Nitrogen dioxide and mortality: review and meta-analysis of long-term studies

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ABSTRACT Exposure to ambient nitrogen dioxide (NO_2) has been linked to increased mortality in several epidemiological studies but the question remains of whether NO_2 is directly responsible for the health effects or is only an indicator of other pollutants, including particulate matter. The aim of the present review was to provide pooled estimates of the long-term effects of NO_2 on mortality, which are potentially useful for health impact assessment.

We selected 23 papers, published from 2004 to 2013, evaluating the relationship between NO_2 and mortality, also including an assessment of the effect of particulate matter exposure. A random-effects meta-analysis was carried out on 19 studies.

The pooled effect on mortality was 1.04 (95% CI 1.02–1.06) with an increase of 10 $\mu g \cdot m^{-3}$ in the annual NO_2 concentration and 1.05 (95% CI 1.01–1.09) for particulate matter <2.5 µm in diameter ($PM_{2.5}$) (10 $\mu g \cdot m^{-3}$). The effect on cardiovascular mortality was 1.13 (95% CI 1.09–1.18) for NO_2 and 1.20 (95% CI 1.09–1.31) for $PM_{2.5}$. The NO_2 effect on respiratory mortality was 1.03 (95% CI 1.02–1.03) and 1.05 (95% CI 1.01–1.09) for $PM_{2.5}$. Four bipollutant analyses with particulate matter and NO_2 in the same models showed minimal changes in the effect estimates of NO_2.

There is evidence of a long-term effect of NO_2 on mortality as great as that of $PM_{2.5}$. An independent effect of NO_2 emerged from multipollutant models.

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This article has supplementary material available from erj.ersjournals.com

Received: July 05 2013 | Accepted after revision: Dec 19 2013 | First published online: Feb 20 2014

Conflict of interest: None declared.

Introduction

The long-term effects on mortality of exposure to nitrogen dioxide (NO_2) were assessed in the World Health Organization (WHO) Air Quality Guidelines in 2005 [1] and the overall evidence was considered limited, given the small number of studies available. Neither the development of chronic diseases nor lung cancer was clearly associated with NO_2 in any of the studies taken into consideration in the 2005 WHO report. However, short-term mortality studies, studies of the impairment of lung function growth in children and investigations of recurrent respiratory problems all suggested a NO_2 effect. Recent documents on traffic exposure from the American Thoracic Society [2] and the Health Effects Institute [3] considered NO_2, among other traffic-related pollutants, and concluded that there is evidence suggesting that these pollutants have a causal role in mortality and the development of chronic respiratory diseases.

The recent review of the health effects of air pollution by a WHO working group (Review of Evidence on Health Aspects of Air Pollution (REVIHAAP)) [4] evaluated the most recent studies on long-term exposure to NO_2 and both natural (non-accidental)/total and cause-specific mortality. The question of whether NO_2 is directly responsible for the health effects or is only an indicator of other pollutants was specifically considered, including particulate matter and specific constituents such as metals, polycyclic aromatic hydrocarbons and other organic matter. The conclusion was that it is difficult ''to judge the independent effects of NO_2 in the long-term studies because, in those investigations, the correlations between concentrations of NO_2 and other pollutants are often high, so that NO_2 might represent a mixture of traffic-related pollutants. In this case, chamber studies do not apply and toxicological evidence is limited. However, some epidemiological studies do suggest associations of long-term NO_2 exposures with respiratory and cardiovascular mortality and with children's respiratory symptoms and lung function that were independent of [particulate matter] mass metrics. As with the short-term effects, NO_2 in these studies may represent other constituents. Despite this, the mechanistic evidence, particularly on respiratory effects, and the weight of evidence on short-term associations suggest a causal relationship.'' In other words, the WHO working group suggested that the response to the causality question cannot be derived only from the epidemiological studies but requires the integration of exposure science, toxicology and human studies.

At the time of the REVIHAAP project, a quantitative systematic review of the long-term effects on mortality of NO_2 was not available. A subsequent review by HOEK *et al.* [5], considering the association between several pollutants and long-term mortality, showed a significant association of all-cause mortality with NO_2 in 15 longitudinal studies. Their work did not permit comparison of the effects of NO_2 with those of other pollutants, namely particulate matter, when the same studies were considered or when the results of the multipollutant models were available.

To contribute to the discussion of the role of NO_2 from an epidemiological perspective and to provide pooled estimates of the long-term effects of NO_2 on mortality, which are potentially useful for assessing the health impact of air pollution, we systematically examined the studies that investigated the long-term effects on mortality of both outdoor NO_2 and particles in the same population, and carried out a meta-analysis of both NO_2 and particulate matter effects.

Methods

Systematic review

The search strategy focused on studies reporting NO_2 (or nitrogen oxides (NO_x)) effects on total and specific-cause mortality. The databases PubMed, Web of Science and LUDOK were searched. The search was restricted to the period from January 2004 to January 2013 and to studies in humans. Search terms were ''nitrogen dioxide'' (as ''nitrogen dioxide/adverse effects'' Medical Subject Headings in PubMed and as ''nitrogen oxides'' in LUDOK), together with ''cohort study'' or ''case–control study'', and ''mortality'', ''cardiovascular mortality'' and ''respiratory mortality''.

Articles were included if long-term effects on natural/total or specific (cardiovascular or respiratory) mortality were assessed and the estimates of association with the pollutants were reported; the exposure needed to have been measured as at least an annual mean concentration in the study area. Papers were excluded when short-term effects were estimated, no health effect was measured, incidence or prevalence was assessed, no original data were analysed or, finally, when no control for confounding due to individual factors was carried out. Reviews and methodological papers were excluded as well. Studies carried out on groups restricted to at-risk people were, however, not excluded.

The first screening of the papers was carried out by checking the titles and abstracts. The texts of the remaining papers were read in full, so as to select those that also included effect estimates for particulate matter $<10\,\mu m$ in diameter (PM_{10}) or $PM_{2.5}$, total suspended particles (TSPs) and black smoke.

The following characteristics were reported for each paper: authors, location and year of publication; study population; study design and study period (including both the enrolment and the follow-up period, if it was required by the study design); exposure assessment, including the method employed to assess levels, the mean concentration of pollutants and their exposure metrics; traffic exposure indicators (such as distance from the nearest high-traffic road and traffic density); and effect estimates of both total and specific-cause mortality.

Meta-analysis

Before pooling data, we converted the effect estimates related to NO_x into NO_2 effects, using a conversion factor of 0.75 [6]. The NO_2 concentrations expressed in parts per billion were converted into micrograms per cubic metre; we used a conversion factor of 1.88 (at 25°C and 1013 mb) for both NO_2 and NO_x. The effects related to TSP were converted into PM10 effects, using a factor of 0.75 [7], and the effects related to PM10 were converted into PM2.5 effects, using a factor of 0.7 [8]. Diesel particulate matter and black smoke were analysed as indicators of PM2.5 [9]. In addition, the effects expressed as interquartile (or quintile or percentile) differences were converted into effects of a 10-$\mu g \cdot m^{-3}$ increase of each pollutant.

We used hazard ratios, odds ratios and relative risks (RRs) of mortality in a random-effects meta-analysis [10] (Stata, version 10; StataCorp, College Station, TX, USA), thus including the risk estimates in the pooled analysis irrespective of the study design. The risk estimates and 95% confidence intervals from each study were reported, having been adjusted for all the factors the authors assessed as confounders. The standard error of the effect was calculated from the risk estimates and the confidence intervals (SE = (ln RR - ln lowest limit CI)/1.96 or SE=(ln highest limit CI - ln RR)/1.96). The random pooled effects were reported as hazard ratios, as most studies used Cox's survival analysis. We assessed heterogeneity across studies by using the Chi-squared test (Cochran's Q) and inconsistency (I^2) [11], which represents the proportion of total variation in effect estimates due to heterogeneity across the studies. Finally, we stratified the results by geographical area (Asia, North America and Europe).

To compare the relative effects of NO_2 and PM2.5, we carried out a meta-analysis using the interquartile range (IQR) as the exposure metric instead of the fixed increment (10 $\mu g \cdot m^{-3}$). We included only studies where the IQRs were available or could be derived by using the mean concentrations and their standard deviations. The pooled estimate was obtained for a median IQR of all the single IQRs from those reported or specifically estimated.

Results

Our initial search led to the selection of a total of 180 articles that were seen to deal with NO_2 (or NO_x) exposure. We excluded 146 articles on the basis of their titles and abstracts: nine reviews, 12 articles that did not analyse original data, two methodological studies, eight with study designs that made it impossible to adjust for individual confounders, 33 articles that did not assess health effects or human outcomes, 46 articles dealing with short-term effects, 26 articles with long-term effects other than mortality, one article studying only lung cancer mortality and nine articles with a mean exposure to air pollution <1 year. Thereafter, from the 34 studies that had been identified as warranting reading in full, we selected 23 studies [12–34] that assessed particulate matter effects in addition to NO_2 or NO_x effects. All the selected studies and their characteristics are reported in detail in online supplementary table S1. The 11 excluded studies are listed in table S2.

14 studies [12, 13, 17, 19–24, 26, 30–32, 34] evaluated natural or total mortality; 17 studies [12, 13, 15, 17–20, 22, 23, 25–29, 32–34] considered cardiovascular mortality (including four studies [13, 23, 32, 34] assessing cardiopulmonary mortality); 13 studies considered respiratory mortality [12–14, 16, 17, 19, 20, 22, 23, 26, 28, 32, 34] (including the four with cardiopulmonary mortality as a single outcome [13, 23, 32, 34], and a further one [14] that assessed lung cancer and respiratory mortality as a single outcome). Only 10 studies [12, 13, 17, 19, 20, 22, 23, 26, 32, 34] evaluated both total and cause-specific mortality, although data were not always exhaustively reported. There were 21 cohort studies [12–24, 26–28, 30–34] and two case–control studies [25, 29].

Cohort studies involved adults of both sexes but one study selected only adult males [20] and four selected only adult females [13, 19, 27, 32], and two further articles [28, 33] studied both males and females but separately. Among the other studies, one studied only females [29]. Five studies focused on specific at-risk groups: patients with stroke [21], respiratory diseases [22], hypertension [24] and first myocardial infarction [25, 29].

Several confounders were assessed in each article. Sex, age and social class (identified by different indicators such as education, deprivation index, occupation, area-level socioeconomic position (SEP) or income) were included in most studies. Only DONG *et al.* [14], KATANODA *et al.* [16] and LIPFERT *et al.* [24] included no

DOI: 10.1183/09031936.00114713

SEP indicator in their final analysis. Habitual smoking was frequently adjusted for [15–17, 21, 26, 27, 32, 33], whereas comorbidities were rarely assessed [12, 18, 21].

Asia was represented by four studies (one from Japan [16] and three from China [14, 15, 17]), North America contributed nine studies (two from Canada [18, 22] and seven from the USA [19, 20, 23, 24, 30, 31, 33]) and Europe contributed 10 studies [12, 13, 21, 25–29, 32, 34].

NO_2 was a main exposure in almost all studies, but two [17, 24] used NO_x. The exposure metric was mostly expressed in micrograms of NO_2 per cubic metre, but one study from Asia [16], one from Canada [22] and all seven studies from the USA [19, 20, 23, 24, 30, 31, 33] reported the mean levels of NO_2 in parts per billion. The unit increase of NO_2 was usually reported (16 studies from all the geographic areas) as interquartile or centile range rather than as per 10 $\mu g \cdot m^{-3}$ [12, 13, 18–20, 22–25, 26–29, 31–33].

The mean yearly level of NO_2 was 41.6 $\mu g \cdot m^{-3}$ in Asia, 42.2 $\mu g \cdot m^{-3}$ in North America and 36.2 $\mu g \cdot m^{-3}$ in Europe.

Particulate matter was studied for the most part as PM10 (11 studies) [13–15, 19–21, 25, 27–29, 32] with the effect estimated per 10 $\mu g \cdot m^{-3}$ (four studies [14, 15, 19, 21]) or IQR, expressed in micrograms per cubic metre. PM2.5 was studied in 11 cases [12, 16, 18, 22–24, 26, 30, 31, 33, 34] with exposure expressed most frequently as an IQR increase of concentrations (six studies [12, 18, 22, 23, 31, 33]). TSPs (expressed per 10 $\mu g \cdot m^{-3}$) was the particle metric in one study [17]. The mean yearly level of PM2.5 was 87.4 $\mu g \cdot m^{-3}$ in Asia, 14.8 $\mu g \cdot m^{-3}$ in North America and 25.4 $\mu g \cdot m^{-3}$ in Europe.

The correlation between NO_2 (or NO_x) and PM10 was reported in eight studies out of 11, with values ranging from 0.5 [13] to 0.9 [14, 15, 29]. The correlation between NO_2 and PM2.5 was reported in nine studies out of 11, with values ranging from 0.3 in the Japanese study [16] and in the Californian (USA) study [33] to 0.88 in the Norwegian study [28]. The study using only TSPs [17] did not indicate the correlation with NO_2.

Traffic exposure indicators were reported in nine publications. Two evaluated proximity to a road with heavy traffic and traffic density [12, 26], the studies in Toronto, Canada [22] and in Germany (the Study on the Influence of Air Pollution on Lung, Inflammation and Aging) [13, 27, 32] investigated only proximity to traffic, and the US Veterans Affairs studies [24, 30, 31] evaluated traffic density only.

We included 19 studies in the meta-analysis. Four of the 23 included in the systematic review have been further excluded from the meta-analysis, as three of them reported more complete analyses 3 years later [29–31] and one other [14] reported mortality only for respiratory diseases and lung cancer as a single outcome. In contrast, two of the included papers [28, 33] carried out separate analyses for males and females, thus producing 21 effect estimates actually analysed.

TABLE 1 Pooled[#] effects of nitrogen dioxide (NO_2) and particulate matter <2.5 μm in diameter (PM2.5) (per 10 $\mu g \cdot m^{-3}$) on natural and cause-specific mortality by region of the world

	Total or natural mortality			Cardiovascular mortality			Respiratory mortality		
	Studies n	RR (95% CI)	I^2 %	Studies n	RR (95% CI)	I^2 %	Studies n	RR (95% CI)	I^2 %
NO_2									
All countries	12	1.041 (1.019–1.064)	89	18[¶,+]	1.133 (1.088–1.180)	98	9[+,§,f]	1.024 (1.017–1.032)	0
Asia	1	1.020 (1.000–1.030)		2	1.588 (0.675–3.740)	99	2	1.023 (1.010–1.036)	47
North America	5	1.027 (0.987–1.069)	95	7	1.031 (0.999–1.065)	67	3	1.005 (0.975–1.035)	0
Europe	6	1.066 (1.029–1.104)	72	9	1.059 (1.032–1.086)	79	4	1.029 (1.013–1.045)	0
PM2.5									
All countries	11[##]	1.045 (1.007–1.088)	87	17[¶,+,##]	1.196 (1.091–1.31)	98	8[+,§,f,##]	1.050 (1.009–1.094)	61
Asia	1	1.000 (0.980–1.020)		2	1.380 (0.761–2.502)	99	2	1.034 (0.943–1.134)	50
North America	4	1.047 (1.035–1.106)	83	6	1.047 (0.992–1.106)	71	2	1.046 (0.990–1.104)	0
Europe	6	1.071 (1.021–1.124)	62	9	1.188 (1.091–1.295)	42	4	1.075 (1.009–1.146)	40

RR: relative risk; I^2: inconsistency. [#]: results from random-effects meta-analyses. [¶]: two studies (one from North America [33] and one from Europe [28]) were included twice with separate groups of males and females. [+]: four studies (one from America [23] and three from Europe [13, 32, 34]) estimated cardiopulmonary mortality as a whole; they were included in the pooled cardiovascular mortality estimate and excluded from the pooled respiratory mortality estimate. [§]: one study from China [14] was excluded because it estimated lung cancer together with respiratory mortality. [f]: one study from Europe [28] was included twice with separate estimates for males and females. [##]: one study from the USA [22] could be included because it did not present particulate matter effect estimates.

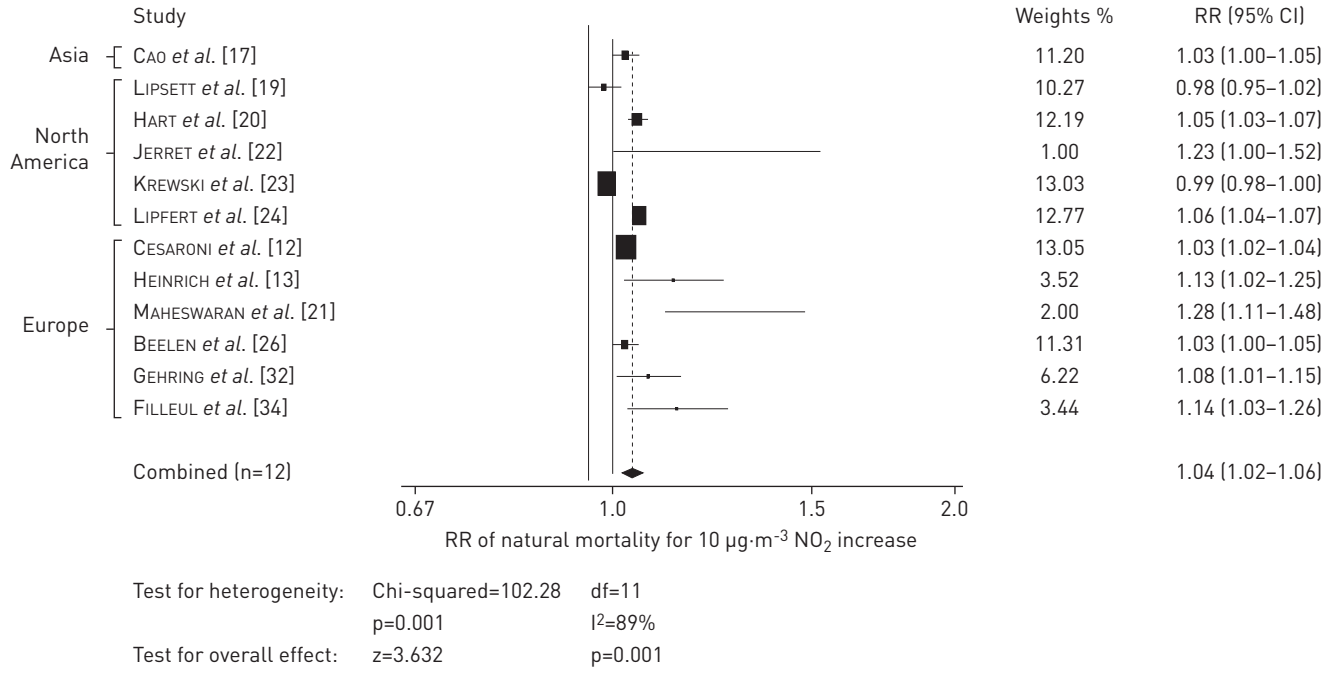

FIGURE 1 Relative risks (RR) of natural mortality with increasing chronic exposure to nitrogen dioxide (NO_2). df: degrees of freedom; I^2: inconsistency.

The pooled estimate of natural/total mortality was performed for 12 studies [12, 13, 17, 19–24, 26, 32, 34], the pooled estimate of cardiovascular mortality made use of 18 effect estimates [12, 13, 15, 17–20, 22, 23, 25–28, 32, 33, 34] (as two studies analysed males and females separately), the meta-analytic estimate of respiratory mortality included nine effect estimates [12, 16, 17, 19, 20, 22, 26, 28] (as one study analysed males and females separately [28]).

The pooled estimate of NO_2 effects on natural/total mortality (table 1 and fig. 1) was RR 1.04 (95% CI 1.02–1.06) per 10 $\mu g \cdot m^{-3}$ NO_2 and the corresponding effect of $PM_{2.5}$ was RR 1.05 (95% CI 1.01–1.09) per 10 $\mu g \cdot m^{-3}$ $PM_{2.5}$. However, there was high heterogeneity across the studies in North America: the NO_2 effects were not statistically significant while the effects of $PM_{2.5}$ were larger and statistically significant (in spite of the fact that the levels of $PM_{2.5}$ observed there were the lowest we found). The greatest effect on natural/total mortality was observed in Europe for both NO_2 and $PM_{2.5}$ but heterogeneity observed across the studies was still high. It should be noticed that a part of this heterogeneity could be explained by just one study [21] on stroke patients.

The pooled estimate of the NO_2 effect (table 1 and fig. 2) on cardiovascular mortality was RR 1.13 (95% CI 1.09–1.18), while the effect of $PM_{2.5}$ was RR 1.20 (95% CI 1.09–1.31), per 10 $\mu g \cdot m^{-3}$ of each pollutant. In Asia, the effects of both NO_2 and $PM_{2.5}$ were greater than those in North America and Europe. Notably, the results were affected by high heterogeneity in all areas. In Asia, where the overall NO_2 estimate did not attain statistical significance (and heterogeneity showed values near 99%), one study [15] provided very high effect estimates, much greater than for the other studies. In North America, the study in Toronto [22], carried out on patients from a respiratory clinic, provided effects on cardiovascular mortality as high as 64% for NO_2, in contrast with a mean increase of 1% in the other studies.

To better evaluate the heterogeneity of the pooled effect estimates of NO_2 on total and cardiovascular mortality, we conducted subgroup analyses considering studies not involving at-risk groups, *i.e.* excluding the studies by Maheswaran *et al.* [21], Jerret *et al.* [22], Lipfert *et al.* [24] and Rosenlund *et al.* [25], and studies with better exposure assessment (land use regression (LUR), dispersion models or other methods at the address level) [12, 18–22, 25, 26, 28]. The heterogeneity of the effects generally remained in these subgroups when they were considered separately (table S3). However, when we restricted our analysis to studies conducted in the general population (not on at-risk groups) and with a better exposure assessment, the heterogeneity for cardiovascular mortality decreased ($p=0.690$ and $I^2=0\%$, based on seven studies) corresponding to an effect estimate of 1.03 for NO_2.

DOI: 10.1183/09031936.00114713

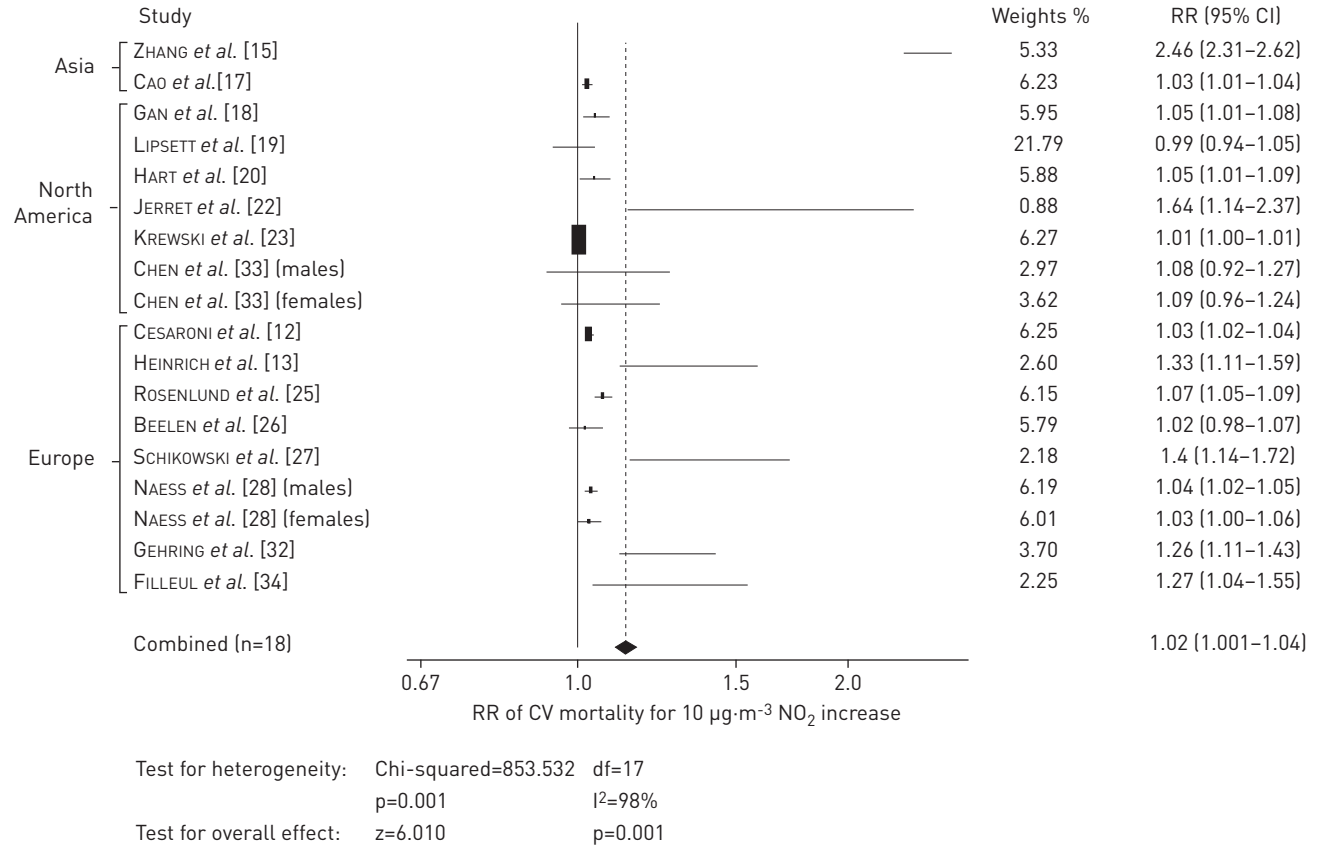

FIGURE 2 Relative risks (RRs) of cardiovascular (CV) mortality with increasing chronic exposure to nitrogen dioxide (NO_2). df: degrees of freedom; I^2: inconsistency.

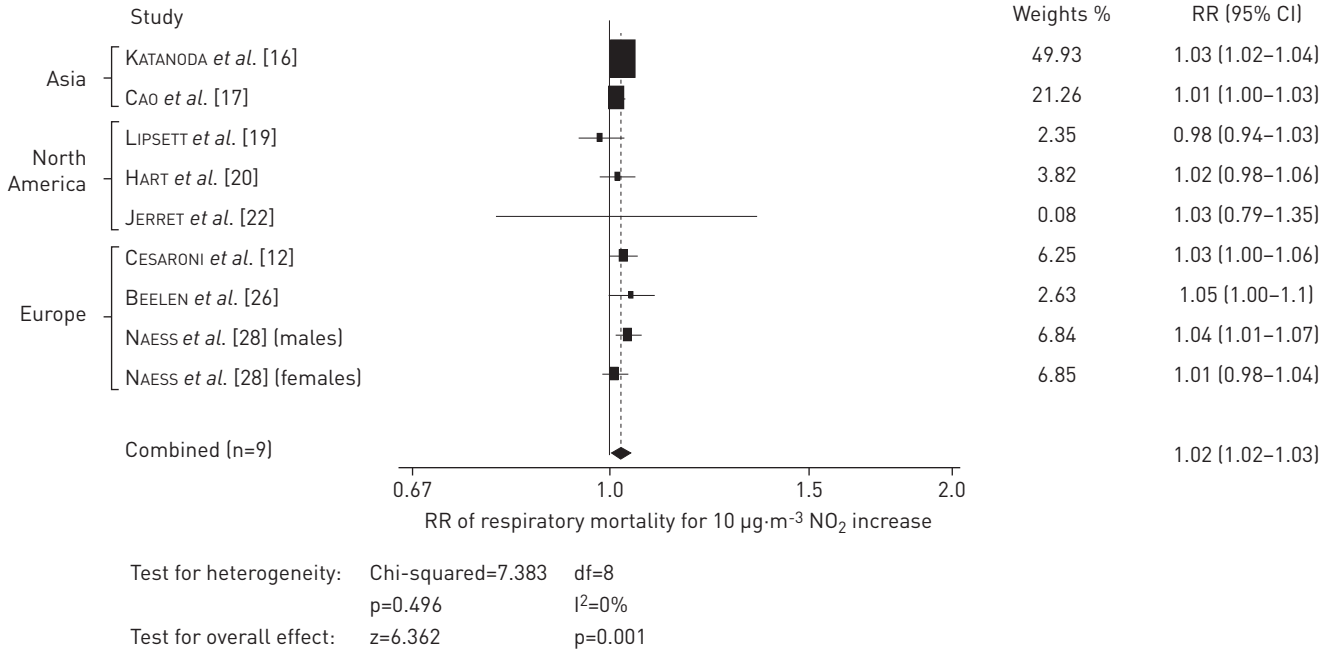

FIGURE 3 Relative risks (RRs) of respiratory mortality with increasing chronic exposure to nitrogen dioxide (NO_2). df: degrees of freedom; I^2: inconsistency.

TABLE 2 Pooled[#] effects of nitrogen dioxide (NO_2) and particulate matter <2.5 μm in diameter ($PM_{2.5}$) on natural and specific-cause mortality

	Total or natural mortality				Cardiovascular mortality				Respiratory mortality			
	Increment $\mu g \cdot m^{-3}$	Studies n	RR (95% CI)	I^2 %	Increment $\mu g \cdot m^{-3}$	Studies n	RR (95% CI)	I^2 %	Increment $\mu g \cdot m^{-3}$	Studies n	RR (95% CI)	I^2 %
NO_2	10	10[¶]	1.040 (1.015–1.065)	91	10	15[+,§,*f*]	1.152 (1.094–1.213)	98	10	7[##,¶¶]	1.024 (1.010–1.038)	0
	14.6		1.059 (1.022–1.096)		17.9		1.288 (1.174–1.413)		15		1.036 (1.015–1.058)	
$PM_{2.5}$	10	9[¶,++]	1.050 (1.024–1.078)	78	10	14[+,§,*f*,¶¶]	1.228 (1.084–1.39)	98	10	6[##,¶¶,++]	1.062 (1.022–1.104)	11
	5.8		1.029 (1.014–1.045)		7		1.155 (1.058–1.259)		7		1.043 (1.015–1.072)	

RR: relative risk; I^2: inconsistency. [#]: random estimates. [¶]: two studies [17, 34] were excluded as they did not give interquartile range (IQR) estimates or dispersion parameters to estimate them. [+]: three studies [17, 25, 34] were excluded as they did not give IQR estimates or dispersion parameters to estimate them. [§]: two studies [28, 33] were included twice with separate groups of males and females. [*f*]: two studies from Europe [13, 32] estimated cardiopulmonary mortality as a whole; they were included in the pooled, cardiovascular mortality estimate and excluded from the respiratory mortality pooled estimate. [##]: three studies [16, 17, 34] were excluded as they did not give IQRs or dispersion parameters to estimate them. [¶¶]: one study from Europe [28] was included twice with separate groups of males and females. [++]: one study from Canada [22] could be included as it did not present the particulate matter effect estimates.

DOI: 10.1183/09031936.00114713

The pooled estimate of NO_2 effects (table 1 and fig. 3) on respiratory mortality was RR 1.02 (95% CI 1.02–1.03) while the PM2.5 effect was RR 1.05 (95% CI 1.01–1.09). The results for NO_2 were homogeneous across the studies. The large effect of PM2.5 on respiratory mortality was notable in Europe, where an effect of 8% was detected with a heterogeneity as high as 40%.

The results of the pooled estimates using IQRs as the exposure metric are presented in table 2. The number of studies with available estimates is slightly lower than for the analysis using a fixed increment, as four of them [16, 17, 25, 34] did not provide IQR data or figures to estimate them. The median IQRs were different for the outcomes considered and the variability of NO_2 levels was greater than for PM2.5 (*e.g.* 14.1 $\mu g \cdot m^{-3}$ for NO_2 and 5.4 $\mu g \cdot m^{-3}$ for $PM_{2.5}$ in natural/total mortality studies). The estimates based on IQRs showed greater effects of NO_2 than of PM2.5 on total mortality (6% *versus* 3% for PM2.5) and on cardiovascular mortality (29% *versus* 16% for PM2.5). The effects were similar for respiratory mortality (4% for both NO_2 and PM2.5).

The results of the multipollutant analyses (as assessed in the original papers) are reported in table S4. Only seven studies [12, 17, 18, 20, 22, 24, 32] among those included in the meta-analysis performed bipollutant analyses, including those by GEHRING *et al.* [32], JERRETT *et al.* [22] and LIPFERT *et al.* [24], who analysed the NO_2 effects only with a traffic indicator. CAO *et al.* [17] in Asia and CESARONI *et al.* [12] in Europe found no change in the effects of NO_2 on total mortality when it was analysed together with particulate matter, whereas the reduced effect observed by HART *et al.* [20] in the USA relates to a model including three pollutants (NO_2, PM10 and sulfur dioxide). Among the three studies that analysed NO_2 and particulate matter effects on cardiovascular mortality [17, 18, 20], two [18, 20] found independent effects of NO_2. Three studies analysed NO_2 effects with a traffic indicator: those by LIPFERT *et al.* [24] in the USA and JERRET *et al.* [22] in Toronto found reduced effects of NO_2 on total and cardiovascular mortality, but no change was reported in the European study [32].

Discussion

We found similar risk estimates for total mortality in studies investigating the long-term effects of both NO_2 and PM2.5 (4% *versus* 5% increase, respectively) using an exposure metric of 10 $\mu g \cdot m^{-3}$. In Europe, there was a 7% increase in total mortality for both NO_2 and fine particles. HOEK *et al.* [5], in their recent review, found a worldwide pooled estimate of 5.5% (95% CI 3.1–8.0%) for NO_2; their study, unlike the present one, included estimates from studies that did not analyse particles. We found greater effects of PM2.5 than of NO_2 for cardiovascular (20% *versus* 13%) and respiratory (5% *versus* 2%) mortality, per 10 $\mu g \cdot m^{-3}$ of pollutants. Per IQR, estimates of cardiovascular mortality were larger for NO_2 than for PM2.5 (29% *versus* 16%). In the recent review by HOEK *et al.* [5], NO_2 effects ranging from 3% to 36% per 10 $\mu g \cdot m^{-3}$ have been reported for cardiovascular mortality in European cohorts as well as an effect of 12% for respiratory mortality. All our estimates were rather heterogeneous, with the sole exception of respiratory mortality. In the few studies with bipollutant models, the effects of NO_2 and particles (of different sizes) seemed independent.

The comparison of studies carried out in worldwide geographical areas is beset by many challenges. The methods used to assess exposure play an important role. The NO_2 levels observed in the USA, Asia and Europe are very similar. In contrast, PM2.5 levels are lower in the USA than in Europe, and both are much lower in Asia. The methods used in assessing exposure (such as dispersion modelling, LUR models and inverse distance weighting) as well as the methods used in pollution monitoring (such as the number and location of fixed monitors as well as the daily sampling intervals) could all influence the quality of the exposure assessment causing a possible differential misclassification. However, as lower levels of PM2.5 in the USA produced effects on total mortality comparable to those observed in Europe, a different toxicity of the fine particle components between areas could also play a role.

The length of the exposure (or modelling) period was not always taken sufficiently into consideration. This is an important drawback when risk estimates for long-term mortality are to be compared. The length of exposure assessment ranged from 1 [21, 23] to 12 years [15] when reported as measured levels of pollutants, and from 2 to 40 years when estimated by means of models (mainly dispersion or LUR models). In addition, length of exposure is mostly assessed on the basis of length of residence, assuming that the pollution levels monitored in a shorter time than residence period represents the real exposure of the whole residence period. Only a few investigations have addressed the long-term stability of the spatial contrasts in the exposures used in the epidemiological analyses [35, 36]

Latency differs among studies, often not consistently with the study design. Three studies [15, 17, 19] had an overlap between the exposure and the follow-up periods; others started the follow-up in the same year the pollutant exposure was assessed [24, 28], although they prolonged the follow-up for up to 30 years. In a few studies [12, 20, 21, 34] exposure was assessed in a few years included within the follow-up or even after the follow-up [22]. Finally, eight studies [16, 18, 23, 25, 26, 27, 32, 33] carried out a follow-up from at least

1 year after the assessed exposure for up to 6–20 years thereafter. Therefore, the differences in assessing the temporal relationship between exposure and outcome across the studies represent a possible challenge for the interpretation of the results. Doubts could also arise when the exposure has been assessed after starting follow-up, since strong assumptions were necessary about the length of residence and the corresponding levels of pollutants.

Some methodological choices in carrying out the meta-analysis need to be discussed. The first relevant point for the interpretation of the results is the reliability of the meta-analytic estimates by geographic area. The European and North American estimates for total and cardiovascular mortality, as well as the European estimates for respiratory mortality, are more reliable than Asian estimates because at least four articles were included. However, the heterogeneity across the studies affects the reliability [10, 37] of the estimates of total and cardiovascular mortality, in all areas and for both pollutants. The sole exception is the effect of NO_2 on respiratory mortality in both Europe and the USA (with three studies). It should be noted that the heterogeneity for cardiovascular mortality was strongly reduced when we restricted to studies with a better quality of exposure assessment and evaluating the entire population and not at at-risk groups [38].

A second methodological point for the interpretation of results is related to the pollutant exposure metric for analysing the effects of different pollutants. Using a fixed increase of 10 $\mu g \cdot m^{-3}$ in pollutant levels makes it possible to compare the effects of a single pollutant across different studies and different countries. In contrast, if one wishes to compare the effects of two or more pollutants, one needs another metric that takes into account the different distributions of pollutant levels. A pollutant with a broader exposure distribution will show smaller effect estimates per unit increase than a pollutant with a less wide exposure distribution in the same population. The additional meta-analyses we performed using the IQRs of NO_2 and $PM_{2.5}$ as the exposure metric show that the NO_2 effects on mortality are larger than those of $PM_{2.5}$, at least for total and cardiovascular mortality.

There is obviously a need for caution in drawing conclusions about the independence of NO_2 effects from $PM_{2.5}$ effects from the multipollutant models. First, not all countries were included (*e.g.* Asia was less represented in this analysis). Second, the high correlation between NO_2 and $PM_{2.5}$ (around 0.7–0.8) in these studies still suggests the possibility that the NO_2 effects could be due in part to confounding from particulate matter. Third, uncertainty is also due to the limited number of bipollutant estimates available. It should be noted, however, that only minimal difference between the single-pollutant and the multipollutant results for NO_2 were found.

Finally, although this study cannot respond exhaustively to the causality question about NO_2, given that it was a meta-analysis, it contributes to the scientific debate on this topic because it assesses the respective role of NO_2 and $PM_{2.5}$ as they emerge from prospective studies [37] and provides a pooled effect estimate to be used for future impact health assessment.

In conclusion, the magnitude of the long-term effects of NO_2 on mortality is at least as important as that of $PM_{2.5}$. These results hold when using either 10 $\mu g \cdot m^{-3}$ or IQR as the metric of choice. The results of the multipollutant models suggest that the role of NO_2 is independent of that of particles. All these elements may be useful when discussing the causality issue for NO_2. Moreover, NO_2 is the only widely regulated pollutant (apart from carbon monoxide) that indicates exposure to combustion pollution. Health impact assessments relying only on $PM_{2.5}$, and not considering NO_2, would be neglecting an important source of the adverse effects of today's pollution mixture.

Acknowledgements

We thank K. Christenfeld (freelance medical editor, Rome, Italy) for editorial revision and S. Ricci (Dept of Epidemiology, Regional Health System, Lazio Region, Rome, Italy) for her help with the figures.

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