Exhaled Nitric Oxide in Children with Asthma and Short-Term $PM_{2.5}$ Exposure in Seattle

Therese F. Mar,[1] Karen Jansen,[1] Kristen Shepherd,[2] Thomas Lumley,[2] Timothy V. Larson,[3] and Jane Q. Koenig[1]

[1]Department of Environmental Health and Occupational Sciences, [2]Department of Biostatistics, and [3]Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA

The objective of this study was to evaluate associations between short-term (hourly) exposures to particulate matter with aerodynamic diameters < 2.5 μm ($PM_{2.5}$) and the fractional concentration of nitric oxide in exhaled breath (Fe_{NO}) in children with asthma participating in an intensive panel study in Seattle, Washington. The exposure data were collected with tapered element oscillation microbalance (TEOM) $PM_{2.5}$ monitors operated by the local air agency at three sites in the Seattle area. Fe_{NO} is a marker of airway inflammation and is elevated in individuals with asthma. Previously, we reported that offline measurements of Fe_{NO} are associated with 24-hr average $PM_{2.5}$ in a panel of 19 children with asthma in Seattle. In the present study using the same children, we used a polynomial distributed lag model to assess the association between hourly lags in $PM_{2.5}$ exposure and Fe_{NO} levels. Our model controlled for age, ambient NO levels, temperature, relative humidity, and modification by use of inhaled corticosteroids. We found that Fe_{NO} was associated with hourly averages of $PM_{2.5}$ up to 10–12 hr after exposure. The sum of the coefficients for the lag times associated with $PM_{2.5}$ in the distributed lag model was 7.0 ppm Fe_{NO}. The single-lag-model Fe_{NO} effect was 6.9 [95% confidence interval (CI), 3.4 to 10.6 ppb] for a 1-hr lag, 6.3 (95% CI, 2.6 to 9.9 ppb) for a 4-hr lag, and 0.5 (95% CI, –1.1 to 2.1 ppb) for an 8-hr lag. These data provide new information concerning the lag structure between $PM_{2.5}$ exposure and a respiratory health outcome in children with asthma. *Key words:* airway inflammation, asthma, children, exhaled nitric oxide, particulate matter less than or equal to 2.5 μm, short-term exposure. *Environ Health Perspect* 113:1791–1794 (2005). doi:10.1289/ehp.7883 available via *http://dx.doi.org/* [Online 8 August 2005]

Most studies of relationships between particulate matter (PM) air pollution and health are based on 24-hr PM measurements. This approach has been driven mainly by the availability of 24-hr gravimetric monitors operated by the U.S. Environmental Protection Agency. However, there currently are several continuous PM monitors in use for documenting community exposure, and these data allow investigators to ask questions about very short-term (hourly) lags between health outcomes and PM exposure. It is important to understand the interval between exposure and health event (lag) as fully as possible because this may help our understanding of both the mechanisms underlying the event and the source of the PM.

Nitric oxide levels in airways are suggestive of the degree of airway inflammation and injury (Yates 2001; Bates and Silkoff 2003). The fractional concentration of NO in exhaled breath (Fe_{NO}) is easy to measure in exhaled breath and is a noninvasive lung measurement used to diagnose asthma (Jones et al. 2001; Kharitonov and Barnes 2000; Zeidler et al. 2004). Fe_{NO} is elevated in subjects with asthma, is elevated during an asthmatic attack (Jones et al. 2001; Silvestri et al. 2001; Yates 2001), and is reduced when subjects with asthma are treated with anti-inflammatory medications such as inhaled corticosteroids (ICS) (Beck-Ripp et al. 2002). Recently, we reported an association between 24-hr average PM with aerodynamic diameters < 2.5 μm ($PM_{2.5}$) and Fe_{NO} in children with asthma participating in a panel study in Seattle, Washington (Koenig et al. 2003). We observed an approximately 4-ppb average increase in Fe_{NO} for a 10-μg/m^3 increase in $PM_{2.5}$. Earlier studies also found that community outdoor air was associated with changes in Fe_{NO} (Van Amsterdam et al. 1999, 2000). More recently, Fe_{NO} has been associated with PM exposure in adults with cardiovascular and respiratory disease in Steubenville, Ohio (Adamkiewicz et al. 2004) and in adults with respiratory disease in Seattle (Jansen et al. 2004). The Steubenville study evaluated short-term exposures using moving-average data to reflect cumulative exposures. They reported associations between cumulative average $PM_{2.5}$ up to 12 hr before the Fe_{NO} measurement (Adamkiewicz et al. 2004).

The objective of this study was to compare short-term (hourly) exposures to PM with Fe_{NO} concentrations in children with asthma and to compare these short-term results with the earlier results. Our hypothesis was that short-term lags would show stronger associations with Fe_{NO} than would 24-hr average lags. Defining the most likely interval between exposure and Fe_{NO} response would be useful for designing future studies.

Materials and Methods

This research was part of an intensive exposure assessment and health effects panel study of susceptible subpopulations in Seattle from 1999 through 2002 (Koenig et al. 2003; Liu et al. 2003). Nineteen children, 6–13 years of age, were recruited from a local asthma and allergy clinic. All had physician-diagnosed asthma and were prescribed asthma medications daily or regularly. Each subject in the panel was asked to participate for a 10-day monitoring session in the winter of 2000–2001 and the spring of 2001. Fourteen children participated in the Fe_{NO} study during the winter heating season, and 15 children participated during spring. Ten participated in both seasons. Approximately half of the children were prescribed ICS therapy. The remainder was prescribed only inhaled albuterol as needed.

Exposure data. Hourly $PM_{2.5}$ data were collected at three fixed sites within the Seattle area by the local air agency with tapered element oscillating microbalances (TEOMs; Rupprecht and Patashnick Co./Thermo Electron, East Greenbush, NY). Descriptive statistics on covariate measurements are given in Table 1.

The average concentration of $PM_{2.5}$ from the TEOM monitors for all subjects stratified by season and ICS use are shown in Figure 1. Average $PM_{2.5}$ concentrations vary with exposure lag. $PM_{2.5}$ concentrations are higher in the winter sessions compared with spring sessions, with winter peaks occurring in the late evening/early morning hours (Fe_{NO} measurements were taken at or about 1600 hr Pacific standard time; see Figure 2). There is little difference in $PM_{2.5}$ exposure between ICS users and nonusers.

Exhaled NO. Fe_{NO} was collected as described in a previous report (Koenig et al. 2003). Briefly, all children participated for

Address correspondence to J.Q. Koenig, Department of Environmental Health and Occupational Sciences, Box 357234, Room F561a, University of Washington, Seattle, WA 98195-7234 USA. Telephone: (206) 543-2026. Fax: (206) 685-3990. E-mail: Jkoenig@u.washington.edu

This work was funded by the U.S. Environmental Protection Agency (EPA) (CR82717701), the Northwest Research Center for Particulate Air Pollution and Health (EPA grant CR827355), and National Institute for Environmental Health Sciences grant P30 ES07033.

This report has been subjected to agency review and approved for publication. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

The authors declare they have no competing financial interests.

Received 21 December 2004; accepted 8 August 2005.

10 continuous days of air pollution monitoring and health measurements. Exhaled breath was collected in a Mylar balloon at approximately 1600 hr each day using an offline FE_{NO} protocol. Exhaled breath was measured with a chemiluminescent nitrogen oxide analyzer (model 200A; API, San Diego, CA). Children were asked to refrain from eating for 1 hr before the exhaled breath collection. Pulmonary function testing was conducted after the exhaled breath because a deep inspiration may affect FE_{NO} values (Deykin et al. 1998). Subject characteristics and FE_{NO} measurements are presented in Table 2.

Statistical analysis. We assessed the association between short-term effects of particulate air pollution and FE_{NO} using a polynomial distributed lag (pdl) model for $PM_{2.5}$ up to 48 hr after exposure. The pdl model allows air pollution effects at many different lags to be estimated in the same model. The model assumes that the air pollution effect varies smoothly with lag, and approximates this smooth variation by a polynomial curve. The pdl model with 3 degrees of freedom is estimated by Poisson regression using a transformed set of three exposure variables that are not highly collinear. The three estimated coefficients specify the polynomial curve, which in turn gives associations at all lags. In addition to estimating the air pollution effect over many lags, the model can be used to estimate the total air pollution effect by summing the estimates at each lag (Schwartz 2000).

Pdl models are used with time-series data where the effects of a regressor are distributed over time. This type of model constrains the coefficients to follow a polynomial that reduces the number of parameters and therefore reduces the effects of collinearity in the lag variables. Similar models have been used to look at the effect of daily lags in air pollution exposure and mortality (Goodman et al. 2004; Schwartz 2000).

Equation 1 describes the model that was used for the analysis. Each pollution variable was modeled as a difference between the daily $PM_{2.5}$ level and the average exposure of the subject during his or her session because we are primarily interested in a within-subject, within-session effect. This model also included a term to account for the ambient concentrations of NO that could potentially contaminate our FE_{NO} measurements. Koenig et al. (2003) used a similar model to look at the within-subject effects of daily increases in $PM_{2.5}$ and FE_{NO}. Model estimates were obtained using the linear mixed-effect equations and the generalized least squares (GLS) estimator in Stata (version 6.0; StataCorp, College Station, TX). As a sensitivity analysis, model estimates were also obtained using a generalized estimating equations (GEE) with an exchangeable working correlation matrix and robust standard errors.

$$\begin{aligned} E[Y] = {} & B_0 + b_i + B_1\left(Z1_{ids} - \overline{Z1}_{is}\right) + B_2\text{med}_i \\ & + B_3\text{med}_i \times \left(Z1_{ids} - \overline{Z1}_{is}\right) \\ & + B_4\left(Z2_{ids} - \overline{Z2}_{is}\right) \\ & + B_5\text{med}_i \times \left(Z2_{ids} - \overline{Z2}_{is}\right) \\ & + B_6\left(Z3_{ids} - \overline{Z3}_{is}\right) \\ & + B_7\text{med}_i \times \left(Z3_{ids} - \overline{Z3}_{is}\right) \\ & + B_8\left(Z4_{ids} - \overline{Z4}_{is}\right) \\ & + B_9\text{med}_i \times \left(Z4_{ids} - \overline{Z4}_{is}\right) \\ & + B_{10}\left(W_{ids} - \overline{W}_{is}\right) + B_{11}RH \\ & + B_{12}\text{temp} + B_{13}\text{age}, \end{aligned} \quad [1]$$

where

$$Z1 = \sum_{n=1}^{24} PMlag_n,$$

$$Z2 = \sum_{n=1}^{24} n \times PMlag_n,$$

$$Z3 = \sum_{n=1}^{24} n^2 \times PMlag_n, \text{ and}$$

$$Z4 = \sum_{n=1}^{24} n^3 \times PMlag_n,$$

W is the ambient NO concentration, *ids* is the PM reading for individual i on day d during session s, *is* is the mean PM reading for a subject during a session, i is the mean PM reading for a subject during all of their sessions, med_i is an indicator variable for medication use (constant for each subject), and RH is relative humidity.

The coefficients for each lag term were obtained using

$$\text{Lag } n\gamma_n = B_1 + nB_4 + n^2B_6 + n^3B_8. \quad [2]$$

Table 1. Summary statistics for daily averages of temperature, relative humidity, and ambient NO.

	Minimum	Maximum	Mean ± SD
Temperature (°F)	33	68.7	44.5 ± 6.5
Relative humidity (%)	55.3	96.5	78.6 ± 10.1
Ambient NO (ppb)	0.003	0.099	0.018 ± 0.023

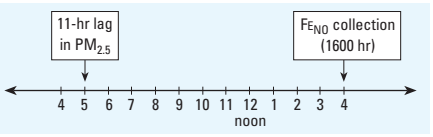

Figure 2. Schematic of real-time and hourly lags (0400 hr to 1600 hr) in $PM_{2.5}$ relative to FE_{NO} collection.

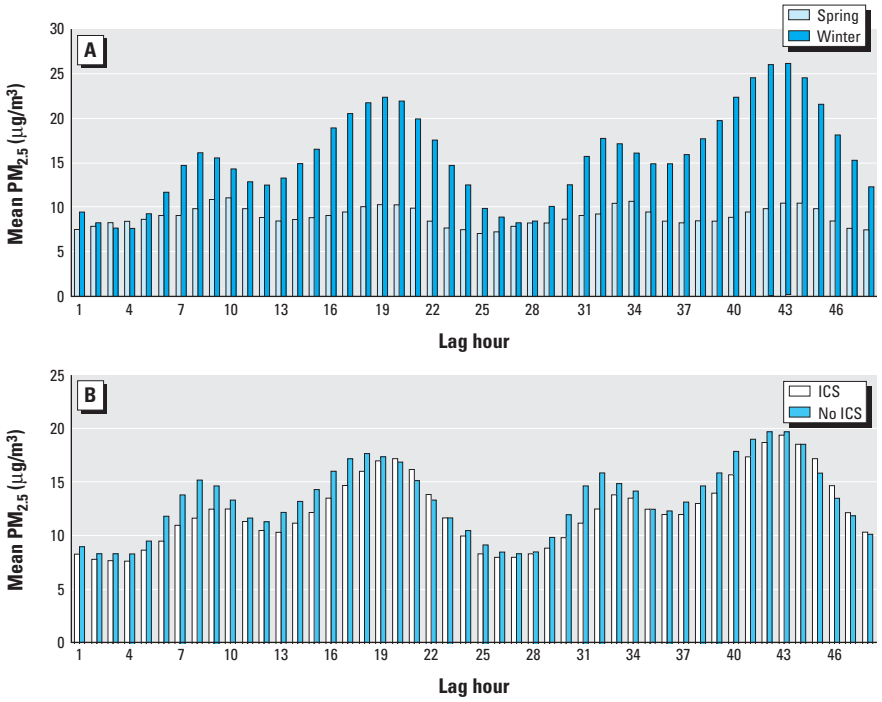

Figure 1. Comparison of mean $PM_{2.5}$ for all subjects stratified by season (*A*) or ICS medication use (*B*).

Results

The results of the polynomial distributed model for the short-term effect of $PM_{2.5}$ on FE_{NO} in subjects not taking ICS are shown in Figure 3A. Significant increases in FE_{NO} associated with $PM_{2.5}$ can be observed in the first 11 hr after exposure. There is also some suggestion of an increase in FE_{NO} between 38 and 41 hr after exposure. The overall effect of a prolonged exposure to $PM_{2.5}$ is obtained by summing up the estimated effects at each time lag. The sum of all the lag coefficients (β) over 48 hr was 7.0 ppb FE_{NO} per 10-μg/m^3 increase in $PM_{2.5}$.

The short-term effects of $PM_{2.5}$ on FE_{NO} for subjects who were prescribed ICS medications are shown in Figure 3B. In general, we found no association between FE_{NO} and $PM_{2.5}$ in subjects prescribed ICS. However, a very small association was observed from the 18-hr lag to the 30-hr lag. This small increase in FE_{NO} (ranging from 0.16 to 0.23 ppb per 10-μg/m^3 increase in $PM_{2.5}$) would not be of clinical significance. For ICS users, the overall effect of $PM_{2.5}$ over 48 hr is a 0.3-ppb increase in FE_{NO} per 10-μg/m^3 increase in $PM_{2.5}$.

The association between FE_{NO} and $PM_{2.5}$ averaged over 1 hr at various lags was also analyzed in a single-lag, linear mixed-effects regression model. These results are shown in Table 3. With the single-lag model where $PM_{2.5}$ was averaged over 1 hr, we found that 7.0-ppb and 6.3-ppb increases in FE_{NO} were associated with $PM_{2.5}$ lagged 1 and 4 hr, respectively, in subjects not taking ICS. No association was found in subjects taking ICS. No associations were found with a $PM_{2.5}$ exposure 8 hr previous in either group of children (Table 3).

We also tested for the lag structure in these data using a GEE model that controls for autocorrelations in the data (Figure 4). The distributed lag pattern was similar to that with the linear-effects model; however, associations between FE_{NO} and $PM_{2.5}$ dropped out for the earliest hourly lags (exposures at 1 and 2 hr before breath collection).

Discussion

The objective of this study was to evaluate the temporal relationship between prior exposure to $PM_{2.5}$ and increases in FE_{NO} in the airways of children with asthma. Using a pdl model, we found that FE_{NO} was associated with hourly averaged $PM_{2.5}$ exposure up to 10–12 hr before the health measurement in subjects not prescribed ICS. The overall effect was a 7-ppb increase in FE_{NO} associated with a 10-μg/m^3 increase in $PM_{2.5}$ relative to each subject's mean $PM_{2.5}$ exposure.

The advantage of using the pdl model is the ability to reduce the collinearity in the individual lags, allowing a better understanding of the relative contribution of individual lags and, in this case, the short-term effect of $PM_{2.5}$ exposure on FE_{NO}. The similarity in results from the analyses using the linear-effects model with the GLS estimator and those using the GEE model strengthens our confidence in these results (Table 3). It is apparent from Figure 1 that associations between $PM_{2.5}$ and FE_{NO} during the 48-hr period of analysis were not predicted by the average $PM_{2.5}$ concentration during that period, but rather by exposures up to 11 hr before FE_{NO} collection. These results are dependent on the pdl model used; different models (e.g., first- and second-degree pdl) may show associations with slightly different time patterns.

Additionally, using a single lag at specific time periods (1, 4, and 8 hr before FE_{NO} collection) for the children not prescribed ICS, we found a 7-ppb increase in FE_{NO} for a 10-μg/m^3 increase in $PM_{2.5}$ exposure 1 hr earlier and a 6.3-ppb increase associated with an 10-μg/m^3 increase in $PM_{2.5}$ 4 hr earlier. The estimate of FE_{NO} increase is similar to that seen in the pdl model; however, the multiple-hour curve gives more complete information. The limitation of using a single-lag model is that the estimated $PM_{2.5}$ effect at each of the lag hours could be confounded by the effect of other lag hours. Our single-lag model was based on 1 hr averaged $PM_{2.5}$ rather than a running average of $PM_{2.5}$ for a cumulative exposure effect. Although the pdl model is the preferred model, both the single-lag and the pdl models resulted in similar effect estimates. The results from our study are consistent with those reported by Adamkiewicz et al. (2004), who found increases in FE_{NO} significantly associated with $PM_{2.5}$ exposures up to 12 hr previously. That study, however, used individual hourly lag models.

The results from our analysis using the third-degree pdl model indicate that the effect of $PM_{2.5}$ on FE_{NO} is not just immediate but may have an effect up to 11 hr after exposure. Because in our study FE_{NO} was measured at approximately 1600 hr each day, this would indicate that $PM_{2.5}$ exposure from 0500 hr to 1600 hr (the time of FE_{NO} measurement) is the relevant period of exposure. Using our time line, this would suggest that sources that predominate during daytime hours are most important.

Table 2. Age and FE_{NO} values stratified by age, sex, and medication use.

	No.	Age (mean ± SD)	FE_{NO} Minimum	FE_{NO} Maximum	FE_{NO} Mean ± SD
Sex					
Female	5	11.2 ± 1.3	5	48.1	13.3 ± 6.3
Male	14	8.2 ± 1.7	5.3	79.8	16.2 ± 10.7
Medication use					
ICS	9	9.7 ± 1.4	5.3	79.8	12.7 ± 7.7
No ICS	10	8.3 ± 2.4	5	72.1	18.4 ± 11.0

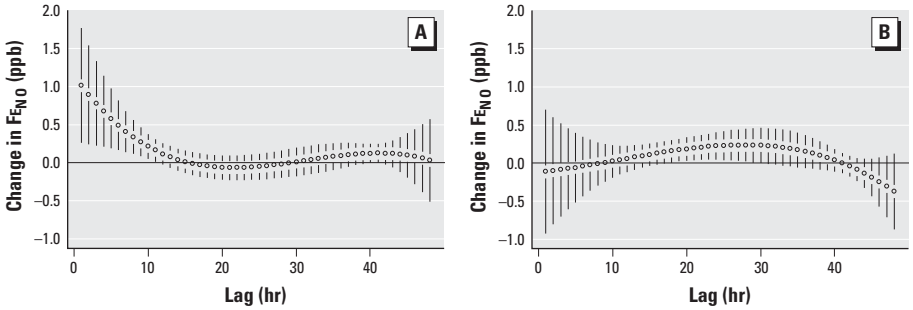

Figure 3. Change in FE_{NO} per 10-μg/m^3 increase in $PM_{2.5}$ (*A*) in subjects not prescribed ICS and (*B*) in subjects prescribed ICS therapy. TEOM readings were averaged from three central sites (Lynnwood, Lake Forest Park, and Kent) for hourly lags from 1 to 48. Model adjusted for temperature, relative humidity, and age. One-hour averaged $PM_{2.5}$ concentrations ranged from 8.3 μg/m^3 at 3-hr lag to 15.2 at 8-hr lag, suggesting that short time-lag periods rather than peak values may determine this health outcome. Error bars indicate 95% confidence intervals.

Table 3. Short-term effects of air pollution on FE_{NO} from the linear-effects model.

Metric	Medication use	Change in FE_{NO}	95% Confidence interval	*p*-Value
1-hr lag	No meds	6.99	3.43 to 10.55	0
	Meds	–0.18	–3.33 to 2.97	0.911
4-hr lag	No meds	6.30	2.64 to 9.97	0.001
	Meds	–0.77	–4.58 to 3.04	0.691
8-hr lag	No meds	0.46	–1.18 to 2.11	0.58
	Meds	0.40	–1.94 to 2.74	0.736

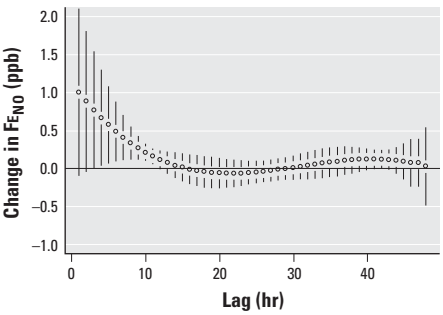

Figure 4. Change in FE_{NO} per 10-μg/m^3 increase in $PM_{2.5}$ in subjects not prescribed ICS therapy. TEOM readings averaged from three sites using GEE model. Error bars indicate 95% confidence intervals.

This is one of the first studies to report short-term temporal relationships between $PM_{2.5}$ and health outcomes in children with asthma. In another short-term study, hourly averages of PM were associated with respiratory symptoms in children with asthma (Delfino et al. 1998). More recently, that group, using personal monitors, reported that associations between PM and lung function derived from 1- or 8-hr $PM_{2.5}$ averages did not differ from associations based on 24-hr averages (Delfino et al. 2004). These findings add more information about the relationship between PM exposure and respiratory effects and may be useful for clinicians and patients. This information also may be informative for researchers in their experiment design efforts.

The relatively wide range of exposure lags associated with increased FE_{NO} in children with asthma that we observed suggests that more than one mechanism may be underlying changes in respiratory NO induced by air pollution. Rapid responses are associated with nervous system changes through nerve receptors or synaptic mediators, whereas delayed responses are sometimes attributed to up-regulation of gene expression and enzyme synthesis. These actions are compatible with up-regulation of NO, which has several roles in the lung (Deykin and Kharitonov 2003). Coincidentally, a recent study of allergen challenges in subjects with asthma found that FE_{NO} was initially decreased after exposure but increased 48 hr after exposure (Ricciardolo et al. 2003). Perhaps air pollution interactions in the airways differ from those of proteins such as allergens.

In conclusion, in this study we present additional data for the use of lag structure selection in epidemiologic studies of air pollution, an area that has received considerable attention. Future studies using sequential measurements of FE_{NO} will allow us to better identify the sources of and mechanisms underlying this health outcome.

References

Adamkiewicz G, Ebelt S, Syring M, Slater J, Schwartz J, Suh H, et al. 2004. Association between air pollution exposure and exhaled nitric oxide in an elderly panel. Thorax 58:242–245.

Bates CA, Silkoff PE. 2003 Exhaled nitric oxide in asthma: from bench to bedside. J Allergy Clin Immunol 111:256–262.

Beck-Ripp J, Griese M, Arenz S, Koering C, Pasqualoni B, Bufler P. 2002. Changes of exhaled nitric oxide during steroid treatment of childhood asthma. Eur Respir J 19:1015–1019.

Delfino R, Quintana P, Floro J, Gastanaga V, Samimi B, Kleinman M, et al. 2004. Associations of FEV1 in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. Environ Health Perspect 112:932–941.

Delfino RJ, Zeigr RS, Seltzer JM, Street DH. 1998. Symptoms in pediatric asthmatics and air pollution: differences in effects by symptom severity, anti-inflammatory medication use, and particulate averaging time. Environ Health Perspect 106:751–761.

Deykin A, Halpern O, Massro AF, Draxen JM, Israel E. 1998 Expired nitric oxide after bronchoprovocation and repeated spirometry in patients with asthma. Am J Respir Crit Care Med 157:769–775.

Deykin A, Kharitonov SA. 2003. Nitric oxide. In: Asthma and COPD (Barnes P, Drazen J, Rennard S, Thomson N, eds). New York:Academic Press, 307–314.

Goodman PG, Dockery DW, Clancy L. 2004. Cause-specific mortality and the extended effects of particulate pollution and temperature exposure. Environ Health Perspect 112:179–185.

Jansen K, Koenig JQ, Larson TV, Fields C, Mar TF, Stewart J, et al. 2004 Nitric oxide in subjects with respiratory disease is associated with PM2.5 and black carbon in Seattle [Abstract]. Am J Respir Crit Care Med 169:A282.

Jones SL, Kittelson J, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, et al. 2001. The predictive values of exhaled nitric oxide measurements in assessing changes in asthma control. Am J Respir Crit Care Med 164:738–743.

Kharitonov SA, Barnes PJ. 2000. Clinical aspects of exhaled nitric oxide. Eur Respir J 16:781–792.

Koenig JQ, Jansen K, Mar TF, Lumley T, Kaufman J, Trenga CA, et al. 2003. Measurement of offline exhaled nitric oxide in a study of community exposure to air pollution. Environ Health Perspect 111:1625–1629.

Liu L-JS, Box M, Kalman D, Kaufman J, Koenig JQ, Larson T, et al. 2003. Exposure assessment of particulate matter for susceptible populations in Seattle, WA. Environ Health Perspect 111:909–918.

Ricciardolo FLM, Timmers MC, Sont JK, Folkerts G, Sterk PJ. 2003. Effect of bradykinin on allergen induced increase in exhaled nitric oxide in asthma. Thorax 58:840–845.

Schwartz J. 2000. The distributed lag between air pollution and daily death. Epidemiology 11:320–326.

Silvestri M, Sabatini F, Spallarossa D, Fregonese L, Battistini E, Biraghi MG, et al. 2001. Exhaled nitric oxide levels in non-allergic and allergic mono- or poly-sensitised children with asthma. Thorax 56:857–862.

Van Amsterdam JG, Nierkens S, Vos SG, Opperhuizen A, van Lovernen H, Steerenberg PA. 2000. Exhaled nitric oxide: a novel biomarker of adverse respiratory health effects in epidemiological studies. Arch Environ Health 55:418–423.

Van Amsterdam JG, Verlaan BPJ, van Lovernen H, Elzakker BGV, Vos SG, Opperhuizen A, et al. 1999. Air pollution is associated with increased level of exhaled nitric oxide in nonsmoking healthy subjects. Arch Environ Health 54:331–335.

Yates DH. 2001. Role of exhaled nitric oxide in asthma. Immunol Cell Biol 79:178–190.

Zeidler MR, Kleerup EC, Tashkin DP. 2004. Exhaled nitric oxide in the assessment of asthma. Curr Opin Pulm Med 101:31–36.