}$ in those with a parental history of atopy and was inversely associated in those without such a parental history. In our study all children were recruited on the basis of having a family history of asthma, and bell-shaped responses were observed for both outcomes. The atopic status of our family members is not known.

It is noteworthy that in this study in Sydney, the allergen concentration in the fifth quintile ($>23.4 \mu\text{g/g}$ Der p 1) is higher than is consistently found in many other places in the world, and such a bell-shaped relationship might be more evident in this population than elsewhere.

Our study showed no clear relationship of mite allergen exposure to eczema at age 5 years. Although mite allergy is commonly associated with eczema, such causal associations lack consistency.¹⁴ A large United Kingdom cohort study recently reported a tendency toward a nonsignificant, bell-shaped relationship between early mite exposure and eczema at age 7 years.¹⁶

In this study the relationships between exposure and outcomes were only significant when the exposure timeframe covered the

entire 5-year period, although similar trends were evident for the first 18 months. This suggests that the effects of exposure continue over this time. Additionally, a more reliable estimate of mite exposure can be obtained with frequent measurements over a 5-year period, which is a major strength of this study. Given that repeated measures in children's beds show variation over time,⁴⁰ partly driven by changes with season and bed type,²² the use of a single measure to represent allergen exposure in other studies might limit their ability to detect any associations with disease outcomes.

Significant and consistent associations with clinical outcomes were only found when exposure was expressed as allergen concentration; this is despite allergen concentration, quantity of allergen in reservoir dust, and aeroallergen all being correlated. There is a history of debate about the most clinically relevant measurement to describe exposure with reservoir samples, and most, but not all, studies have used allergen concentration in preference to total quantities of allergen. Although generally indices of reservoir concentrations have only weakly correlated with acute measures of aeroallergens,⁴¹ a recent study found acute aeroallergen measured with nasal samplers correlated to reservoir concentrations in bed dust samples,⁴² but only the latter correlated to asthma severity.⁴³ The consistency of findings relating to reservoir allergen concentrations in our study supports the use of this index.

In conclusion, our analysis of clinical allergic outcomes at age 5 years in a large cohort of at-risk children who had their mite allergen exposure measured at regular intervals from birth onward showed that the prevalence of sensitization and asthma increased with increasing allergen exposure up to an intermediate level but then markedly decreased in the most highly exposed subgroup. This phenomenon was most evident when allergen exposure was assessed over the entire time period and when exposure was measured in units of allergen concentration. The immune mechanisms underlying the attenuation of the expression of asthma at high levels of mite allergen exposure in early childhood are of deep interest because they might indicate new mechanisms that offer the possibility of controlling the disease.

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Clinical implications: The immune mechanisms underlying the attenuation of the expression of asthma at high levels of mite allergen exposure in early childhood are of deep interest because these offer the possibility of controlling the disease.

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RESULTS

Distribution of exposure measured over time as both total quantity of allergen and as settling airborne allergens over the study period

For exposure measured as the quantity of allergen and for the settling aeroallergen collected with Petri dishes, the distribution of exposure also did not differ substantially over the 3 time periods (data not shown). The cutoff points for the quintiles of quantities of allergen over the 5-year period are shown in Fig E3.

Reliability of the 3 measurements of exposure and the correlation between them

The reliability within households of the 3 different measures of exposure over the course of the study was determined as ICCs. For the 13 dust samples, when measured in units of allergen concentration, the ICC was 0.39, whereas when measured as the quantity of allergen, the ICC was 0.33. The ICC for the 3 settling aeroallergen samples was 0.16. When comparing these different measures of expressing exposure, there was a strong correlation between the allergen concentration and the quantity of allergen per sample ($R = 0.79$, $P < .001$). Both of these were significantly but weakly correlated with samples collected in Petri dishes ($R = 0.21$, $P < .001$ and $R = 0.14$, $P < .001$) for concentration and quantity of allergen, respectively.

Clinical outcomes when using time-weighted average quantity of allergen from birth to 18 months or birth to 5 years as the measure of exposure

When the 5 clinical outcomes were determined for each quintile of time-weighted average quantity of allergen from

birth to 5 years (Fig E3) or as the time-weighted average quantity of allergen from birth to 18 months (data not shown), no significant associations were observed for the overall group for any outcomes. The only significant interaction was in the effect of the HDM intervention groups and quantity of allergen averaged from birth to 18 months on asthma at 5 years ($P = .026$). Here, in the active HDM intervention group, the lowest quintile of quantity of allergen was associated with the lowest prevalence of asthma ($P = .016$). No other interactions were significant (all $P > .06$), and no other associations between quintiles of quantity of allergen averaged over either time period and outcomes at 5 years were significant (all $P > .16$). Furthermore, there were no significant linear relationships between quantities of allergen averaged over either time period and any of the clinical outcomes at 5 years (all $P > .08$).

Clinical outcomes when using settling airborne allergen at 18 months or as the average of 3 samples over 5 years as the measure of exposure

When the 5 clinical outcomes were determined for each quintile of settling airborne exposure, as measured with Petri dishes, there were no significant associations for any of the clinical outcomes at 5 years (all $P > .1$) and exposure. For exposure at 1 month, the prevalence of eczema tended to be lower in the fourth exposure quintile, whereas other clinical outcomes tended to be lower in the fifth exposure quintile. When the average exposure over the 3 periods was used, there was a less clear pattern for the 5 clinical outcomes (data not shown).

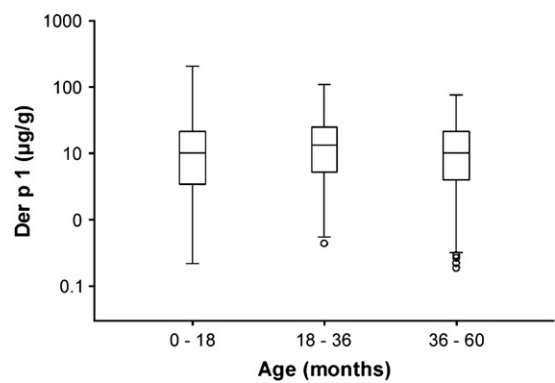


FIG E1. Box-and-whisker plots of the distribution of the time-weighted average mite allergen concentrations for the periods of birth to 18 months, 18 months to 3 years, and 3 years to 5 years. The *upper and lower horizontal lines* show the maximum and minimum observations (excluding outliers), the *box* shows the boundaries of the 25% and 75% quartiles and the median, and the *open circles* denote outliers that are more than 3 times the IQR from the 25% quartile.

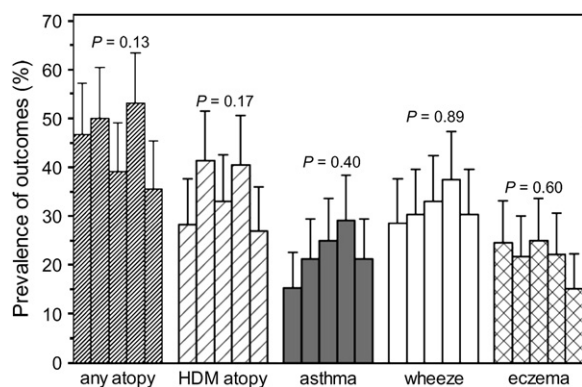


FIG E2. The prevalence of the 5 clinical outcomes at 5 years of age is shown for each quintile of time-weighted average mite allergen concentration over the period of birth to 18 months. The quintile cutoff points are 3.1, 6.84, 14.25, and 23.62 μg of Der p 1/g of dust. The bars show the 95% CIs.

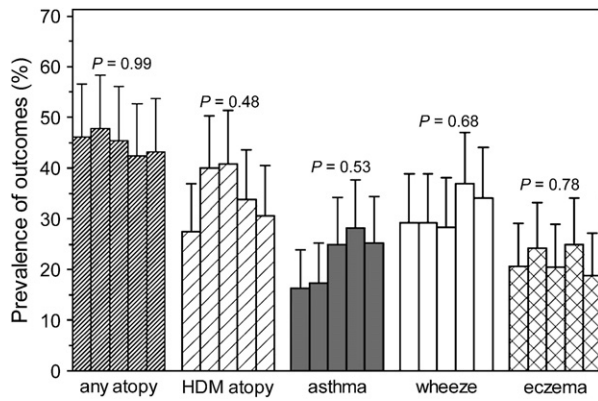


FIG E3. The prevalence of the 5 clinical outcomes at 5 years of age is shown for each quintile of time-weighted average quantity of mite allergen over the period of birth to 5 years. The quintile cutoff points are 0.33, 0.94, 1.96, and 3.95 μg of Der p 1 per sample. The bars show the 95% CIs.

TABLE E1. Distribution of social and housing factors among the children at age 5 years according to quintile of mite exposure as allergen concentration ($\mu\text{g/g}$)

	Quintile					P value#
	1	2	3	4	5	
Threshold Der p 1 ($\mu\text{g/g}$)	0-<3.5	≥ 3.5 -<7.8	≥ 7.8 -<13.5	≥ 13.5 -<23.4	>23.4	
N (458)	91	92	92	92	91	
Female sex	43 (47.3%)	43 (46.7%)	42 (45.7%)	45 (48.9%)	57 (62.6%)	.13
HDM intervention	77 (84.6%)	62 (67.4%)	44 (47.8%)	22 (23.9%)	18 (19.8%)	<.001
Diet intervention	42 (46.2%)	45 (48.9%)	52 (56.5%)	42 (45.7%)	53 (58.2%)	.28
Dog ownership*	48 (52.7%)	56 (60.9%)	52 (56.5%)	62 (67.4%)	56 (61.5%)	.32
Cat ownership†	8 (8.8%)	11 (12.0%)	10 (10.9%)	19 (20.7%)	19 (20.9%)	.05
Mattress (>3 y)‡	69 (75.8%)	69 (75.0%)	73 (79.3%)	70 (76.1%)	76 (83.5%)	.62
Any mold§	42 (46.2%)	55 (59.8%)	55 (59.8%)	63 (68.5%)	59 (64.8%)	.03
Occupant						
A	7 (7.7%)	5 (5.4%)	4 (4.3%)	2 (2.2%)	6 (6.6%)	.060
B	26 (28.6%)	21 (22.8%)	18 (19.6%)	24 (26.1%)	25 (27.5%)	
C	58 (63.7%)	66 (71.7%)	70 (76.1%)	66 (71.7%)	60 (65.9%)	
Household dampness¶						
<0.4 g/m ³	52 (59.8%)	53 (58.2%)	36 (40.4%)	54 (58.7%)	47 (52.2%)	.05
≥ 0.4 g/m ³	35 (40.2%)	38 (41.8%)	53 (59.6%)	38 (41.3%)	43 (47.8%)	

*Number of subjects ever answering yes to owning a dog during the study.

†Number of subjects ever answering yes to owning a cat during the study.

‡Number of subjects ever reported to be using mattresses older than 3 years during the study.

§Number of subjects ever having mold sighted in their bedrooms during the study.

||The number of times 4 or more persons occupied a house in only less than 3 visits (A), 3 or more to less than 10 visits (B), and 10 or more visits (C).

¶The difference between on-site indoor and outdoor absolute humidity averaged over 6 monthly assessments from age 3 to 5 years and classified as greater than or less than the mean value (NB: n = 449).

#P value for χ^2 test.