

Variations in Peak Expiratory Flow Measurements Associated to Air Pollution and Allergic Sensitization in Children in Sao Paulo, Brazil

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Background In the last 20 years, there has been an increase in the incidence of allergic respiratory diseases worldwide and exposure to air pollution has been discussed as one of the factors associated with this increase. The objective of this study was to investigate the effects of air pollution on peak expiratory flow (PEF) and FEV1 in children with and without allergic sensitization.

Methods Ninety-six children were followed from April to July, 2004 with spirometry measurements. They were tested for allergic sensitization (IgE, skin prick test, eosinophilia) and asked about allergic symptoms. Air pollution, temperature, and relative humidity data were available.

Results Decrements in PEF were observed with previous 24-hr average exposure to air pollution, as well as with 3–10-day average exposure and were associated mainly with PM₁₀, NO₂, and O₃ in all three categories of allergic sensitization. Even though allergic sensitized children tended to present larger decrements in the PEF measurements they were not statistically different from the non-allergic sensitized. Decrements in FEV1 were observed mainly with previous 24-hr average exposure and 3-day moving average.

Conclusions Decrements in PEF associated with air pollution were observed in children independent from their allergic sensitization status. Their daily exposure to air pollution can be responsible for a chronic inflammatory process that might impair their lung growth and later their lung function in adulthood. Am. J. Ind. Med. © 2012 Wiley Periodicals, Inc.

KEY WORDS: air pollution/adverse effects; peak expiratory flow; children; allergic sensitization; linear models; PM₁₀; O₃; NO₂

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INTRODUCTION

The incidence of allergic respiratory diseases has been increasing worldwide in the last few decades, in urban areas as well as rural areas [Wyler et al., 2000; Nicolai, 2002; Etzel, 2003; D'Amato et al., 2010]. Allergic respiratory diseases are determined by a combination of genetic and environmental factors [Johnson et al., 2002]. As it is not probable that the gene pool has changed significantly during these last decades [Bracken et al., 2002], environmental factors as exposure to aeroallergens, tobacco smoke, and air pollution may be associated with the observed increase [Kramer et al., 2000; Schwartz et al., 2000; Kay, 2001; Salvi, 2001; Wamboldt et al., 2002; Nicolai et al., 2003; Jerrett et al., 2008].

Exposure to indoor aeroallergens (dust mite, cat allergen, mold) and tobacco smoke has been associated with allergic sensitization, the development of allergic respiratory diseases and worsening of asthma symptoms [Mannino et al., 2002; Morkjaroenpong et al., 2002; Viegi et al., 2004]. Experimental and epidemiological studies have also shown that there is a significant association between urban levels of air pollution and the increase in allergic respiratory diseases [Davies et al., 1998; Trasande and Thurston, 2005]. One explanation is that air pollution is associated with airway inflammation [Holguin et al., 2007] and can enhance the airway allergic response to aeroallergens [Salvi, 2001].

Panel studies of lung function have been extensively used to investigate the health effects of air pollution. It has been described that lung function in children decreases with exposure to PM₁₀, SO₂, NO₂, and O₃, considering the 24-hr average concentration before the Peak Expiratory Flow (PEF) measurements as well as moving averages from 2 to 10 previous days [Pope et al., 1991; Pope and Dockery, 1992; Gielen et al., 1997; van der Zee et al., 1999].

The city of Sao Paulo, Brazil, has 10 million inhabitants, and the main source of air pollution is traffic related, with a light motor vehicle fleet of 7 million. Since 1979, with the implementation of the Alcohol National Program (PROALCOOL) in Brazil, hydrated ethanol has been used as a fuel for vehicles. Later, it was also added to pure gasoline forming a mixture named "gasohol". This mixture consists of 20–25% anhydrous ethyl alcohol and 78% pure gasoline [CETESB, 2011]. In 2004, vehicles that used hydrated ethanol comprised 14.5% of the vehicle fleet in Sao Paulo Metropolitan Region; the ones that used gasohol represented 69.5%. Flex-fuel vehicles, the ones that can use either gasohol or hydrated ethanol, represented only 1%. Motorcycles represented 9.3% and diesel run vehicles, 5.8% [CETESB, 2011].

Air pollution is monitored by the State of Sao Paulo Environmental Agency (CETESB) in 20 air monitoring

stations located throughout the metropolitan area of São Paulo. The pollutants that are continuously monitored are: PM₁₀, SO₂, NO₂, NO, CO, and O₃. One of the monitoring stations is located in the backyard of a public school. In this study we chose to investigate the effects of air pollution on PEF measurements of students, attending that school, who were allergic-sensitized and who were not allergic-sensitized.

MATERIAL AND METHODS

A panel study was conducted from April 7th to July 7th, 2004 in Sao Paulo, Brazil. The school chosen for the study has an air pollution monitoring station in its backyard. All 112 students from this school aged 9–11-years-old were invited to participate. The study protocol was approved both by the Institutional Review Boards at the Faculdade de Medicina da Universidade de São Paulo in Sao Paulo, Brazil and at the Mount Sinai School of Medicine in New York, USA. Once their parents signed the informed consent, children were enrolled in the study.

This study protocol included measurements of height and weight at the beginning and at the end of the study period, daily records of school absences, of respiratory symptoms, and full spirometry. During the study, children answered the ISAAC questionnaire [1998], had their blood collected for dosage of serum IgE and total blood count, skin prick test, and nasal smear. Daily air pollution and weather data were obtained during the study.

Spirometry

Students who received authorization from their parents were trained in the use of pneumotachs (SpiroCard – Medgraphics Cooperation, St Paul, MN, USA) to perform lung function measurements for a period of 15 school days. These values were not used in the analyses as they were considered learning period. Spirometry was performed in the morning of every school day in the presence of the researchers. Measurements were performed at the same time of the day for each child. In this report, the results of the PEF and of the forced expiratory volume in one second (FEV1) were used.

Skin Prick Test

Allergy skin prick tests were performed using a positive control (histamine), a negative control (saline solution) and common allergen extracts (mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and *Blomia tropicalis*), dog (*Canis familiaris*), cat (*Felis domesticus*), cockroach (*Periplaneta americana*) and a pool of fungus (*Aspergillus fumigatus*, *Alternaria alternata*, *Cladosporium herbarium*, *Chaetomium globosum*,

Mucor mucedo, *Pullularia pullulans*, *Penicillium notatum*) from the laboratory IPI ASAC BRASIL). No child had received antihistamines orally for 3 days before the test. Briefly, after cleansing the forearm skin, a drop of each allergen was placed at 3 cm intervals, the skin was then lightly punctured with a 1mm polymethacrylate lancet for about 10 seconds in a way that no bleeding occurred. Reading took place after 15–20 minutes. The mean diameter of a wheal formed by the allergen was calculated by its longest diameter and the largest diameter perpendicular to it. Any reaction greater than 3 mm was considered positive. If there was a reaction with the negative control, its mean diameter was subtracted from the other reaction wheal [Dreborg, 1993].

Blood Samples

Blood was collected from a peripheral vein in Vacutainer™ tubes and was taken to the Hospital das Clínicas Laboratory for the dosage of total IgE and total blood count. This last exam was performed on an automated hematology analyzer that quantified the number of erythrocytes, leukocytes, and thrombocytes. Leukocytes count included mononuclear cells (lymphocytes and monocytes) and granulocytic cells (neutrophils, eosinophils, and basophils). The leukocytes results were given as percentage and as an absolute number per cubic millimeter. In this study, blood eosinophilia was defined as an increase in eosinophils to more than 700 cells per cubic millimeter of blood.

Nasal Smear

Nasal smear samples were taken from both nasal cavities by wiping the surface of the inferior turbinate with cotton tipped applicator. The samples were immediately smeared over a standard glass slide, and transported to the Laboratory to be fixed, stained, and examined. Cells were identified and counted (neutrophils, eosinophils, basophils, goblet cells, mastocytes). The result was expressed as percentages. For eosinophils the proportion was expressed as a percentage of the total non-squamous cell count. For this study, eosinophilia was considered when the percentage of eosinophils was higher than 30%.

Allergic Sensitization Categories

Children were categorized in three groups: 0 (children who presented no symptoms of allergies and normal values of total IgE, eosinophils in total blood count and lower than 30% eosinophils in nasal smear, no positive reaction to skin prick test), 1 (any symptoms of allergies or one test positive – IgE, prick test, eosinophilia in blood or nasal smear), 2 (symptoms of allergies and all tests

positive – IgE, prick test, eosinophilia in blood and nasal smear). This way, we created 2 main distinct groups: Non allergic-sensitized (0) and allergic-sensitized (2), and one intermediate group (1) where the allergic sensitization was not as clear as in group 2, but was not negative as in group 0.

Air Pollution and Weather

The State of Sao Paulo Environmental Agency (CETESB) provided daily hourly concentrations of particulate matter with aerometric diameter less than 10 μm (PM₁₀), nitrogen dioxide (NO₂), nitrogen oxide (NO), carbon monoxide (CO), sulfur dioxide (SO₂), ozone (O₃), temperature, and relative humidity from the air pollution monitoring station located at the school, from April 1st to July 7th, 2004.

Statistical Analysis

The association between air pollutants and the selected lung function parameters was evaluated by regression analysis based on the generalized estimating equations (GEE) proposed by Liang and Zeger [1993], [Zeger et al., 1988].

Given that data collected sequentially in time are most likely to exhibit serial correlation, the assumption made was that peak flow measurements within individual children were not independent. Therefore, we used a random effects model. All statistical analyses were performed with STATA 8.0 software (2003). A *P*-value of <0.05 was considered significant.

Within each child, daily measures of PEF and FEV1 were centred by the child-specific mean for the appropriate period of measurement (month), giving a deviation-from-the-mean measure of PEF and FEV1. This allowed for varying baseline lung function between children. We regressed child-specific deviation-from-mean PEF and FEV1 on date (allowing for the possibility of linear growth during the study period), dummies for school absences lagged by 1 day (1 = present, 2 = without medical reason, 3 = ill (non-respiratory), 4 = ill (respiratory) with 0 as reference (weekends and holidays)), minimum temperature (°C) measure, minimum relative humidity (%), and pollution measure, allowing for first-order serial correlation [Cochrane and Orcutt, 1949] and computing robust standard errors. This regression procedure yielded a coefficient for the pollution measure pooled across children. The coefficient was estimated by way of generalised least squares, as estimation of the parameter of a first-order autoregressive process was performed, the estimate of which was subsequently required for the estimation of the variance-covariance matrix of the errors.

RESULTS

Children Characteristics and Exams

The collection of data from the children comprised 60 school days (from April 7th to July 7th, 2004). Of the 112 eligible children, 96 (85.7%) were authorized by their parents to participate in the study. Sixty-three (65.6%) of them were males. Fifty children were 9 years old (52.1%), 39 were 10 (40.6%), and only seven were 11 years old (7.3%). Mean height and weight increased by 1.2 cm and 1.8 kg, respectively, during the study (Table I). Twenty-one (22.1%) [95 tested] children presented eosinophils above 30% of the total non-squamous cell count in the nasal smear exam. Of the 93 children who had their blood collected, eleven (11.8%) presented eosinophils in total blood count above normal limits. Forty-seven children presented high levels of IgE, 17 (18.3%) above 1,000 UI/ml. Fifty-six children (58.9%) [94 tested] had at least one positive reaction to the skin prick test. The most common reactions were to house mites (56.8%) and to cat (18.9%).

Seventy-four children (77.1%) were living in the same house for more than 2 years. There was no stationary source of air pollution near any of the children's houses. The presence of mold on the wall of the bedroom was referred by 22 children (22.9%). Forty-nine (51.04%) children owned either dog or cat. Also, 49 children were exposed to second-hand tobacco smoke at home.

When asked about respiratory and allergic symptoms without the presence of any respiratory disease, 66 children cited sneeze (68.8%), 62 cough (64.6%), and 55 pruritus in the eye (57.3%). Sixteen children (16.7%) said they had had wheeze in the last year (Table I).

Spirometry

PEF average was 276.4 L/min (SD 40.7) for 9-year-old children, 284.4 L/min (SD 49.3) for 10 years old, and 316.0 L/min (SD 77.9) for 11 years old. There was no statistical difference between sexes (males = 283.3 L/min (SD 48.0), females 281.0 L/min (SD 49.0). Mean PEF increased during the study from 270.4 to 284.7 L/min.

Forced expiratory volume in 1 s average was 1.82 L (SD 0.32) for 9-year-old children, 1.90 L (SD 0.40) for 10 years old, and 2.14 L (SD 0.50) for 11 years old. Also, there was no statistical difference between sexes (males = 1.89 L (SD 0.38), females 1.84 L (SD 0.37). Mean FEV decreased during the study from 1.92 L in April to 1.83 L in July.

Allergic Sensitization Categories

Thirty-one children were classified as Category 0 (%), thirty-six as Category 1 (%), and twenty-eight as

TABLE I. Baseline Characteristics of the 96 Schoolchildren

Characteristic	Data	Min	Max
Sex (M/F) ^a	63 (M) 33 (F)		
Age (years) (m ± SD) ^b	9.6 ± 0.63	9	11
Height 1 ^c (cm) (m ± SD)	140.6 ± 8.31	125.5	169.0
Height 2 ^d (cm) (m ± SD)	141.8 ± 8.38	126.5	170.5
Weight 1 ^c (kg) (m ± SD)	36.1 ± 9.10	21.5	63.3
Weight 2 ^d (kg) (m ± SD)	37.9 ± 9.55	22.0	66.0
BMI 1 ^c (m ± SD)	18.0 ± 3.07	13.2	30.7
BMI 2 ^d (m ± SD)	18.6 ± 3.24	13.3	31.8
IgE (IU/mL) (m ± SD)	684.9 ± 1282.47	6	8950
Eosinophils (thousand/mm ³) (blood) (m ± SD)	0.36 ± 0.45	0	2.6
Eosinophils (nasal smear) (%) (m ± SD)	17.7 ± 27.57	0	98
Prick test (N/P) ^e	38/56		
PEF (L/min) (m ± SD)			
April	270.4 ± 45.46	181.8	393.9
May	283.6 ± 49.36	171.5	441.5
June	291.9 ± 53.11	161.4	478.3
July	284.7 ± 55.95	163.1	452.7
FEV1 (L)			
April	1.92 ± 0.36	1.01	3.25
May	1.86 ± 0.38	0.70	3.29
June	1.84 ± 0.39	0.45	3.27
July	1.83 ± 0.37	0.74	2.85
Allergen exposure:			
Tobacco smoke (Y/N) ^f	47/49		
Dog (Y/N)	45/51		
Cat (Y/N)	9/87		
Mold (Y/N)	22/74		
Allergy Symptoms:			
Runny or Blocked Nose (Y/N)	27/69		
Cough (Y/N)	62/34		
Sneeze (Y/N)	66/30		
Pruritus in the eye (Y/N)	55/41		
Eczema (Y/N)	4/92		
Rhinitis (Y/N)	6/90		
Wheeze (Y/N)	16/80		
Doctor diagnosed asthma (Y/N)	6/90		

^aM, male; F, female.

^bm ± SD, mean ± standard deviation.

^c1, first day of the study.

^d2, Last day of the study.

^eN/P, Negative/Positive.

^fY/N, Yes/No.

Category 2 (%). In Category 0, total IgE levels averaged 57.4 UI/ml, mean eosinophils in blood were 143.3/mm³, and mean percentage of eosinophils in nasal smear was 1.5%. In Category 1, following the same order, the results were 481.5 UI/ml, 322.9/mm³, and 10.9%. In Category 2, they were 1,611.5 UI/ml, 559.3/mm³, and 41.2%.

Two children did not collect blood, so their total IgE and complete blood count were unavailable. But they could be categorized according to the nasal smear eosinophil percentage, skin prick test, and questionnaire.

It is important to notice that there was no eosinophilia, either in the blood or nasal smear, which occurred without the presence of high IgE levels or at least one positive skin prick test.

Air Pollution and Weather Variables

Data from pollutants and weather variables were obtained from April 1st to July 7th, 2004, 6 days before the beginning of the spirometry tests as we needed prior days pollutants concentrations to calculate moving averages. Ozone, PM₁₀, and NO₂ exceeded air quality primary standards during the study. Temperature was mild, with an average of 18.5°C (lowest 9.9°C). Average relative humidity was 78.2%. A summary of air pollution concentrations and weather during the period studied is shown in Table II. All pollutants measurements were significantly correlated (Table III).

Air Pollution, PEF, and FEV1–Regression Analyses

Considering the previous 2- and 24-hr exposure, all pollutants were associated with declines in PEF and in FEV1 measurements in the study period (Tables IV and V). School absence related to respiratory symptoms and exposure to tobacco smoke could be confounders to the effects of air pollution, in the regression analysis we controlled for school absence and tobacco smoke exposure, both were not associated with PEF or FEV1 variations (results not shown). Other time lags as 3-day, 5-day, 7-day, and 10-day moving average of PM₁₀, NO₂, and O₃ were also negatively associated with PEF, but not with the

TABLE III. Pearson Correlation Between the 24 hr Concentration of the Pollutants, Temperature and Relative Humidity in the Period of Study

	CO	PM ₁₀	NO	NO ₂	SO ₂	O ₃	TEMP
PM ₁₀	0.73*						
NO	0.69*	0.62*					
NO ₂	0.51*	0.59*	0.51*				
SO ₂	0.62*	0.75*	0.87*	0.60*			
O ₃	0.01	0.31*	−0.07	0.40*	0.07		
Temp.	0.02	0.05	−0.14	0.02	−0.14	0.22**	
Rel.humid.	−0.16	−0.36*	0.10	−0.22**	−0.02	−0.57*	−0.01

* $P < 0.01$.

** $P < 0.05$.

other pollutants—CO, SO₂, and NO—(results not shown). Using distributed lag models up to 30 days we verified that exposure to NO₂ was significantly associated with declines in PEF until a lag of 21 days. The effects of O₃ and PM₁₀ began to diminish after 18 days and 10 days, respectively (Figs. 1–3). Percent variations in PEF and FEV1 measurements for the 25–75% interquartile range in PM₁₀, NO₂, NO, SO₂, CO, and O₃ were calculated as they represent a more realistic scenario for that monitoring station and thus the air the children were exposed to.

We used two-pollutant models to investigate whether the larger effect of ozone could be explained by its association with other pollutants. For short exposure (previous 2-hr mean), two-pollutant models indicated that PM₁₀ had the most robust effect on lung function, remaining statistically significant with SO₂, NO₂, NO, and CO ($P < 0.001$). Ozone was not used in two-pollutant model for short exposure as its mean values were below 6 µg/m³ and the GEE regression showed non-significant coefficients. For previous 24-hr exposure, PM₁₀ did not remain significant with any pollutant in two-pollutant models. NO₂, O₃, and CO maintained statistical significance at $P < 0.05$, with smaller effects on PEF than in one pollutant model. When two-pollutant models were used for 3–10-day moving average, NO₂ and O₃ remained statistically significant with robust effects on PEF. PM₁₀ was not significant with NO₂ or O₃ (these results are available as supporting material online). We also regressed O₃ with absolute high levels of O₃ in earlier periods to test for interaction; however, no interaction was seen with O₃ and O₃ absolute levels in earlier periods (results not shown).

Declines in FEV1 (Table V) were similar to PEF but smaller in magnitude; however, the percentage variations associated with the pollutants 25–75% IQR were higher for previous 24-hr exposure and 3-day moving average than for PEF (Table V). Considering 5-day and 7-day

TABLE II. Twenty-Four Hour Average (Standard Deviation) and Percentiles of the Pollutants, Temperature, and Relative Humidity in the Period of Study

Variable	Days	Average(SD)	10%	25%	50%	75%	90%
CO(ppm)	99	3.0(0.95)	2.1	2.4	2.9	3.3	4.35
PM ₁₀ (µg/m ³)	95	48.8(17.21)	29.4	35.9	45.6	58.8	73.9
SO ₂ (µg/m ³)	99	23.0(8.56)	12.9	14.9	23.1	29.4	34.4
O ₃ (µg/m ³)	99	63.2(38.21)	18.6	34.9	60.6	83.7	108.3
NO ₂ (µg/m ³)	97	131.4(47.90)	77.5	90.9	126.7	158.9	192.0
NO(µg/m ³)	97	183.4(86.68)	87.0	118.6	161.4	247.6	308.9
Temp.(°C)	99	18.5(3.06)	14.2	16.2	18.7	20.9	22.4
Rel.humid.(%)	99	78.4(7.67)	66.0	73.6	79.4	84.4	86.9

TABLE IV. Estimates of the Association Between Air Pollutant Exposures and PEF in 96 Schoolchildren

Pollutant and time lag	Coef. ^a (95% CI)	Coef ^b (95% CI)	P-value
Previous 2-hr average			
CO	−1.50 (−2.35 to −0.64)	−0.48 (−0.75 to −0.20)	0.001
PM ₁₀	−0.11 (−0.14 to −0.07)	−0.89 (−1.13 to −0.57)	<0.0001
SO ₂	−0.08 (−0.15 to −0.02)	−0.41 (−0.77 to −0.10)	0.009
NO	−0.01 (−0.01 to 0.00)	−0.17 (−0.17 to 0.00)	0.005
NO ₂	−0.09 (−0.13 to −0.05)	−2.16 (−3.12 to −1.20)	<0.0001
Previous 24-hr average			
CO	−1.89 (−3.24 to −0.53)	−0.60 (−1.03 to −0.17)	0.006
PM ₁₀	−0.05 (−0.11 to 0.00)	−0.40 (−0.89 to 0.00)	0.065
SO ₂	−0.03 (−0.12 to 0.05)	−0.15 (−0.61 to 0.26)	0.443
NO	−0.01 (−0.02 to 0.00)	−0.17 (−0.34 to 0.00)	0.044
NO ₂	−0.07 (−0.12 to −0.02)	−1.68 (−2.88 to −0.48)	0.003
O ₃	−0.10 (−0.18 to −0.01)	−4.56 (−8.21 to −0.46)	0.024
3-day moving average			
PM ₁₀	−0.11 (−0.19 to −0.03)	−0.89 (−1.54 to −0.24)	0.008
NO ₂	−0.13 (−0.19 to −0.07)	−3.12 (−4.57 to −1.68)	<0.0001
O ₃	−0.17 (−0.23 to −0.11)	−2.93 (−3.97 to −1.90)	<0.0001
5-day moving average			
PM ₁₀	−0.32 (−0.46 to −0.17)	−2.59 (−3.72 to −1.38)	<0.0001
NO ₂	−0.26 (−0.36 to −0.16)	−6.25 (−8.65 to −3.84)	<0.0001
O ₃	−0.21 (−0.29 to −0.14)	−3.62 (−5.00 to −2.41)	<0.0001
7-day moving average			
PM ₁₀	−0.26 (−0.43 to −0.10)	−2.10 (−3.48 to −0.81)	0.002
NO ₂	−0.29 (−0.39 to −0.18)	−6.97 (−9.37 to −4.33)	<0.0001
O ₃	−0.29 (−0.38 to −0.21)	−5.00 (−6.55 to −3.62)	<0.0001
10-day moving average			
PM ₁₀	−0.17 (−0.37 to 0.02)	−1.38 (−2.99 to 0.16)	0.081
NO ₂	−0.28 (−0.40 to −0.16)	−6.73 (−9.61 to −3.84)	<0.0001
O ₃	−0.29 (−0.41 to −0.16)	−5.00 (−7.07 to −2.76)	<0.0001

^aCoefficients represent the expected change in PEF associated with one $\mu\text{g}/\text{m}^3$ or one ppm increase in each air pollutant level, adjusted for temperature, relative humidity, school absences.

^bCoefficients represent the expected percent change in PEF associated with 25–75% IQR of each air pollutant, adjusted for temperature, relative humidity, school absences.

moving averages, the association with PM₁₀ and NO₂ was significant but not with ozone. After this period, all associations were not statistically significant (results not shown).

Air Pollution, PEF Variations, and Allergic Sensitization

As the coefficients generated in the regression analyses for FEV1 were very small and the effect was not as long as we observed with PEF, we analyzed the association of allergic sensitization only with PEF variations.

Children of all three categories of allergic sensitization exhibited declines in PEF measurements associated with increases in pollutant concentration in the regression analysis (Table VI).

Percent variation in PEF measurements for a 10 $\mu\text{g}/\text{m}^3$ increase in PM₁₀, O₃, and NO₂ concentration for category 0 ranged from −1.44 to −0.06%. For categories 1 and 2 the declines ranged from −0.83 to −0.03%, and from −1.58 to −0.08%, respectively.

It is important to notice that decrements in PEF were larger when the pollutant concentrations were averaged over 3, 5, 7, and 10 days, independent from the children allergic sensitization status.

Considering the 25–75% interquartile range in O₃ in the period studied, children in the non-sensitized category presented declines in PEF measurements of 2.8, 4.0, 6.2, 5.9% for exposures to 3-day, 5-day, 7-day, and 10-day moving average, respectively. For the same moving averages and pollutant, children in the category 2 (most

TABLE V. Estimates of the Association Between Air Pollutant Exposures and FEV1 in 96 Schoolchildren

Pollutant and time lag	Coef. ^a (95% CI)	Coef. ^b (95% CI)	P-value
Previous 2-hr average			
CO	−0.007 (−0.013 to −0.005)	−0.33 (−0.64 to −0.23)	0.035
PM ₁₀	−0.001 (−0.001 to 0.000)	−0.65 (−1.08 to −0.22)	0.003
SO ₂	0.000 (0.000 to 0.000)	−0.08 (−0.36 to 0.19)	0.541
NO	0.000 (0.000 to 0.000)	0.07 (−0.21 to 0.28)	0.704
NO ₂	0.000 (−0.001 to 0.000)	−1.64 (−2.80 to −0.44)	0.008
O ₃	−0.001 (−0.001 to 0.000)	−1.90 (−3.31 to −0.49)	0.008
Previous 24-hr average			
CO	−0.034 (−0.051 to −0.018)	−1.65 (−2.44 to −0.86)	<0.0001
PM ₁₀	−0.001 (−0.002 to −0.001)	−1.70 (−2.61 to −0.81)	<0.0001
SO ₂	−0.001 (−0.002 to 0.000)	−0.85 (−1.54 to −0.16)	0.016
NO	0.000 (0.000 to 0.000)	−0.76 (−1.45 to 0.00)	0.040
NO ₂	−0.001 (−0.001 to 0.000)	−2.18 (−3.67 to −0.69)	0.004
O ₃	−0.002 (−0.003 to −0.001)	−4.65 (−6.81 to −2.48)	<0.0001
3-day moving average			
PM ₁₀	−0.002 (−0.003 to −0.001)	−2.49 (−3.59 to −1.38)	<0.0001
NO ₂	−0.001 (−0.002 to −0.001)	−5.27 (−7.53 to −3.02)	<0.0001
O ₃	−0.001 (−0.002 to −0.001)	−3.84 (−5.45 to −2.22)	<0.0001
5-day moving average			
PM ₁₀	−0.001 (−0.002 to 0.000)	−1.63 (−2.84 to −0.42)	0.008
NO ₂	−0.001 (−0.001 to 0.000)	−2.98 (−5.35 to −0.58)	0.014
O ₃	0.000 (−0.001 to 0.000)	−0.57 (−2.06 to −0.91)	0.452
7-day moving average			
PM ₁₀	−0.003 (−0.005 to −0.001)	−3.48 (−5.58 to −1.37)	0.001
NO ₂	−0.002 (−0.003 to −0.001)	−5.82 (−9.13 to −2.51)	0.001
O ₃	0.000 (−0.001 to 0.001)	−0.73 (−2.79 to 1.30)	0.481

^aCoefficients represent the expected change in FEV1 associated with one $\mu\text{g}/\text{m}^3$ or one ppm increase in each air pollutant level, adjusted for temperature, relative humidity, school absences.

^bCoefficients represent the expected percent change in FEV1 associated with 25–75% IQR of each air pollutant, adjusted for temperature, relative humidity, school absences.

sensitized) presented declines in PEF measurements of 4.8, 4.3, 6.2, and 7.8%.

DISCUSSION

The results of our study show that exposure to urban air pollution was associated with PEF decrements in children regardless of their allergic sensitization status. This effect occurred within hours or days of exposure. The association was strong for all pollutants except for PM₁₀ 24-hr average that reached borderline significance and for SO₂ where the association was not significant. Also the strongest associations were seen with 3–10-day moving averages.

We hypothesized that children who were classified in the third group (high total IgE concentrations, high blood and nasal smear eosinophils, at least one positive reaction to the skin prick test, and symptoms of allergy) would present larger decrements in the PEF measurements

associated with exposure to air pollution. The results did not confirm our hypothesis even though those children tended to present larger decrements in the PEF measurements, they were not statistically different than those of the non-allergic sensitized. This result is in agreement with a review of panel air pollution studies [Ward and Ayres, 2004] that did not find children diagnosed with asthma to be more susceptible to the effects of air pollution than children who were not asthmatic as some studies suggest.

Our results on PEF variations are also similar to studies published previously [Pope et al., 1991; Pope and Dockery, 1992; Neas et al., 1995; Gielen et al., 1997; Gold et al., 1999; van der Zee et al., 1999; Epton et al., 2008], bearing in mind that comparisons with other studies are difficult due to differences in the study design and in the way the outcomes are defined and measured.

The number of children with medical diagnosed asthma in this study was too small (six children) to compare

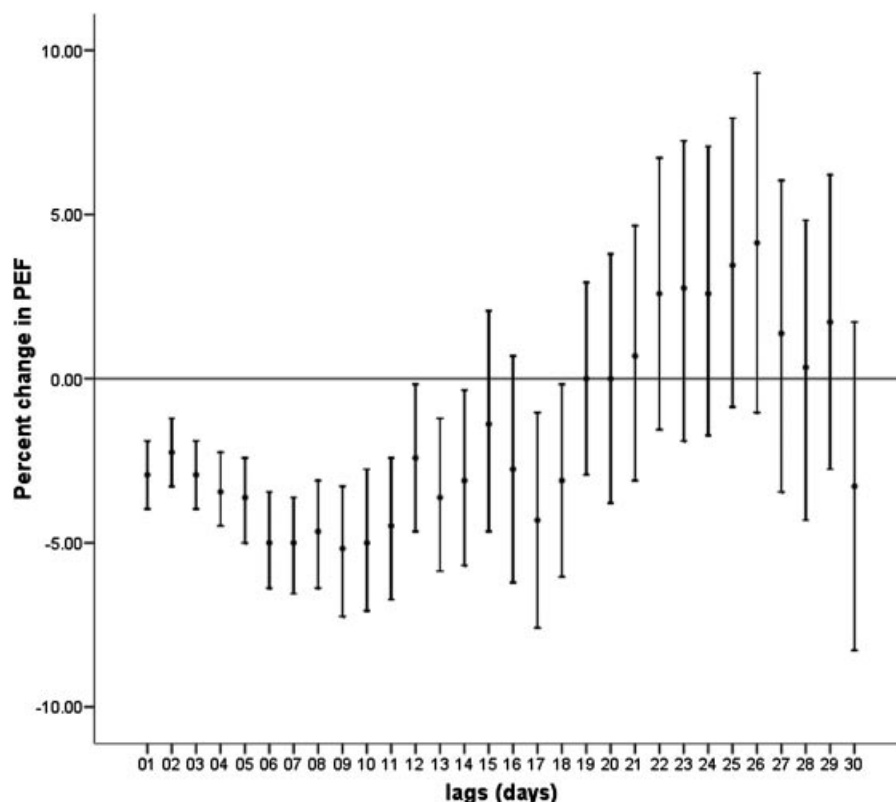


FIGURE 1. Mean (95% confidence interval) peak expiratory flow percent change associated with O_3 lagged from 1 to 30 days.

their results with children without medical diagnosed asthma. But only 31 (32.6%) children could be considered non-allergic sensitized, the other children presented signs and/or exams results that pointed to some level of sensitization, enabling us to categorize them in three groups (non-sensitized, less sensitized, and more sensitized). Several studies show that air pollution has a larger effect in children with allergic symptoms or children diagnosed with asthma. A panel study conducted with 459 children showed that children with bronchial hyperresponsiveness and relatively high levels of total IgE were more susceptible to air pollution [Boezen et al., 1999]. Also in children with a history of wheeze, there is a significant association between PEF decrements and same day ozone concentration [Jalaludin et al., 2000]. In this study, children who were not allergic sensitized presented similar PEF decrements as the ones with any degree of allergic sensitization.

When we look at panel studies of children who had medical diagnosed asthma, we find that ozone seems to be the pollutant that is more associated with declines in the PEF measurements, the occurrence of asthma attacks, respiratory infections, medication use, and shortness of breath [Romieu et al., 1997; Just et al., 2002; Mortimer

et al., 2002; Villeneuve et al., 2006; Dales et al., 2009]. In the present study, ozone was the pollutant associated more consistently with larger decrements in PEF and even though there was no statistical difference among the categories, the children categorized in the “more sensitized group” presented even larger decrements. Percent variation in PEF measurements for category 0 for a $10 \mu\text{g}/\text{m}^3$ increase in PM_{10} , O_3 , and NO_2 concentration ranged from -1.44 to -0.06% . For categories 1 and 2 the declines ranged from -0.83 to -0.03% , and from -1.58 to -0.08% , respectively.

In 2004, when the study was performed, ozone exceeded the national air quality standard ($160 \mu\text{g}/\text{m}^3$) in 62 days. It is important to notice that Sao Paulo has a significant fleet of light duty vehicles using hydrated ethanol and “gasohol” (22% anhydrous ethyl alcohol and 78% pure gasoline). Ethanol, a cleaner-burning fuel than gasoline, is responsible for the increase in the emissions of aldehydes, especially acetaldehyde and formaldehyde, which are among the main precursors of ozone in Sao Paulo [Orlando et al., 2010]. In the last 10 years, SP has experienced an improvement in the levels of most air pollutants, but levels of ozone have not improved as the others. As its formation depends on a chain of reactions

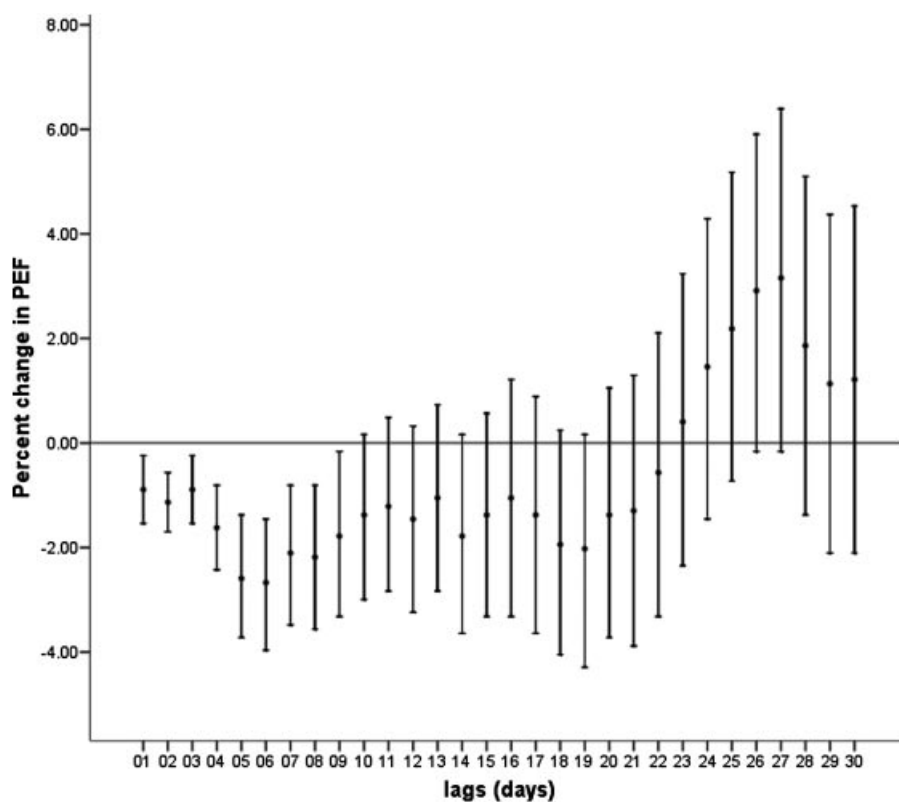


FIGURE 2. Mean (95% confidence interval) peak expiratory flow percent change associated with PM₁₀ lagged from 1 to 30 days.

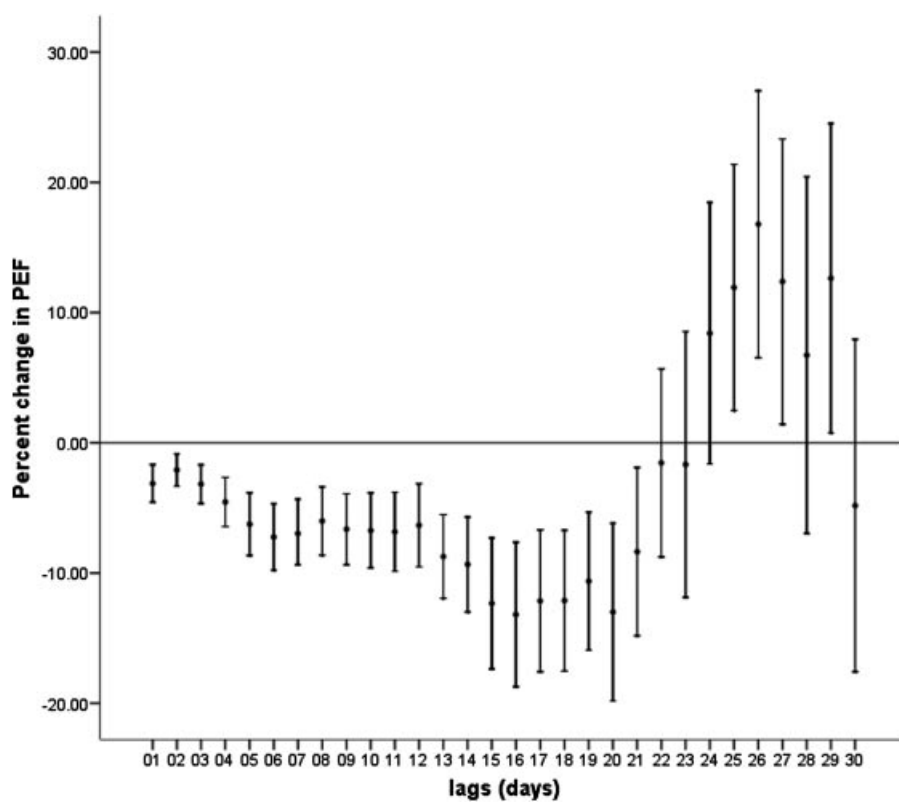


FIGURE 3. Mean (95% confidence interval) peak expiratory flow percent change associated with NO₂ lagged from 1 to 30 days.

TABLE VI. Estimates of the Association Between Percent Change in PEF Associated With 25–75% IQR of Each Air Pollutant in 96 Schoolchildren Stratified by Allergic Sensitization Categories

Pollutant and time lag	Category 0, Coeff. ^a (95% CI)	Category 1, Coeff. (95% CI)	Category 2, Coeff. (95% CI)
Previous 2-hr average			
PM ₁₀	−1.62 (−2.73 to −0.52)*	−1.76 (−2.55 to −0.98)*	−2.01 (−3.63 to −0.40)*
NO ₂	−0.77 (−1.95 to 0.40)	−1.80 (−2.80 to −0.80)*	−2.05 (−4.11 to 0.01)
CO	−0.26 (−0.70 to 0.19)	−0.57 (−0.89 to −0.26)*	−0.51 (−1.21 to 0.18)
SO ₂	−0.13 (−0.53 to 0.27)	−0.24 (−0.58 to 0.10)	−0.45 (−1.06 to 0.17)
Previous 24-hr average			
PM ₁₀	−1.72 (−3.12 to −0.33)*	−0.52 (−2.06 to 1.02)	−0.70 (−2.95 to 1.55)
NO ₂	−1.83 (−3.01 to −0.65)*	−1.56 (−3.02 to −0.10)*	−0.55 (−3.02 to 1.92)
O ₃	−0.30 (−1.18 to 0.58)	−0.14 (−0.83 to 0.55)	−1.74 (−2.98 to −0.50)*
CO	−0.59 (−1.23 to 0.06)	−0.93 (−1.49 to −0.38)*	−0.12 (−1.20 to 0.96)
SO ₂	−0.11 (−0.76 to 0.53)	−0.21 (−0.72 to 0.30)	−0.23 (−1.40 to 0.93)
3-day moving average			
PM ₁₀	−2.98 (−5.62 to −0.33)*	−2.20 (−4.08 to −0.33)*	−2.35 (−5.59 to 0.89)
NO ₂	−4.91 (−7.16 to −2.66)*	−2.25 (−4.36 to −0.14)*	−2.43 (−6.18 to 1.31)
O ₃	−2.69 (−4.37 to −1.01)*	−1.88 (−3.25 to −0.51)*	−4.80 (−6.83 to −2.77)*
5-day moving average			
PM ₁₀	−4.18 (−7.56 to −0.80)*	−3.16 (−6.62 to 0.30)	−5.74 (−10.98 to −0.49)*
NO ₂	−8.41 (−11.63 to −5.19)*	−5.02 (−8.52 to −1.52)*	−6.17 (−10.77 to −1.58)*
O ₃	−3.95 (−5.88 to −2.02)*	−3.11 (−4.73 to −1.50)*	−4.26 (−7.35 to −1.18)*
7-day moving average			
PM ₁₀	−5.79 (−10.48 to −1.09)*	−2.79 (−7.38 to 1.79)	−6.50 (−12.08 to −0.91)*
NO ₂	−9.79 (−13.81 to −5.76)*	−5.67 (−9.92 to −1.41)*	−5.77 (−10.82 to −0.72)*
O ₃	−6.17 (−8.76 to −3.58)*	−3.67 (−5.84 to −1.49)*	−6.12 (−9.26 to −2.99)*
10-day moving average			
PM ₁₀	−3.74 (−10.82 to 3.34)	−0.92 (−5.43 to 3.59)	−5.96 (−12.42 to 0.48)
NO ₂	−9.30 (−14.99 to −3.61)*	−5.32 (−9.41 to −1.24)*	−6.31 (−11.85 to −0.76)*
O ₃	−5.88 (−9.23 to −2.52)*	−2.39 (−5.81 to 1.03)	−7.71 (−11.92 to −3.50)*

^aCoefficients represent the expected percent change in PEF associated with 25–75% IQR of each air pollutant, adjusted for temperature, relative humidity, school absences.

* $P < 0.05$.

that include the presence of volatile organic compounds (VOCs), NO_x, and sunlight, it is unlikely that that this picture will change in a near future.

Even though the decrements in lung function measurements found in most studies are modest enough not to produce respiratory symptoms, there is evidence that exposure to urban air pollution contributes to retard lung growth in children [He et al., 1993, 2010; Gauderman et al., 2007] and that ozone may be an important factor [Frischer et al., 1999] as it has been demonstrated experimentally that airway inflammation persists after repeated ozone exposure [Jorres et al., 1996].

In conclusion, our results add more evidence that air pollution has a deleterious effect on children's lung function. Decrements in PEF and FEV1 were observed with previous 24-hr average exposure to air pollution, as well as with 3–10-day average exposure and were associated mainly with PM₁₀, NO₂, and O₃. Even though allergic

sensitized children tended to present larger decrements in the PEF measurements when exposure to O₃ and PM₁₀ were considered, these decrements were not statistically different than the decrements of the non-allergic sensitized. Given that children are exposed to this air on a daily basis, this exposure can lead to a chronic inflammatory process that might impair their lung growth and further their lung function in adulthood.

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Table I. Two-pollutant models estimates of the association between previous 2-h air pollutant average exposures and PEF in 96 schoolchildren.

Single pollutant	Two-pollutant	Coef. (95% CI)	p-value
PM₁₀ -0.11 (-0.14 to -0.07)	PM₁₀	-0.11 (-0.15 to -0.06)	0.000
	NO₂	0.00 (-0.04 to 0.05)	0.873
	PM₁₀	-0.13 (-0.19 to -0.07)	0.000
	NO	0.00 (0.00 to 0.01)	0.294
	PM₁₀	-0.12 (-0.17 to -0.07)	0.000
	SO₂	0.04 (-0.02 to 0.10)	0.214
	PM₁₀	-0.11 (-0.18 to -0.05)	0.001
	CO	0.28 (-1.24 to 1.79)	0.721
NO₂ -0.09 (-0.13 to -0.05)	NO₂	-0.07 (-0.12 to 0.02)	0.008
	NO	0.00 (-0.01 to 0.01)	0.924
	NO₂	-0.07 (-0.12 to -0.03)	0.001
	SO₂	0.01 (-0.05 to 0.07)	0.639
	NO₂	-0.03 (-0.08 to 0.01)	0.126
	CO	-1.10 (-2.24 to 0.03)	0.057
NO -0.01 (-0.01 to 0.00)	NO	-0.01 (-0.01 to 0.00)	0.014
	SO₂	0.01 (-0.05 to 0.07)	0.750
	NO	0.02 (0.00 to 0.03)	0.007
	CO	-4.79 (-7.36 to -2.22)	0.000
SO₂ -0.08 (-0.15 to -0.02)	SO₂	0.01 (-0.04 to 0.07)	0.617
CO -1.50 (-2.35 to -0.64)	CO	-1.65 (-2.67 to -0.63)	0.001

Table II. Two-pollutant models estimates of the association between previous 24-h air pollutant average exposures and PEF in 96 schoolchildren.

Single pollutant	Two-pollutant	Coef. (95% CI)	p-value
PM₁₀ -0.05 (-0.11 to 0.00)	PM₁₀	-0.03 (-0.16 to 0.11)	0.713
	NO₂	-0.02 (-0.12 to 0.07)	0.613
	PM₁₀	-0.03 (-0.09 to 0.02)	0.246
	NO	-0.01 (-0.02 to 0.00)	0.022
	PM₁₀	-0.05 (-0.11 to 0.00)	0.068
	O₃	-0.03 (-0.06 to 0.00)	0.057
	PM₁₀	-0.05 (-0.11 to 0.01)	0.083
NO₂ -0.05 (-0.11 to 0.00)	SO₂	-0.02 (-0.11 to 0.06)	0.588
	PM₁₀	-0.03 (-0.08 to 0.03)	0.393
	CO	-2.06 (-3.45 to -0.67)	0.004
	NO₂	-0.05 (-0.09 to -0.01)	0.024
	NO	-0.01 (-0.02 to 0.00)	0.122
	NO₂	-0.04 (-0.09 to 0.00)	0.031
	O₃	-0.05 (-0.08 to -0.02)	0.001
NO -0.01 (-0.02 to 0.00)	NO₂	-0.05 (-0.09 to -0.01)	0.011
	SO₂	0.00 (-0.09 to 0.08)	0.923
	NO₂	-0.04 (-0.08 to 0.00)	0.045
	CO	-1.44 (-2.77 to -0.11)	0.034
	NO	-0.01 (-0.02 to 0.00)	0.009
	O₃	-0.07 (-0.10 to -0.04)	0.000
	NO	-0.03 (-0.05 to -0.01)	0.011
O₃ -0.10 (-0.18 to -0.01)	SO₂	0.23 (0.02 to 0.43)	0.031
	NO	0.00 (-0.01 to 0.02)	0.543
	CO	-2.51 (-4.38 to -0.64)	0.008
	O₃	-0.06 (-0.10 to -0.03)	0.000
	CO	-2.78 (-4.26 to -1.29)	0.000
	O₃	-0.04 (-0.07 to -0.01)	0.009
	SO₂	-0.04 (-0.12 to 0.05)	0.391
SO₂ -0.10 (-0.18 to -0.01)	CO	0.07 (-0.02 to 0.17)	0.126
	CO	-2.44 (-4.00 to -0.88)	0.002
		-1.89 (-3.24 to -0.53)	

Table III. Two-pollutant models estimates of the association between air pollutant and moving average exposures and PEF in 96 schoolchildren.

Single pollutant	Two-pollutant	Coef. (95% CI)	p-value
3-day moving average			
PM₁₀	PM₁₀	0.15 (-0.02 to 0.32)	0.078
-0.11 (-0.19 to -0.03)	NO₂	-0.21 (-0.34 to -0.08)	0.002
NO₂	PM₁₀	-0.07 (-0.16 to 0.01)	0.079
-0.13 (-0.19 to -0.07)	O₃	-0.15 (-0.22 to -0.09)	0.000
O₃	PM₁₀	-0.11 (-0.17 to -0.05)	0.001
-0.17 (-0.23 to -0.11)	O₃	-0.16 (-0.21 to -0.10)	0.000
5-day moving average			
PM₁₀	PM₁₀	0.07 (-0.15 to 0.29)	0.523
-0.32 (-0.46 to -0.17)	NO₂	-0.29 (-0.44 to -0.15)	0.000
NO₂	PM₁₀	-0.15(-0.31 to 0.02)	0.084
-0.26 (-0.36 to -0.16)	O₃	-0.16(-0.24 to -0.08)	0.000
O₃	NO₂	-0.19(-0.31 to -0.08)	0.001
-0.21 (-0.29 to -0.14)	O₃	-0.10(-0.18 to -0.02)	0.018
7-day moving average			
PM₁₀	PM₁₀	0.23(-0.01 to 0.47)	0.057
-0.26 (-0.43 to -0.10)	NO₂	-0.39(-0.55 to -0.23)	0.000
NO₂	PM₁₀	0.17(-0.03 to 0.36)	0.096
-0.29 (-0.39 to -0.18)	O₃	-0.36(-0.46 to -0.26)	0.000
O₃	NO₂	-0.15(-0.29 to -0.01)	0.030
-0.29 (-0.38 to -0.21)	O₃	-0.18(-0.29 to -0.07)	0.001
10-day moving average			
PM₁₀	PM₁₀	0.35(0.08 to 0.63)	0.012
-0.17 (-0.37 to 0.02)	NO₂	-0.43(-0.60 to -0.26)	0.000
NO₂	PM₁₀	0.22(0.02 to 0.42)	0.028
-0.28 (-0.40 to -0.16)	O₃	-0.38(-0.51 to -0.26)	0.000
O₃	NO₂	-0.19(-0.33 to -0.05)	0.008
-0.29 (-0.41 to -0.16)	O₃	-0.17(-0.31 to -0.02)	0.022

Table IV. Estimates of the association between PEF and air pollutant exposures in 96 schoolchildren stratified by allergic sensitization categories.

Pollutant and time lag	Category 0	Category 1	Category 2
	Coeff.** (95% CI)	Coeff. (95% CI)	Coeff. (95% CI)
Previous 2-h average			
PM ₁₀	-0.10 (-0.16 to -0.03)*	-0.10 (-0.15 to -0.06)*	-0.12 (-0.21 to -0.02)*
NO ₂	-0.03 (-0.08 to 0.02)	-0.08 (-0.12 to -0.03)*	-0.09 (-0.17 to 0.00)
CO	-0.81 (-2.21 to 0.59)	-1.81 (-2.80 to -0.82)*	-1.61 (-3.79 to 0.58)
SO ₂	-0.03 (-0.10 to 0.05)	-0.05 (-0.11 to 0.02)	-0.09 (-0.21 to 0.03)
Previous 24-h average			
PM ₁₀	-0.10 (-0.18 to -0.02)*	-0.03 (-0.12 to 0.06)	-0.04 (-0.17 to 0.09)
NO ₂	-0.08 (-0.13 to 0.03)*	-0.07 (-0.13 to -0.004)*	-0.02 (-0.13 to 0.08)
O ₃	-0.02 (-0.07 to 0.03)	-0.01 (-0.05 to 0.03)	-0.10 (-0.17 to -0.03)*
CO	-1.84 (-3.88 to 0.20)	-2.93 (-4.67 to -1.21)*	-0.38 (-3.78 to 3.01)
SO ₂	-0.22 (-0.15 to 0.10)	-0.04 (-0.14 to 0.06)	-0.05 (-0.27 to 0.18)
3-day moving average			
PM ₁₀	-0.17 (-0.33 to -0.02)*	-0.13 (-0.24 to -0.02)*	-0.14 (-0.32 to 0.05)
NO ₂	-0.21 (-0.30 to -0.11)*	-0.10 (-0.18 to -0.01)*	-0.10 (-0.26 to 0.06)
O ₃	-0.16 (-0.25 to -0.06)*	-0.11 (-0.19 to -0.03)*	-0.28 (-0.40 to -0.16)*
5-day moving average			
PM ₁₀	-0.24 (-0.44 to -0.05)*	-0.18 (-0.38 to 0.02)	-0.33 (-0.64 to -0.03)*
NO ₂	-0.35 (-0.48 to -0.22)*	-0.21 (-0.35 to -0.06)*	-0.26 (-0.45 to -0.07)*
O ₃	-0.23 (-0.34 to -0.12)*	-0.18 (-0.27 to -0.09)*	-0.25 (-0.43 to -0.07)*
7-day moving average			
PM ₁₀	-0.34 (-0.61 to -0.06)*	-0.16 (-0.43 to 0.10)	-0.38 (-0.70 to -0.05)*
NO ₂	-0.41 (-0.58 to -0.24)*	-0.24 (-0.41 to -0.06)*	-0.24 (-0.45 to -0.03)*
O ₃	-0.36 (-0.51 to -0.21)*	-0.21 (-0.34 to -0.09)*	-0.36 (-0.54 to -0.17)*
10-day moving average			
PM ₁₀	-0.22 (-0.63 to 0.19)	-0.05 (-0.31 to 0.21)	-0.35 (-0.72 to 0.03)
NO ₂	-0.39 (-0.62 to -0.15)*	-0.22 (-0.39 to -0.05)*	-0.26 (-0.49 to -0.03)*
O ₃	-0.34 (-0.54 to -0.15)*	-0.14 (-0.34 to 0.06)	-0.45 (-0.69 to -0.20)*

* $p < 0.05$

** Coefficients represent the expected change in PEF associated with one unit increase in each air pollutant level, adjusted for temperature, relative humidity, and school absences.