

# Exhaled breath malondialdehyde as a marker of effect of exposure to air pollution in children with asthma

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**Background:** Assessment of the adverse effects of oxidative stress related to air pollution is limited by the lack of biological markers of dose to the lungs.

**Objective:** We evaluated the use of exhaled breath condensate (EBC) malondialdehyde as a biomarker of exposure to traffic-related pollution in children with asthma as part of a panel study in Mexico City.

**Methods:** Standard spirometry and collection of EBC and nasal lavage were performed. Environmental monitoring sites were located within 5 km of the children's homes and schools. Data were analyzed by using generalized estimating equations.

**Results:** A total of 480 samples of malondialdehyde were obtained from 107 patients with asthma, with a median level of 18.7 (interquartile range [IQR], 12.4-28.7) nmol. Ambient particulates less than 2.5  $\mu\text{g}/\text{m}^3$  and ozone levels on the day of sampling were significantly associated with higher malondialdehyde levels. A 14.2- $\mu\text{g}/\text{m}^3$  (IQR) increase in 8-hour moving average particulates less than 2.5  $\mu\text{g}/\text{m}^3$  in size was associated with a 1.12-nmol increase in malondialdehyde and a 15.9-ppb (IQR) increase in 8-hour moving average ozone with a 1.16-nmol increase in malondialdehyde. Malondialdehyde levels were inversely associated with forced vital capacity and FEV<sub>1</sub> and positively associated with IL-8 levels in nasal lavage.

**Conclusion:** Exhaled breath condensate malondialdehyde was related to both air pollution exposure and changes in lung function and inflammatory markers. (J Allergy Clin Immunol 2007;119:1016-1022.)

**Key words:** Asthma, clinical immunology, environment, epidemiology, pediatrics

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## Abbreviations used

EBC: Exhaled breath condensate

FVC: Forced vital capacity

PM<sub>2.5</sub>: Particulates less than 2.5  $\mu\text{g}/\text{m}^3$  in size

ROS: Reactive oxygen species

Oxidative stress appears to play an important role in the pathogenesis of asthma. Recent studies have reported increased levels of hydrogen peroxide and nitric oxide in the exhaled breath of patients with asthma.<sup>1</sup> Air pollutants have the ability to drive free radical reactions, inducing oxidative stress in the lung when endogenous antioxidant defenses are overwhelmed.<sup>1,2</sup> The resultant inflammatory response appears to be particularly strong among susceptible individuals, including patients with asthma.

Oxygen radicals that are not neutralized by the antioxidant defenses react with polyunsaturated fatty acid residues in phospholipids, resulting in the production of reactive aldehydes. The most abundant of these, malondialdehyde,<sup>3</sup> has been previously evaluated as a biomarker of pulmonary oxidative stress in occupational settings.<sup>4-6</sup> A key issue for risk assessment of air pollutants is the characterization of the dose reaching the target organ.<sup>6</sup> Although a theoretical exposure dose can be calculated from the level of the pollutant, the time spent in the contaminated environment, and the subject's minute ventilation, there are significant limitations to this approach, especially in accurate measures of personal pollutant exposure. Therefore, a biomarker that is relatively easy to collect and measure and that reflects the effects of the exposure on the lungs would prove useful in epidemiologic studies of traffic related air pollutants and respiratory health.

Exhaled breath condensate (EBC), a fluid formed by cooling exhaled air, has been proposed as a simple, safe, and noninvasive technique for obtaining samples from the lower respiratory tract to assess airway inflammation and is of particular interest in pediatrics. Several oxidative stress biomarkers are measurable in EBC.<sup>7</sup> The aim of the current study was to evaluate whether malondialdehyde levels in EBC could be used to assess air pollutant exposure in children with asthma better. In the current study, the use of malondialdehyde as a biomarker of pollutant exposure was evaluated by showing first that malondialdehyde levels were related to pollutant exposure and second that malondialdehyde levels were related to end-organ effects, namely lung function and secretion of inflammatory cytokines in the nose.

## METHODS

See this article's [Methods](#) in the Online Repository at [www.jacionline.org](http://www.jacionline.org) for expanded methodology.

Our study population included 107 children with asthma living in Iztapalapa and Nezahualcoyotl, 2 areas of Mexico City with high population and traffic densities, recruited from the allergy clinic of the Hospital Infantil Federico Gómez. The study was conducted as a panel study from January 28 to October 10, 2004. Children were followed for 8 weeks average (minimum, 2, to maximum, 16 weeks) during the study period and attended the clinic each 2 weeks during this time. The study protocol was approved by the ethics committees of the Hospital and of the Instituto Nacional de Salud Pública, and parents gave written consent.

Parents completed a baseline questionnaire on sociodemographic characteristics, health, and risk factors for asthma. Asthma was categorized clinically on the basis of symptom pattern as intermittent, mild persistent, or moderate persistent according to the National Heart, Lung, and Blood Institute (NHLBI) classification.<sup>8</sup> At each clinic visit, standard spirometry was measured, exhaled breath samples collected, and nasal lavage performed (see [Methods](#) in the Online Repository). Parents provided information about respiratory symptoms over the preceding 2 weeks.

Malondialdehyde was measured in 480 randomly selected exhaled breath samples (4–8 per child) using the method described by Lärstad et al,<sup>9</sup> and IL-8 was measured in nasal washes by using commercially available ELISA kits. We attempted to measure IL-4, IL-10, and TNF- $\alpha$ , but all samples were below the limit of detection, and these data are not presented. We also measured the pH of exhaled breath condensate<sup>7,10</sup> (see [Methods](#) in the Online Repository).

## Pollutant exposure assessment

Measurements of NO<sub>2</sub>, SO<sub>2</sub>, particulates less than 2.5  $\mu\text{g}/\text{m}^3$  in size (PM<sub>2.5</sub>), ambient ozone, and climatic variables were obtained from government air monitoring stations located in the study area. The home of each participating child was georeferenced and data from the closest monitoring station assigned to the child. All children attended public schools located close to their homes, and no monitoring station was more than 5 km from a child's home or school. Concentrations of SO<sub>2</sub> were low and not considered further. Traffic count was measured at major intersections in the study area ( $n = 52$  points) for an average of 4.5 hours on a randomly selected day by both manual count and radar measurements and used to represent traffic load. The type of vehicle was also recorded (heavy load or regular). The traffic load assigned to each individual child came from the monitoring point closest to the child's home.

## Statistical analyses

*Association of malondialdehyde levels with pollution exposure.* To study the association of traffic-related pollutants (ozone, NO<sub>2</sub>, and PM<sub>2.5</sub>) with the malondialdehyde content of the EBC, several times of pollution exposure (ie, 1-hour maximum, 24-hour average, 8-hour moving average) were modeled. Preliminary examination (data not shown) revealed strong correlations between different pollutants and exposure metrics (see [Methods](#) in the Online Repository). Therefore, we used 8-hour moving average (maximum) for ozone, NO<sub>2</sub>, and PM<sub>2.5</sub> as exposure metrics in our final models. Pearson correlations were determined between air pollutant levels and various climatic variables.<sup>11</sup> Malondialdehyde levels were not normally distributed and were log-transformed. One hundred nineteen (24.8%) of the samples had nondetectable levels of malondialdehyde and were replaced in the analyses by random numbers between 0 and 4.1 nmol (the detection limit) with a normal distribution as described by Anemiyi.<sup>12</sup>

Generalized estimating equation models with a normal link<sup>13</sup> were used to evaluate the association between levels of air pollutant and malondialdehyde levels. These models are specially developed to account for autocorrelation in the data because of including multiple measurements (average, 4.9) per child and to allow for the use of time-dependent covariates. Models were adjusted for potential confounding factors including sex, school shift (morning or afternoon turn), minimum temperature, and chronological time. Other variables such as age, outdoor activities, and exposure to environmental tobacco smoke (smoking at home of the father and mother and number of cigarettes smoked) were not significant confounders and were not included in the final models. In

models including traffic count, we also account for the distance between the home and the traffic count device located on the main avenue closest to the home. We also evaluated the potential modifying effect of asthma severity on the relationship between pollutant exposure and malondialdehyde levels.

*Association of malondialdehyde levels with health outcomes.* Generalized estimating equation models were used to study the association between malondialdehyde levels and pulmonary function (forced vital capacity [FVC] and FEV<sub>1</sub>) and IL-8 levels in nasal lavage. We also calculated the predicted FEV<sub>1</sub> and FVC using the equation proposed by Pérez-Padilla et al<sup>14</sup> for Mexican children and calculated the association between malondialdehyde levels and changes in FEV<sub>1</sub>% and FVC%. IL-8 levels were not normally distributed and were log-transformed. Models were adjusted for sex, body mass index, and chronological time when studying lung function and for sex and chronological time only in models including IL-8 given that body mass index was not significantly related to IL-8 levels. Other potential confounding factors including atopy, age, and exposure to tobacco smoke were examined in preliminary analyses and did not modify the association.

Analyses were conducted using Stata software.<sup>15</sup>

## RESULTS

### Study population

[Table I](#) presents the characteristics of the study population. The mean age of participants was 9.5 years. Fifty-six (52.3%) of the children were classified as having mild intermittent, 27.1% mild persistent, and 19.6% moderate persistent asthma. Eighty-nine (89.9%) of the children had positive skin prick test results, with the most common sensitivities to house dust mite (*Dermatophagoides pteronyssinus*; 62.6%), cat (Fel d 1; 23.2%), and cockroach (*Blattella americana*; 12.1%). Malondialdehyde was detected in the EBC of 75.2% (361/480) samples. The median and interquartile range of malondialdehyde were 18.7 (25th percentile [Q25] = 12.4, 75th percentile [Q75] = 28.7) nmol. At baseline, FVC, and FEV<sub>1</sub> were 2.4 L (SD, 0.8) and 1.9 L (SD, 0.6), respectively.

### Pollution exposure data

The 8-hour moving average PM<sub>2.5</sub> ranged from 4.2 to 89.5  $\mu\text{g}/\text{m}^3$ , with a mean of 27.4  $\mu\text{g}/\text{m}^3$ . The 8-hour moving average of NO<sub>2</sub> ranged from 13.9 to 73.5 ppb with a mean of 35.3 ppb. The 8-hour moving average ozone level ranged from 9.8 to 60.7 ppb, with a mean of 31.1 ppb ([Table II](#)). The correlation between PM<sub>2.5</sub> and ozone was  $r = 0.48$  ( $P < .001$ ). The correlations between ozone and NO<sub>2</sub> and PM<sub>2.5</sub> were  $r = 0.44$  ( $P < .001$ ) and  $r = 0.54$  ( $P < .001$ ), respectively.

### Associations between air pollutants and malondialdehyde levels

[Table III](#) presents the associations between traffic-related pollutant concentrations and malondialdehyde levels in EBC on the day of sampling. After adjusting for sex, school shift (morning or afternoon), minimum temperature, and chronological time of the observation, statistically significant associations were seen ([Table III](#)). An increase of 14.2  $\mu\text{g}/\text{m}^3$  in 8-hour moving average PM<sub>2.5</sub> levels (equal to 1 interquartile range) was associated with a 1.12-nmol increase in malondialdehyde. The association was significant on the day of sampling but not with other lags. An increase of 15.9 ppb in 8-hour moving average (maximum) ozone levels was associated with a 1.16-nmol increase in malondialdehyde. Similar results were also observed with 1-day lag and 2-day cumulative ozone exposure. For both PM<sub>2.5</sub> and ozone, other exposure metrics provided comparable results (see [Methods](#) in the

Online Repository). When PM<sub>2.5</sub> and ozone were both entered in the regression models, only the effect of ozone remained significant ( $P = .04$ ). We also observed a 1.19-nmol increase in malondialdehyde for an interquartile increase in the heavy vehicle load accounting for the distance of the children's home to the closest avenue. When either PM<sub>2.5</sub> or ozone and traffic count was included in the same model, both the pollutant and traffic count remained significantly associated with malondialdehyde levels (see [Methods](#) in the Online Repository). When traffic count was broken into tertiles, a significant positive trend was seen with EBC malondialdehyde levels (test of trend,  $P = .02$ ; [Fig 1](#)).

### Associations between malondialdehyde levels and health outcomes

Malondialdehyde levels were inversely related to FEV<sub>1</sub> and FVC and directly related to IL-8 levels in nasal lavage ([Table IV](#)). For an increase of an interquartile range of malondialdehyde (log scale), FEV<sub>1</sub> decreased by 26 mL (1.3% [0.51% to 2.18%]), and FVC decreased by 51 mL (2.1% [0.09% to 4.13%]). When lung function was expressed as percent predicted, FEV<sub>1</sub>% decreased by 1.6% and FVC% by 2.8% for an interquartile range increase in malondialdehyde. Nasal lavage IL-8 levels (log scale) increased by 0.21 pg/mL for an interquartile range increase in malondialdehyde (log scale; [Fig 2](#)).

## DISCUSSION

The results of the current study show that malondialdehyde levels in EBC are increased in relation to increased exposure to traffic-related pollutants. In addition, these increased levels of malondialdehyde are associated with lower lung function and increased levels of the inflammatory cytokine IL-8 in nasal lavage. These data suggest that malondialdehyde level in EBC is a suitable biomarker of effect of traffic-related pollutants and could be used as a noninvasive marker in epidemiologic studies in children.

Traffic-related pollution is a complex mixture of air toxicants including particulate matter, products of incomplete fuel combustion, and secondary pollutants such as ozone, a highly reactive gas that induces oxidative stress in the lung, especially when the antioxidant defenses have been overwhelmed. Oxidative stress in the lungs results in an influx of inflammatory cells to the lung with the subsequent generation and release of large quantities of free radicals and reactive oxygen species (ROSs). The release of lipid oxidation products (aldehydes) in the lining fluids after exposure to ozone has been reported.<sup>16</sup> Similarly, oxidative stress appears to underlie the toxicity of particulate matter.<sup>17</sup> Intratracheal instillation of residual oil fly ash leads to the generation of aldehydes, possibly associated with transition metals such as Fe, Ni, and V and organic compounds such as polycyclic aromatic hydrocarbons that are capable of redox cycling.<sup>18</sup> Particulate matter may also alter mitochondrial function, including nicotinamide adenine dinucleotide phosphate-oxidase, and activate inflammatory cells capable of generating ROSs and reactive nitrogen species.<sup>19</sup> Diesel exhaust has been associated in animal and human studies with oxidative stress responses; increased secretion of proinflammatory cytokines, chemokines, and adhesion molecules<sup>20,21</sup>; and exacerbation of allergic responses in the airway.<sup>22</sup> In Mexico, most of the heavy load vehicles use diesel fuel. Exposure to these pollutants is therefore likely to cause oxidative stress, particularly in

**TABLE I.** Characteristics of the study population, Mexico City, 2004

| Variables                              | Percent (n = 107) |            |
|----------------------------------------|-------------------|------------|
| Asthma severity                        |                   |            |
| Mild intermittent                      | 52.3              |            |
| Mild persistent                        | 27.1              |            |
| Moderate persistent                    | 19.6              |            |
| Atopic                                 | 89.9              |            |
| Allergy test positive                  |                   |            |
| Cat                                    | 23.2              |            |
| Dog                                    | 12.1              |            |
| Dust mite                              | 62.6              |            |
| Cockroach                              | 12.1              |            |
| Smoking at home                        |                   |            |
| Father                                 | 55.9              |            |
| Mother                                 | 37.0              |            |
| Average cigarettes per day             |                   |            |
| 1-5                                    | 92.3              |            |
| 6-10                                   | 7.7               |            |
| 11-15                                  | 0.0               |            |
| Other                                  | 20.7              |            |
| Pets at home                           | 59.4              |            |
| Presence of dampness in house          | 42.1              |            |
| Presence of cockroach in last 12 mo    | 20.6              |            |
| Male                                   | 64.5              |            |
|                                        | Mean              | SD         |
| Age (y)                                | 9.5               | 2.1        |
| Weight (kg)                            | 38.1              | 12.1       |
| Height (cm)                            | 137.5             | 14.0       |
| Age at diagnosis (y)                   | 8.9               | 2.0        |
| Mother education (y)                   | 9.8               | 3.1        |
| Baseline lung function and IL-8 levels |                   |            |
| FVC (L)                                | 2.4               | 0.8        |
| FEV <sub>1</sub> (L)                   | 1.9               | 0.6        |
| IL-8 (pg/mL)                           | 210.0             | 110.0      |
| Malondialdehyde                        |                   |            |
| No. of samples                         | 480               |            |
| Mean of samples by children            | 5.0               | 2.7        |
| Median (nmol/L)*                       | 18.7              | 12.4, 28.7 |
| Median by asthma severity (nmol/L)*    |                   |            |
| Mild intermittent                      | 19.9              | 12.7, 27.2 |
| Mild persistent                        | 16.4              | 12.4, 28.7 |
| Moderate persistent                    | 18.7              | 10.1, 31.3 |

\*Median (Q25, Q75).

children with asthma, because they are likely to have pre-existing airway inflammation and have been shown to have a "compromised lung lining fluid antioxidant status."<sup>2</sup>

A significant problem limiting studies of the health effects of exposure to traffic-related pollutants is the availability of a simple assessment of exposure dose—that is, the amount of pollutant reaching the lungs. Although the dose can theoretically be calculated from the pollutant concentration, the time in contact with the contaminated environment, and the individual's minute ventilation, there are substantial difficulties with determining the exposure of individuals to ambient pollutants. In addition, the lung has a substantial antioxidant defense capacity that varies with the age of the child and is subject to genetic variation.<sup>23,24</sup> Thus, a biomarker of effect—that is, one that becomes positive once the oxidant stimulus exceeds the lung's antioxidant defenses and ROS are available to cause oxidative stress injury—is attractive. In the current study, malondialdehyde could not be detected

**TABLE II.** Air pollution levels from the Mexico City monitoring network during the study period (January to October 2004)

|                                            | Mean | SD   | Minimum | Maximum | Median (Q25, Q75)  |
|--------------------------------------------|------|------|---------|---------|--------------------|
| 4.5 h Average vehicular flow of heavy load | 42.9 | 39.0 | 2.8     | 128.9   | 22.4 (14.4, 65.1)  |
| O <sub>3</sub> (ppb)                       |      |      |         |         |                    |
| 24-h Average                               | 24.4 | 7.9  | 7.0     | 44.8    | 24.1 (17.9, 30.7)  |
| 8-h Moving average (maximum)               | 31.1 | 10.3 | 9.8     | 60.7    | 31.4 (22.5, 38.3)  |
| 1-h Maximum                                | 85.7 | 32.4 | 22.0    | 183.0   | 87.2 (61.5, 107.5) |
| NO <sub>2</sub> (ppb)                      |      |      |         |         |                    |
| 24-h Average                               | 34.7 | 8.3  | 12.9    | 66.4    | 34.2 (29.5, 39.7)  |
| 8-h Moving average (maximum)               | 35.3 | 8.9  | 13.9    | 73.5    | 34.9 (29.6, 40.5)  |
| 1-h Maximum                                | 63.7 | 19.6 | 26.0    | 120.0   | 61.2 (51.1, 74.9)  |
| PM <sub>2.5</sub> (μg/m <sup>3</sup> )     |      |      |         |         |                    |
| 24-h Average                               | 25.7 | 10.6 | 4.8     | 85.2    | 24.9 (19.3, 30.9)  |
| 8-h Moving average (maximum)               | 27.4 | 11.5 | 4.2     | 89.5    | 26.6 (19.5, 33.8)  |
| 1-h Maximum                                | 54.1 | 23.1 | 10.0    | 162.0   | 51.4 (39.0, 65.6)  |
| Temperature minimum (°C)                   | 11.7 | 2.7  | 0.6     | 16.4    | 12.6 (10.6, 13.5)  |

**TABLE III.** Malondialdehyde and exposure to pollutants in children with asthma residing in Mexico City, 2004

| LNMDA*                                                              | Coefficient | SE   | P value |
|---------------------------------------------------------------------|-------------|------|---------|
| Model 1                                                             |             |      |         |
| 8-h Moving average (maximum) O <sub>3</sub> (ppb)                   | 0.151       | 0.07 | .021    |
| Model 2                                                             |             |      |         |
| 8-h Moving average (maximum) NO <sub>2</sub> (ppb)                  | 0.056       | 0.05 | .261    |
| Model 3                                                             |             |      |         |
| 8-h Moving average (maximum) PM <sub>2.5</sub> (μg/m <sup>3</sup> ) | 0.110       | 0.05 | .045    |
| Model 4                                                             |             |      |         |
| 4.5-h Average vehicular flow of heavy load                          | 0.178       | 0.08 | .019    |
| Model 5                                                             |             |      |         |
| 8-h Moving average (maximum) O <sub>3</sub> (ppb)                   | 0.145       | 0.07 | .043    |
| 8-h Moving average (maximum) PM <sub>2.5</sub> (μg/m <sup>3</sup> ) | 0.056       | 0.06 | .353    |

\*LNMDA, Log (malondialdehyde).

Models (1, 2, 3 and 5) were adjusted for sex, school shift (morning or afternoon turn), minimum temperature, and chronological time. Model 1 includes 480 measurements of 107 subjects with asthma. O<sub>3</sub> coefficient is calculated for a change of 15.9 ppb (interquartile range). Model 2 includes 477 measurements of 107 subjects with asthma. NO<sub>2</sub> coefficient is calculated for a change of 11 ppb (interquartile range). Model 3 includes 448 measurements of 91 subjects with asthma. PM<sub>2.5</sub> coefficient is calculated for a change of 14.2 μg/m<sup>3</sup> (interquartile range). Model 4 includes 343 measurements of 72 subjects with asthma; the vehicular flow of heavy load coefficient is calculated for a change of 50.6 vehicles (interquartile range). The model was adjusted for sex, shift of the vehicular count (morning or afternoon), distances from house to the vehicular count, and chronological time. Model 5 includes both O<sub>3</sub> and PM<sub>2.5</sub> levels, and the coefficients are calculated for a change of 15.9 ppb for O<sub>3</sub> and 14.22 μg/m<sup>3</sup> for PM<sub>2.5</sub>, and includes 448 measurements of 91 subjects with asthma.

in 24.8% of EBC samples. One plausible explanation for this is that the pollutant exposure in these children on these occasions was not sufficient to overwhelm their antioxidant defenses. An interesting point is that we were able to show by using repeated measurements of malondialdehyde levels in 107 children with asthma that malondialdehyde levels varied with exposure to air pollutants, in particular ozone and PM<sub>2.5</sub>. This supports the fact that malondialdehyde levels are at least in part related to pollutant exposure. To be a confounding, any other factor would have to vary closely with air pollution exposure and affect malondialdehyde levels.

The accepted mechanism by which exposure to traffic-related pollutants is thought to adversely affect health is by inducing oxidative stress. Thus, measuring a biomarker of oxidative stress injury in the lung is logical. Several markers of oxidative stress

have been proposed, including hydrogen peroxide, eicosanoids, and aldehydes. One of the latter, malondialdehyde, is one of the final products of the lipid peroxidation process in the cells caused by free radicals<sup>3</sup> and is generated mainly by arachidonic acid and docosahexaenoic acid. Previous studies conducted in adults exposed in occupational<sup>6</sup> settings or to ambient particulate have suggested that malondialdehyde may be a good indicator of the oxidative potential of air pollutants.<sup>25</sup> Higher malondialdehyde levels has been observed in the serum of women exposed to high levels of particulate matter related to burning biomass fuel.<sup>26</sup> Malondialdehyde in the plasma of cement plant workers was negatively correlated with FEV<sub>1</sub> and erythrocyte reduced glutathione.<sup>4</sup> High levels of malondialdehyde have also been observed in the EBC of workers exposed to cobalt and tungsten.<sup>6</sup> Diesel-exposed toll collectors have been reported to have significantly higher plasma malondialdehyde and nitrite+nitrate levels than office workers, suggesting that malondialdehyde and nitrite+nitrate levels may be used as biological markers of oxidative stress related to diesel exhaust exposure.<sup>5</sup> Although malondialdehyde is not a specific product of lipid peroxidation and the reliability of using malondialdehyde as a marker of oxidative stress *in vivo* has been questioned,<sup>27</sup> with the advent of sensitive techniques measuring malondialdehyde with HPLC separation and fluorescence detection,<sup>7,9,28,29</sup> such as used in the current study, reliable data have been obtained. Although malondialdehyde represents only approximately 1% of lipid peroxides, it can be used as a marker of oxidative stress in clinical settings.<sup>7,26,29</sup> A recent study comparing children with asthma and control children reported detectable levels of malondialdehyde and erythrocyte reduced glutathione in exhaled breath in the children with asthma that increased during asthma exacerbation.<sup>28</sup> The levels of malondialdehyde found in the current study were in the range of those previously reported in children with asthma.<sup>28</sup> Asthma severity was not related to the levels of malondialdehyde and did not modify the effect of exposure to pollutant. This might be because our sample did not include children with severe asthma (very rare in Mexico) and only 21 children were classified as having moderate asthma. Therefore, our sample was likely to have been too small to detect an effect. Another possible explanation is that our children with asthma were well controlled. The effect we demonstrated on lung function with a relatively greater effect on FVC than on FEV<sub>1</sub> is more consistent with a chronic effect on lung growth than with an acute effect causing airway obstruction.

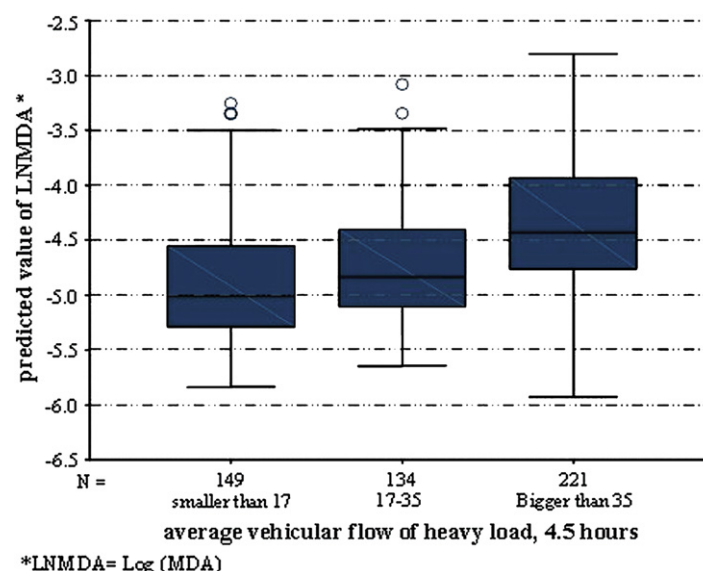


**FIG 1.** Malondialdehyde (MDA) levels in children with asthma living in Mexico City according to heavy load traffic flow (in tertiles).

Collection of EBC is of particular interest in pediatrics because it is a simple, noninvasive technique for collecting samples from the lower respiratory tract. It is also suitable for use in epidemiologic studies in field settings using the R-tube technique we used. Previous studies have shown that the concentration of aldehydes in EBC is not affected by sampling time, expiratory flow, total exhaled air volume, or total condensate volume. The R-tube technique we used was acceptable to the children we studied and resulting in the collection of 2-mL EBC samples within 10 to 15 minutes.

Before discussing the implications of our findings, some technical issues need to be considered. Our measure of pollutant exposure was based on fixed monitoring stations located within 5 km of the children's homes and schools. Therefore, some degree of misclassification might be present. However, in a validation study conducted in a similar population of children with asthma using personal monitors, we observed a significant association between personal exposure and ambient ozone levels ( $P < .01$ ).<sup>30</sup> In the current study, we measured  $PM_{2.5}$  mass, which is thought to be a poor surrogate for the biologically effective dose of particulate matter.<sup>31</sup> However, a recent study reporting health effects in children with asthma did not find a difference when using the concentration of ultrafine particulates,  $PM_{10}$ , or black smoke to represent exposure.<sup>32</sup> Relying on particle mass, which is not necessarily related to particle number, might explain the relatively small association we observed between  $PM_{2.5}$  and malondialdehyde levels. In addition, the degree of spatial variation of  $PM_{2.5}$  is likely to be larger than for ozone, adding to the misclassification of exposure to this pollutant.<sup>33</sup> If present, misclassification should be random and would result in an underestimation of the associations of air pollutants and malondialdehyde. The fact that heavy load traffic count was also positively associated with an increase in malondialdehyde and that distance of the children's homes from the closest avenue was inversely related to malondialdehyde (decrease in malondialdehyde levels of 0.99 nmol per 100 meters;  $P = .20$ ) supports the use of this biomarker as a surrogate of exposure to traffic air pollutant.

**TABLE IV.** Relation between lung function and IL-8 with malondialdehyde (LNMDA) in children with asthma residing in Mexico City, 2004

| IL-8/lung function*                | Coefficient | SE    | P value |
|------------------------------------|-------------|-------|---------|
| FEV <sub>1</sub>                   | -0.026      | 0.008 | .002    |
| FVC                                | -0.051      | 0.025 | .041    |
| Percent predicted FEV <sub>1</sub> | -1.575      | 0.895 | .079    |
| Percent predicted FVC              | -2.802      | 1.125 | .013    |
| IL-8 (LnIL-8)                      | 0.206       | 0.099 | .037    |

LNMDA, Log (malondialdehyde).

\*IL-8, log IL-8 (LnIL-8), FEV<sub>1</sub>, and FVC models include 450 measurements of 107 subjects with asthma adjusted for sex, body mass index, and chronological time. Model IL-8 includes 426 measurements of 104 subjects with asthma and was adjusted for sex and time. All coefficients are calculated for a change of 2.36 units logarithmic of malondialdehyde (interquartile range).

The data from the current study strongly suggest that malondialdehyde in EBC is a suitable biomarker for studying the health effects of exposure to traffic-related pollutants. First, we have shown an association between several indices of exposure to traffic-related pollutants and malondialdehyde levels in EBC. In addition, we observed a significant inverse correlation between malondialdehyde levels in exhaled breath and the pH levels ( $r = 0.20$ ;  $P = .001$ ). The measurement of pH levels is considered a good marker of airway disease, and lower pH values have been associated with eosinophilic inflammation of the airways in asthma.<sup>34</sup> Second, we have shown that malondialdehyde levels in EBC were significantly related to lower lung function and to an increase in the proinflammatory cytokine IL-8 measured in nasal lavage. The fact that data from the current study show significant relationships between malondialdehyde levels and 2 different biological effects, measured in 2 different media, namely lower lung function as an index of lung dose and higher IL-8 levels in nasal lavage, an index of nasal dose, gives considerable reassurance that measuring malondialdehyde in EBC is a suitable biomarker of traffic-related pollutants in children with

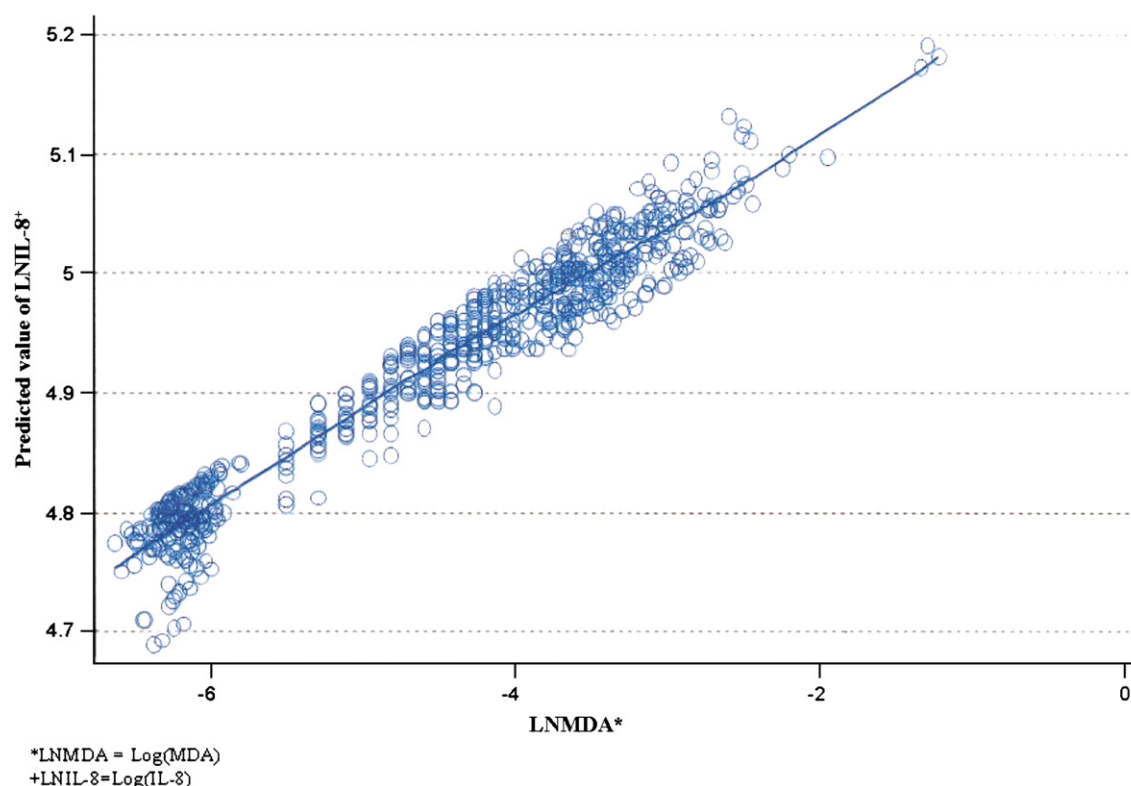


**FIG 2.** IL-8 levels in children with asthma living in Mexico City according to malondialdehyde levels in EBC.  
MDA, Malondialdehyde.

asthma. Besides, although respiratory symptoms were not our primary outcomes, we also observed that malondialdehyde levels were related to an increase in wheezing and bronchodilator use with a 3-day lag ( $P = .10$  and  $P = .04$ , respectively). Because our study population was recruited from an allergy clinic, the rate of atopy was high. Our results therefore can only be extrapolated to a similar population.

In summary, the data from the current study have shown that malondialdehyde levels in EBC can be used as a biomarker of effect of traffic-related pollutants and that increased malondialdehyde levels are associated with lower lung function and increased secretion of IL-8 in the nose. To our knowledge, our study is the first that evaluates the use of malondialdehyde in exhaled breath as a biomarker of effect of traffic-related air pollutants in a free living population of children with asthma. This marker of oxidative stress might be a useful tool in epidemiologic studies of air pollution conducted in susceptible children such as patients with asthma.

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**Clinical implications: EBC malondialdehyde can be used as a biomarker of the effect of traffic-related pollutants and is suitable to monitoring exposures in children with asthma.**

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## METHODS

### Study population

Our study population included children with asthma living in Iztapalapa and Nezahualcoyotl, 2 areas of Mexico City with high population and traffic densities. Children ( $n = 107$ ) were recruited from those attending the allergy clinic of the Hospital Infantil Federico Gómez. The study was conducted as a panel study from January to October 2004. Children were followed for 8 weeks average (minimum, 2, to maximum, 16 weeks) during the study period and attended the clinic each 2 weeks during this time. The parents completed a baseline questionnaire on sociodemographic characteristics and health and risk factors for asthma. Asthma was categorized clinically on the basis of symptom pattern as intermittent, mild persistent, or moderate persistent according to the NHLBI classification.<sup>E1</sup> The study protocol was approved by the ethical committees of the Hospital Infantil Federico Gómez and the Instituto Nacional de Salud Publica, and parents gave written consent.

During follow-up, the children attended the clinic each 2 weeks to measure lung function and provide exhaled breath samples, and nasal lavage was performed. Visits occurred between 10:00 AM and 2:00 PM. Parents provided information about their child's respiratory symptoms during the previous 2 weeks.

### Spirometry

Lung function was measured with the child standing using a portable spirometer (EasyOne, Medizintechnik AG, Zurich, Switzerland) in accordance with the American Thoracic Society recommendations.<sup>E2</sup> FVC and FEV<sub>1</sub> were obtained from the 3 best acceptable forced expiratory maneuvers and expressed in absolute terms.

### Skin prick test

Skin prick tests were undertaken in all children. A wheal of 2 mm or greater in the presence of a positive histamine control and a negative uncoated control constituted a positive skin test result. The test included 24 aeroallergens. A positive skin test to at least 1 allergen was considered indicative of atopy.

### EBC collection

Exhaled breath condensate was collected by using the R-tube, in which the condensation chamber is a disposable polypropylene tube connected to an exhalation valve.<sup>E3</sup> Exhaled breath is cooled by placing an aluminium cooling sleeve over the disposable polypropylene tube.<sup>E3</sup> This technique allows the collection of a large number of samples in a relatively simple and efficient manner. Samples were obtained following the recommendations of the American Thoracic Society/European Respiratory Society Task Force.<sup>E3,E4</sup> Participants were asked to breathe tidally through a mouthpiece connected to the R-tube for 10 to 15 minutes to collect approximately 2 mL EBC. Samples were aliquoted and frozen to  $-70^{\circ}\text{C}$  within 15 minutes of collection.

### Malondialdehyde

Malonaldehyde was measured in 480 randomly selected EBC samples obtained from 4 to 8 EBC samples per child by fluorescence detection as described by Lärstad et al.<sup>E5</sup> Briefly, EBC samples were reconstituted with thiobarbituric acid (Sigma, St Louis, Mo). Malondialdehyde tetrabutylammonium salt and 85% orthophosphoric acid were from Fluka (Buchs, Switzerland). HPLC-grade acetonitrile was provided by Merck (Darmstadt, Germany). Water was purified with a Millipore Alpha-Q system (Bedford, Mass). The equipment used for HPLC analysis was a Nucleosil 100 column ( $\text{C}_{18}$ ,  $150 \times 4.6$  mm internal diameter, particle size 5  $\mu\text{m}$ ; Macherey-Nagel, Düren, Germany), a PU-980 HPLC pump, and a FP-920 fluorescence detector (Jasco, Tokyo, Japan). The excitation and emission wavelengths for the thiobarbituric acid (TBA)-malondialdehyde adduct were 532 nm and 553 nm, respectively. Samples were injected with an ASTED 233XL laboratory robot equipped with a 30  $\mu\text{L}$  Rheodyne 7010 injection valve (Gilson, Villiers-le-Bel, France). Registration of chromatograms and peak area determinations were achieved with HPLC-HP Chemstation software (Hewlett-Packard, Sverige, Sweden). The mobile phase was acetonitrile, 20 mmol/L potassium

phosphate buffer, pH 6.8 (20:80 vol/vol), and the flow rate was 1.3 mL/min. Samples were prepared by adding 50  $\mu\text{L}$  EBC, or purified water for standards, to 450  $\mu\text{L}$  TBA reagent. Derivatization was performed in a water bath at  $95^{\circ}\text{C}$  for 60 minutes. After cooling in ice for 5 minutes, samples were allowed to reach room temperature for about 30 minutes until analysis. The method has a detection limit of 4.1 nmol. Within-assay and between-assay precision for standards is 2.2% and 4.1%, respectively.

### pH in exhaled breath

Stable pH was obtained after deaeration/decarbonation of breath condensate samples by bubbling with argon (350 mL/min) for 10 minutes. Samples were frozen at  $-70^{\circ}\text{C}$  within 15 minutes of collection. pH was measured by using a pH meter (Corning 240; Science Products Division, New York, NY) with a 0 to 14 pH range as described previously.<sup>E6,E7</sup>

### Nasal lavage

Nasal lavage was performed with the subjects sitting with a closed nasopharynx while tilting their neck back  $45^{\circ}$  from the vertical. Five milliliters of warmed ( $37^{\circ}\text{C}$ ) normal saline was instilled into each nostril by pipette. After 10 seconds, during which the subjects shake their heads softly from side to side, they bring their heads forward, expelling the wash fluid into a plastic receptacle. The fluid is then transferred to a separate tube.<sup>E8</sup> The subjects then perform as many as 4 subsequent nasal washes at 30-second intervals, with each wash being collected into a separate tube.

### IL-8

IL-8 was measured in nasal wash by using commercially available ELISA kits according to the manufacturer's instructions in the Laboratory of David Diaz-Sanchez (University of California, Los Angeles).

### Pollution exposure assessment

We obtained measurements of nitrogen dioxide ( $\text{NO}_2$ ; chemiluminescence, analyzer-model 1200 MCA; Advanced Pollution Instrumentation, Mexico City, Mexico), sulfur dioxide ( $\text{SO}_2$ ; ultraviolet fluorescence analyzer, analyzer-model 100; Advanced Pollution Instrumentation),  $\text{PM}_{2.5}$  particulates (mass median diameter  $< 2.5 \mu\text{m}$ ; Tapered Element Oscillating Microbalance sampler; Patashnik and Rupprecht), ambient ozone (UV photometry analyzer-model 400; Advanced Pollution Instrumentation) and climatic variables from 5 government air monitoring stations located in the study area (Cerro de la Estrella, Merced, Universidad Autonoma Metropolitana Iztapalapa, Hangares, and La Perla). The home of each participating child was georeferenced and the closest monitoring station assigned to the child. All children attended public schools located close to their homes, and no monitoring station was more than 5 km from a child's home or school. Concentrations of  $\text{SO}_2$  were low and were excluded from further analysis. Traffic count was measured in the main avenues of the study area at intersection points ( $n = 52$  points) for an average of 4.5 hours on a randomly selected day by manual count and radar measurements. Daily averages of 4.5 hours were obtained for each point and assumed to be representative of traffic load. Average traffic count was assigned to each child on the basis of the proximity of this residence. The type of vehicle was specified (heavy load or regular).

### Statistical analysis

*Association of malondialdehyde levels with pollution exposure.* We first studied the association of traffic-related pollutants (ozone,  $\text{NO}_2$ , and  $\text{PM}_{2.5}$ ) on the day of sampling and preceding days with the MDA content of the EBC. Several indices of pollution exposure (ie, 1-hour maximum, 24-hour average, 8-hour moving average) were modeled. However, because these pollutants were strongly correlated (Table E1), we used 1-hour maximum ozone levels and 8-hour moving average  $\text{NO}_2$  and  $\text{PM}_{2.5}$  as exposure values in our final models. Pearson correlations were determined between air pollutant levels and various climatic variables.<sup>E9</sup> Malondialdehyde levels were not normally distributed and were log-transformed. One hundred nineteen (24.8%) of the

samples had nondetectable levels of malondialdehyde. To include these samples in the analyses, we generated random numbers between 0 and 4.1 nmol (the detection limit) with a normal distribution as described by Anemiya.<sup>E10</sup>

Because we had repeated EBC samples for each child (4.9 on average), we used generalized estimating equation models with a normal link<sup>E11</sup> to evaluate the association between levels of air pollutant and malondialdehyde levels. These models are specially developed to account for autocorrelation in the data and to allow for the use of time dependent covariates. A key feature of this approach is that it models the marginal distribution with autocorrelation in the variance. This gives a coefficient with the usual interpretation.<sup>E11</sup>

Analyses of the within-subjects and between-subjects variation of malondialdehyde measurements showed that 27% of the variation occurred between subjects and 48% within subjects.

Models were adjusted for potential confounding factors including sex, school shift (morning or afternoon turn), minimum temperature, and chronological time. Other variables such as age and exposure to environmental tobacco smoke were not significant and did not alter the results.

## RESULTS

Fig E1 presents the daily variation of ozone and PM<sub>2.5</sub> ambient levels and malondialdehyde levels in exhaled breath of children with asthma.

Table E2 presents the association of air pollution measurements and malondialdehyde levels. The association between malondialdehyde levels and different exposure metrics of pollutants (24-hour average, 8-hour moving average, or 1-hour maximum) were very similar for ozone and PM<sub>2.5</sub>.

Table E3 presents results of multipollutant models and models including 1 pollutant and traffic count as predictors of malondialdehyde levels in exhaled breath. When PM<sub>2.5</sub> and ozone were both entered in the regression models, only the effect of ozone remained significant ( $P = .04$ ). When either PM<sub>2.5</sub> or ozone and

traffic count were included in the same model, both the pollutant and traffic count remained significantly associated with malondialdehyde levels.

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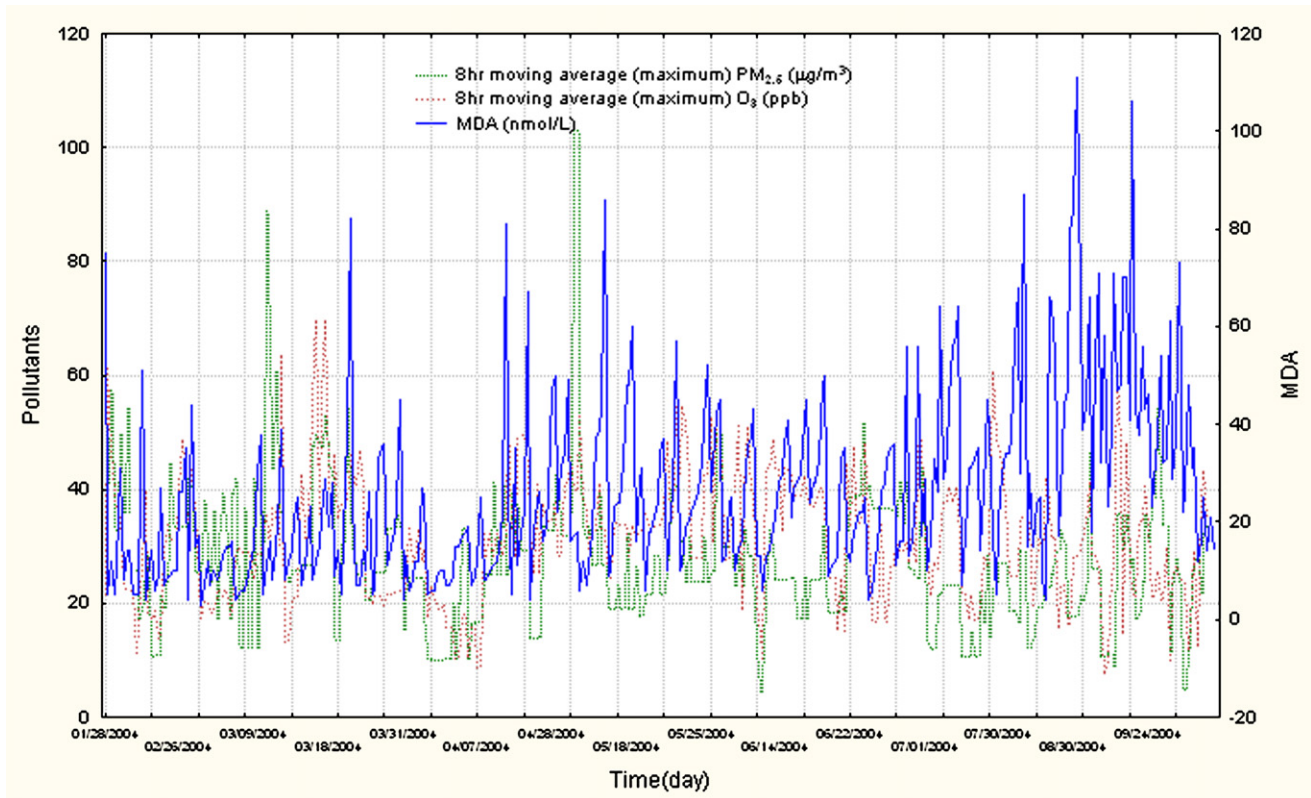


FIG E1. Daily levels of  $O_3$ ,  $PM_{2.5}$ , and malondialdehyde (MDA) during the study period.

**TABLE E1.** Correlation matrix between individual air pollutants

|                      | O <sub>3</sub> mx            | NO <sub>2</sub> mx    | PM <sub>2.5</sub> mx  | O <sub>3</sub> av     | NO <sub>2</sub> av    | PM <sub>2.5</sub> av  | O <sub>3</sub> ma     | NO <sub>2</sub> ma    | PM <sub>2.5</sub> ma |
|----------------------|------------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|
| O <sub>3</sub> mx    | 1                            |                       |                       |                       |                       |                       |                       |                       |                      |
| NO <sub>2</sub> mx   | <b>0.464</b><br><b>0.000</b> | 1                     |                       |                       |                       |                       |                       |                       |                      |
| PM <sub>2.5</sub> mx | 0.388<br><b>0.000</b>        | 0.529<br><b>0.000</b> | 1                     |                       |                       |                       |                       |                       |                      |
| O <sub>3</sub> av    | 0.811<br><b>0.000</b>        | 0.395<br><b>0.000</b> | 0.338<br><b>0.000</b> | 1                     |                       |                       |                       |                       |                      |
| NO <sub>2</sub> av   | 0.521<br><b>0.000</b>        | 0.821<br><b>0.000</b> | 0.476<br><b>0.000</b> | 0.383<br><b>0.000</b> | 1                     |                       |                       |                       |                      |
| PM <sub>2.5</sub> av | 0.463<br><b>0.000</b>        | 0.638<br><b>0.000</b> | 0.835<br><b>0.000</b> | 0.447<br><b>0.000</b> | 0.583<br><b>0.000</b> | 1                     |                       |                       |                      |
| O <sub>3</sub> ma    | 0.866<br><b>0.000</b>        | 0.451<br><b>0.000</b> | 0.371<br><b>0.000</b> | 0.983<br><b>0.000</b> | 0.465<br><b>0.000</b> | 0.480<br><b>0.000</b> | 1                     |                       |                      |
| NO <sub>2</sub> ma   | 0.519<br><b>0.000</b>        | 0.813<br><b>0.000</b> | 0.443<br><b>0.000</b> | 0.356<br><b>0.000</b> | 0.976<br><b>0.000</b> | 0.518<br><b>0.000</b> | 0.436<br><b>0.000</b> | 1                     |                      |
| PM <sub>2.5</sub> ma | 0.481<br><b>0.000</b>        | 0.656<br><b>0.000</b> | 0.864<br><b>0.000</b> | 0.443<br><b>0.000</b> | 0.592<br><b>0.000</b> | 0.987<br><b>0.000</b> | 0.480<br><b>0.000</b> | 0.540<br><b>0.000</b> | 1                    |

av, 24-h Average; ma, 8-h moving average (maximum); mx, 1-h maximum.

**TABLE E2.** Malondialdehyde and exposure to pollutants in children with asthma residing in Mexico City, 2004

| <b>LNMDA*</b>                                | <b>Interquartile range</b> | <b>Coefficient</b> | <b>SE</b> | <b>P value</b> |
|----------------------------------------------|----------------------------|--------------------|-----------|----------------|
| Model O <sub>3</sub> (ppb)                   |                            |                    |           |                |
| 24-h Average                                 | 12.7                       | 0.153              | 0.07      | .026           |
| 8-h Moving average (maximum)†                | 15.9                       | 0.151              | 0.07      | .021           |
| 1-h Maximum                                  | 46.0                       | 0.170              | 0.06      | .009           |
| Model NO <sub>2</sub> (ppb)                  |                            |                    |           |                |
| 24-h Average                                 | 10.3                       | 0.042              | 0.05      | .408           |
| 8-h Moving average (maximum)                 | 11.0                       | 0.056              | 0.05      | .261           |
| 1-h Maximum                                  | 23.9                       | 0.004              | 0.05      | .931           |
| Model PM <sub>2.5</sub> (μg/m <sup>3</sup> ) |                            |                    |           |                |
| 24-h Average                                 | 11.6                       | 0.088              | 0.05      | .082           |
| 8-h Moving average (maximum)                 | 14.2                       | 0.110              | 0.05      | .045           |
| 1-h Maximum                                  | 26.8                       | 0.054              | 0.05      | .253           |

\*LNMDA, Log (malondialdehyde). All models were adjusted for sex, school shift (morning or afternoon turn), minimum temperature, and chronological time. Model for O<sub>3</sub> includes 480 measurements of 107 subjects with asthma. Model for NO<sub>2</sub> includes 477 measurements of 107 subjects with asthma. Model for PM<sub>2.5</sub> includes 448 measurements of 91 subjects with asthma. All coefficients are calculated for a change of an interquartile range.

†Corresponds to the daily maximum of the 8-hour moving averages.

**TABLE E3.** Malondialdehyde and exposure to pollutants and traffic count in children with asthma residing in Mexico City, 2004

| <b>LNMDA*</b>                                                       | <b>Interquartile range</b> | <b>Coefficient</b> | <b>SE</b> | <b>P value</b> |
|---------------------------------------------------------------------|----------------------------|--------------------|-----------|----------------|
| Model 1, two pollutants                                             |                            |                    |           |                |
| 8-h Moving average (maximum) O <sub>3</sub> (ppb)†                  | 15.9                       | 0.145              | 0.07      | .043           |
| 8-h Moving average (maximum) PM <sub>2.5</sub> (μg/m <sup>3</sup> ) | 14.2                       | 0.056              | 0.06      | .353           |
| Model 2, O <sub>3</sub> (ppb) and traffic count                     |                            |                    |           |                |
| 8-h Moving average (maximum)                                        | 15.9                       | 0.151              | 0.07      | .026           |
| 4.5-h Average vehicular flow of heavy load                          | 50.6                       | 0.149              | 0.08      | .049           |
| Model 3, PM <sub>2.5</sub> (μg/m <sup>3</sup> ) and traffic count   |                            |                    |           |                |
| 8-h Moving average (maximum)                                        | 14.2                       | 0.120              | 0.06      | .033           |
| 4.5-h Average vehicular flow of heavy load                          | 50.6                       | 0.159              | 0.08      | .040           |

\*LNMDA, Log (malondialdehyde). Model 1 was adjusted for sex, school shift (morning or afternoon turn), minimum temperature, and chronological time, and includes 448 measurements of 91 subjects with asthma. Models 2 and 3 include 397 measurements of 75 subjects with asthma. Both models were adjusted for shift of the vehicular count (morning or afternoon), distances from house to vehicular count, minimum temperature, and chronological time. All coefficients are calculated for a change of an interquartile range.

†Corresponds to the daily maximum of the 8-hour moving averages.