ERJ Express. Published on March 15, 2006 as doi: 10.1183/09031936.06.00143905

Short-term effects of nitrogen dioxide on mortality: an analysis within the APHEA project

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Running title: Effects of nitrogen dioxide on mortality

Abstract

We investigated the short-term effects of nitrogen dioxide (NO_2) on total, cardiovascular and respiratory mortality in 30 European cities participating in the APHEA-2 project.

The association was examined using hierarchical models implemented in two stages. In the first stage data from each city were analyzed separately, whereas in the second stage the city specific air pollution estimates were regressed on city specific covariates to obtain overall estimates and to explore sources of possible heterogeneity.

We found a significant association of NO_2 with total, cardiovascular and respiratory mortality, with stronger effects on cause-specific mortality. There was evidence of confounding in respiratory mortality with black smoke and sulphur dioxide. The effect of NO_2 on total and cardiovascular mortality was observed mainly in western and south European cities and was larger when smoking prevalence was lower and household gas consumption was higher. The effect of NO_2 on respiratory mortality was higher in cities with larger proportion of elderly persons in the population and higher levels of PM_{10}.

The results of this large study are consistent with an independent effect of NO_2 on mortality, but the role of NO_2 as a surrogate of other unmeasured pollutants cannot be completely ruled out.

Key words: Air pollution, heterogeneity, modelling, mortality, nitrogen dioxide.

Introduction

Many epidemiological studies have documented adverse short-term effects of different types of air pollution on health outcomes in recent years (1-5). The pollution indicators used were mainly ambient particles (4,6,7) but gaseous pollutants such as NO_2, O_3 and CO have also been shown to have adverse effects on mortality and morbidity (3,8,9,10). The results from epidemiological studies have led to revisions of air quality guidelines and standards and scheduled dates for regular revisions in the future (11-13).

Nitrogen oxides (NO_x), primarily nitric oxide (NO), are produced from high-temperature combustion, such as when fuel is burned in motor vehicles and power plants. Nitric oxide, once emitted, relatively rapidly reacts with ozone or oxygen to form nitrogen dioxide (NO_2). This usually dominating part is known as secondary NO_2, but the transformation mainly takes part already close to the source. Primary NO_2 emissions are particularly important from diesel vehicles, and this fraction seems in Europe to be increasing. Due to non-linearities in the oxidation reactions and variations in the background ozone levels, NO_x has a stronger temporal correlation than NO_2 has with other combustion products (exhaust particles, CO, SO_2) emitted at the same time from cars and other sources. Indoor air can also be contaminated with high levels of NO_2, since unventilated heaters and gas stoves also emit substantial amounts of NO_2.

In spite of laboratory, clinical, and epidemiological research, the health effects of NO_2 exposure on humans are not well understood. The toxicological evidence suggests that increased susceptibility to infection, functional deficits from effects on airways, and

deterioration of the status of persons with chronic respiratory conditions, including asthmatics, are of potential concern. NO_2 is a highly reactive, nitrogen-centred free radical, poorly water-soluble gas deposited peripherally in the lungs. It is absorbed along the entire respiratory tract, but exposure studies indicate that the major target site for the action of NO_2 is the terminal bronchioles. The main mechanism of NO_2 toxicity has been suggested to involve lipid peroxidation in cell membranes and various actions of free radicals on structural and functional molecules. Antioxidants such as ascorbic acid and a-tocopherol appear to play a protective role (14). NO_2 induces an airway inflammation probably restricted to the smaller airways and the terminal bronchioles, at least after a single exposure (15). The main effect of NO_2 in human exposure studies has been on bronchial responsiveness, usually seen at concentrations of at least 1800 $\mu g/m^3$ in healthy subjects and about 200-500 $\mu g/m^3$ in patients with asthma (16) or COPD (17). NO_2 also has an amplifying effect on the asthmatic response to allergen exposure. A short (15-30 min) exposure to 500 $\mu g/m^3$ seems to increase the reaction. Some data suggest that exposure to NO_2 at concentrations occurring in highly trafficked areas (15 min at 500 $\mu g/m^3$) can enhance allergic inflammatory reaction in the airways without causing symptoms or pulmonary dysfunction (18).

Most epidemiological studies on the health effects of NO_2 focused on morbidity rather than mortality. We have investigated the short term effects of NO_2 on mortality within the Air Pollution and Health: A European Approach 2 (APHEA-2) project that uses an extensive European database from 30 European cities. Special attention was paid to efforts to distinguish the effects of NO_2 per se from confounding or modifying effects of other pollutants, such as particles.

Data and Methods

Data

APHEA-2 is a multi-center project including 30 cities across Europe and associated regions which studies short-term health effects of air pollution. Data were collected on daily counts of all-cause mortality excluding deaths from external causes (International Classification of Disease ICD-9>800), cardiovascular mortality (ICD-9: 390-459) and respiratory mortality (ICD-9:460-519). The data covered at least 3 consecutive years for each city within the years 1990 to 1997. In all, 2,893,430 deaths occurred in the studied cities during that period. Details about the data have been published elsewhere (5).

Daily air pollution measurements were provided by the monitoring networks established in each town participating in the APHEA-2 project (5). Time series data on daily temperature (^{o}C, daily mean) and relative humidity (%) were used to control for the potential confounding effects of weather. External information on influenza epidemics or other unusual events (heat waves, strikes, etc) was also collected, if available (5).

Table 1 presents descriptive characteristics of the data. The total population exposed is more than 60 million. The Netherlands is considered as one urban area because of its relative small size and dense population. The mean daily total number of deaths ranged from 6 to 342. For respiratory mortality, daily counts ranged from <1 to 29. The mean levels of NO_2 (1h max) ranged from 46 $\mu g/m^3$ to 155 $\mu g/m^3$. In the various cities, the correlation between NO_2 and PM_{10} ranged from 0.11 to 0.69, between NO_2

and black smoke (BS) it ranged from 0.11 to 0.78, between NO_2 and SO_2 from 0.15 to 0.87 and between NO_2 and O_3 from -0.21 to 0.31. For the four Polish cities included in the analysis (i.e. Cracow, Poznan, Lodz, Wroclaw) only NO_2 cumulative 24h measurements were available. In order to include them in the analysis, the NO_2-1h values were estimated as 1.64 times the 24h values, were 1.64 is the average of the ratio between the two measurements in the cities that provided both. There was a substantial variability among all cities in the study in the levels of all pollutants, as well as in the mean daily temperature and humidity.

Methods

Within the APHEA-2 project we analyzed the maximum hourly NO_2 concentration of each day (NO_2-1h) rather than the average concentration of NO_2 over 24 hours (NO_2-24h) since more cities provided measurements for the former. The maximum daily 1 and 24-hour NO_2 concentrations are highly correlated (the correlation coefficient ranges from 0.80 to 0.94, with a median of 0.90). In the present study we decided a priori to use the average of lags 0 and 1 for NO_2, for all cities, since there is evidence that the average of two days' pollution correlates better with mortality than a single day's exposure (19). Furthermore, this approach avoids potential bias which could result from selectively reporting the most significant lags. In this analysis we assumed a linear dose-response relationship between NO_2-1h and mortality. We based this assumption on previously published results from the APHEA-2 project indicating that the dose response relationship could be adequately approximated by a linear association (20, 21). To investigate the NO_2 effect over a larger span of days and examine the shape of the association with each analyzed health outcome we fitted polynomial distributed lag models for the NO_2, using lags 0 to 5 (19).

For the analysis, we used a hierarchic modeling approach. First, we fit regression models in each city separately to allow specific control for seasonal effects, weather and other potential confounders. We then used the individual city results in a second-stage analysis to obtain overall estimates and to investigate potential effect modifiers.

Individual city analysis

The pollution-mortality associations for each city were investigated using Poisson regression models allowing for overdispersion (22). We used smooth functions to control for potential confounding effects of seasonality, long-term trends and meteorological variables (mean daily temperature and mean daily relative humidity). A linear term for the pollutant was introduced in the model. We used the penalized regression splines as smoothing functions, as implemented by Wood in R (23). We also included dummy variables for the day of the week effect, holidays and influenza epidemics.

We followed the general methodological guidelines developed within the framework of the APHEA-2 project, described in detail elsewhere (22). One additional feature is the use of penalized regression splines instead of the non-parametric function loess as smoothing functions to control for possible confounding. The smooth functions of time serve as a proxy for any time-dependent outcome predictors or confounders with long term trends and seasonal patterns not explicitly included in the model. Hence we remove long term trends and seasonal patterns from the data to guard against this confounding by omitted variables. Weather variables, which are potential confounders, were included explicitly. In particular, same day temperature and humidity and a lagged value of these meteorological variables were included in the

models. We used thin plate regression splines as basis functions for the penalized regression splines (24). Based on our experiences from the previous analyses of the APHEA-2 data, we chose the number of basis functions to be 40 for the time variable and 10 for the weather variables. We then chose the smoothing parameters that minimized the absolute value of the sum of partial autocorrelations (PAC's) of the residuals from lags three to 30. To account for serial correlation in the residuals where it remained in the final model, autoregressive terms were added into the model as appropriate (25). In the special case of the small cities (and especially in cause-specific mortality) we required a minimum one degree of freedom per year.

We used the APHEA-2 method for influenza control, including a dummy variable taking the value of one when the seven-day moving average of the respiratory mortality was greater than the 90-th percentile of its city-specific distribution. Since influenza control as described was based on the distribution of respiratory mortality, we included the influenza dummy variable only when we analyzed total and cardiovascular mortality (22). Based on previously published results (26, 27) there is no indication that omitting control for influenza when we analyzed respiratory mortality would influence the association between air pollution and mortality. It is unclear why the specific time within a winter that an epidemic occurs in a particular city should have much to do with air pollution levels and hence confound the relation under investigation. Nevertheless, to further explore the potential confounding effects of influenza on respiratory mortality we also analyzed respiratory mortality for days below the 90^{th} percentile of its distribution.

To evaluate how sensitive our results are to the choice of the degree of smoothing we also applied models with +/- 25% of the degrees of freedom for the time smooth that were chosen based on the PAC criterion.

To investigate potential confounding effects of the daily levels of other pollutants, we also applied two pollutant models, i.e. we included in the model NO_2 and alternatively PM_{10} (24h-mean), SO_2 (24h-mean) or O_3 (maximum of 8h-means).

Some studies have demonstrated the effects of air pollution on mortality are spread over more than two days (28, 29). To examine this question, we fit a separate distributed lag model for each city. We used a cubic polynomial distributed lag with lags out to five days before the deaths, which has proven adequate in past studies (28). Therefore we estimated in each city the coefficients for the cubic polynomial that define the shape of the distributed lag.

Second-stage analysis

We assumed the city specific effect estimates (for the mean of lags 0 and 1) to be normally distributed around an overall estimate. We estimated fixed effects pooled regression coefficients by weighted regression of city-specific estimates on potential effect modifiers (at city level) with weights inversely proportional to their city-specific variances. If substantial heterogeneity remained among city-results beyond the variation explained by the effect modifiers, random-effects regression models were applied. In these models, it was assumed that the true city-specific coefficients are a sample of independent observations from the Normal distribution with mean equal to the random effects pooled estimate and variance equal to the between-cities

variance. The random variance component was estimated using the method of moments (30). In order to combine the city specific coefficients from the polynomial distributed lags models, we used the multivariate maximum likelihood method (31).

Results

Table 2 shows the percentage increase in the daily number of deaths associated with 10 μg/m^3 increase in NO_2 levels, as well as the corresponding figures adjusting for seasonality using +/-25% of the number of degrees of freedom determined by the PAC criterion. Because there was significant heterogeneity in the single-city results, pooled estimates using random effects models are also shown. For 10 μg/m^3 increase in the daily NO_2 concentrations, the increase for the total deaths was 0.30% (95% confidence intervals (CIs): 0.22%, 0.38%), for cardiovascular mortality the associated increase was 0.40% (0.29%, 0.52%) and for respiratory mortality 0.38% (0.17%, 0.58%). When we adjusted for seasonality using more degrees of freedom, the associated effect in all studied outcomes decreased by less than 13% compared with the baseline model. When we adjusted for seasonality using fewer degrees of freedom, the associated effect in all studied outcomes increased by less than 16%. When we analyzed respiratory mortality restricted to days below the 90th percentile of its distribution, the pooled effect was slightly increased (associated estimate 0.41% CI: (0.25%, 0.58%)), supporting the hypothesis of absence of confounding by influenza epidemics.

Figure 1 shows the increase in total mortality and its 95% CIs associated with an increase of 10 μg/m^3 in the levels of NO_2 using the average of 0, 1 (black lines) or polynomial distributed lag models for lags 0-5 (red lines) for each city included in the analysis as well as the combined results. We did not apply distributed lag models in

Bucharest because of systematically missing exposure data. Statistically significant results for single cities ranged from 0.20% in Madrid to 1.14% in Wroclaw for the baseline model, and from 0.32% in Paris to 1.30% in Wroclaw for the distributed lags model. The overall increase in total mortality from the distributed lags model was higher by 23% compared with the effect from the baseline model.

Figure 2 shows the per city and combined increase in cardiovascular mortality and its 95% CIs associated with an increase of 10 $\mu g/m^3$ in the levels of NO_2, when we analyzed either two (black lines) or six (red lines) days of exposure. Statistically significant results for single cities ranged from 0.33% in Tel-Aviv to 1.58% in Wroclaw for the baseline model, and from 0.58% in Netherlands to 1.97% in Wroclaw for the distributed lags model. The overall increase in cardiovascular mortality from the distributed lags model was higher by 22% compared with the effect from the baseline model.

Figure 3 shows the per city and combined increase in respiratory mortality and its 95% CIs associated with an increase of 10 $\mu g/m^3$ in the levels of NO_2, when we analyzed either two (black lines) or six days (red lines) of exposure. Statistically significant results for single cities ranged from 0.92% in Torino to 2.88% in Ljubliana for the baseline model, and from 0.98% in Milano to 5.25% in Geneva for the distributed lags model. The overall increase in respiratory mortality from the distributed lags model was higher by 45% compared with the effect from the baseline model.

Figure 4 presents the shape of the association of total and respiratory mortality with NO_2 over 6 days (lags 0 to 5) combined for all cities using a cubic polynomial distributed lag model. The shape of association with cardiovascular mortality is not displayed since it was analogous to the one observed for total mortality. In the case of total mortality the highest effects are observed in lags 1 and 2. From there on, there is a decrease in the effect but in the final lag 5 the effect of the pollutant on mortality appears to increase again. This S-shape is not so apparent in the case of respiratory mortality where the effect of NO_2 seems to persist over more days, and this pattern is reflected in the higher size of the cumulative exposure effect compared with 0-1 lags seen for this outcome.

Table 3 presents results from two-pollutant models, adjusting in turn for the confounding effects of black smoke (BS), PM_{10}, SO_2 and O_3. NO_2 associations with total and cardiovascular mortality are not confounded by any of these pollutants. The association with respiratory mortality was substantially confounded by BS and more so by SO_2 levels. When adjusting for BS, the estimated increase in respiratory mortality associated with an increase of 10 $\mu g/m^3$ in the levels of NO_2 was reduced by 32%, and it even decreased by 50% and became non-significant when adjusting for SO_2.

We investigated the observed heterogeneity in the effect estimates of NO_2 by examining potential effect modifiers in second stage regression models. Potential effect modifiers used in the APHEA-2 analysis included variables describing the air pollution level and mix in each city, the health status of the population, the geographical area and the climatic conditions (5). Table 4 shows the resulting

estimated NO_2 effect (that is, the increase in mortality and its 95% CIs per 10 $\mu g/m^3$ increase in the daily levels of NO_2) for two cities characterized by a value of the effect modifier equal to the first and the third quartile of the respective distribution. Among the potential effect modifiers, only those explaining more than 10% of the heterogeneity are presented.

When investigating the source of heterogeneity in the association between NO_2 and total and cardiovascular mortality, the most important effect modifier was the geographical area (defined as Western, South and Central Eastern European cities), followed by the prevalence of smoking in the city. More specifically in cities with lower prevalence of smoking the effect of NO_2 on total and cardiovascular mortality was greater. The highest effect of NO_2 on total and CVD mortality was in north-western cities, followed closely by the effect in southern European cities, while there was only a small and non-significant effect in eastern cities. Concerning cardiovascular mortality there was additional evidence that household consumption of natural gas acted as an effect modifier, with higher NO_2 effect where the consumption was higher. Finally, in the association with respiratory mortality the most important effect modifier were the median levels of PM_{10} followed by the proportion of the elderly (i.e. above 65 years) in the population of the city. In cities with high median PM_{10} levels and a high proportion of elderly there was a stronger effect of NO_2 on respiratory mortality.

Discussion

In studies published during the recent decades NO_2 has been associated with decrease in lung function, increase in respiratory symptoms and increase in asthma and COPD

hospital admissions. Most of the epidemiological studies of short-term effects of NO_2 on health were focused on symptoms reported in diaries, on hospitalization for respiratory diseases, and on decrease of pulmonary function (32-35). A few studies have been conducted on the effects of photochemical air pollution on mortality (36-39). We have used the most extensive European database available today to investigate the potential effects of NO_2 on mortality.

We have found significant adverse health effects of NO_2 on total, cardiovascular and respiratory mortality, with stronger effects on cause-specific mortality. These findings complement those previously reported from APHEA-1, which was the first part of the APHEA project and included a smaller number of cites (i.e only 6 compared with 30 cites included in the APHEA-2 analysis). As part of that analysis, Touloumi et al (3) have reported significant positive associations between NO_2 and daily total deaths. In that first part of the APHEA analysis an increase per 10 $\mu g/m^3$ in NO_2 was associated with 0.26% increase in total mortality compared with 0.30% increase reported in our results. Kinney and Ozkaynak (36) also found significant association of NO_2 with total and cardiovascular mortality in Los Angeles County. However, no significant association was observed for respiratory deaths. The authors suggested that the small number of deaths from respiratory causes may have limited the power to detect small pollution effects. In our analysis, where power is gained by use of multiple locations, there is a stronger effect on respiratory mortality compared with total mortality. However, the associated standard error is larger and the effect decreases and becomes non-significant when controlling for BS and SO_2. Finally, in the analysis of 20 U.S. cities within the NMMAPS project no consistent pattern of association between total mortality and NO_2 was found (40). The investigators found a positive but not

statistically significant effect of NO_2 at lag 0 and at lag1 but a highly statistically significant result at lag 2. The difference between the NMMAPS and APHEA findings may be attributed to the varying air pollution sources and mixture in Europe and the U.S.

In this study, the cumulative effect over six days was larger by about 22% for total and cardiovascular mortality and by 45% for respiratory mortality compared with the average exposure over two days. This indicates that previous estimates of NO_2 effects may in fact represent an underestimation if they take into account only very short-term health effects. Moreover, when the shape of the association between mortality and NO_2 is considered, two different patterns can be distinguished: total and cardiovascular mortality is clearly more affected by NO_2 levels on the two previous days, whereas for respiratory mortality the effects are more evenly distributed over the 6 previous days. This difference may be explained by differences in the biological mechanisms underlying the health effects of NO_2.

Although the above results indicate adverse effects of NO_2 on mortality, the independence of its effect from those of other pollutants is still unclear. A single pollutant could act as a marker of a pollution mixture. Hence, NO_2 could be a marker of other pollutants generated by vehicle exhausts such as particles. We have tried to estimate the independent effects of pollutants using two pollutant models, for those pollutants for which data were available. We have found no evidence of confounding for total and cardiovascular mortality. There was evidence of confounding by BS and SO_2 in the effect on respiratory mortality. A possible explanation is that the BS- and SO_2- adjusted effect estimates of NO_2 on respiratory mortality may reflect, to a larger

extent, the effects from sources other than traffic. BS is more specific for traffic related particles than PM_{10} and provides a means of addressing the question of particle composition. It was impossible to control for indices for which no measurements were available such as number of particles. Further study focusing on exposure to mixtures including NO_2, BS and SO_2 is needed to further our understanding of the etiological mechanism through which the pollutants affect mortality and in particular respiratory mortality.

In order to further investigate the independence of the NO_2 effects and contribute to the ongoing discussion on the possible confounding effect of smaller particle sizes we have used the $PM_{2.5}$ median level for nine of the analyzed cities as potential effect modifier (41). The $PM_{2.5}$ median level did not act as significant effect modifier and reduced the heterogeneity by less than 10 % in each of the studied outcomes. Besides if NO_2 truly reflects $PM_{2.5,}$ then in cities with higher ratio $PM_{2.5}/PM_{10}$ we would expect higher NO_2/PM_{10} levels. This scenario was not verified in four cities with relevant data (Athens, Birmingham, Helsinki, Amsterdam -RUPIOH project, unpublished data). Finally in these four cities the correlations between PM_{10} and $PM_{2.5}$ ranged from 0.58 to 0.95. These high correlations indicate that controlling for PM_{10}, as in our two-pollutants analysis, is largely equivalent to controlling for $PM_{2.5}$. However, the data used in this approach are limited to only a few cities.

Seaton et al (42) suggest that NO_2 is a surrogate for particle numbers. Their hypothesis is illustrated using data only from Aberdeen where the correlation of NO_2 with PM_{10} is 0.45, with $PM_{2.5}$ 0.55 and with particle numbers 0.89. However, this correlation is fairly high. In a recent three-city study (43), the correlation between

ultrafine particles and NO_2 were 0.49, 0.72 and 0.82. Therefore, in many cases it is possible to separate the effects of NO_2 and ultrafine particles. Using information from four European cities (RUPIOH project, unpublished data) in our analysis that provided relevant data the correlation between NO_2 and PM_{10} ranged from 0.38 to 0.54, between NO_2 and $PM_{2.5}$ from 0.43 to 0.63, between NO_2 and particle numbers from 0.53 to 0.72 and between PM_{10} and particle numbers from 0.19 to 0.54. Hence in our data the associations between NO_2 and particle numbers are not so high to make the distinction of the effects impossible as in the case of Seaton et al. It should be noted that among these four cities, the one with the highest NO_2-particle numbers correlation (Helsinki) had the lowest estimated NO_2 effect on total mortality.

We investigated several environmental and social factors that might explain variation between cities in the effect estimates. It was found that when smoking prevalence is lower, the effect of NO_2 on total and cardiovascular mortality is larger. A possible explanation may be that smoking acts as a competing risk, harvesting the population of susceptible individuals, but this issue needs further investigation. Besides the effect of NO_2 on total and cardiovascular mortality was observed mainly in western and south European cities. In more than half of the eastern European cities involved in the analysis the NO_2-1h levels were in the lower range of the observed distribution. Domestic gas consumption as an indicator of domestic NO_2 exposure acted as potential effect modifier in cardiovascular mortality, indicating that the effect of outdoor NO_2 exposure increased with higher indoor exposure. The risk contrast is not so pronounced and it is difficult to believe that indoor pollution levels act as an effect modifier for the association of outdoor NO_2 levels and mortality, unless the slope becomes steeper at higher cumulative exposures. Another likely explanation may lie

in the association of higher gas consumption in the more developed cities with characteristics of air pollution sources (eg more traffic). Finally the effect of NO_2 on respiratory mortality was higher in cities with larger proportion of elderly persons in the population, for whom there is evidence of increased susceptibility to other pollutants. For cities with higher PM_{10} levels larger NO_2 effects were estimated which may mean that the two pollutants act synergically. One limitation of our second stage analysis is that there is a noticeable difference in the effects between Eastern European and other cities. Hence any variables, such as the mortality rate, etc that strongly differ between the two regions are likely to appear as significant explanatory factors of the heterogeneity in the effect estimates. Or alternatively, if the effect modifiers identified are indeed true modifiers, their distribution across Europe may result in these marked geographical differences.

In conclusion, our results confirm those previously reported about the adverse effects of NO_2 on mortality and complement them by investigating potential confounding by other pollutants and possible effect modification. The results of this large study are consistent with an independent effect of NO2 on mortality but the role of NO_2 as a surrogate of other unmeasured pollutants cannot be completely ruled out. Since the short-term effects of NO_2 on respiratory mortality may be confounded by other vehicle-derived pollutants, further investigation is needed to enhance our understanding of the underlying biological mechanism.

Acknowledgments:

This work was funded through two grants from the European Commission (E.C.) Environment and Climate Programme (Contract numbers: ENV4-CT97-0534 and QLK4-CT-2001-30055).

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Figure legends

Figure 1. Percent increase in total mortality and its 95% confidence intervals associated with an increase of 10 $\mu g/m^3$ in the levels of NO_2, using the average of lags 0-1 (black line) or polynomial distributed lags models for lags 0-5 (red line). The boxes represent the inverse of the squared standard error.

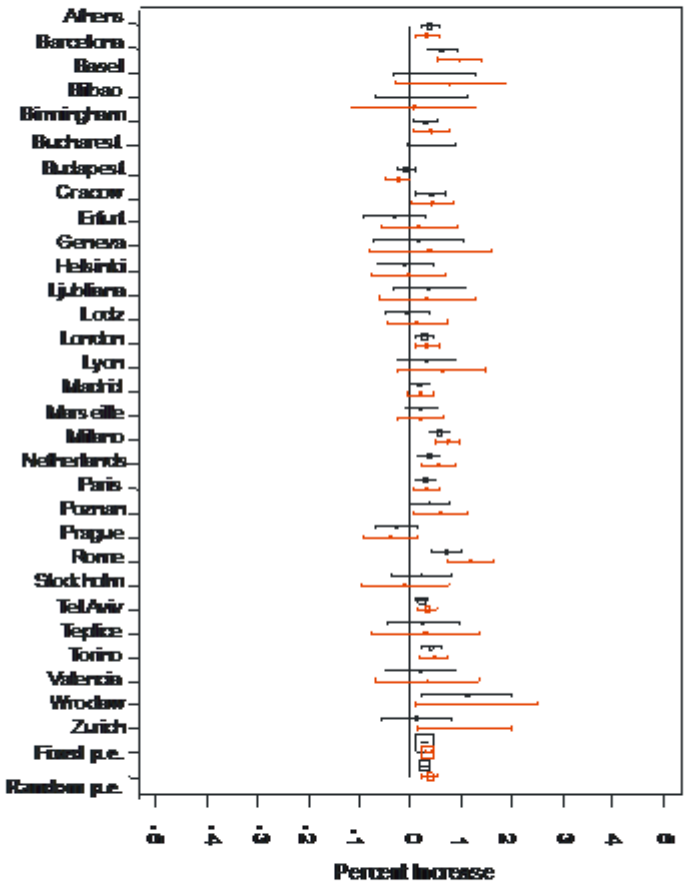


Figure 2. Percent increase in cardiovascular mortality and its 95% confidence intervals associated with an increase of 10 μg/m^3 in the levels of NO_2, using the average of lags 0-1 (black line) or polynomial distributed lags models for lags 0-5 (red line). The boxes represent the inverse of the squared standard error.

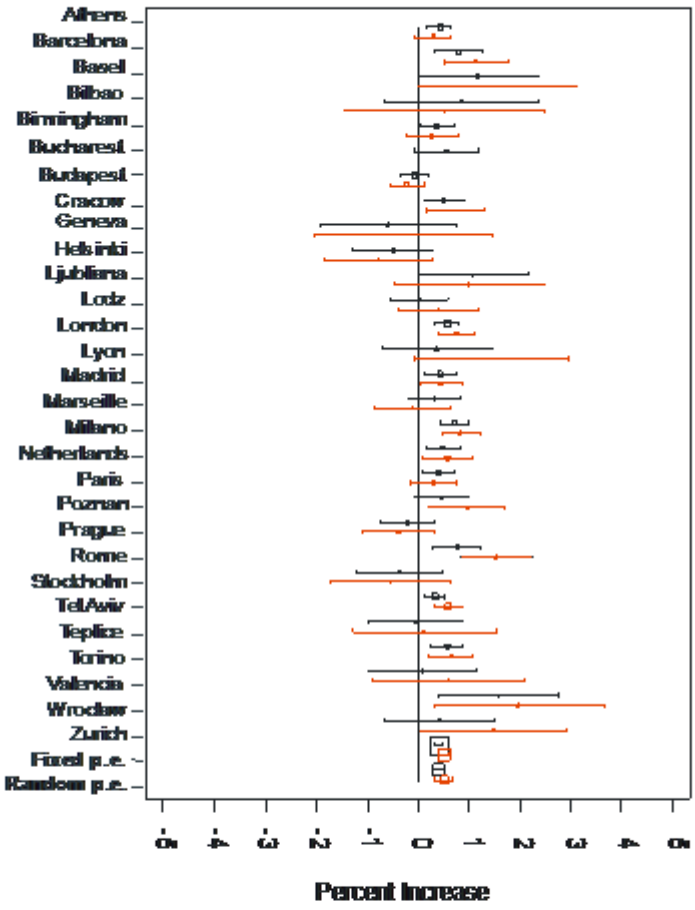


Figure 3. Percent increase in respiratory mortality and its 95% confidence intervals associated with an increase of 10 $\mu g/m^3$ in the levels of NO_2, using the average of lags 0-1 (black line) or polynomial distributed lags models for lags 0-5 (red line). The boxes represent the inverse of the squared standard error.

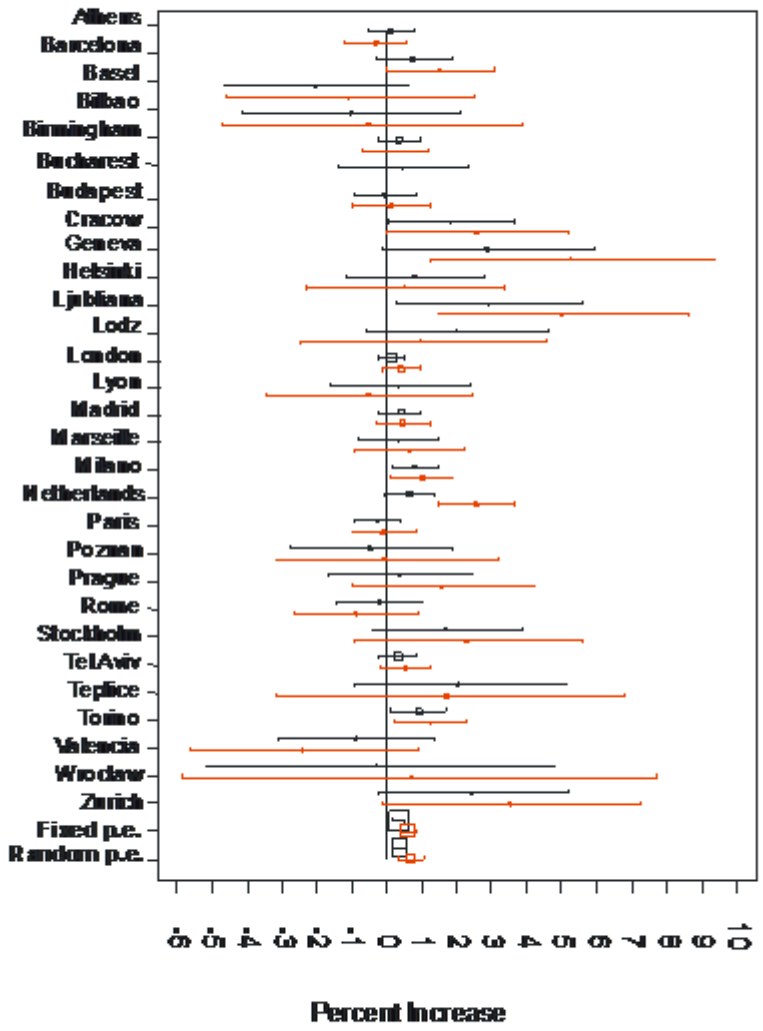
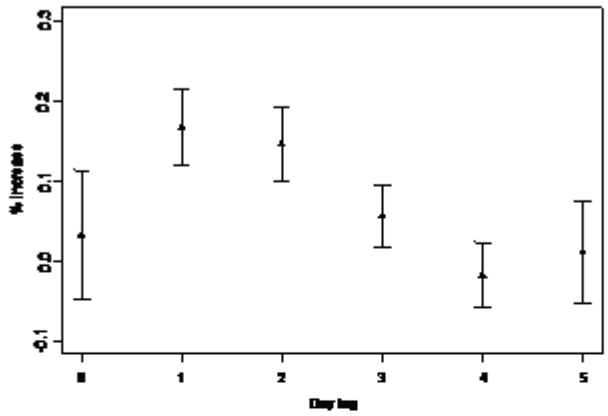
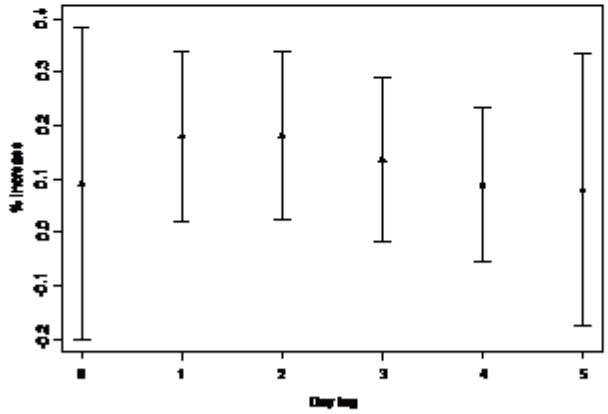


Figure 4. Shape of the association of total and respiratory mortality with NO_2 over 6 days (lags 0 to 5) summarized over all cities using a cubic polynomial distributed lag model.



a) Total mortality



b) Respiratory mortality

Table 1. City Descriptive Data on the Study Period, Population, Exposure (NO_2) and Outcome (Daily Number of Deaths)

CITY	Study period	Population X 1000	Mean No of deaths per day			NO_2-1h ($\mu g/m^3$) Mean (10^{th}-90^{th} centile)
			Total	CVD	Respiratory	
Athens	1/92-12/96	3,073	73	36	5	129.9 (84.3-187.0)
Barcelona	1/91-12/96	1,644	40	16	4	91.1 (63.2-124.7)
Basel	1/90-12/95	360	9	4	1	65.9 (44.2-92.1)
Bilbao	4/92-3/96	667	15	5	1	78.7 (58.1-101.6)
Birmingham	1/92-12/96	2,300	61	28	9	74.5 (49.3-99.5)
Budapest	1/92-12/95	1,931	80	40	3	131.9 (88.0-185.6)
Bucharest	1/92-12/96	2,100	71	38	4	50.5 (31.4-85.5)
Cracow	1/90-12/96	746	18	10	0	*79.4 (37.7-132.0)
Erfurt	1/91-12/95	216	6	-	-	76.0 (36.0-119.0)
Geneva	1/90-12/95	317	6	2	0	79.2 (54.1-111.4)
Helsinki	1/93-12/96	828	18	9	2	62.4 (40.4-87.0)
Ljubljana	1/92-12/96	322	7	3	0	80.0 (47.5-115.0)
Lodz	1/90-12/96	828	30	17	1	*66.4 (40.1-96.4)
London	1/92-12/96	6,905	169	71	29	94.8 (67.1-128.5)
Lyon	1/93-12/97	416	9	3	1	107.2 (75.1-143.2)
Madrid	1/92-12/95	3,012	61	22	6	122.9 (83.7-174.7)
Marseille	1/90-12/95	855	22	8	2	119.6 (80.9-163.1)
Milano	1/90-12/96	1,343	29	11	2	154.8 (104.7-217.8)
Netherlands	1/90-9/95	15,400	342	140	29	53.1 (32.8-74.9)
Paris	1/91-12/96	6,700	124	38	9	84.0 (55.1-118.5)
Poznan	1/90-12/96	582	17	9	1	*81.1 (45.1-119.7)
Prague	2/92-12/96	1,213	38	22	1	60.7 (37.7-86.5)
Rome	1/92-12/96	2,775	56	23	3	147.6 (111.6-189.2)
Stockholm	1/90-12/96	1,126	30	15	3	47.6 (31.2-64.2)
Tel-Aviv	1/91-12/96	1,141	27	12	2	139.7 (57.5-254.9)
Teplice	1/90-12/97	625	18	10	1	59.7 (40.9-81.7)
Torino	1/90-12/96	926	21	9	1	132.4 (78.2-199.6)
Valencia	1/94-12/96	753	16	6	2	116.5 (60.8-170.3)
Wroclaw	1/90-12/96	643	15	9	1	*46.2 (29.5-63.7)
Zurich	1/90-12/95	540	13	6	1	70.2 (46.9-97.4)

* NO_2-1h values were estimated as 1.64*NO_2-24h values

Table 2. Pooled estimates for the increase in mortality associated with an increase of 10 μg/m^3 in NO_2 (average of lags 0 and 1 of the 1h-maxima of NO_2), for different choices of the number of degrees of freedom used for seasonality control.

	Total mortality			**CVD mortality**			**Respiratory mortality**		
	Degrees of freedom			**Degrees of freedom**			**Degrees of freedom**		
	-25%	**Baseline model***	**+25%**	**-25%**	**Baseline model***	**+25%**	**-25%**	**Baseline model***	**+25%**
Model	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**	**%increase (95% CI)**
Fixed effects	0.34 (0.29,0.39)	0.30 (0.25,0.35)	0.27 (0.22,0.32)	0.45 (0.37,0.52)	0.41 (0.34,0.49)	0.37 (0.30,0.45)	0.40 (0.23,0.57)	0.34 (0.17,0.51)	0.31 (0.13,0.48)
Random effects	0.33 (0.24,0.42)	0.30 (0.22,0.38)	0.27 (0.19,0.36)	0.43 (0.32,0.55)	0.40 (0.29,0.52)	0.37 (0.25,0.49)	0.44 (0.24,0.65)	0.38 (0.17,0.58)	0.33 (0.13,0.52)

*i.e. minimizing the absolute value of the sum of the partial autocorrelations of the final model's residuals from lags 3 to 30.

Table 3. Pooled estimates for the increase in mortality associated with an increase of 10 μg/m^3 in NO_2 (average of lags 0 and 1 of the 1h-maxima of NO_2), adjusting alternatively for the other pollutants (average of lags 0 and 1).

	Total Mortality		**CVD mortality**		**Respiratory mortality**	
	Fixed effects	**Random effects**	**Fixed effects**	**Random effects**	**Fixed effects**	**Random effects**
Other pollutant	**% increase (95% CI)**	**% increase (95% CI)**	**% increase (95% CI)**	**% increase (95% CI)**	**% increase (95% CI)**	**% increase (95% CI)**
None	0.30 (0.25,0.35)	0.30 (0.22,0.38)	0.41 (0.34,0.49)	0.40 (0.29,0.52)	0.34 (0.17,0.51)	0.38 (0.17,0.58)
BS	0.33 (0.23,0.42)	0.33 (0.23,0.42)	0.44 (0.31,0.58)	0.44 (0.31,0.58)	0.28 (-0.02,0.58)	0.26 (-0.12,0.65)
PM10	0.27 (0.20,0.34)	0.27 (0.16,0.38)	0.35 (0.24,0.45)	0.35 (0.21,0.50)	0.37 (0.13,0.61)	0.37 (0.08,0.67)
SO_2	0.26 (0.20,0.33)	0.26 (0.18,0.34)	0.37 (0.27,0.46)	0.33 (0.20,0.47)	0.16 (-0.06,0.39)	0.19 (-0.07,0.45)
O_3-8h	0.34 (0.27,0.40)	0.33 (0.22,0.43)	0.45 (0.36,0.54)	0.42 (0.27,0.58)	0.34 (0.14,0.53)	0.38 (0.13,0.63)

Table 4. Results of second stage regression models, investigating the role of potential modifiers* of the estimated effects** of NO_2 (average of lags 0 and 1 of the 1h-maxima of NO_2) on mortality.

Effect modifier in the model+	Total mortality		CVD mortality		Respiratory mortality	
	Estimated increase at the 25th percentile$	Estimated increase at the 75th percentile$	Estimated increase at the 25th percentile$	Estimated increase at the 75th percentile$	Estimated increase at the 25th percentile$	Estimated increase at the 75th percentile$
	% increase (95% CI)	% increase (95% CI)	% increase (95% CI)	% increase (95% CI)	% increase (95% CI)	% increase (95% CI)
Prevalence of smoking	0.36 (0.30,0.42)	0.25 (0.19,0.31)	0.48 (0.39,0.56)	0.35 (0.27,0.43)		
Proportion of Elderly					0.17 (-0.07,0.41)	0.50 (0.28,0.73)
Natural gas consumption in the household			0.32 (0.20, 0.43)	0.40 (0.32, 0.48)		
PM_{10} median levels					0.22 (0.01, 0.43)	0.49 (0.26, 0.73)
Geographical area Western cities Eastern South	0.35 (0.28,0.43) 0.09 (-0.04,0.21) 0.33 (0.25,0.41)		0.49 (0.38,0.60) 0.16 (-0.01,0.34) 0.44 (0.32,0.56)			

* These are variables characterizing each city. Only effect modifiers reducing the heterogeneity by >10% are presented.

** Effect estimates used from first stage models, are based on the chosen number of degrees of freedom.

$ Increase in mortality per 10μg/m^3 increase in the daily NO_2 concentration, estimated using fixed effects model, for a city whose level of the corresponding effect modifier equals to the 25th and the 75th percentile, respectively of the distribution of this effect modifier.

\+ The effect modifiers were included alternatively in the model.