Distinguishing the associations between daily mortality and hospital admissions and nitrogen dioxide from those of particulate matter: a systematic review and meta-analysis

I C Mills,1 R W Atkinson,2 H R Anderson,2,3 R L Maynard,4 D P Strachan2

ABSTRACT

Objectives: To quantitatively assess time-series studies of daily nitrogen dioxide (NO2) and mortality and hospital admissions which also controlled for particulate matter (PM) to determine whether or to what extent the NO2 associations are independent of PM.

Design: A systematic review and meta-analysis.

Methods: Time-series studies—published in peer-reviewed journals worldwide, up to May 2011—that reported both single-pollutant and two-pollutant model estimates for NO2 and PM were ascertained from bibliographic databases (PubMed, EMBASE and Web of Science) and reviews. Random-effects summary estimates were calculated globally and stratified by different geographical regions, and effect modification was investigated.

Outcome measures: Mortality and hospital admissions for various cardiovascular or respiratory diseases in different age groups in the general population.

Results: 60 eligible studies were identified, and meta-analysis was conducted on 23 outcomes. Two-pollutant model study estimates generally showed that the NO2 associations were independent of PM mass. For all-cause mortality, a 10 µg/m3 increase in 24-hour NO2 was associated with a 0.78% (95% CI 0.47% to 1.09%) increase in the risk of death, which reduced to 0.60% (0.33% to 0.87%) after control for PM. Heterogeneity between geographical region-specific estimates was removed by control for PM. Heterogeneity between geographical region-specific estimates was removed by control for PM.

Conclusions: The association between short-term exposure to NO2 and adverse health outcomes is largely independent of PM mass. Further studies should attempt to investigate whether this is a generic PM effect or whether it is modified by the source and physicochemical characteristics of PM. This finding strengthens the argument for NO2 having a causal role in health effects.

INTRODUCTION

Outdoor air pollution has long been established as a hazard to human health, with particulate matter (PM) regarded as the most plausible toxicant in the mixture of ambient air pollutants. The epidemiological evidence has consistently shown adverse associations between chronic and short-term exposure to PM and mortality and morbidity from cardiovascular and respiratory disease, and this is supported by experimental evidence.6 While the epidemiological evidence also shows relationships between nitrogen dioxide (NO2) and adverse health effects, concerns have been expressed repeatedly about the causal nature of these associations.7–11 It has been asserted...
that the \( \text{NO}_2 \) associations do not reflect adverse effects of \( \text{NO}_2 \) itself but, rather, reflect the health effects of other air pollutants, mainly \( \text{PM} \) or other components of the complex mixture of traffic-related air pollutants. Primarily, this is due to the strong correlations between \( \text{NO}_2 \) and other combustion-derived air pollutants, especially \( \text{PM} \). The extent of these correlations varies from city-to-city and over time, due to variations in emission sources. Scepticism also exists because of limited experimental evidence (controlled human exposure and animal toxicology studies) for \( \text{NO}_2 \), which, to date, has focused largely on respiratory endpoints and has generally employed concentrations of \( \text{NO}_2 \) well above current ambient levels.\(^2\)\(^-\)\(^9\) In light of the uncertainties regarding \( \text{NO}_2 \) and the stronger evidence for associations between \( \text{PM} \) and health, many researchers and policymakers have adopted a view that the epidemiological associations of \( \text{NO}_2 \) reflect adverse health effects of \( \text{PM} \).

In an earlier paper, we reviewed the time-series evidence associating daily concentrations of \( \text{NO}_2 \) with daily mortality and emergency hospital admissions.\(^{12}\) In this study, we assess the subset of time-series studies, reporting all-year estimates of \( \text{NO}_2 \) from both single-pollutant and two-pollutant models adjusted for \( \text{PM} \) to determine whether the \( \text{NO}_2 \) associations are attenuated after adjustment for \( \text{PM} \).

**METHODS**

The full method and a priori protocols governing the identification of studies and effect estimates for the systematic review have been described previously;\(^{12-14}\) but a synopsis, along with aspects unique to this review, is provided below.

**Identification of studies for review**

Three bibliographic databases were searched to identify peer-reviewed time-series studies of \( \text{NO}_2 \) and daily mortality or hospital admissions indexed up to May 2011. No restriction on language was applied. The literature search strategy is described in the online supplementary material, and the following inclusion criteria were used: papers must (1) have had a minimum of 1 year of data; (2) been based on the general population; (3) have controlled for important confounding factors, including season and meteorological factors; and (4) have reported sufficient quantitative information, in numeric format, to enable the calculation of standardised effect estimates and standard errors for use in quantitative analysis. Two authors of the review—ICM and RWA—undertook the literature search.

**Data extraction and coding**

Data from each relevant study were entered into a Microsoft Access database (Microsoft Office 2010, Microsoft Corporation). These included:

A. Citation details of each paper;
B. All-year single-pollutant and two-pollutant model estimates of \( \text{NO}_2 \) adjusted for \( \text{PM} \);
C. Single-pollutant and two-pollutant model estimates of \( \text{PM} \) adjusted for \( \text{NO}_2 \) reported in studies providing data for \( \text{NO}_2 \);
D. Season-specific estimates of \( \text{NO}_2 \), including those adjusted for \( \text{PM} \), from studies reporting all-year estimates;
E. Descriptive (outcome, diagnosis (International Classification of Diseases codes), age, etc) and quantitative data (pollution increment and averaging time etc) associated with each estimate, and needed for calculating standardised estimates expressed as the percentage change (and 95% CI) in the mean number of daily events associated with a 10 µg/m\(^3\) increase in \( \text{NO}_2 \) (or \( \text{PM} \));
F. Correlations between concentrations of \( \text{NO}_2 \) and \( \text{PM} \);
G. Effect modifiers for investigating of sources of heterogeneity in all-year estimates.

Time-series studies often report results for different time lags (in days) between exposure and health events, and they vary in the lag for the reported results. We identified, for each outcome/disease/age/averaging time combination from each study, a pair of estimates of \( \text{NO}_2 \) that is from a single-pollutant model and a corresponding estimate adjusted for \( \text{PM} \) for the same lag, to enable comparison of the \( \text{NO}_2 \) association before and after adjustment for \( \text{PM} \). To avoid selection bias, we developed an a priori protocol for identifying the principal lag for each outcome/disease/age/averaging time combination for use in our review. This was the lag highlighted by the author or stated a priori, and if this was not clear, because several lagged model estimates were reported, we chose (1) the lag with the highest statistical significance, regardless of the estimate being positive or negative, or (2) the lag with the largest estimate, again, irrespective of its direction. If only results from cumulative or distributed lag models—that is, lags averaged over several days—were reported in a study, these were used. In some instances, a different lag was investigated in two-pollutant models. In such cases, the lagged estimate from the two-pollutant model was coded according to the same algorithm and the (additional) corresponding single-pollutant estimate for the same lag was coded in our database.

Processing of data also included classifying each study into the geographical region, as the WHO region, in which the study was conducted, as well as categorising the various metrics of \( \text{PM} \) controlled for in two-pollutant models; see online supplementary material for details.

**Statistical analyses**

A similar procedure to that outlined in our earlier paper was used for meta-analysis,\(^{12}\) but with some modifications, in order to identify a pair of estimates of \( \text{NO}_2 \) for each pollutant/outcome combination from each study. We applied an a priori protocol to select estimates for meta-analysis to avoid selection bias and duplication of studies from the same population. We gave priority to
Meta-analysis was undertaken for available combination available for an outcome/disease/age/averaging time regarded as being evidence of high heterogeneity.17 C. We conducted additional meta-analyses for NO2 B. Corresponding NO2 estimates adjusted for any PM A. Single-pollutant NO2 estimates relating to two-pollutant was assessed using the I2 statistic,16 with I2 statistics >50% heterogeneity between WHO region summary estimates multicity estimates being pooled to produce a global estimate and WHO region-specific summary estimates. Heterogeneity between WHO region summary estimates was assessed using the I2 statistic,16 with I2 statistics >50% regarded as being evidence of high heterogeneity.17 Meta-analysis was undertaken for: A. Single-pollutant NO2 estimates relating to two-pollutant models; B. Corresponding NO2 estimates adjusted for any PM metric: i. if within a study, several estimates of NO2 adjusted for different individual PM metrics were available, a NO2 estimate was selected according to the following order of priority of PM metric used in adjustment: PM10, PM2.5, Black Smoke, PM10–2.5; ii. if, having applied the protocol, a NO2 estimate was not selected for a city because several were available due to different PM metrics used to adjust the NO2 effect in different studies, the NO2 estimate was chosen in the order of priority of the PM metrics listed above. C. We conducted additional meta-analyses for NO2 adjusted for specific metrics of particles, for example, NO2 adjusted for PM10 and separately for PM2.5, and so on, to determine whether the NO2 associations showed different sensitivity to control for different PM metrics. All analyses were conducted in STATA (STATA/SE V.11. StataCorp, Texas, USA).

RESULTS
Sixty studies provided estimates of both (1) NO2, single-pollutant, and (2) NO2 adjusted for PM: a list of references is provided in the online supplementary material. Table 1 presents a summary of these 60 time-series studies stratified by the PM metric controlled for in regression models, broad disease categories, WHO regions in which the studies were conducted, single-city and multicity study designs, and by averaging time (24-hour and 1 hour).

There were 36 and 24 studies of daily mortality or hospital admissions, respectively, and 13 studies used a multicity design. The majority of the studies were conducted in the WHO regions European A and Western Pacific region B, and most used 24-hour NO2. Forty of the 60 studies controlled for the effects of daily PM10 in the regression models for NO2, and a much smaller number of studies used other particle size fractions or constituents of PM. Eight studies of mortality and two of hospital admissions reported estimates of NO2, each adjusted for a different PM metric. None of the studies investigated the influence of carbon on the NO2 associations, and four studies controlled for the effects of ultrafine particles.

**NO2 and all-cause mortality**

Figure 1 shows all available (32 pairs) single-pollutant and two-pollutant estimates for 24-hour NO2 and daily all-cause mortality in all ages. In the majority of studies, daily NO2 was positively and significantly associated with increases in the risk of death, including after controlling for daily PM. In many of the studies, the NO2 estimates were not greatly reduced in size, changed direction or lose statistical significance after adjustment for PM. In general, the NO2 estimates appeared robust to adjustment for PM at both high and low correlations between concentrations of NO2 and PM.

Fifteen (of 32) pairs of estimates for 24-hour NO2 and all-cause mortality, which represented 26 cities from five WHO regions, were selected for meta-analysis (see online supplementary figure S1). The random-effects single-pollutant summary estimate for all-cause mortality was 0.78% (95% CI 0.47% to 1.09%) per 10 µg/m3 increase in NO2. There was evidence of high heterogeneity (I2=66.9%) between the WHO region-specific estimates, which ranged from 0.48% for WHO region America A to 1.41% for South East Asia B (see online supplementary table S1). The overall estimate was comparable to the single-pollutant summary estimate of 0.71% (95% CI 0.43% to 1.00%) calculated from the larger body of time-series evidence analysed in our previous paper.12 After adjustment for daily PM, all-cause mortality remained positively and significantly associated with 24-hour NO2: 0.60% (95% CI 0.33% to 0.87%) per 10 µg/m3 increase in NO2, and there was no evidence of heterogeneity (I2=0%) between the region-specific estimates.

Control for specific PM metrics did not greatly alter the relationship of 24-hour NO2 with all-cause mortality (table 2). With the exception of NO2 adjusted for PM10, and to a lesser extent PM2.5, meta-analyses for NO2 adjusted for the remaining PM metrics were limited to
findings from a multicity Canadian study by Burnett et al.\textsuperscript{18}—see figure 1.

Six pairs of estimates were available for meta-analysis for all-cause mortality and 1 hour NO\textsubscript{2} adjusted for PM (see online supplementary figure S2). Thirty of the 36 cities represented by these estimates were in Europe. Meta-analysis of four pairs of estimates resulted in an overall estimate of 0.32% (95% CI −0.02% to 0.66%) for a 10 µg/m\textsuperscript{3} increment in 1 hour NO\textsubscript{2} and 0.20% (95% CI −0.24% to 0.65%) following adjustment for PM (see online supplementary table S2). High heterogeneity was observed between the WHO region-specific estimates. In contrast with findings for 24-hour measures, the summary estimate for 1 hour NO\textsubscript{2} for WHO region European A was little affected by adjustment for PM\textsubscript{10} (or Black Smoke) —see online supplementary table S2. Table 3 provides meta-analysis results for all-cause mortality and 1 hour NO\textsubscript{2} adjusted for different PM metrics. Control for PM\textsubscript{10} led to attenuation of the estimate and loss of statistical significance, while the association was robust to control for Black Smoke and visibility (measured as black suspended particles, BSP).

**NO\textsubscript{2} and mortality from specific causes**

NO\textsubscript{2} estimates adjusted for PM were available for several specific causes of death in all ages: all cardiovascular (see online supplementary figures S3 and S4), all respiratory (see online supplementary figure S5), stroke (see online supplementary figure S6), cardiac (see online supplementary figure S7), ischaemic heart disease, dysrhythmia, chronic obstructive pulmonary (COPD) disease including asthma and upper respiratory infections (see online supplementary figure S8). Sufficient numbers of estimates for meta-analysis were available for all cardiovascular (see online supplementary table S3), all respiratory (see online supplementary table S4) and stroke (see online supplementary table S5) mortality.

Eight studies providing 14 pairs of estimates showed positive associations between all cardiovascular deaths and 24-hour NO\textsubscript{2}, including after adjustment mainly for PM\textsubscript{10} (see online supplementary figure S3). However,
attenuation of estimates and loss of statistical significance was observed in the few studies with control for PM2.5 or Black Smoke. Meta-analysis of 10 pairs of estimates found a 1.07% (95% CI 0.43% to 1.72%) increase in the risk of death from all cardiovascular diseases per 10 µg/m³ increase in 24-hour NO2 (see online supplementary table S3 and figure S9). This was attenuated (0.82% (95% CI 0.22% to 1.42%))—see online supplementary table S3—following adjustment for PM, but comparable to our earlier result (0.88% (95% CI 0.63% to 1.13%).12 Control of the NO2 association with all cardiovascular mortality for specific PM metrics showed an association that was robust to adjustment for PM10 (table 2). There were too few estimates to permit meta-analysis for other PM metrics controlled for in the studies. The available data for 1 hour NO2 and all cardiovascular mortality were sparse and limited to two studies representing 29 European cities that showed positive NO2 associations that were robust to adjustment for both PM10 and Black Smoke (see table 3 and online supplementary figure S4).

Evidence for all respiratory mortality and 24-hour NO2 adjusted for PM came from six cities (see online supplementary figure S5). Meta-analysis produced a 1.42% (95% CI 0.64% to 2.21%) increased risk of all respiratory deaths per 10 µg/m³ increase in 24-hour NO2 (see online supplementary table S4 and figure S10). The corresponding estimate adjusted for particles was attenuated (1.13% (95% CI 0.75% to 1.42%)) derived from the larger body of time-series evidence examined in our previous paper.12 There was no evidence of heterogeneity (I²=0%) between the geographic specific estimates either before or after adjustment for PM (see online supplementary table S4). Evidence for associations between all respiratory mortality and 1 hour NO2 came solely from the multicity APHEA II study of 29 European cities,19 which showed a positive association that was robust to adjustment for PM10 but not Black Smoke (table 3).

PM and mortality
Meta-analyses were undertaken separately for PM adjusted for the different averaging times of NO2 to allow comparison with the relevant meta-analyses for NO2, using data from the same studies, cities and time periods. Figure 2 shows positive, single-pollutant

---

**Figure 1** All available studies providing two-pollutant model estimates for meta-analysis for all-cause mortality, all ages, 24-hour NO2, 1000×ln (RR) approximates to a percentage change per 10 µg/m³. *Single-pollutant model estimate for days with both NO2 and visibility (coefficient of haze, COH) data in Burnett et al.18 [RMID 3000]. NO2, single-pollutant NO2 adjusted for PM.
### Table 2
Random-effects summary estimates (as percentage change (95% CIs)) for mortality or hospital admissions associated with a 10 µg/m³ increase 24 hour average pollution

<table>
<thead>
<tr>
<th></th>
<th>All SC/MC*</th>
<th>Selected SC/MC (cities)†</th>
<th>24-hour NO₂</th>
<th>24-hour PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Single-pollutant</td>
<td>Adjusted for PM</td>
</tr>
<tr>
<td>All-cause mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>13/3</td>
<td>4/1 (21)</td>
<td>0.92 (0.58 to 1.72)</td>
<td>0.85 (0.52 to 1.18)</td>
</tr>
<tr>
<td>PM₂.₅</td>
<td>2/3</td>
<td>2/1 (14)</td>
<td>0.53 (0.42 to 0.64)</td>
<td>0.57 (0.24 to 0.89)</td>
</tr>
<tr>
<td>PM₁₀–₂.₅</td>
<td>0/3</td>
<td>0/1 (12)</td>
<td>0.62 (0.19 to 1.06)</td>
<td>0.73 (0.28 to 1.18)</td>
</tr>
<tr>
<td>Visibility</td>
<td>0/1</td>
<td>0/1 (12)</td>
<td>0.60 (0.34 to 0.87)</td>
<td>0.66 (0.33 to 1.00)</td>
</tr>
<tr>
<td>All cardiovascular mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>10/0</td>
<td>4/0 (8)</td>
<td>0.99 (0.49 to 1.49)</td>
<td>0.87 (0.28 to 1.46)</td>
</tr>
<tr>
<td>All respiratory mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>7/0</td>
<td>2/0 (5)</td>
<td>1.44 (0.63 to 2.27)</td>
<td>1.15 (0.47 to 1.84)</td>
</tr>
<tr>
<td>All respiratory hospital admissions, children (5–14 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>0/1</td>
<td>0/1 (5)</td>
<td>5.95 (1.74 to 10.33)</td>
<td>6.56 (3.08 to 10.17)</td>
</tr>
<tr>
<td>Cardiac hospital admissions, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>2/1</td>
<td>2/1 (7)</td>
<td>0.93 (0.46 to 1.40)</td>
<td>0.75 (−0.13 to 1.64)</td>
</tr>
<tr>
<td>BS</td>
<td>0/1</td>
<td>0/1 (4)</td>
<td>0.68 (0.17 to 1.20)</td>
<td>0.36 (−0.65 to 1.38)</td>
</tr>
<tr>
<td>TSP</td>
<td>0/1</td>
<td>0/1 (6)</td>
<td>1.03 (0.45 to 1.61)</td>
<td>1.08 (0.43 to 1.72)</td>
</tr>
</tbody>
</table>

*Numbers of available pairs of single-city (SC)/multi-city (MC) estimates from all studies.
†Numbers of pairs of pooled (from single-city estimates) and multicity estimates used to calculate the overall summary estimate across WHO regions. Estimates were selected for meta-analysis from all those available. The number of cities represented by the summary estimates is given in brackets.
‡The results for visibility (measured as coefficient of haze (COH units)) are not comparable to other PM results.

BS, Black Smoke; NO₂, nitrogen dioxide; PM, particulate matter.

### Table 3
Random-effects summary estimates (as percentage change (95% CIs)) for mortality or hospital admissions associated with a 10 µg/m³ increase in air pollution

<table>
<thead>
<tr>
<th></th>
<th>All SC/MC*</th>
<th>Selected SC/MC (cities)†</th>
<th>1 hour NO₂</th>
<th>24-hour PM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Single-pollutant</td>
<td>Adjusted for PM</td>
</tr>
<tr>
<td>All-cause mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>2/1</td>
<td>2/1 (32)</td>
<td>0.22 (−0.15 to 0.60)</td>
<td>0.10 (−0.40 to 0.61)</td>
</tr>
<tr>
<td>BS</td>
<td>0/2</td>
<td>0/1 (30)</td>
<td>0.30 (0.22 to 0.38)</td>
<td>0.33 (0.23 to 0.43)</td>
</tr>
<tr>
<td>Visibility</td>
<td>0/1</td>
<td>0/1 (4)</td>
<td>0.63 (0.21 to 1.05)</td>
<td>0.52 (0.05 to 1.00)</td>
</tr>
<tr>
<td>All cardiovascular mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>1/1</td>
<td>0/1 (29)</td>
<td>0.40 (0.29 to 0.51)</td>
<td>0.35 (0.21 to 0.49)</td>
</tr>
<tr>
<td>BS</td>
<td>1/1</td>
<td>0/1 (29)</td>
<td>0.40 (0.29 to 0.51)</td>
<td>0.44 (0.31 to 0.57)</td>
</tr>
<tr>
<td>All respiratory mortality, all ages</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>0/1</td>
<td>0/1 (29)</td>
<td>0.38 (0.17 to 0.59)</td>
<td>0.37 (0.08 to 0.66)</td>
</tr>
<tr>
<td>BS</td>
<td>0/1</td>
<td>0/1 (29)</td>
<td>0.38 (0.17 to 0.59)</td>
<td>0.26 (−0.12 to 0.64)</td>
</tr>
<tr>
<td>All respiratory hospital admissions, children (&lt;5 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM₁₀</td>
<td>1/1</td>
<td>1/1 (6)</td>
<td>0.77 (−0.59 to 2.15)</td>
<td>0.13 (−0.09 to 0.35)</td>
</tr>
<tr>
<td>BS</td>
<td>0/1</td>
<td>0/1 (4)</td>
<td>1.62 (0.41 to 2.84)</td>
<td>4.85 (0.41 to 9.50)</td>
</tr>
<tr>
<td>All respiratory hospital admissions, elderly (65+years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visibility</td>
<td>0/1</td>
<td>0/1 (4)</td>
<td>1.42 (0.79 to 2.06)</td>
<td>1.21 (0.47 to 1.95)</td>
</tr>
<tr>
<td>Cardiac hospital admissions, elderly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visibility</td>
<td>0/1</td>
<td>0/1 (4)</td>
<td>1.21 (0.84 to 1.58)</td>
<td>0.73 (0.31 to 1.16)</td>
</tr>
</tbody>
</table>

*Numbers of available pairs of single-city (SC)/multi-city (MC) estimates from all studies.
†Numbers of pairs of pooled (from single-city estimates) and multicity estimates used to calculate the overall summary estimate across WHO regions. Estimates were selected for meta-analysis from all those available. The number of cities represented by the summary estimates is given in brackets.
‡The results for visibility (measured as black suspended particles (10⁻⁴/m)) are not comparable to other PM results.

BS, Black Smoke; NO₂, nitrogen dioxide; PM, particulate matter.
associations between various mass metrics of PM and all-cause mortality. In the majority of studies, attenuation of estimates was observed following control for 24-hour NO2. Estimates for ultrafine particles and all-cause mortality were robust to adjustment for 24-hour NO2 (see online supplementary figure S11), but the data came from three studies conducted in the same city—Erfurt, Germany. Results of meta-analysis for all-cause mortality and PM metrics are shown in tables 2 and 3 for adjustment for 24-hour and 1 hour NO2, respectively. In contrast to the results for NO2, the summary estimates for PM were attenuated, in most cases by more than half, and CIs overlapped zero. Evidence of high heterogeneity between region-specific summary estimates for PM10 and all-cause mortality was identified (see online supplementary table S6). Summary estimates for deaths from all cardiovascular or all respiratory diseases and PM were also sensitive to control for NO2 (see tables 2 and 3; online supplementary figures S12, S13, tables S7 and S8 for region-specific results).

**NO2 and hospital admissions**

Few cause-specific and age-specific combinations of hospital admissions for 24-hour or 1 hour NO2 with control for PM had sufficient numbers of estimates for meta-analysis—all respiratory diseases in children and the elderly, asthma in children, and cardiac disease in all ages and the elderly—and half were based solely on a multiplicity estimate from a single study.

Positive associations were identified between all respiratory hospital admissions in different age groups and 24-hour or 1 hour NO2, which remained after control for PM (see tables 2 and 3; online supplementary figures S12, S13, tables S7 and S8 for region-specific results).

![Figure 2](http://bmjopen.bmj.com/)

**Figure 2** All studies providing two-pollutant model estimates for all-cause mortality, all ages, PM adjusted for 24-hour NO2. PM, particulate matter. — PM, single-pollutant — PM adjusted for NO2.
category (table 2) identified positive estimates that were attenuated and CIs overlapped zero after controlling for PM\textsubscript{10} and Black Smoke. One multicity study of four Australian cities provided evidence for the relationship between 1 hour NO\textsubscript{2} and cardiac admissions in the elderly. The association (1.21% (95% CI 0.84% to 1.58%)) was weakened by control for BSP (an indicator of fine particles), but remained statistically significant (0.73% (95% CI 0.31% to 1.16%)).

**Sources of variation in NO\textsubscript{2} estimates**

We examined season-specific NO\textsubscript{2} estimates of mortality from studies that reported all-year estimates to explore possible effect modification by season. Some studies, mainly from Western Europe, Canada and the USA, reported stronger associations between daily mortality and NO\textsubscript{2} in the summer months (see online supplementary figures S20–S22). The extent of the correlations between concentrations of NO\textsubscript{2} and PM in the different seasons is unclear because very few studies reported these data, and only one study reported season-specific estimates adjusted for PM. Similarly, limited evidence is available on which to base an assessment of seasonal variation of associations between hospitalisation for cardiovascular and respiratory diseases and 24-hour NO\textsubscript{2} (see online supplementary figure S23).

We explored reasons for the observed high heterogeneity by ranking study estimates for all-cause mortality and 24-hour NO\textsubscript{2} (from the full data set)\textsuperscript{12} by different potential effect modifiers (see online supplementary figures S24–S27). None of the variables used to represent the pollution and meteorological environments in the cities examined accounted for the observed between-study variability.

**DISCUSSION**

Sixty time-series studies of NO\textsubscript{2} were used to determine whether NO\textsubscript{2} is associated with daily mortality or hospital admissions independently of daily PM. In general, our results demonstrate that after controlling for PM, daily NO\textsubscript{2} remained significantly associated with increases in the risk of adverse health outcomes. The evidence appears clearest for daily deaths from all causes and from all cardiovascular and all respiratory diseases, and for all respiratory hospital admissions, outcomes for which more co-pollutant estimates were available. Robustness of the NO\textsubscript{2} associations to control for PM was observed at both high and low correlations between NO\textsubscript{2} and PM, and no clear relationship could be discerned between the correlations and changes in the size of the adjusted NO\textsubscript{2} estimates. In contrast to the results for NO\textsubscript{2}, the associations between daily PM and the main mortality outcomes (all cause, all cardiovascular, all respiratory) were very sensitive to the inclusion of NO\textsubscript{2} in two-pollutant models.

Two/multipollutant models are increasingly being used to draw conclusions about whether or not NO\textsubscript{2} is independently associated with adverse health outcomes. This comprehensive review provides systematic evaluation and formal meta-analysis of the full body of two-pollutant estimates of NO\textsubscript{2} adjusted for PM, across several cause-specific and age-specific health outcomes, both globally and by different geographical regions. While earlier reviews\textsuperscript{7–8 13} 20–23 included some assessment of these data, they were either limited in scope to specific health outcomes, and/or examined two-pollutant and multipollutant model NO\textsubscript{2} estimates together, or did not undertake meta-analysis whatsoever. Another key strength of this review is the protocol-led approach to identifying and assembling studies and estimates, which aimed to minimise selection bias in the different stages of the review.

The subset of studies of NO\textsubscript{2} analysed in this paper were generally comparable to the studies examined in our earlier paper in terms of the magnitudes of summary estimates and overlap in CIs.\textsuperscript{12} For example, the single-pollutant summary estimates for all-cause mortality, the outcome with the most data, were similar across both data sets, suggesting that the studies reporting two-pollutant model estimates were typical of the wider body of time-series evidence of NO\textsubscript{2}.

While evidence of NO\textsubscript{2} associations which are robust to control for PM mass has been identified, it is possible that there may be some residual confounding by PM. The components of PM—primary combustion particles, for example, ultrafine particles or Black Carbon—which have been proposed as the real causal agents of the NO\textsubscript{2} associations, were not included in co-pollutant models of NO\textsubscript{2} because concentration data for these pollutants were either unavailable or sparse, reflecting the fact that these PM metrics are not routinely measured. PM\textsubscript{10} was by far the most used metric—in 67% of the studies. Summary estimates of NO\textsubscript{2} were generally robust to adjustment for PM\textsubscript{10}. However, PM\textsubscript{10} may not adequately reflect the toxic component of PM because it reflects a number of sources that do not include combustion/traffic and that, are not shared with NO\textsubscript{2}. Where the data permitted meta-analysis, robustness of the NO\textsubscript{2} associations to adjustment for PM\textsubscript{2.5} and Black Smoke was observed. Few data were available to permit an assessment of the extent to which the NO\textsubscript{2} associations are sensitive to control for combustion-derived particles such as Black Carbon or ultrafine particles. This has also been noted by others.\textsuperscript{7–8 24}

Given that the sources and composition of PM vary by location, and hence its toxicity, it cannot be assumed that PM represents the same thing in each study (city/country). In view of the differential toxicity of PM, it is preferable to examine individual studies that used more than one particle metric to investigate possible confounding of the NO\textsubcript{2} associations by PM when answering the research question, because they ‘tested’ the robustness of the NO\textsubscript{2} associations to different fractions/components of the ambient aerosol in the same location.
Unfortunately, such studies were few in number (8), but their findings support the view that the associations of NO₂ with major health outcomes are robust to adjustment for PM measured in different ways.

We observed confounding of the associations between daily PM and mortality outcomes by NO₂. This suggests that NO₂, rather than the PM metrics examined, is a better predictor of the observed mortality effects in the cities examined. An alternative interpretation may be that daily variation in NO₂ in the cities better represents the mortality effects of daily variations in the complex urban air pollution mixture or an unknown toxic entity than the metrics of PM used in the analyses. Some caution is, however, needed in drawing conclusions about the analysis of PM estimates because it only reflects a subset of the available studies on PM. Whether the results are a feature of the subset of studies examined is unclear, and formal meta-analysis of the full body of PM estimates, similar to the current review, is warranted. This may provide further insights into whether the different fractions/components of PM might show different sensitivity to adjustment for NO₂.

Our results for PM are in contrast with the predominant views in the literature: although confounding of the PM-mortality associations by NO₂ has been observed in some time-series studies and noted in reviews, the general consensus is that the PM-mortality estimates are robust to adjustment for co-pollutants. The associations have been regarded as reflecting a causal relationship, and experimental evidence has been used to support this. There is a lack of experimental evidence for NO₂ at current ambient concentrations and for cardiovascular endpoints, and this has contributed to uncertainty regarding whether NO₂ is causally related to health.

We also found evidence of high heterogeneity between the geographic specific summary estimates of NO₂, which suggests that it cannot be assumed that the results for one city (region) represent the results for all cities (regions). For all-cause mortality and 24-hour NO₂, the high heterogeneity between WHO region-specific estimates was completely removed after control for PM (% from 66.9% to 0%), suggesting that some study estimates were a bit extreme in comparison with others in the meta-analysis, but were less so after adjustment for PM. Geographical variation in effect estimates may be due to variations in population characteristics and in pollution sources, mixtures and ambient concentrations. However, none of the variables used to represent the pollution and meteorological environments in the cities examined accounted for the high between-study variability we observed. Further work is therefore required to investigate potential explanations for the heterogeneity.

Results from the studies published since our literature search cut-off are summarised and discussed in the online supplementary appendix 1. The studies indicate that, in general, the associations between NO₂ and mortality and hospital admissions remain after control for PM. This is in keeping with the findings set out in this paper.

In addition to the issue of confounding, studies have examined the potential for factors (eg, season, socioeconomic status, age, etc) to modify the relationship between daily NO₂ and mortality or hospital admissions. Few studies have, however, examined modification of the associations of NO₂ with health by particulate air pollution. The available evidence suggests that the size of an NO₂ association may be dependent on concentrations of PM. However, studies have also observed the potential for daily NO₂ to modify the relationship between PM and mortality. The few available data on this issue come largely from the USA and Europe, but interaction between NO₂ and PM (on cardiac hospitalisation) has also been observed in Hong Kong. Further research on this aspect of the NO₂–PM issue is needed.

Our review supports the conclusions of recent narrative reviews but also provides meta-analytical estimates based on two-pollutant model estimates of NO₂ from the worldwide data. Taken together with the recent quantitative reviews of cohort studies on long-term exposure to NO₂ and mortality, and of short-term exposure to NO₂ and respiratory symptoms in children with asthma from panel studies, the evidence suggests a need for re-evaluation of the approach to health risk assessment (hazard identification and health impact assessment) for air pollution, an activity that has long been dominated by PM. The current review suggests that the relationship between temporal variations in PM and mortality may not be as robust to control for NO₂ as previously thought. We note also that attenuation of PM–mortality estimates following control for NO₂ has been observed in long-term exposure studies. These findings could have implications for the calculation of health impacts attributable to these pollutants and for possible double counting of effects.

In summary, we identified evidence of associations between NO₂ and adverse health outcomes that are independent of PM mass. However, there was limited evidence on adjustment of the NO₂ associations for primary combustion particles that are thought to be responsible for the NO₂ associations. Therefore, some uncertainty remains regarding possible confounding and health impact assessments should reflect this.

Author affiliations
1 Public Health England, Centre for Radiation, Chemical and Environmental Hazards, Oxfordshire, UK
2 Population Health Research Institute and MRC-PHE Centre for Environment and Health, St George’s, University of London, London, UK
3 MRC-PHE Centre for Environment and Health, King’s College London, London, UK
4 University of Birmingham, Birmingham, UK

Acknowledgements We wish to thank the authors of peer-reviewed papers who responded to our requests for study specific results.

Contributors ICM, RWA, HRA, RLM and DPS contributed to the design of the study and to the drafting of the paper, and have seen and approved the final version. ICM and RWA undertook the literature search. ICM read all papers, checked data prior to meta-analysis and carried out all analyses. RWA and ICM produced the statistical code in STATA, used by ICM in the analyses. ICM is responsible for the overall content as lead author of the paper.
Funding This work was supported by Public Health England (formerly Health Protection Agency).

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

REFERENCES