

**EXPOSURE TO TRAFFIC-RELATED PARTICLES AND ENDOTOXIN  
DURING INFANCY IS ASSOCIATED WITH WHEEZING AT AGE THREE**

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Funding: This research was supported by NIEHS R01 11170

Running Title: Traffic Exposure and Childhood Wheeze

Descriptor Number: 118

Word Count: 4,138

**AT A GLANCE COMMENTARY:**

Scientific Knowledge on the Subject: Prior studies suggest that exposure to diesel exhaust particles (DEP) results in the production of reactive oxygen species (ROS). In addition, murine models have demonstrated a synergistic production of ROS upon co-exposure to DEP and endotoxin. Previous studies of traffic-related pollution and wheezing during childhood have not examined exposure during early infancy to traffic-

related particles in combination with indoor endotoxin which may lead to increased persistent wheezing in at-risk children.

What This Study Adds to the Field: This study provides evidence that exposure to traffic-related particles is associated with childhood wheeze and that a synergistic relationship exists between co-exposure to traffic-related particles and endotoxin during infancy and persistent wheezing at age three.

This manuscript has an online data supplement, which is accessible in this issue's table of content online at [www.atsjournals.org](http://www.atsjournals.org).

## ABSTRACT

**Rationale:** Murine models demonstrate a synergistic production of reactive oxygen species upon co-exposure to diesel exhaust particles and endotoxin.

**Objectives:** It was hypothesized that co-exposure to traffic-related particles and endotoxin would have an additive effect on persistent wheezing during early childhood.

**Methods:** Persistent wheezing at age 36 months was assessed in the Cincinnati Childhood Allergy and Air Pollution Study, a high-risk birth cohort. A time weighted average exposure to traffic-related particles was determined by applying a land-use regression model to the homes, daycares, and other locations where children spent time from birth through age 36 months. Indoor levels of endotoxin were measured from dust samples collected prior to age 12 months. The relationship between dichotomized ( $< / \geq 75^{\text{th}}$  percentile) traffic-related particle and endotoxin exposure and persistent wheezing, controlling for potential covariates, was examined.

**Measurements and Main Results:** Persistent wheezing at age 36 months was significantly associated with exposure to increased levels of traffic-related particles prior to age 12 months (OR = 1.75, 95%CI 1.07 – 2.87). Co-exposure to endotoxin had a synergistic effect with traffic exposure on persistent wheeze (OR = 5.85, 95% CI 1.89-18.13) after adjustment for significant covariates.

**Conclusions:** The association between traffic-related particle exposure and persistent wheezing at age 36 months is modified by exposure to endotoxin. This finding supports prior toxicological studies demonstrating a synergistic production of ROS after co-exposure to DEP and endotoxin. The effect of early versus later exposure to traffic-

related particles, however, remains to be studied due to the high correlation between exposure throughout the first three years of life.

**Words in Abstract:** 250

**KEY WORDS:** particles, diesel, land-use regression, wheeze, allergies, asthma, children, endotoxin, air pollution

**ABBREVIATIONS:**

PM	particulate matter
DEP	diesel exhaust particle(s)
ROS	reactive oxygen species
CCAAPS	Cincinnati Childhood Allergy and Air Pollution Study
SPT	skin prick test
LUR	land-use regression
GIS	geographic information system
HCDOES	Hamilton County Department of Environmental Services
LPS	lipopolysaccharide
EC	elemental carbon
ECAT	elemental carbon attributable to traffic

## INTRODUCTION

Toxicological and epidemiologic studies have demonstrated a consistent association between exposure to air pollution and exacerbation of existing asthma (1,2). Proximity to major roads, a surrogate of traffic exposure, has been associated with decreased lung growth (3), increased asthma symptoms (4), increased airway inflammatory markers, including exhaled nitric oxide (eNO) (5), and increased oxidative stress markers (6). The association between exposure to air pollution and development of asthma, however, is less clear though recent research has found incident asthma to be associated with exposure to traffic-related air pollution (7). Air pollution in urban areas is a complex mixture of particles and gas-phase pollutants arising from a myriad of sources. The association between traffic-related air pollution and respiratory health effects in children is of interest due to the toxicological effects of the air pollution mixture arising from mobile sources, i.e. gasoline and diesel combustion engines (8). In particular, fine and ultrafine particulate matter (PM<sub>2.5</sub> and PM<sub>0.1</sub>, respectively), is derived primarily from vehicular exhaust and, in contrast to PM<sub>10</sub>, has a larger fraction of elemental and organic carbon (9). Diesel exhaust particles (DEP), a model particulate air pollutant, are a major component of PM<sub>2.5</sub>, particularly in urban areas where diesel exhaust is the largest single source of airborne PM from vehicles (10,11). As such, DEP have been widely studied with respect to adverse respiratory health effects (10) where it has been demonstrated that they are associated with increased inflammatory cells, increased cytokine levels, decreased macrophage function, and increased airway resistance (10). Though the mechanisms by which DEP exert their toxicological effects remain unknown, the heterogeneous mixture of diesel exhaust is likely associated with the generation of

reactive oxygen species (ROS) and inflammation. Laboratory studies have shown DEP to also have immune adjuvant properties, enhancing production of allergen specific IgE (12,13,14,15,16) and production of T<sub>H</sub>2 cytokines including IL-4, IL-5, IL-10, and IL-13 (12,17).

Animal and human studies have shown that inhalation of endotoxin induces airway inflammation (18,19), and the proinflammatory effect of endotoxin is enhanced by concomitant exposure to DEP (20). High exposure to endotoxin has been associated with wheezing in children (21). A murine experimental model has shown that co-exposure to DEP and endotoxin are additive in forming oxygen free radicals in lungs (18), resulting in enhanced neutrophilic lung inflammation and proinflammatory cytokines (22). Paradoxically, endotoxin may also suppress cytokine production after stimulation by diesel exhaust particles (23).

Exposure to DEP and endotoxin either alone or in combination may modify immune responses early in life and may be important in the subsequent development of allergic respiratory disorders in childhood. At birth, the infant immune system is biased toward T<sub>H</sub>2 immune responses that can initially be manifested in the first two years of life by atopic dermatitis and food allergies (24,25). It has been hypothesized that failure of the immune system to modify or suppress T<sub>H</sub>2 biased cellular cytokine responses can lead to development of atopic clinical phenotypes including allergic rhinitis and asthma. Thus, there may be periods of life, particularly in the first year, when air pollutants, indoor endotoxin, pets and aeroallergen exposures may either modify or enhance development of allergic disorders in childhood (26).

The Cincinnati Childhood Allergy and Air Pollution Study (CCAAPS) is a prospective birth cohort study of children born to atopic parents in the greater Cincinnati metropolitan area (27). A previous report found that infants exposed to high levels of traffic-related particles were significantly more likely to have parental reported wheezing without a cold prior to age one (28). This follow-up study examines estimated levels of exposure to traffic-related particles and co-exposure to indoor endotoxin. The central hypothesis of this study was that traffic-related particle exposure during early childhood increases the risk of persistent wheezing at age three, and this effect is modified by exposure to indoor endotoxin. Some of these studies have previously been reported in the form of an abstract (29).

## **METHODS**

***Study Population*** Detailed information regarding the study's objectives, recruitment methods, air monitoring, and protocols is available (27,30,31). Children enrolled in the study were identified from birth records. Infants were eligible for study recruitment if their residence at time of birth was either less than 400 m from a major road (defined as  $\geq$  1000 trucks / day) or more than 1500 m from a major road. Additionally, all enrolled infants had at least one atopic parent confirmed by symptoms and skin prick test (SPT) to 15 aeroallergens (27).

Children were clinically evaluated annually at ages one, two and three, receiving a SPT and physical exam. Parents were administered a questionnaire gathering information on parental and child health in the previous year and environmental exposures including environmental tobacco smoke and pets. History of locations where

the child spent eight or more hours per week (e.g. home, daycare, relative's home) from birth through age three were collected. Children who had completed the clinical examination at age 36 months were included in this report.

A home visit was conducted prior to the child's first birthday (average age of 8 months) and included a detailed environmental assessment and house dust sample collection. House dust samples were collected from the floor of the child's primary activity room identified by the parents as the room where the child spent most of his/her daytime. Participants were requested not to clean the floor for at least 1 day prior to dust sampling. The majority of infants spent their daytime in either the living room (56%) or the family room (36%). Dust samples were collected using a vacuum cleaner (Filter Queen Majestic, HMI Industries, Inc, Seven Hills, OH) at a flow rate of 800 L/min. A custom-made cone shaped high-efficiency particulate air filter trap (Midwest Filtration, Cincinnati, OH) was attached to the nozzle of the vacuum cleaner. All home visits and dust samples were conducted by trained teams (32). After collection, dust samples were stored desiccated at -20°C until further analysis.

Endotoxin concentrations were determined by the limulus amoebocyte lysate test (Associates of Cape Cod Inc, Falmouth, MA) in all samples according to methods described by Milton et al. (33). All glassware and materials used were endotoxin and pyrogen-free. Intraassay mean coefficient of variation (CV) ranged from 2.1% - 4.6% (SD, 1.5-4.2), and the interassay mean CV was 16.1% (SD, 9) (34). A total of 37 samples were below the lower limit of detection (6 EU/mg of dust). Endotoxin levels below the LOD were analyzed as LOD/2 (34,35).

**Health Outcomes** *Recurrent wheezing* at age 36 months was defined as parental report of two or more wheezing episodes in the previous 12 months at the 36 month clinic visit.

*Persistent wheezing* at age 36 months was designated if the child was reported by his/her parent to have had two or more wheezing episodes in the previous 12 months at both their 36 and 24 month clinic visit. A child was also considered to have persistent wheezing at age 36 months if the parent reported at the 36 month visit that his/her child had been diagnosed with asthma by their private physician. *Persistent allergic wheezing* required a positive SPT to at least one aeroallergen at age 36 months while *persistent non-allergic wheeze* was defined as a negative SPT to all aeroallergens at age 36 months.

Children were also classified as having increased risk of future asthma based on an *Asthma Predictive Index (API)* proposed by Castro-Rodriguez et al. (36) and modified by Guilbert et al. (37). Children were considered to have a positive API if they were reported to have recurrent wheezing at age 36 months and met at least 1 of 3 major criteria (parental asthma history, allergic sensitization to  $\geq 1$  aeroallergen, and eczema) or 2 of 3 minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg). Eczema was defined as either physician-diagnosed eczema on physical examination or parental report of frequent skin scratching for more than 6 months accompanied by red spots, raised bumps, or rough, dry, scaly skin.

**Traffic-Related Particle Exposure** Each participating child's average daily exposure to traffic-related particles was calculated using a land-use regression (LUR) model as described in Ryan et al. (38). Ambient air sampling was conducted at a total of 27 sampling sites in the greater Cincinnati area from December 2001 through December 2006. The average daily level of sampled elemental carbon attributable to traffic

(ECAT), a marker of traffic-related particles, was determined for each sampling site as described in the online supplement. Regression models were developed to relate this marker of traffic-related particles measured at the 27 sampling sites with land-use and traffic variables. The final LUR model had an  $R^2$  equal to 0.73 and contained independent variables related to elevation, truck intensity within 300 m, length of bus routes within 300 m, and wind direction (38).

Individual traffic exposure was calculated as a child's time weighted average daily exposure during the following periods: birth to 6 months, 7 to 12 months, 13 to 24 months, and 25 to 36 months. This exposure metric was determined by first geocoding all addresses where the child was reported to have spent more than eight hours per week within each time period and deriving a time-weighted microenvironmental exposure estimate (38). All geocoding and geographic information systems (GIS) were conducted using EZLocate (TeleAtlas, Lebanon, NH) and ArcGIS 9.0 (Environmental Systems Research Institute, Redlands, Calif).

***Statistical Analysis*** The correlation and distribution of average daily exposure to ECAT was examined for each time period (0-6, 7-12, 13-24, 25-36). Exposure to ECAT and endotoxin were highly skewed (Figure 1).and subsequently dichotomized using the 75<sup>th</sup> percentile (average daily exposure to ECAT  $\geq$  /  $<$  0.41  $\mu\text{g}/\text{m}^3$ ) to define high/low exposure. Univariate analyses were conducted to assess the association between environmental exposures (ECAT, ETS, endotoxin, visible mold in the home) and potential covariates (race, household income, gender, parental history of asthma, daycare attendance, report of an upper respiratory condition in the previous 12 months, report of a lower respiratory condition in the previous 12 months, breastfeeding) with recurrent

wheeze, persistent wheeze, and asthma predictive index at age 36 months. ETS exposure was categorically defined as parental report of at least one current smoker residing in the household at age 36 months. Parental history of asthma (yes/no) was determined by parental report of either the biological mother or biological father ever having been diagnosed by a physician with asthma. Upper respiratory conditions (yes/no) were defined as parental report of at least one of the following: ear infection, sinus infection, strep throat, tonsillitis, colored drainage in the previous 12 months reported at the 36 months visit. Lower respiratory condition (yes/no) was defined as parental report of at least one of the following: whooping cough, croup, viral infections, bronchitis/bronchiolitis, respiratory flu, or pneumonia in the previous 12 months reported at the 36 month visit. Visible mold was categorically defined as present/absent based upon the home visit prior to age 12 months if any sign of visible mold was observed in the home. Breastfeeding was dichotomized (yes/no) by parental report of breastfeeding for  $\geq 4$  weeks after birth. First-order interaction products between environmental exposures were also examined. Environmental exposures, covariates, and first-order interactions significant at the 10% level ( $p < 0.1$ ) were initially included in multivariate logistic regression models for each outcome. As race and income were significantly correlated ( $p < 0.01$ ), race was selected for consideration in the multivariate models. Multivariate models included, a priori, exposure to ECAT and endotoxin. Additional significant environmental exposures, covariates, and first-order interactions remaining in each final multivariate model were chosen using backward elimination with variables remaining in the model having a p-value  $< 0.1$ .

## RESULTS

The CCAAPS cohort enrolled 762 children at age one. Of these, 82% (n = 624) completed the age three clinical examination, questionnaire, and SPT and were included in this analysis. The average age of the child at the time of their age three study visit was 36.6 (SD 2.3) months. The prevalence of persistent wheezing, recurrent wheezing, and positive asthma predictive index was 13% (n = 82), 17 % (n = 103), and 16 % (n = 97), respectively. Of those children defined as having persistent wheeze at age 36 months (n = 82), persistent wheezing was defined by parental report of wheezing episodes for 83% (n = 68) and personal physician diagnosis for 17% (n = 14). Of those children with a positive asthma predictive index, 87% (n = 71) were reported to have persistent wheezing at age three. Of those with persistent wheezing, 42 had a concurrent positive SPT (persistent allergic wheeze) while the rest (n = 40) had non-allergic persistent wheezing. Children who completed the age three clinic visit were similar to those who did not with respect to gender, race, and parental history of asthma but were less likely to have ETS exposure and an annual household income less than \$20,000 (p < 0.05).

Indoor endotoxin values were available for 77% (n = 483) of the 624 children completing the age three examination. Of these 483, the prevalence of persistent wheezing was 14% (n = 66), i.e. 16 subjects reporting persistent wheeze were removed from analyses examining endotoxin due to lack of indoor endotoxin data. Children having indoor endotoxin measurements were significantly more likely to be Caucasian, have a household income  $\geq$  \$20,000 per year, and be breastfed and less likely to be exposed to ETS (p < 0.05). There were no significant differences in this subset with

respect to gender, parental history of asthma, daycare attendance, upper respiratory conditions, lower respiratory conditions, and prevalence of persistent wheezing.

The mean average daily exposure to ECAT at ages 6, 12, 24, and 36 months was 0.39 (SD 0.14), 0.39 (SD 0.14), 0.38 (SD 0.14), and 0.38 (SD 0.12)  $\mu\text{g}/\text{m}^3$ , respectively and was significantly correlated throughout each time period (Table 1). Therefore, further analyses were conducted using the average daily ECAT exposure during the first 12 months of life as this likely represents a critical time period of development and corresponds to the time of endotoxin exposure assessment.

The prevalence of persistent wheeze, recurrent wheeze, and positive asthma predictive index was examined by quartile of exposure (Table 2). The univariate association between persistent wheeze, recurrent wheeze, and asthma predictive index was significantly elevated only among children exposed to the highest quartile of average daily exposure to ECAT ( $\geq / < 0.41 \mu\text{g}/\text{m}^3$ ) (Table 2). Subsequent analyses utilized this dichotomization to define high/low exposure, i.e.  $\geq / < 75^{\text{th}}$  percentile of average daily ECAT exposure.

Subject characteristics and the results of univariate analyses are presented in Table 3. Significant univariate associations were observed between all wheezing outcomes and ECAT exposure (Table 3). Exposure to ETS, parental history of asthma, gender, race, and respiratory infections (upper and lower) were associated with all outcomes and considered for inclusion in the multivariate model (Table 3). Significant first-order interactions were observed for ECAT and endotoxin exposure with persistent wheezing and a positive asthma predictive index (Table 3). The effect modification of ECAT exposure on persistent wheeze, recurrent wheeze, and asthma predictive index by

high levels of endotoxin in the home is illustrated in Figure 2. Among children exposed to low levels of endotoxin in the home, the prevalence of persistent wheeze was 11% in those exposed to low levels of ECAT and 18% among children exposed to high levels of ECAT. In the presence of high endotoxin in the home, the prevalence of persistent wheeze remained the same when exposed to low levels of ECAT (11%) but significantly increased to 36% among children co-exposed to high levels of endotoxin and ECAT (Figure 2).

The results of the final multivariate logistic regression models are presented in Table 4. After backward elimination, exposure to ETS, parental history of asthma, gender, and having had a lower respiratory condition in the previous 12 months remained significant in the final model (Table 4) for persistent wheeze. The interaction between ECAT and endotoxin exposure also remained significant after adjustment. The association between persistent wheeze and exposure to ECAT was significantly increased in the presence of high endotoxin (OR = 5.85, 95% CI 1.89 – 18.13) when compared to those with low ECAT and endotoxin (Table 4). In order to examine the sensitivity of the dichotomized ECAT exposure, the continuous estimates of ECAT exposure and endotoxin (log-transformed) were entered into the model in lieu of the categorized variables and the interaction between endotoxin and ECAT remained significant ( $\beta = 0.94$ ,  $p = 0.06$ ). Significant associations were also observed between recurrent wheeze at age 36 months and ETS, parental history of asthma, gender, and lower respiratory conditions, though ECAT, endotoxin, and the interaction between ECAT and endotoxin did not remain significant in the final model. Exposure to ECAT, ETS, visible mold in the home, gender, and lower respiratory conditions were significant in the multivariate

model with asthma predictive index, though the interaction between ECAT and endotoxin was not (Table 4).

The associations between persistent allergic and non-allergic wheezing and ECAT were also examined (Table S1, online supplement). Persistent allergic wheeze was associated, though not significantly, with exposure to high ECAT (OR = 2.11, 95% CI 0.97 – 4.61), in comparison to children without persistent allergic wheeze (i.e. no current wheezing or current wheezing without current allergic sensitization) after adjustment for endotoxin, gender, parental asthma, race, lower respiratory conditions, and breastfeeding. The interaction between ECAT and endotoxin did not remain significant in the final multivariate model. In the multivariate model examining children with persistent non-allergic wheeze in comparison to children without persistent non-allergic wheeze (i.e. no current wheezing or current wheezing and a positive SPT) a significant effect modification was observed between high ECAT and endotoxin (OR = 3.76, 95% CI 1.01 – 14.03) after adjustment for gender, parental history of asthma, and lower respiratory conditions.

## **DISCUSSION**

This study found a significant association between exposure to traffic-related particles in the first year of life and persistent wheeze at age three. Furthermore, to our knowledge, this report is the first to follow-up on the animal and human experimental studies (17,19) relating combined exposure to traffic particles and endotoxin with respiratory effects on a cohort of children. A synergistic interaction between estimated traffic-related particle exposure and endotoxin in the home resulted in increased

persistent wheezing, particularly non-allergic, at age 36 months. These results support the hypothesis proposed by Yeatts et al. (39) that diverse environmental exposures (e.g, air pollutants, ETS, indoor contaminants, aeroallergens, viral infections) may exert combined effects on the airways occurring at different time points throughout life ultimately determining clinical outcomes.

Endotoxin is a component of the cell wall of gram-negative bacteria and likely stimulates the maturing immune system to develop T<sub>H</sub>1-type immune responses. Though some studies have shown that exposure to endotoxin or surrogates of endotoxin is protective against the development of allergic disease in children(40,41), others have found that endotoxin increases risk of wheezing during early childhood (42,43,44). Celedon et al. (45) recently demonstrated that exposure to endotoxin prior to age one was inversely associated with the development of atopy. In this same high risk cohort, however, exposure to high levels of endotoxin during infancy increased the risk for development of asthma and late-onset wheezing at age seven (45). Previously in this birth cohort we reported that exposure to both multiple dogs and high endotoxin during infancy was protective for wheeze at age one (34). The effect of either exposure alone, however, was not significant. In the current study, the definition of persistent wheeze at age three applies to older children with recurrent wheezing reported over a time period of at least two years. The apparent conflicting results between the aforementioned studies related to effects of endotoxin exposure may be due to how the outcomes are defined, different environmental exposures, the population being studied (high-risk atopic versus a general population), and gene-environment interactions. In addition, as endotoxin

exposure was assessed prior to age one, it is not known the current level of endotoxin exposure in the home.

Although persistent wheeze was not significantly associated with endotoxin exposure alone, co-exposure to both endotoxin in the presence of high traffic-related particle exposure resulted in increased risk (Table 4). Interestingly, though persistent allergic and non-allergic wheeze were significantly associated with traffic-related particle exposure, the effect modification of endotoxin was found with respect to persistent non-allergic wheeze. These results are consistent with previous studies showing that inhalation of endotoxin elicits airways inflammation and increases airway hyperresponsiveness to histamine (46,47). Braun-Fahrlander et al. (40) reported that endotoxin exposure in school-age children was protective for atopic wheeze but increased the risk of nonatopic wheeze.

Endotoxin also induces inflammation in the lung and generates free radicals (18). In murine models the combined exposure to DEP and endotoxin has been shown to enhance neutrophilic lung inflammation and work synergistically to promote formation of ROS (15,19). In asthmatic school-age children, McConnell et al. (48) reported that among children who owned a dog (a potential surrogate of endotoxin exposure) the odds ratio for increased bronchitis symptoms per 4.2 ppb increase in NO<sub>2</sub> exposure was 1.49 (95% CI = 1.14 - 1.95) compared to an odds ratio of 1.16 (95% CI = 0.84 - 1.60) among children with no dog in the home. In the current study, the prevalence of persistent wheeze among children exposed to high levels of ECAT but low levels of endotoxin was 18%. The prevalence, however, increased to 36% among children exposed to high levels

of traffic-related particles with concurrent exposure to high levels of endotoxin (Figure 2).

The findings were similar for both persistent wheezing and a positive API (Table 3). As childhood asthma is difficult to diagnose, the API was utilized as a marker of risk for future asthma diagnosis. The API was initially developed for children at age three and had a positive predictive value for asthma at ages 6-13 of 59%, a negative predictive value of 73%, a specificity of 85%, and a sensitivity of 42% (36). Persistent wheeze and a positive API were associated with ECAT exposure in the multivariate models. Exposure to ECAT, however, was not associated with recurrent wheeze at age three. This finding may be a result of transient wheezing, as opposed to persistent wheezing over the course of at least two years, due to factors including viral infections.

A possible limitation of this study is the estimate of elemental carbon attributable to traffic derived from the total sampled EC in ambient PM<sub>2.5</sub>. Exposure to ECAT is likely correlated with exposure to other traffic related air pollution (e.g. NO<sub>2</sub>). Furthermore, the causative agent in the mixture of air pollution exposure to which children are likely exposed is unknown and may include volatile gases, PAHs, and other constituents of air pollution. ECAT is a representative marker of traffic-related particles derived from gasoline and diesel combustion. However, elemental carbon in fine particulate matter is predominately derived from diesel combustion with approximately 75% of diesel PM<sub>2.5</sub> comprised of elemental carbon. In contrast, in the Eastern United States, approximately 4% of the total fine particulate matter composition is comprised of elemental carbon (49). Particulates produced from the combustion of diesel fuel are comprised of an elemental carbon core with more than 450 organic compounds attached

that are proposed to be primarily responsible for the proinflammatory and adjuvant effects observed with DEP exposure (10). Future source apportionment research utilizing eight temperature-resolved organic and elemental carbon fractions will help elucidate the contribution of diesel and gasoline combustion to the total ECAT.

We were also limited in the ability to distinguish the effects of exposure to ECAT during specific time periods of life due to the high degree of correlation between ECAT exposure throughout life. It is possible, given the correlation between estimated ECAT exposure throughout the first three years of life (Table 1), that the observed effects are associated with current exposure. Furthermore, the children enrolled in the CCAAPS cohort are at-risk children, i.e. born to at least one atopic parent. Therefore, the results of this analysis may not be generalizable to children born to non-atopic parents.

In conclusion, this study demonstrates that children exposed to traffic-related particles prior to age 12 months are at increased risk for the development of persistent allergic wheeze at age 36 months. The effect of high traffic-related particle exposure is accentuated in children co-exposed to high levels of endotoxin. These findings support the hypothesis of synergistic interactions between immune function development and potential damage to the infant's developing lung resulting in early onset persistent allergic wheeze. Airway inflammation and remodeling have been suggested as underlying mechanisms of asthma (50) and exposure to DEP and endotoxin, both separately and concurrently, results in the production of ROS and airway inflammation. Early persistent wheezing is a distinct wheezing phenotype and a significant risk factor for the development of asthma at later ages (51). Follow-up of this cohort will confirm

the asthma phenotype with objective measures including pulmonary function testing and the effects of early environmental exposures.

**ACKNOWLEDGEMENTS:** We thank the study participants and their families for their time and effort. We also thank Patrick Reilly, Sherry Stanforth, and Stephanie Maier who assisted with questionnaire administration and clinic visits.

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**FIGURE LEGENDS**

Fig 1. Distribution of estimated traffic-related particle exposure (ECAT)

Fig 2. Prevalence of persistent wheeze (a), recurrent wheeze (b), and positive asthma predictive index (c) by ECAT and endotoxin exposure

Table 1. Correlation of Average Daily ECAT\* Exposure by Child Age

	0-6 Months	7-12 Months	13-24 Months	25-36 Months
0-6 Months	1			
7-12 Months	0.93	1		
13-24 Months	0.86	0.94	1	
25-36 Months	0.70	0.72	0.81	1

\* ECAT: Elemental Carbon Attributable to Traffic

Table 2. Prevalence of Persistent Wheeze, Recurrent Wheeze, and Positive Asthma Predictive Index at Age 36 Months by Quartile of ECAT Exposure

Exposure		Persistent Wheeze		Recurrent Wheeze		Asthma Predictive Index <sup>2</sup>	
Quartile ECAT <sup>1</sup>	Estimated ECAT ( $\mu\text{g}/\text{m}^3$ )	% Wheeze	OR* (95% CI)	% Wheeze	OR* (95% CI)	% Positive	OR* (95% CI)
<25th percentile	$\leq 0.30$	9.0	1	13.5	1	12.2	1
$\geq 25$ th percentile - 50th percentile	0.31 - 0.34	12.2	1.4 (0.7 - 2.9)	14.1	1.1 (0.6 - 2.0)	12.8	1.1 (0.5 - 2.1)
$\geq 50$ th percentile - 75th percentile	0.35 - 0.40	12.8	1.5 (0.7 - 3.1)	16.7	1.3 (0.7 - 2.4)	14.1	1.2 (0.6 - 2.3)
$> 75$ th percentile	$\geq 0.41$	18.6	2.3 (1.2 - 4.6)	21.8	1.8 (1.0 - 3.3)	23.1	2.2 (1.2 - 4.0)

<sup>1</sup> ECAT: Elemental Carbon Attributable to Traffic

<sup>2</sup> Asthma Predictive Index: Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least 1 of 3 major criteria (parental asthma history, allergic sensitization to  $>1$  aeroallergen, and eczema) or 2 of 3 minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

\* Unadjusted odds ratios

Table 3. Unadjusted association between environmental exposures, environmental first-order interactions, and potential covariates with persistent wheezing, current wheezing, and asthma predictive index at age 36 months

Exposure / Covariate	n (%)	OR (95% CI)		
		Persistent Wheeze	Recurrent Wheeze	Asthma Predictive Index <sup>3</sup>
ECAT				
High <sup>1</sup>	158 (25%)	1.75 (1.07 - 2.87)	1.58 (1.00 - 2.49)	1.96 (1.24 - 3.10)
Low	466 (75%)	1	1	1
Endotoxin <sup>2</sup>				
High	121 (25%)	1.25 (0.71 - 2.22)	1.26 (0.74 - 2.14)	1.52 (0.90 - 2.57)
Low	362 (75%)	1	1	1
ETS*				
Present	118 (19%)	1.83 (1.08 - 3.12)	2.02 (1.25 - 3.28)	2.10 (1.28 - 3.43)
Absent	506 (81%)	1	1	1
Visible Mold in the Home				
Present	315 (50%)	1.37 (0.86 - 2.19)	1.46 (0.95 - 2.23)	1.49 (0.96 - 2.30)
Absent	309 (50%)	1	1	1
Parental History of Asthma				
Yes	213 (34%)	3.42 (2.12 - 5.52)	2.17 ( 1.42 - 3.34)	4.41 (2.80 - 6.96)
No	411 (66%)	1	1	1
Gender				
Male	337 (54%)	1.88 (1.15 - 3.07)	1.49 (0.96 - 2.30)	1.62 (1.04 - 2.54)
Female	287 (46%)	1	1	1
Race				
African-American	140 (22%)	1.74 (1.05 - 2.90)	1.06 (0.64 - 1.75)	1.60 (0.99 - 2.59)
Caucasian	484 (78%)	1	1	1
Income				
≤20,000	98 (16%)	1.62 (0.91 - 2.88)	1.27 (0.73 - 2.20)	1.74 (1.02 - 2.97)
>20,000	526 (84%)	1	1	1
Daycare Attendance				
Yes	208 (36%)	0.93 (0.56 - 1.54)	0.99 (0.62 - 1.56)	0.94 ( 0.58 - 1.51)
No	363 (64%)	1	1	1
Upper Respiratory Condition**				
Yes	305 (49%)	1.57 (0.98 - 2.51)	1.82 (1.18 - 2.80)	1.38 (0.89 - 2.13)
No	319 (51%)	1	1	1
Lower Respiratory Condition***				
Yes	176 (28%)	2.38 (1.48 - 3.84)	3.43 (2.22 - 5.31)	2.48 (1.59 - 3.88)
No	448 (72)	1	1	1
Breastfeeding****				
Yes	300 (48%)	0.65 (0.40 - 1.04)	0.77 (0.50 - 1.17)	0.58 (0.37 - 0.91)
No	324 (52%)	1	1	
ECAT * Endotoxin				
High / High	24 (5%)	4.43 (1.72 - 11.37)	1.82 (0.63 - 5.21)	3.38 (1.29 - 8.87)
High / Low	82 (17%)	1.74 (0.89 - 3.39)	1.74 (0.93 - 3.24)	2.18 (1.17 - 4.08)
Low / High	97 (20%)	0.97 (0.47 - 2.00)	1.37 (0.74 - 2.53)	1.61 (0.86 - 3.00)
Low / Low	280 (58%)	1	1	1
ECAT * ETS				
High / Present	44 (7%)	2.12 (0.92 - 4.91)	2.12 (0.98 - 4.56)	3.05 (1.46 - 6.38)
High / Absent	112 (18%)	2.08 (1.16 - 3.71)	1.84 (1.07 - 3.16)	2.08 (1.20 - 3.60)

Low / Present	75 (12%)	2.32 (1.20 - 4.48)	2.54 (1.40 - 4.59)	2.31 (1.24 - 4.32)
Low / Absent	393 (63%)	1	1	1
Endotoxin * ETS				
High / Present	24 (5%)	1.88 (0.60 - 5.91)	2.98 (1.15 - 7.76)	4.11 (1.61 - 10.46)
High / Absent	97 (20%)	1.51 (0.78 - 2.93)	1.23 (0.66 - 2.31)	1.49 (0.80 - 2.79)
Low / Present	58 (12%)	2.89 (1.45 - 5.76)	2.38 (1.23 - 4.61)	2.68 (1.37 - 5.22)
Low / Absent	304 (63%)	1	1	1

<sup>1</sup> High Elemental Carbon Attributable to Traffic (ECAT):  $\geq 75^{\text{th}}$  percentile (0.41  $\mu\text{g}/\text{m}^3$ ), Low ECAT:  $< 75^{\text{th}}$  percentile

<sup>2</sup> Endotoxin values available for 483 homes

<sup>3</sup> Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least 1 of 3 major criteria (parental asthma history, allergic sensitization to  $>1$  aeroallergen, and eczema) or 2 of 3 minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

\*ETS present is defined as parental report of at least one current smoker residing in the household

\*\* Upper respiratory conditions defined as ear infection, sinus infection, tonsillitis, strep throat

\*\*\* Lower respiratory condition defined as whooping cough, croup, viral infection, bronchitis/bronchiolitis, pneumonia, respiratory flu

\*\*\*\* Parental report of breastfeeding  $\geq 4$  weeks

Table 4. Multivariate models for persistent wheeze, recurrent wheeze, and asthma predictive index

Exposure / Covariate	OR (95% CI)		
	Persistent Wheeze	Recurrent Wheeze	Asthma Predictive Index <sup>2</sup>
ECAT <sup>1</sup>			
High	--- <sup>£</sup>	1.59 (0.88 – 2.87)	2.04 (1.15 - 3.63)
Low		1	1
Endotoxin			
High	--- <sup>£</sup>	1.27 (0.72 – 2.26)	1.67 (0.95 - 2.92)
Low		1	1
ETS*			
Present	2.14 (1.07 – 4.27)	2.47 (1.35 – 4.52)	2.31 (1.26 - 4.21)
Absent	1	1	1
Visible Mold in the Home			
Present	1.68 (0.92 – 3.08)	--- <sup>§</sup>	1.77 (1.04 - 3.03)
Absent			1
Parental History of Asthma			
Yes	4.03 (2.25 – 7.23)	2.12 (1.25 – 3.57)	--- <sup>¥</sup>
No	1	1	
Gender			
Male	2.78 (1.51 – 5.11)	2.05 (1.20 – 3.50)	1.83 (1.08 - 3.10)
Female	1		1
Lower Respiratory Condition			
Yes	3.31 (1.82 – 5.99)	3.91 (2.33 – 6.58)	2.61 (1.54 - 4.44)
No	1	1	1
Breastfeeding			
Yes	0.60 (0.33 – 1.09)	--- <sup>§</sup>	0.62 (0.36 - 1.06)
No	1		1
ECAT * Endotoxin			
High / High	5.85 (1.89 – 18.13)	--- <sup>§</sup>	--- <sup>§</sup>
High / Low	1.43 (0.68 – 3.02)		
Low / High	0.88 (0.41 – 1.91)		
Low / Low	1		

<sup>1</sup> High Elemental Carbon Attributable to Traffic (ECAT):  $\geq 75^{\text{th}}$  percentile (0.41  $\mu\text{g}/\text{m}^3$ ), Low ECAT:  $< 75^{\text{th}}$  percentile

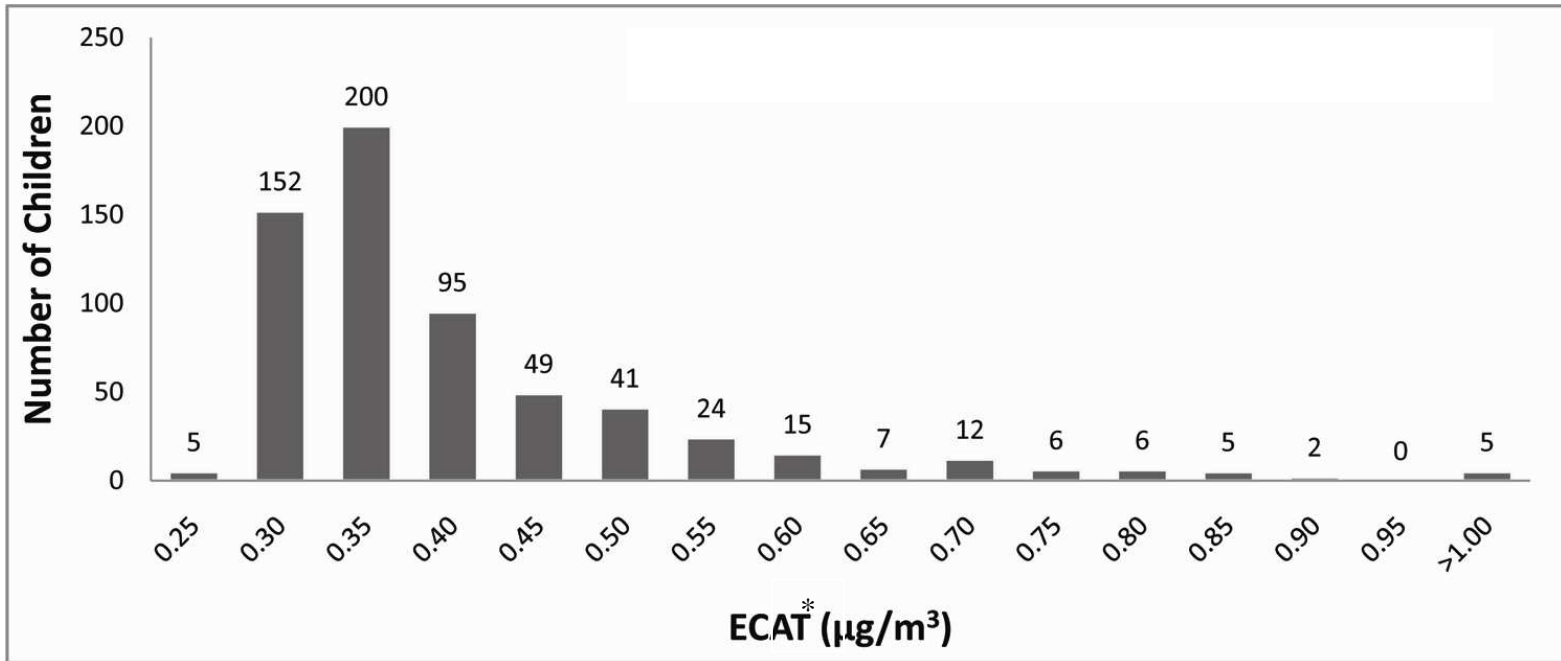
<sup>2</sup> Defined as positive if the child was reported to have recurrent wheezing at age 36 months and met at least 1 of 3 major criteria (parental asthma history, allergic sensitization to  $>1$  aeroallergen, and eczema) or 2 of 3 minor criteria (wheezing without a cold, physician-diagnosed allergic rhinitis, and allergic sensitization to milk or egg).

£ Odds ratios presented for interaction

§ Not included in final multivariate model

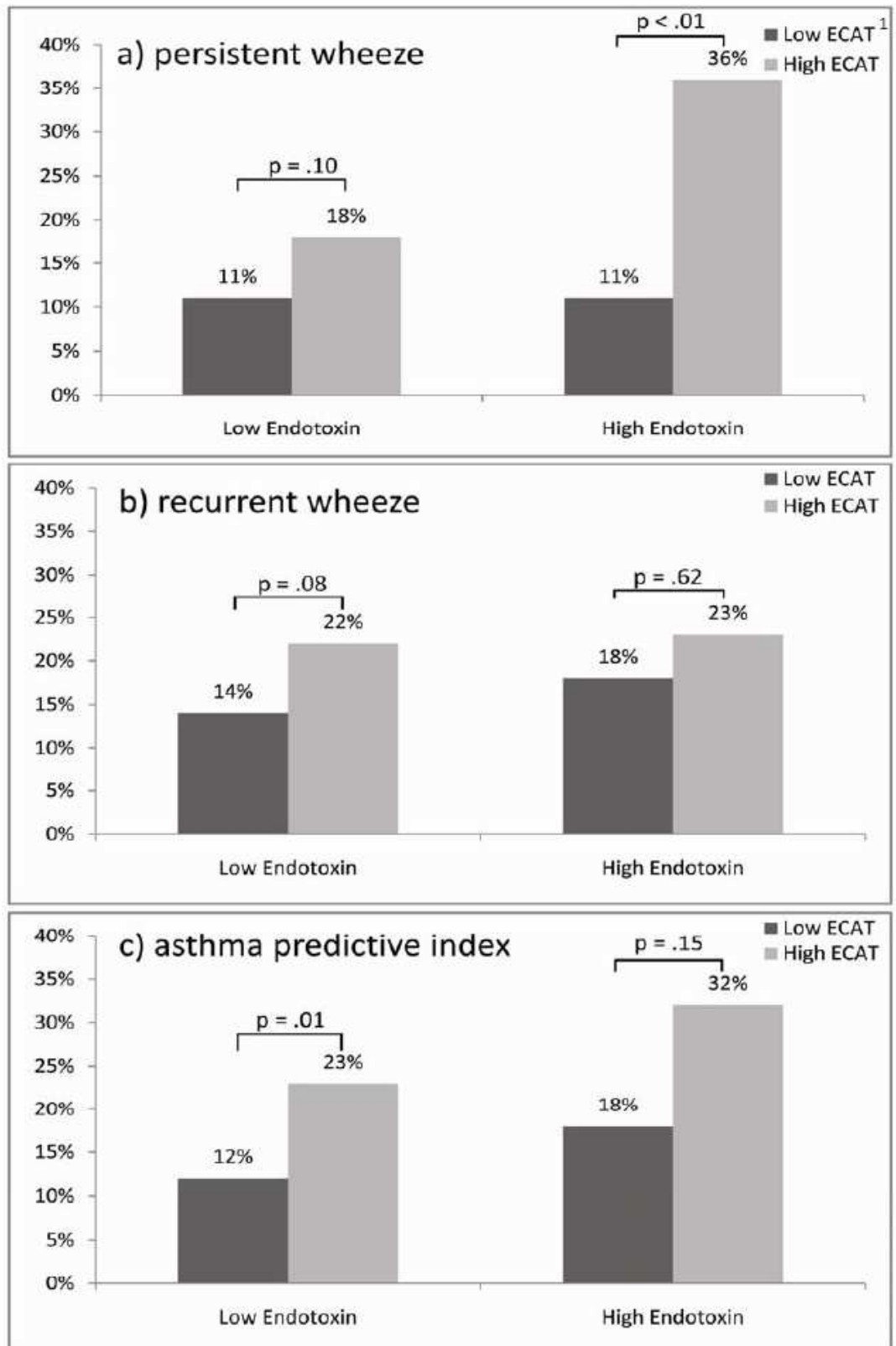
¥ Not included due to incorporation into outcome definition

Figure 1.



\* ECAT: Elemental Carbon Attributable to Traffic

Figure 2.



**EXPOSURE TO TRAFFIC-RELATED PARTICLES AND ENDOTOXIN  
DURING INFANCY IS ASSOCIATED WITH WHEEZING AT AGE THREE**

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ONLINE DATA SUPPLEMENT

**Online Supplement:**

**METHODS**

***Traffic-Related Particle Exposure***

PM<sub>2.5</sub> samples were at 27 sampling sites selected by city area, proximity of pollution sources including highway and state routes, location of the study population, location relative to the major interstate highway corridors, and predominant wind direction (S1). During each measurement cycle, sampling was conducted simultaneously at fur to five sites over different seasons. PM<sub>2.5</sub> samples were collected on 37-mm Teflon membrane filters (nominal pore size, 1 µm; Pall Gellman, Ann Arbor, MI) and 37-mm quartz filters (Whatman, Kent, ME) with Harvard-type Impactors (Air Diagnostics and Engineering, Harrison, ME). Standardized operating procedures for the filter media preparation, gravimetric operations, and sampling were followed (S1, S2). The Teflon filters were analyzed by X-ray fluorescence (XRF) to determine elemental concentrations (Chester Labnet, Tigard, OR) for a total of 38 elements. The quartz filters were sectioned with one half analyzed by the Thermal-Optical Transmittance (TOT) technique using the NIOSH-5040 method (S3) to determine elemental carbon (EC) and organic carbon (OC)

concentrations. The other half was frozen and preserved. Of the total samples, 24% were collocated on Teflon and quartz filters. These were analyzed by the TOT technique to determine EC concentrations. Given the importance of EC to this study, an alternative method, optical reflectance, was used to estimate the EC concentrations from the Teflon filters. The reflectance of ambient aerosols deposited on the Teflon filter was measured by a reflectometer (EEL model 43; Diffusion System Ltd., London, UK). The absorption coefficient ( $A_{bs}$ ) of the aerosol-loaded Teflon filters was calculated according to International Standard ISO 9835 (1993). The absorption coefficient was correlated with EC from NIOSH 5040 thermo-optical transmittance analysis for days when quartz filter sampling was conducted. The EC concentrations in the remaining samples were determined by the reflectance method. Field and laboratory blanks were routinely analyzed, with details reported elsewhere (S4). The multivariate receptor model, UNMIX (S5), was first used to determine source signatures in the airshed. Four different source categories were identified, with one being attributed to traffic sources. The estimated source profiles were compared to those obtained from measurements conducted for cluster sources of trucks and buses (S2). The marker of traffic-related particles, the elemental carbon attributable to traffic (ECAT) was determined for each sampling site by both multivariate receptor modeling, UNMIX and the Chemical Mass Balance (CMB) (S6) model.

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Table S1. Multivariate models for persistent allergic and non-allergic wheeze

Exposure / Covariate (% Frequency)	OR (95% CI)	
	Persistent Allergic Wheeze	Persistent Non-Allergic Wheeze
<b>ECAT*</b>		
High (25%)	2.11 (0.97 - 4.61)	--- <sup>£</sup>
Low (75%)	1	
<b>Endotoxin</b>		
High (25%)	1.12 (0.49 - 2.58)	--- <sup>£</sup>
Low (75%)		
<b>ETS*</b>		
Present (19%)	--- <sup>§</sup>	--- <sup>§</sup>
Absent (81%)		
<b>Visible Mold in the Home</b>		
Present (50%)	--- <sup>§</sup>	--- <sup>§</sup>
Absent (51%)		
<b>Parental History of Asthma</b>		
Yes (34%)	2.32 (1.13 - 4.79)	5.00 (2.22 - 11.28)
No (66%)	1	1
<b>Gender</b>		
Male (54%)	2.10 (0.99 - 4.46)	2.97 (1.25 - 7.05)
Female (46%)	1	1
<b>Lower Respiratory Condition***</b>		
Yes (28%)	3.19 (1.52 - 6.70)	2.77 (1.25 - 6.15)
No (72%)	1	1
<b>Breastfeeding****</b>		
Yes (48%)	0.49 (0.23- 1.06)	--- <sup>§</sup>
No (52%)	1	
<b>Race</b>		
Caucasian	0.41 (0.18 - 0.94)	--- <sup>§</sup>
Non-Caucasian	1	
<b>ECAT * Endotoxin</b>		
High / High (5%)	--- <sup>§</sup>	3.76 (1.01 - 14.03)
High / Low (17%)		0.76 (0.24 - 2.43)
Low / High (20%)		1.00 (0.37 - 2.74)
Low / Low (58%)		1

\* ECAT: Elemental Carbon Attributable to Traffic

£ Odds ratios presented for interaction

§ Not included in final multivariate model

¥ Not included due to incorporation into outcome definition