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

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ABSTRACT: **The short-term effects of nitrogen dioxide (NO_2) on total, cardiovascular and respiratory mortality in 30 European cities participating in the Air Pollution on Health: a European Approach (APHEA)-2 project were investigated.**

The association was examined using hierarchical models implemented in two stages. In the first stage, data from each city were analysed 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.

A significant association of NO_2 with total, cardiovascular and respiratory mortality was found, 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 southern 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 a larger proportion of elderly persons in the population and higher levels of particulate matter with a 50% cut-off aerodynamic diameter of 10 μm.

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

KEYWORDS: **Air pollution, heterogeneity, modelling, mortality, nitrogen dioxide**

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 nitrogen dioxide (NO_2), ozone (O_3) and carbon monoxide (CO), have also been shown to have adverse effects on mortality and morbidity [3, 8–10]. 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. Once emitted, NO relatively rapidly reacts with O_3 or oxygen to form NO_2. This usually dominating part is known as secondary NO_2, but the transformation mainly takes place close to the source. Primary NO_2 emissions are particularly important from diesel vehicles, and this fraction seems to be increasing in Europe. Due to nonlinearities in the oxidation reactions and variations in the background O_3 levels, NO_x has a stronger temporal correlation than NO_2 has with other combustion products (exhaust particles, CO, sulphur dioxide (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,

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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 α-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 $\geqslant$1,800 $\mu g \cdot m^{-3}$ in healthy subjects and ~200–500 $\mu g \cdot m^{-3}$ in patients with asthma [16] or chronic obstructive pulmonary disease (COPD) [17]. NO_2 also has an amplifying effect on the asthmatic response to allergen exposure. A short (15–30 min) exposure to 500 $\mu g \cdot m^{-3}$ seems to increase the reaction. Some data suggest that exposure to NO_2 at concentrations occurring in highly trafficked areas (15 min at 500 $\mu g \cdot m^{-3}$) 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 have focused on morbidity rather than mortality. The short-term effects of NO_2 on mortality within the Air Pollution and Health: A European Approach (APHEA)-2 project, which uses an extensive European database from 30 European cities, were investigated. 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

APHEA-2 is a multicentre 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 $\geqslant$3 consecutive years for each city within the years 1990–1997. In all, 2,893,430 deaths occurred in the cities studied during that period. Details of 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 (°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 >60 million. The Netherlands is considered as one urban area because of its relatively small size and dense population. The mean daily total number of deaths ranged from six to 342. For respiratory mortality, daily counts ranged <1–29. The mean levels of NO_2 (1 h max) ranged 46–155 $\mu g \cdot m^{-3}$. In the various cities, the correlation between NO_2 and particulate matter with a 50% cut-off aerodynamic diameter of 10 μm (PM_{10}) ranged 0.11–0.69, between NO_2 and black smoke (BS) it ranged 0.11–0.78, between NO_2 and SO_2 0.15–0.87, and between NO_2 and O_3 -0.21–0.31.

For the four Polish cities included in the analysis (*i.e.* Cracow, Poznan, Lodz, Wroclaw), only NO_2 cumulative 24-h measurements were available. In order to include them in the analysis, the maximum hourly NO_2 concentration of each day (NO_2 1-h values) was estimated as 1.64 times the 24-h values, where 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 of the levels of all pollutants, as well as in the mean daily temperature and humidity.

METHODS

Within the APHEA-2 project the maximum hourly NO_2 concentration of each day (NO_2 1-h) was analysed rather than the average concentration of NO_2 over 24 h (NO_2 24-h), since more cities provided measurements for the former. The maximum daily 1- and 24-h NO_2 concentrations are highly correlated (the correlation coefficient ranges 0.80–0.94, with a median of 0.90). In the present study, it was 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 2 days' pollution correlates better with mortality than a single day's exposure [19]. Furthermore, this approach avoids potential bias that could result from selectively reporting the most significant lags. In this analysis, a linear dose–response relationship between NO_2 1-h and mortality was assumed. This assumption was based on previously published results from the APHEA-2 project indicating that the dose–response relationship could be adequately approximated by a linear association (E. Samoli; personal communication) [20]. To investigate the effect of NO_2 over a larger number of days and examine the shape of the association with each analysed health outcome, polynomial distributed lag models for the NO_2 were fitted, using lags 0 to 5 [19].

For the analysis, a hierarchical modelling approach was used. First, regression models in each city were fitted separately to allow specific control for seasonal effects, weather and other potential confounders. The individual city results were then used 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 [21]. Smooth functions were used 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. The penalised regression splines were used as smoothing functions, as implemented by WOOD [22]. Dummy variables were also included for the day-of-the-week effect, holidays and influenza epidemics.

The general methodological guidelines developed within the framework of the APHEA-2 project were followed, and are described in detail elsewhere [21]. One additional feature is the

TABLE 1 City descriptive data on the study period, population, exposure (nitrogen dioxide (NO_2)) and outcome (daily number of deaths)

City	Study period month/yr	Population × 1000	Mean number of deaths per day			NO_2 1 h $\mu g \cdot m^{-3}$ #
			Total	CVD	Respiratory	
Athens	01/1992–12/1996	3073	73	36	5	129.9 (84.3–187.0)
Barcelona	01/1991–12/1996	1644	40	16	4	91.1 (63.2–124.7)
Basel	01/1990–12/1995	360	9	4	1	65.9 (44.2–92.1)
Bilbao	04/1992–03/1996	667	15	5	1	78.7 (58.1–101.6)
Birmingham	01/1992–12/1996	2300	61	28	9	74.5 (49.3–99.5)
Budapest	01/1992–12/1995	1931	80	40	3	131.9 (88.0–185.6)
Bucharest	01/1992–12/1996	2100	71	38	4	50.5 (31.4–85.5)
Cracow	01/1990–12/1996	746	18	10	0	79.4 (37.7–132.0)¶
Erfurt	01/1991–12/1995	216	6			76.0 (36.0–119.0)
Geneva	01/1990–12/1995	317	6	2	0	79.2 (54.1–111.4)
Helsinki	01/1993–12/1996	828	18	9	2	62.4 (40.4–87.0)
Ljubljana	01/1992–12/1996	322	7	3	0	80.0 (47.5–115.0)
Lodz	01/1990–12/1996	828	30	17	1	66.4 (40.1–96.4)¶
London	01/1992–12/1996	6905	169	71	29	94.8 (67.1–128.5)
Lyon	01/1993–12/1997	416	9	3	1	107.2 (75.1–143.2)
Madrid	01/1992–12/1995	3012	61	22	6	122.9 (83.7–174.7)
Marseille	01/1990–12/1995	855	22	8	2	119.6 (80.9–163.1)
Milan	01/1990–12/1996	1343	29	11	2	154.8 (104.7–217.8)
The Netherlands	01/1990–09/1995	15400	342	140	29	53.1 (32.8–74.9)
Paris	01/1991–12/1996	6700	124	38	9	84.0 (55.1–118.5)
Poznan	01/1990–12/1996	582	17	9	1	81.1 (45.1–119.7)¶
Prague	02/1992–12/1996	1213	38	22	1	60.7 (37.7–86.5)
Rome	01/1992–12/1996	2775	56	23	3	147.6 (111.6–189.2)
Stockholm	01/1990–12/1996	1126	30	15	3	47.6 (31.2–64.2)
Tel Aviv	01/1991–12/1996	1141	27	12	2	139.7 (57.5–254.9)
Teplice	01/1990–12/1997	625	18	10	1	59.7 (40.9–81.7)
Torino	01/1990–12/1996	926	21	9	1	132.4 (78.2–199.6)
Valencia	01/1994–12/1996	753	16	6	2	116.5 (60.8–170.3)
Wroclaw	01/1990–12/1996	643	15	9	1	46.2 (29.5–63.7)¶
Zurich	01/1990–12/1995	540	13	6	1	70.2 (46.9–97.4)

CVD: cardiovascular disease. #: mean (10th–90th percentile); ¶: the maximum hourly NO_2 concentration of each day was estimated as 1.64 × NO_2 24-h values.

use of penalised regression splines instead of the nonparametric function locally weighted nonparametric smoothers (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 long-term trends and seasonal patterns were removed 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 lagged values of these meteorological variables were included in the models. Thin-plate regression splines were used as basis functions for the penalised regression splines [23]. Based on experiences from the previous analyses of the APHEA-2 data, the number of basis functions was chosen to be 40 for the time variable and 10 for the weather variables. The smoothing parameters that minimised the absolute value of the sum of partial autocorrelations (PACs) of the residuals from lags 3 to 30 were then chosen. To account for serial correlation in the residuals where it remained in the final model, autoregressive terms were added into the model as appropriate [24]. In the special case of the small cities (and especially in cause-specific mortality), a minimum of one degree of freedom per year was required.

The APHEA-2 method was used for influenza control, including a dummy variable taking the value of one when the 7-day moving average of the respiratory mortality was greater than the 90th percentile of its city-specific distribution. Since the influenza control, as described, was based on the distribution of respiratory mortality, the influenza dummy variable was included only when the total and cardiovascular mortality were analysed [21]. Based on previously published results [25, 26], there is no indication that omitting control for influenza when respiratory mortality was analysed 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, respiratory mortality was also analysed for days below the 90th percentile of its distribution.

To evaluate how sensitive the present results are to the choice of the degree of smoothing, models with ±25% of the degrees of freedom for the time smoothing that were chosen based on the PAC criterion were also applied.

To investigate potential confounding effects of the daily levels of other pollutants, two pollutant models were also applied, *i.e.* NO_2 and alternatively PM10 (24-h mean), SO_2 (24-h mean) or O_3 (maximum of 8-h means) were included in the model, and were also applied.

Some studies have demonstrated that the effects of air pollution on mortality are spread over >2 days [19, 27]. To examine this question, a separate distributed lag model was fitted for each city. A cubic polynomial distributed lag with lags to 5 days before the deaths was used, which has proven adequate in past studies [19]. Therefore, the coefficients for the cubic polynomial that define the shape of the distributed lag were estimated in each city.

Second-stage analysis

It was assumed that the city-specific effect estimates (for the mean of lags 0 and 1) to be normally distributed around an overall estimate. 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, were estimated. 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 means 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 [28]. In order to combine the city-specific coefficients from the polynomial distributed lags models, the multivariate maximum likelihood method was used [29].

RESULTS

Table 2 shows the percentage increase in the daily number of deaths associated with 10 $\mu g \cdot m^{-3}$ 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 $\mu g \cdot m^{-3}$ increase in the daily NO_2 concentrations, the increase in 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 adjusted for seasonality using more degrees of freedom, the associated effect in all studied outcomes decreased by <13% compared with the baseline model. When adjusted for seasonality using fewer degrees of freedom, the associated effect in all studied outcomes increased by <16%. When respiratory mortality restricted to days below the 90th percentile of its distribution was analysed, the pooled effect was slightly increased (associated estimate 0.41% (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 $\mu g \cdot m^{-3}$ 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. Distributed lag models in Bucharest were not applied 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 increases in cardiovascular mortality and the 95% CIs associated with an increase of 10 $\mu g \cdot m^{-3}$ in the levels of NO_2, when either two (black lines) or six (red lines) days of exposure were analysed. 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 the 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 increases in respiratory mortality and the 95% CIs associated with an increase of 10 $\mu g \cdot m^{-3}$ in the levels of NO_2, when either 2 (black lines) or 6 days (red lines) of exposure were analysed. Statistically significant results for single cities ranged from 0.92% in Torino to 2.88% in Ljubljana for the baseline model, and from 0.98% in Milan to 5.25% in Geneva for the distributed

TABLE 2 Pooled estimates for the increase in mortality associated with an increase of 10 $\mu g \cdot m^{-3}$ in nitrogen dioxide (NO_2; average of lags 0 and 1 of the 1-h maxima of NO_2) for different choices of the number of degrees of freedom used for seasonality control

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

Data are presented as percentage increase (95% confidence interval). CVD: cardiovascular disease. #: *i.e.* minimising the absolute value of the sum of the partial autocorrelations of the final model's residuals from lags 3 to 30.

FIGURE 1. Percentage increase in total mortality and its 95% confidence intervals associated with an increase of 10 $\mu g \cdot m^{-3}$ in the levels of nitrogen dioxide, 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 SE. Fixed: fixed pooled estimate; Random: random pooled estimate.

FIGURE 2. Percentage increase in cardiovascular mortality and its 95% confidence intervals associated with an increase of 10 $\mu g \cdot m^{-3}$ in the levels of nitrogen dioxide, using the average of lags 0–1 (black lines) or polynomial distributed lags models for lags 0–5 (red lines). The boxes represent the inverse of the squared SE. Fixed: fixed pooled estimate; Random: random pooled estimate.

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 BS, PM10, 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 $\mu g \cdot m^{-3}$ in the levels of NO_2 was reduced by 32%, and it even decreased by 50% and became nonsignificant when adjusting for SO_2.

The observed heterogeneity in the effect estimates of NO_2 was examined using 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 $\mu g \cdot m^{-3}$ increase in the daily levels of NO_2) for two cities characterised 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 >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, southern and central eastern European cities), followed by the prevalence of smoking in the city. More specifically, in cities with a 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 cardiovascular disease mortality was in north-western cities, followed closely

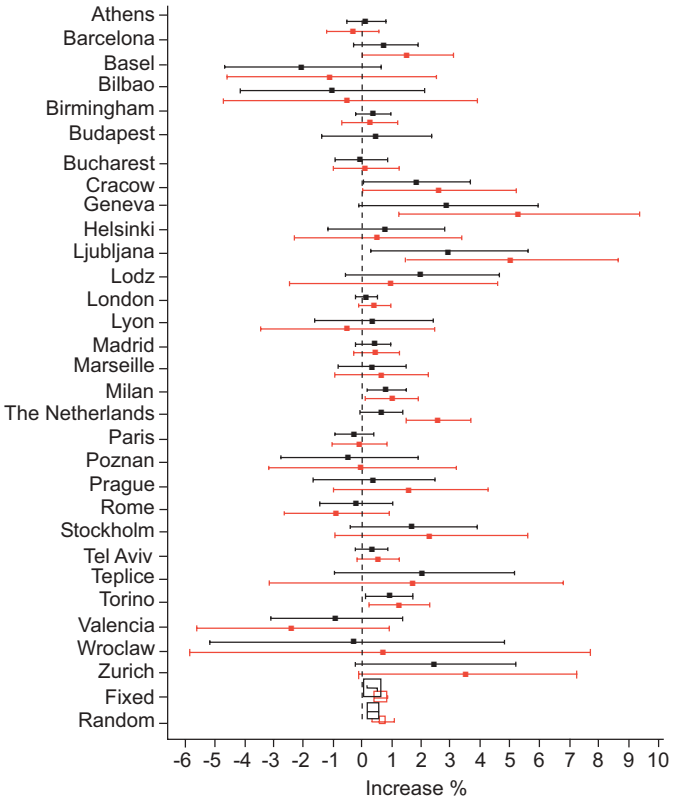

FIGURE 3. Percentage increase in respiratory mortality and its 95% confidence intervals associated with an increase of 10 μg·m^{-3} in the levels of nitrogen dioxide, using the average of lags 0–1 (black line) or polynomial distributed lags models for lags 0–5 (red line). The boxes represent the inverse of the squared SE. Fixed: fixed pooled estimate; Random: random pooled estimate.

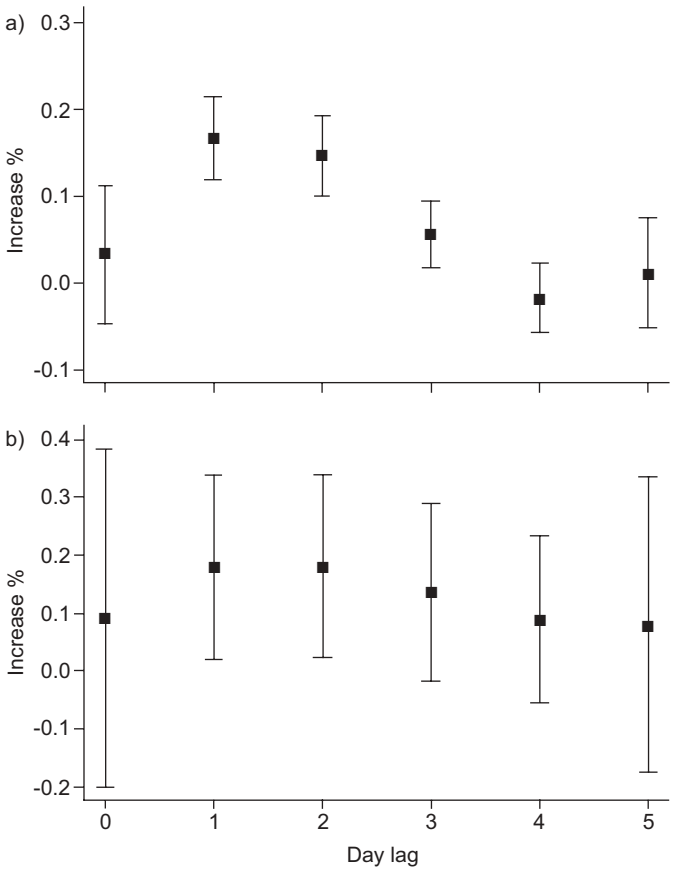

FIGURE 4. Shape of the association of a) total and b) respiratory mortality with nitrogen dioxide over 6 days (lags 0–5) summarised over all cities using a cubic polynomial distributed lag model.

by the effect in southern European cities, while there was only a small and nonsignificant 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 modifiers were the median levels of PM10 followed by the proportion of the elderly (*i.e.* >65 yrs of age) in the population of the city. In cities with high median PM10 levels and a high proportion of elderly people, 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 a decrease in lung function, an increase in respiratory symptoms and an 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 hospitalisation for respiratory diseases, and on decrease of pulmonary function [30–33]. A few studies have been conducted on the effects of photochemical air pollution on mortality [34–37]. The most extensive European database available today to investigate the potential effects of NO_2 on mortality was used.

Significant adverse health effects of NO_2 on total, cardiovascular and respiratory mortality, with stronger effects on cause-specific mortality have been found. 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 six compared with 30 cities 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 μg·m^{-3} in NO_2 was associated with 0.26% increase in total mortality compared with 0.30% increase reported in the present results. KINNEY and OZKAYNAK [34] also found a significant association of NO_2 with total and cardiovascular mortality in Los Angeles County, USA. 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 the present 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 nonsignificant when controlling for BS and SO_2. Finally, in the analysis of 20 US cities within the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) project, no consistent pattern of association between total mortality and NO_2 was found [38]. The

TABLE 3 Pooled estimates for the increase in mortality associated with an increase of 10 μg·m^{-3} in nitrogen dioxide (NO_2; average of lags 0 and 1 of the 1-h maxima of NO_2), adjusting alternatively for the other pollutants (average of lags 0 and 1)

Other pollutant	Total mortality		CVD mortality		Respiratory mortality	
	Fixed effects	Random effects	Fixed effects	Random effects	Fixed effects	Random effects
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 8-h	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)

Data are presented as % increase (95% confidence interval). CVD: cardiovascular disease; BS: black smoke; PM10: particle matter with a 50% cut-off aerodynamic diameter of 10 μm; SO_2: sulphur dioxide; O_3 8-h: maximum daily 8-h O_3 concentration.

investigators found a positive, but not statistically significant, effect of NO_2 at lag 0 and at lag 1, and 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 US.

In this study, the cumulative effect over 6 days was larger by about 22% for total and cardiovascular mortality and by 45% for respiratory mortality compared with the average exposure over 2 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 2 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. An attempt has been made to estimate the independent effects of pollutants using two pollutant models, for those pollutants for which data were available. No evidence of confounding for total and cardiovascular mortality has been found. 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 PM10 and provides a means of addressing the question of particle composition. It was impossible to control for indices for which no measurements were available, for example, the number of particles. Further study focusing on exposure to mixtures including NO_2, BS and SO_2 is needed to further understanding of the aetiological mechanism

TABLE 4 Results of second-stage regression models, investigating the role of potential modifiers[#] of the estimated effects[¶] of nitrogen dioxide (NO_2; average of lags 0 and 1 of the 1-h maxima of NO_2) on mortality

Effect modifier in the model[+]	Total mortality		CVD mortality		Respiratory mortality	
	25th percentile[§]	75th percentile[§]	25th percentile[§]	75th percentile[§]	25th percentile[§]	75th percentile[§]
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)		
PM10 median levels					0.22 (0.01–0.43)	0.49 (0.26–0.73)
Geographical area						
Western cities	0.35 (0.28–0.43)		0.49 (0.38–0.60)			
Eastern cities	0.09 (-0.04–0.21)		0.16 (-0.01–0.34)			
Southern cities	0.33 (0.25–0.41)		0.44 (0.32–0.56)			

Data are presented as % increase (95% confidence interval). CVD: cardiovascular disease; PM10: particulate matter with a 50% cut-off aerodynamic diameter of 10 μm. [#]: Potential modifiers are variables characterising 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. [+]: The effect modifiers were included alternatively in the model. [§]: Estimated increase at the 25th and 75th percentiles. Increase in mortality per 10 μg·m^{-3} increase in the daily NO_2 concentration, estimated using the 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.

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, the $PM_{2.5}$ median level for nine of the analysed cities as potential effect modifier has been used [39]. The $PM_{2.5}$ median level did not act as significant effect modifier and reduced the heterogeneity by <10% in each of the studied outcomes. Besides, if NO_2 truly reflects $PM_{2.5}$, then in cities with a higher $PM_{2.5}/PM_{10}$ ratio, a higher NO_2/PM_{10} level would be expected. This scenario was not verified in four cities with relevant data (Athens, Birmingham, Helsinki, Amsterdam; the Relationship between Ultrafine and fine Particulate matter in Indoor and Outdoor air and respiratory Health (RUPIOH) project, unpublished data). Finally, the correlation between PM_{10} and $PM_{2.5}$ in these four cities ranged 0.58–0.95. These high correlations indicate that controlling for PM_{10}, as in the 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.* [40] 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 [41], 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 (the RUPIOH project, unpublished data) that provided relevant data for the present analysis, the correlation between NO_2 and PM_{10} ranged 0.38–0.54, between NO_2 and $PM_{2.5}$, 0.43–0.63, between NO_2 and particle numbers, 0.53–0.72, and between PM_{10} and particle numbers, 0.19–0.54. Hence, in the present data, the associations between NO_2 and particle numbers are not so high as to make the distinction of the effects impossible, as in the case of SEATON *et al.* [40]. 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.

The present authors investigated heterogeneity in the effect estimates between cities by taking into account several city characteristics, and environmental and social factors as potential effect modifiers. 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. Furthermore, the effect of NO_2 on total and cardiovascular mortality was observed mainly in western and southern European cities. In more than half of the eastern European cities involved in the analysis, the NO_2 1-h 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 characteristic air pollution sources (*e.g.* more traffic). Finally, the effect of NO_2 on respiratory mortality was higher in cities with a 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 synergistically. One limitation of the present study's 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, the present results confirm those previously reported concerning the adverse effects of nitrogen dioxide 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 nitrogen dioxide on mortality, but the role of nitrogen dioxide as a surrogate of other unmeasured pollutants cannot be completely ruled out. Since the short-term effects of nitrogen dioxide on respiratory mortality may be confounded by other vehicle-derived pollutants, further investigation is needed to enhance understanding of the underlying biological mechanism.

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