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Short-term Exposure to Nitrogen Dioxide and Mortality: a systematic review and meta-analysis

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Bachelor of Science
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Abstract

Short-term Exposure to Nitrogen Dioxide and Mortality: a systematic review and meta-analysis

By Mingrui Wang

Background: Ambient air pollution has been characterized as a leading cause of mortality worldwide and has been associated with cardiovascular and respiratory diseases. There is increasing evidence that short-term exposure to nitrogen dioxide (NO2), is related to adverse health effects and mortality.

Methods: We conducted a systematic review of short-term NO2 and daily mortality, which were indexed in PubMed and Embase up to February 2020. We calculated random-effects estimates by different continents and globally, and tested for heterogeneity and publication bias.

Results: We included 79 articles in our quantitative analysis. NO2 and all-cause as well as cause-specific mortality were positively associated in the main analysis. For all-cause mortality, a 10 ppb increase in NO2 was associated with a 1.49% (95%CI 1.24% to 1.75%, I2 = 90.8%, Eggers' test $p < 0.01$, N= 51) increase in the risk of death. For cause-specific mortality, a 10 ppb increase in NO2 was associated with a 1.79% (95%CI 1.45% to 2.12%, I2 = 88.3%, Eggers' test $p < 0.01$, N= 38) increase in cardiovascular mortality and a 2.15% (95%CI 1.56% to 2.74%, I2 = 80.1%, Eggers' test $p = 0.013$, N= 34) increase in respiratory mortality. In the sensitivity analysis, the meta-estimates for all-cause mortality, cardiovascular and respiratory mortality were nearly identical. The heterogeneity would decline to varying degrees through regional and study-type stratification.

Conclusions: This study provides evidence of an association between short-term exposure to NO2 and all-cause, cardiovascular and respiratory mortality. The results are robust based on sensitivity analysis and we provide a possible explanation for the high heterogeneity observed between the regions

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Table of contents

1. Introduction

Ambient air pollution is one of the greatest environmental hazards to human health, with substantial economic and social burden[1]. As a traffic-related air pollutant, nitrogen dioxide (NO_2) is of increasing concern recently[2]. In the past decades, growing epidemiological evidence has indicated the adverse effects of nitrogen dioxide on human health, such as all-cause mortality, cardiovascular disease, respiratory disease and even COVID-19[3-8]. In spite of the uncertain causality[9], these associations that reflect adverse health effects of NO_2 deserve our attention.

Up to now, four meta-analyses have integrated existing studies published prior to September 2018 and reported a relationship between short-term exposure to NO_2 and all-cause or cause-specific mortality[9-12]. These studies did not explore the extremely high heterogeneity in their meta-analyses[9,10]. Recently, an emerging interest in the health effects of NO_2 has motivated the study and publication of NO_2-exposed cohorts that provide a more global representation of the affected populations[7]. Given this increased interest, to date, the latest epidemiological studies on short-term NO_2 have not been incorporated in any systematic review yet, presenting a serious gap in our understanding of the current data.

In this present study, we systematically searched scientific literature worldwide and performed a meta-analysis of all available up-to-date epidemiological studies to examine the association between short-term exposure to NO_2 and mortality endpoints, including all-cause, cardiovascular, and respiratory mortality. We have incorporated evidence from studies that have not been included in previous quantitative synthesis. Our aim is to systematically evaluate the most recent evidence to inform adverse health impact assessment of NO_2 and better frame environmental policy.

2. Methods

2.1 Studies search and selection

This meta-analysis was performed according to the PRISMA guidelines[13]. We searched from both PubMed and EMBASE databases, to identify epidemiology studies that evaluated short-term exposure to NO_2 and mortality. To include the most relevant studies, our search of all-language studies was restricted to those published from January 1, 2006 through February 29, 2020.

We excluded book chapters, commentaries, editor pieces, conference abstracts, review articles, meta-analyses, toxicity studies, in vitro studies, and studies that were not written in English. We also excluded epidemiology studies that did not provide risk estimates for NO_2 exposure, or did not evaluate all-cause, cardiovascular, or respiratory mortality.

Four authors (S.H., H.L., M.W., Y.Q.) independently evaluated titles and abstracts found in the 2 databases (n=1,774). Reference lists of review articles and meta-analyses were also reviewed manually to further identify epidemiology studies of NO_2 exposure and mortality (additional papers retrieved n=1). This resulted in a total of 207 potentially relevant articles for full screen review. The eligibility of each study was independently assessed by two authors (S.H., M.W.) and any discrepancies were resolved through discussion with a third author (Y.Q.). Overall, 84 articles met all the criteria and were included in the final quantitative meta-analysis. Our study selection process is presented in Figure 1 (PRISMA Flowchart). The study protocol was registered at OSF and the link is provided at the bottom of the Figure 1.

2.2 Data extraction

Data extraction and accuracy assessment were done by the four authors stated above. Extracted information was entered into an Excel database, which included titles, authors' names, publication year, country, study design, study period, number of deaths, age range, sex distribution, time period of exposure assessment, exposure assessment method, exposure levels, exposure increment, lag patterns, effect measure, effect estimate and its standard error, and co-pollutant adjustment. For each study, we only extracted the effect estimates from the main model or with the most suitable adjustment of potential confounders. Several studies employed both single- and multiple-pollutant models. In this situation, we extracted estimates from both models, and used estimates from the former in the main analysis and the latter in the sensitivity analysis. In addition, most studies have published different estimates to accommodate various lags. To prevent selection bias within data extraction, only one estimate was selected from each study, according to the following rules: a) if only one lag estimate for a given pollutant/outcome pair is reported, it was included in the analysis. b) If multiple lag-estimates were reported, the selection principle was: 1) the most frequently used lag in all selected studies (e.g. lag 0, lag 1 or lag 0-1); 2) single lags, but not cumulative/distributed lags[14].

2.3 Risk of bias

The risk of bias assessment included the selected studies was performed using a new domain-based Risk of Bias assessment tool from the WHO. The tool's detailed information can be seen in

the WHO website (Risk of bias assessment instrument for systematic reviews informing WHO global air quality guidelines, 2020). There are 13 items grouped in six domains (confounding, selection bias, exposure assessment, outcome measurement, missing data, and selective reporting) in the instrument. Each item could be evaluated as low, moderate and high risk of bias. In the instrument, we could assess the risk of bias on confounding through four critical confounders (temperature, seasonality, long-term trends, day of the week) and two additional confounders (holidays, influenza epidemics). If any critical confounders were not included, the item was judged as having high risk of bias; If all critical confounders were included but all additional confounders were not included, the item was judged as having moderate risk of bias. Otherwise, the item have a low risk of bias. We analyzed the results for separately and didn't consider a single result for the whole article[14]. If any item in one domain was classified as having high risk of bias, the whole domain would be judged as having high risk of bias. Moderate and low risk of bias followed the same rule. At last, according to the domain risk rating, we assess the overall risk rating for each studies. If all domains are low risk of bias, the study could be classified as "Low Risk" study; If there are at least two domains are high risk of bias, the study would be classified as "High Risk" study; Otherwise, the study was "Moderate Risk"[15]. The sensitivity analyses would use these results from the assessment of risk of bias across studies.

2.4 Statistical analysis

Most studies reported risk ratios (RRs) or odds ratios (ORs) along with 95% confidence intervals (CI), though a couple of studies reported excess risks (ER). After data extraction, all effect estimates

were converted to hazard ratios (HRs) per 10 ppb increase in NO_2 concentrations with 95%CI, as for the following equations[14]:

$$HR_{(standardized)} = e^{\ln(HR_{(original)})*\frac{10}{Increment_{(original)}}}$$

Forest plots were used to display the brief study information and HRs in each study graphically. The between-study heterogeneity was evaluated using the I^2 statistic. The heterogeneity was considered "high" if $I^2 \geq 50\%$, and a DerSimonian-Laird method random effects model was used to provide a meta-estimate. Otherwise, a Mantel-Haenszel fixed effects model was used for studies with a "moderate" or "low" heterogeneity. A few stratified analyses were also conducted to assess potential effects modification resulted by either cohorts or research characters. These included study locations— which were divided into four regions including North America, Europe, Asia and South America— and study design types including time-series studies, case-crossover studies and cohort studies.

We conducted a sensitivity analysis to evaluate the robustness of results. "High Risk" studies were excluded in the main analysis according to risk of bias assessment, so we would add them back and rerun meta-analysis as a sensitivity analysis. In addition, we extracted from the multi-pollutant models as the second sensitivity analysis, if both single- and multi-pollutant models were fit. As for publication bias, we used two methods to assess the potential publication bias among the studies according to the symmetry. First, funnel plots offer visual examination on publication bias. Second, we performed bias evaluation by Egger's linear regression test. All statistical analyses were conducted in R version 4.0.1.

3. Results

3.1 Characteristic of the eligible studies

Table 1 presents the brief description of the included studies. We recorded country, author name, publication year, study period, number of events, study type, daily average exposure of NO_2, lag pattern and model type. There were 6, 3, 19 and 56 studies from North America[16], South America[17], Europe[18] and Asia[19] respectively, which covers the exposed population on a global scale. Data used in the studies covered the period from 1981 to 2017, which could sufficiently reflect the NO_2 adverse effects on human health. There were three study types: time-series studies[18], case-crossover studies[20] and cohort studies[21]. We analyzed the associations separately for each study design. According to the lag type in the studies, we summarized lag patterns separately, including single-day, multiple-days and both. Most studies use multiple-days lag or both lag patterns. Finally, we sort out the pollutant number in the model and all studies examined single pollutant model.

3.2 Risk of bias assessment

The summary of the risk of bias assessment is shown in summary plot (Figure 2). In two out of 6 domains (selection bias, selective reporting), the risk of bias was found to be only low or moderate. But in the other four domains, we found a variable proportion of articles having high risk of bias. 19.0% articles were classified as high risk of bias on confounding, and 14.3% articles were judged as high risk of bias on outcome measurement and missing data. Only one article had high risk of

bias on exposure assessment[22]. The main reason for the high risk of bias in the confounding domain was the lack of critical confounders (temperature, seasonality, long-term trends, day of the week). And the reason for the high risk of bias in the outcome measurement domain was the lack of ICD code. As for the missing data domain, the high risk rating was related to the absence of information on the number of missing values in the exposure or imputation methods.

The risk of bias assessment in individual studies is shown in traffic plot (Figure 3). Among them, five studies were judged as overall high risk of bias and they were excluded in the main analyses. And we would add them back in the sensitivity analysis.

3.3 Results of the meta-analysis

There were 51, 38 and 34 studies focused on all-cause mortality, cardiovascular mortality and respiratory mortality, respectively. Most of the studies (68) employed time-series analyses, according to the study type stratification. Besides, seventeen studies employed case-crossover analyses and two studies were cohort studies. Among them, three studies both employed time-series and case-crossover analyses. Regarding regions, most studies (56) were conducted in Asian population, while forty-two studies were conducted in China, accounting for 50% of the total research.

Table 2 presents the pooled effect estimates and heterogeneity for each of the three endpoints of interest. Despite substantial heterogeneity across studies, and the fact that estimates vary by region and study type, the results suggest an association of NO_2 with all three endpoints. There are only one exception with positive but not significant pooled effect estimates (cardiovascular mortality in

North America (HR=1.0023, 95%CI: 0.9966-1.0079) per 10 ppb increase). Two studies estimated the effects with wide confidence intervals.

3.3.1 All-cause mortality

Figure 4 shows all available single pollutant estimates for NO_2 and all-cause mortality in all continents. The overall pooled meta-estimate for all-cause mortality was 1.01 (95%CI: 1.01-1.02, I^2=90.8%, N=51) per 10 ppb increase in short-term NO_2 exposure. In most studies, the results showed NO_2 was associated with increases in the risk of death positively and significantly, especially in Asia. The pooled HRs for studies in Asia (HR=1.02, 95%CI: 1.01-1.02, I^2=91.4%, N=28) was larger than that in North America (HR=1.01, 95%CI: 1.00-1.02, I^2=84.7%, N=5), South America (HR=1.01, 95%CI: 1.00-1.02, I^2=91.5%, N=2) and Europe (HR=1.01, 95%CI: 1.01-1.02, I^2=91.2%, N=16). Heterogeneity was not explained by study region. Figure 5 shows all available single pollutant estimates for NO_2 and all-cause mortality in all study types. The pooled HRs for cohort study (HR=1.03, 95%CI: 1.02-1.05, N=1) were larger than that in time-series studies (HR=1.01, 95%CI: 1.01-1.02, I^2=91.4%, N=44) and case-crossover studies (HR=1.01, 95%CI: 1.00-1.03, I^2=75.9%, N=7). Although the heterogeneity in the many of the studies was still high, we found that the studies that used case-crossover methods had relatively lower heterogeneity.

3.3.2 Cardiovascular mortality

Figure 6 shows all available single pollutant estimates for NO_2 and cardiovascular mortality in all continents. The overall pooled meta-estimate for cardiovascular mortality was 1.02 (95%CI: 1.01-1.02, I^2=88.3%, N=38) per 10 ppb increase in short-term NO_2 exposure. In most studies, the

results showed NO_2 was associated with increases in the risk of death positively and significantly, especially in Asia. The pooled HRs for studies in Asia (HR=1.02, 95%CI: 1.02-1.03, I^2=88.3%, N=31) was larger than that in North America (HR=1.00, 95%CI: 1.00-1.01, I^2=48.1%, N=2), South America (HR=1.02, 95%CI: 1.01-1.02, N=1) and Europe (HR=1.01, 95%CI: 1.00-1.01, I^2=20.7%, N=6). The heterogeneity in European and North American studies was significantly lower than in other continents. Figure 7 shows all available single pollutant estimates for NO_2 and cardiovascular mortality in all study types. The pooled HRs for time-series studies (HR=1.02, 95%CI: 1.01-1.02, I^2=88.2%, N=34) we smaller than that in case-crossover studies (HR=1.02, 95%CI: 1.01-1.04, I^2=87.1%, N=6). In this stratification, we could not find the difference about the heterogeneity.

3.3.3 Respiratory mortality

Figure 8 and 9 show all available single pollutant estimates for NO_2 and respiratory mortality in all continents and in all study types. The overall pooled meta-estimate for respiratory mortality was 1.02 (95%CI: 1.02-1.03, I^2=80.1%, N=34) per 10 ppb increase in short-term NO_2 exposure. Similarly, the results in the majority of studies showed NO_2 was associated with increases in the risk of death positively and significantly. The pooled HRs for studies in Asia (HR=1.02, 95%CI: 1.01-1.03, I^2=77.5%, N=22) was lower than that in South America (HR=1.03, 95%CI: 1.02-1.03, I^2=0.0%, N=2) and Europe (HR=1.03, 95%CI: 1.01-1.05, I^2=83.7%, N=6). The pooled HRs for time-series studies (HR=1.02, 95%CI: 1.01-1.03, I^2=82.2%, N=28) we smaller than that in case-crossover studies (HR=1.03, 95%CI: 1.02-1.04, I^2=0.0%, N=5) and in cohort study (HR=1.08, 95%CI: 1.00-1.16, N=1). The heterogeneity in South American studies was not only lower than

those in other continents, but the studies that used case-crossover also had the lower heterogeneity.

3.3.4 Publication bias

Figure 10-12 show whether small studies with small effect sizes show adequate results through funnel plots. After Egger's liner regression test (all-cause mortality's p value < 0.01, cardiovascular mortality's p value < 0.01, respiratory mortality's p value = 0.013), all three plots are symmetrical, which does not provide evidence for publication bias.

3.3.5 Sensitivity analysis

Table 3 shows the first sensitivity analysis for short-term NO_2 exposure and mortality. After adding back the studies that reported high risk of bias, the meta-estimates and the heterogeneity for all-cause mortality (HR=1.02, 95%CI: 1.01-1.02, I^2=91.0%, N=56), cardiovascular (HR=1.02, 95%CI: 1.01-1.02, I^2=89.2%, N=40) and respiratory mortality (HR=1.02, 95%CI: 1.02-1.03, I^2=81.6%, N=35) were nearly identical. Table 4 shows the second sensitivity analysis for short-term NO_2 exposure and mortality. In the multi-pollutant models, the meta-estimates and the heterogeneity for all-cause mortality (HR=1.02, 95%CI: 1.01-1.02, I^2=84.5%, N=17), cardiovascular (HR=1.01, 95%CI: 1.01-1.02, I^2=77.9%, N=10) and respiratory mortality (HR=1.02, 95%CI: 1.01-1.03, I^2=47.7%, N=14) were smaller than the main analysis.

4. Discussion

This review identified 84 various study type studies reporting mortality effects of short-term exposure to NO_2, including studies from all over the world. Our analyses showed positive associations between short-term NO_2 exposure and all cause, respiratory and cardiovascular mortality, and confirmed the conclusion of previous systematic reviews of the adverse effects of NO_2 on human health. Compared to the most recent meta-analyses[9, 10], we also considered the case-crossover and cohort studies. In addition, there were evidence of high heterogeneity between estimates for the three endpoints.

In specific, our quantitative assessment observed increased hazard ratio of all-cause mortality of 1.49% (95%CI: 1.24, 1.75), per 10 ppb increases in NO_2. Meanwhile, the HRs of cardiovascular mortality and respiratory mortality increased 1.79% (95%CI: 1.45, 2.12) and 2.15% (95%CI: 1.56, 2.74), per 10 ppb increases in NO_2. All results showed significant positive associations between short-term NO_2 exposure and the three types of mortality. According to the data above, ambient NO_2 increases the excess risk of cardiovascular mortality and respiratory mortality more than all-cause mortality. In addition, the adverse effects of NO_2 on a specific mortality are different among the different continents. The HRs of all-cause mortality increased 1.30% (95%CI: 0.33, 2.28), 1.22% (95%CI: 0.29, 2.15), 1.09% (95%CI: 0.58, 1.60) and 1.81% (95%CI: 1.44, 2.18) in North America, South America, Europe and Asia per 10 ppb increases in NO_2. Meanwhile, we observed increased HRs of cardiovascular mortality of 0.23% (95%CI: -0.34, 0.79), 1.64% (95%CI: 1.05, 2.23), 0.65% (95%CI: 0.36, 0.95) and 2.38% (95%CI: 1.91, 2.85) in North America, South America, Europe and

Asia per 10 ppb increases in NO_2; The HRs of respiratory mortality of 2.56% (95%CI: 1.81, 3.32), 2.82% (95%CI: 0.98, 4.66) and 1.97% (95%CI: 1.28, 2.65) in South America, Europe and Asia per 10 ppb increases in NO_2. The excess risk of all-cause mortality and cardiovascular mortality are larger in Asia than in other continents, and respiratory mortality in Asia is smaller than in other continents.

We also observed high heterogeneity between the different continents. Most I-square values are over 80%, which indicate that there are some subgroups of studies present in the meta-analysis and we cannot assume that the results for one region could represent the results for other regions. Due to the study design differences between time-series studies and case-crossover studies, we choose study design types as a subgroup. According to the results, the heterogeneity in the case-crossover studies decreased by 16% to 100%, compared to in the time-series studies, except cardiovascular mortality. Due to the correlation between the air pollutant of interest and weather, which are various among different studies, the sensitivity of time series analysis could be influenced, the case-crossover design is used as an alternative to time series analysis[23]. Therefore, compared to time-series studies, the case-crossover studies are more resistant to time-in varying confounders and relevant variables. Further research is required to explain the high heterogeneity in the time-series studies because the current stratification could not provide an adequate plausible explanation. Meteorological conditions, study design or the selected population in different continents may cause such high heterogeneity in this meta-analysis.

Besides heterogeneity, this review also considers publication bias caused by small studies. According to the funnel plots, most of the estimates were distributed symmetrically due to Egger's

test, which indicate that there are no missing small studies with small estimates. Owing to relatively large sample size, the publication bias has been eliminated and we could assume that this review has included enough studies. Besides, the results of the sensitivity analysis were similar with the main analysis and proved the robustness of the main meta-analysis.

This review provides up to date meta-analytic estimates for NO_2 both worldwide and specific regions. A key strength of the review is to perform a meta-analysis and estimates including the most studies all over the world, which also provides a possible explanation about the high heterogeneity among studies. Taken together with the recent systematic reviews of long-term exposure to NO_2 and mortality[24] the evidence suggests that we need a approach to risk assessment for air pollution, partly caused by NO_2. The previous and current reviews show the robust relationship between NO_2 exposure and mortality, no matter exposure time period or mortality types. Indeed, other ambient pollutants, such as $PM_{2.5}$, may cause the increase in mortality, but our findings could confirm the health impacts and possible double counting of effects attributable to NO_2.

As one of important ambient air pollutants, exposure to NO_2 in high-intensity, confined space has caused adverse effects to humans, including death. Ambient NO_2 exposure may increase the risk of respiratory disease through the pollutant's interaction with the immune system[25]. Therefore, the high hazard ratio on respiratory mortality could be explained by the above mechanism.

In summary, we identified evidence of associations between short-term NO_2 exposure and adverse health outcomes. However, there were limited explanations on heterogeneity of the NO_2 associations with mortality, especially in the time-series studies. Therefore, some uncertainties remain regarding possible confounding and other factors influencing the studies.

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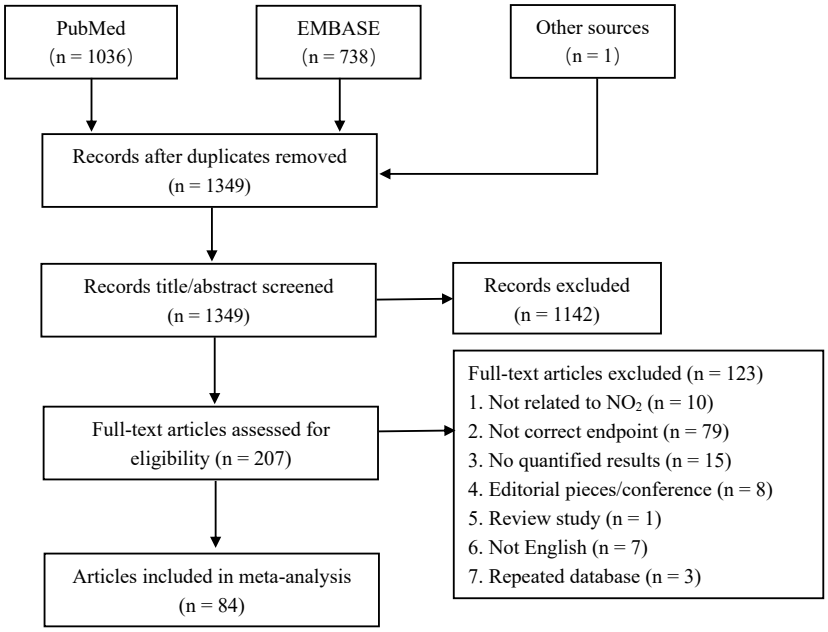
Figures



Protocol Link: https://osf.io/k3wcu/?view_only=e3b5d39f869a407e9432cfd952481b29

Figure 1. Flowchart of selection of eligible studies

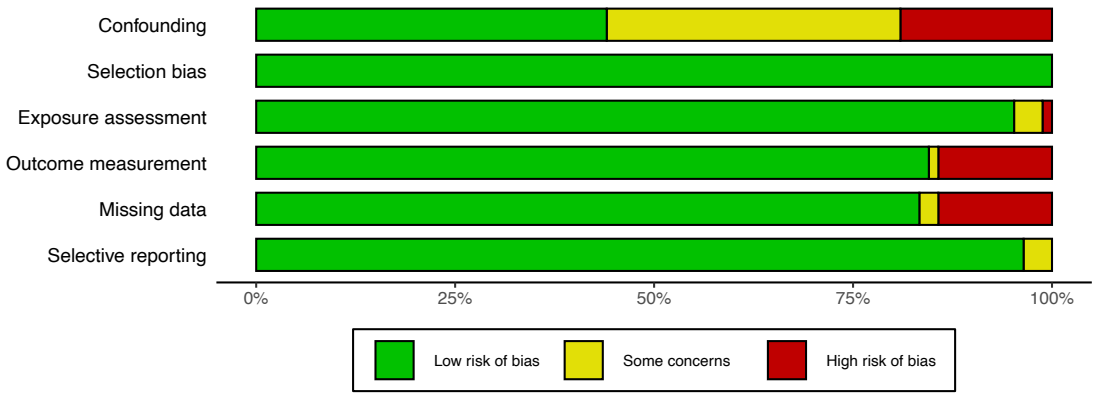


Figure 2. Summary of the Risk of Bias assessment

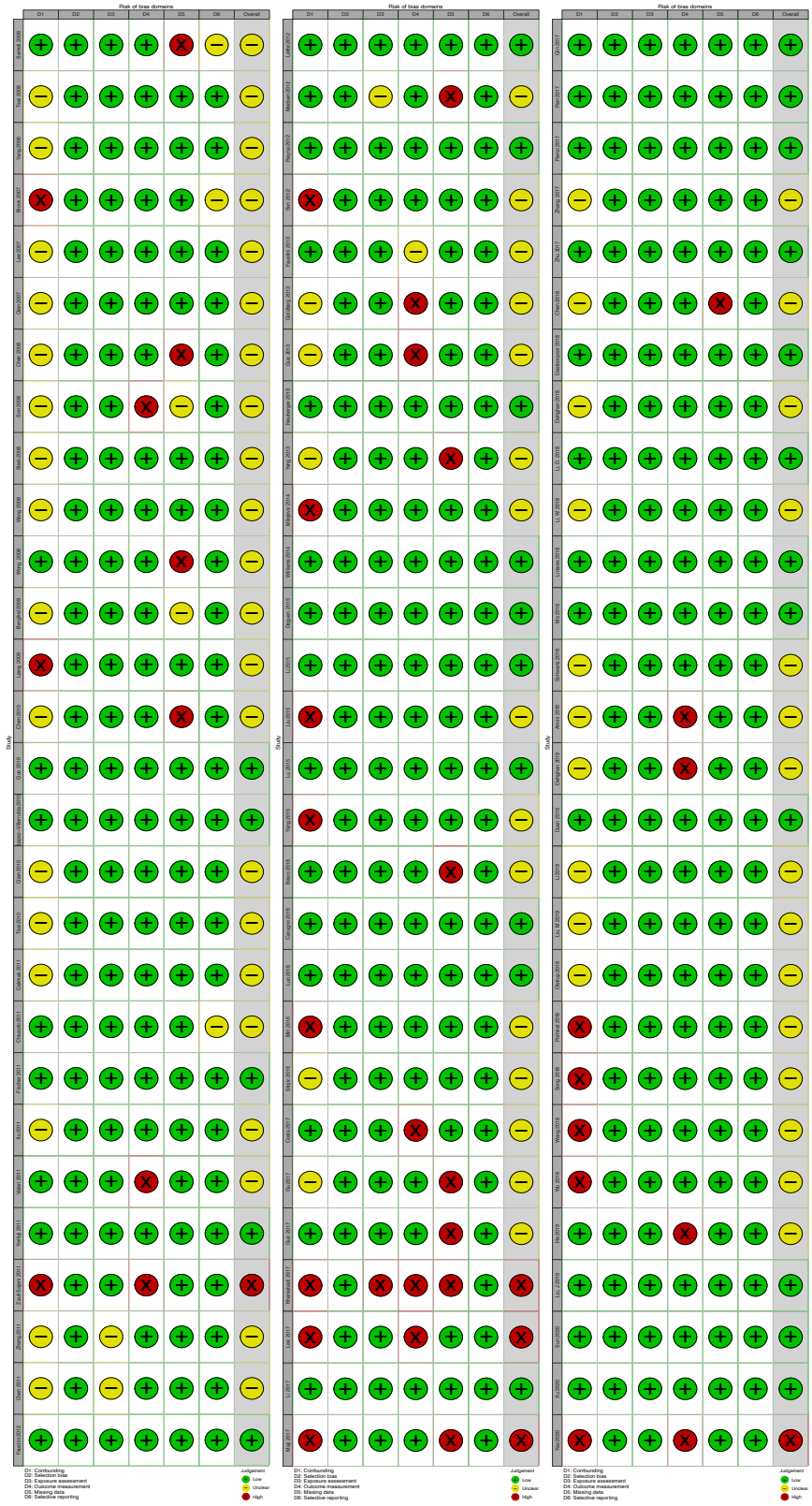


Figure 3. Risk of Bias rating for each studies

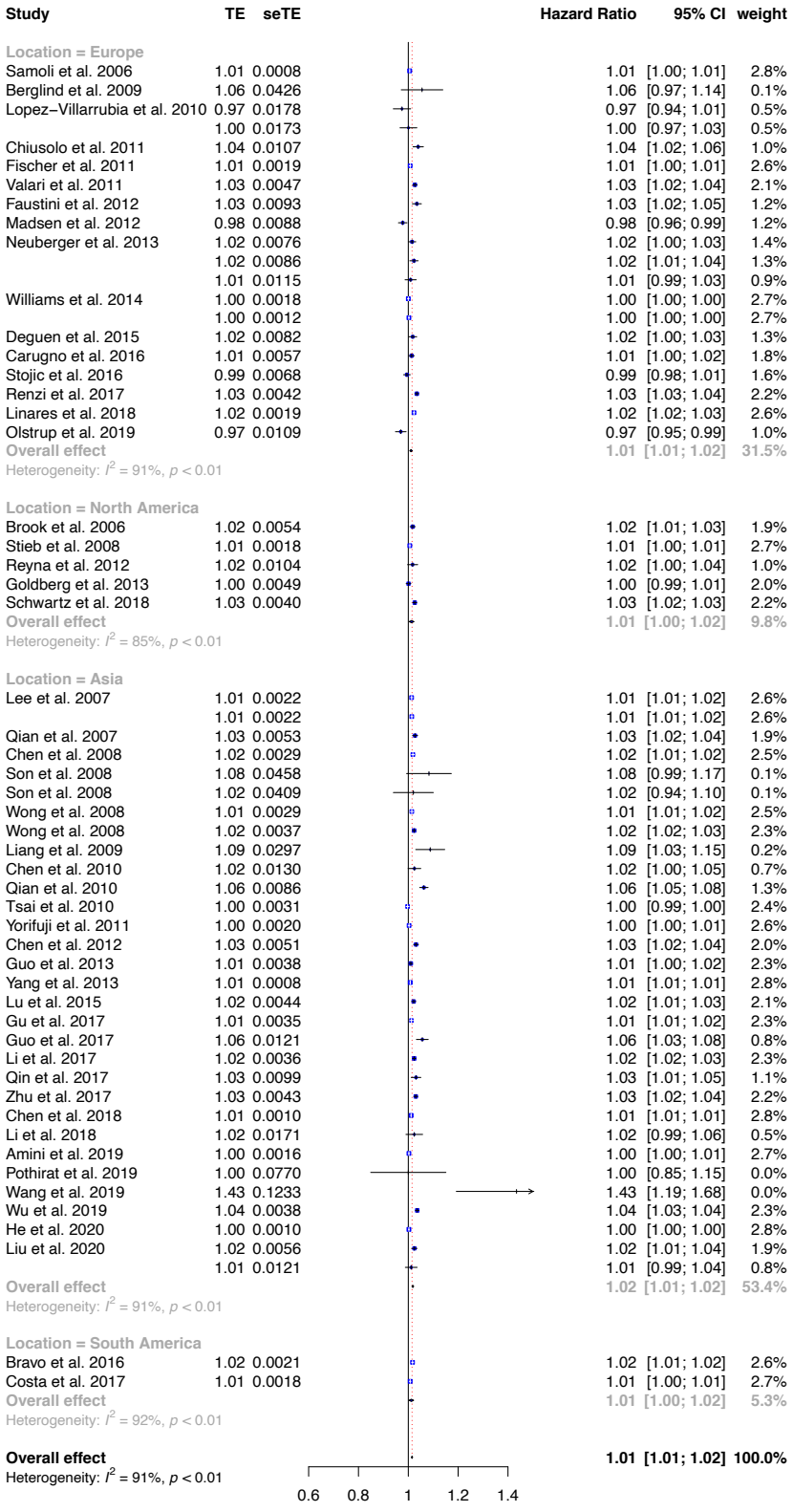


Figure 4. All available studies providing single-pollutant model estimates for meta-analysis for all-cause mortality in the regional stratification.

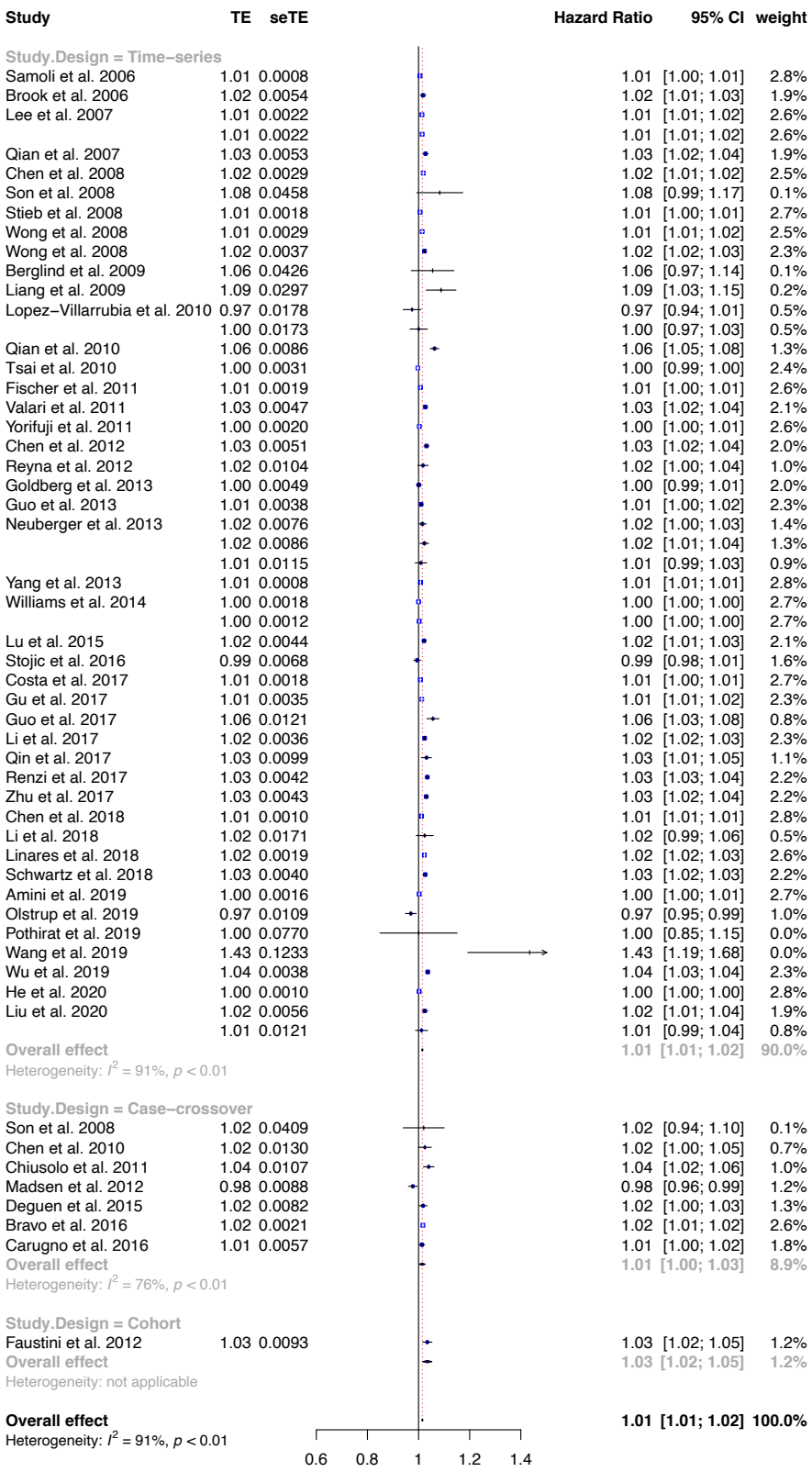


Figure 5. All available studies providing single-pollutant model estimates for meta-analysis for all-cause mortality in the study type stratification.

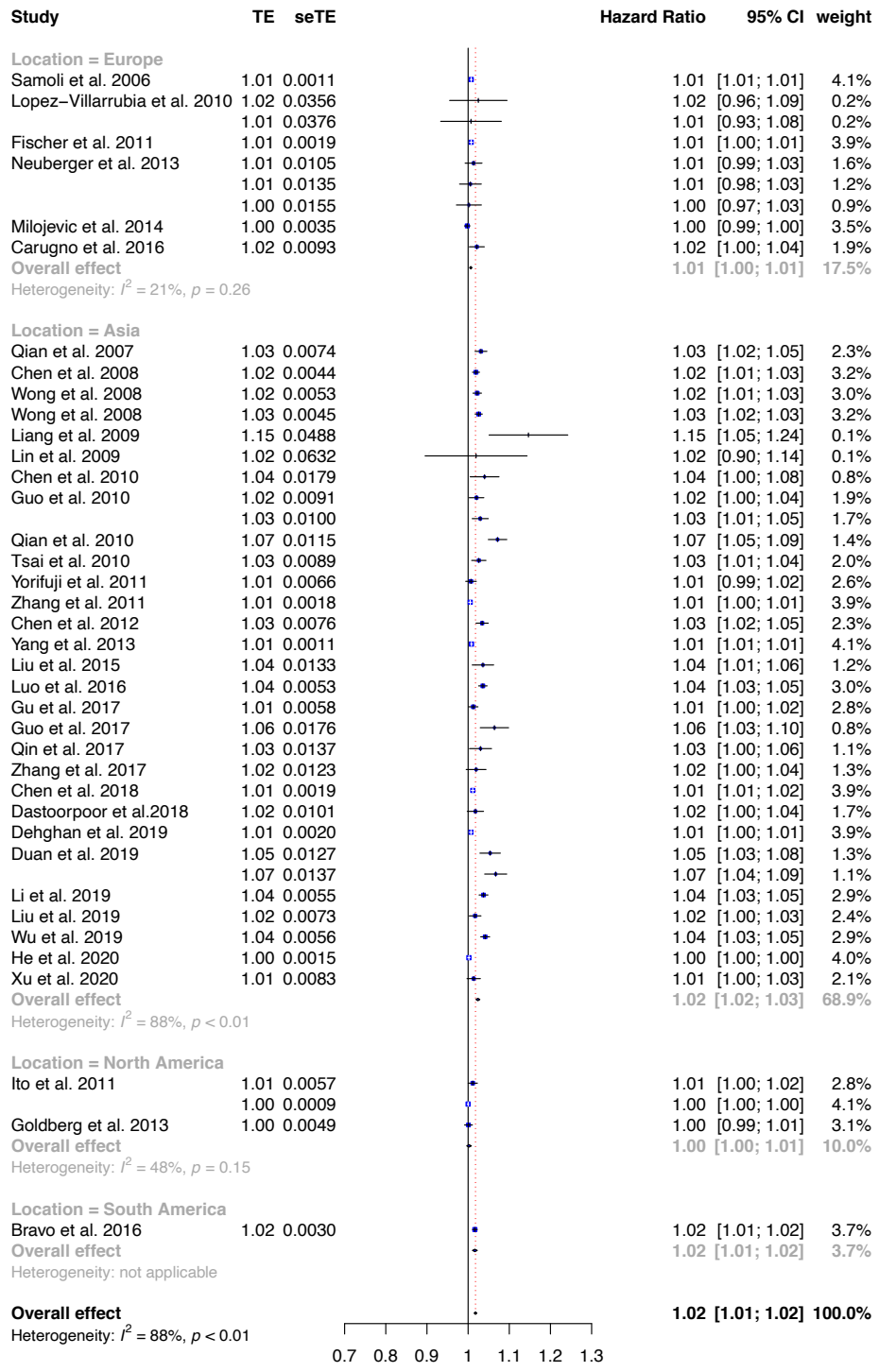


Figure 6. All available studies providing single-pollutant model estimates for meta-analysis for cardiovascular mortality in the regional stratification.

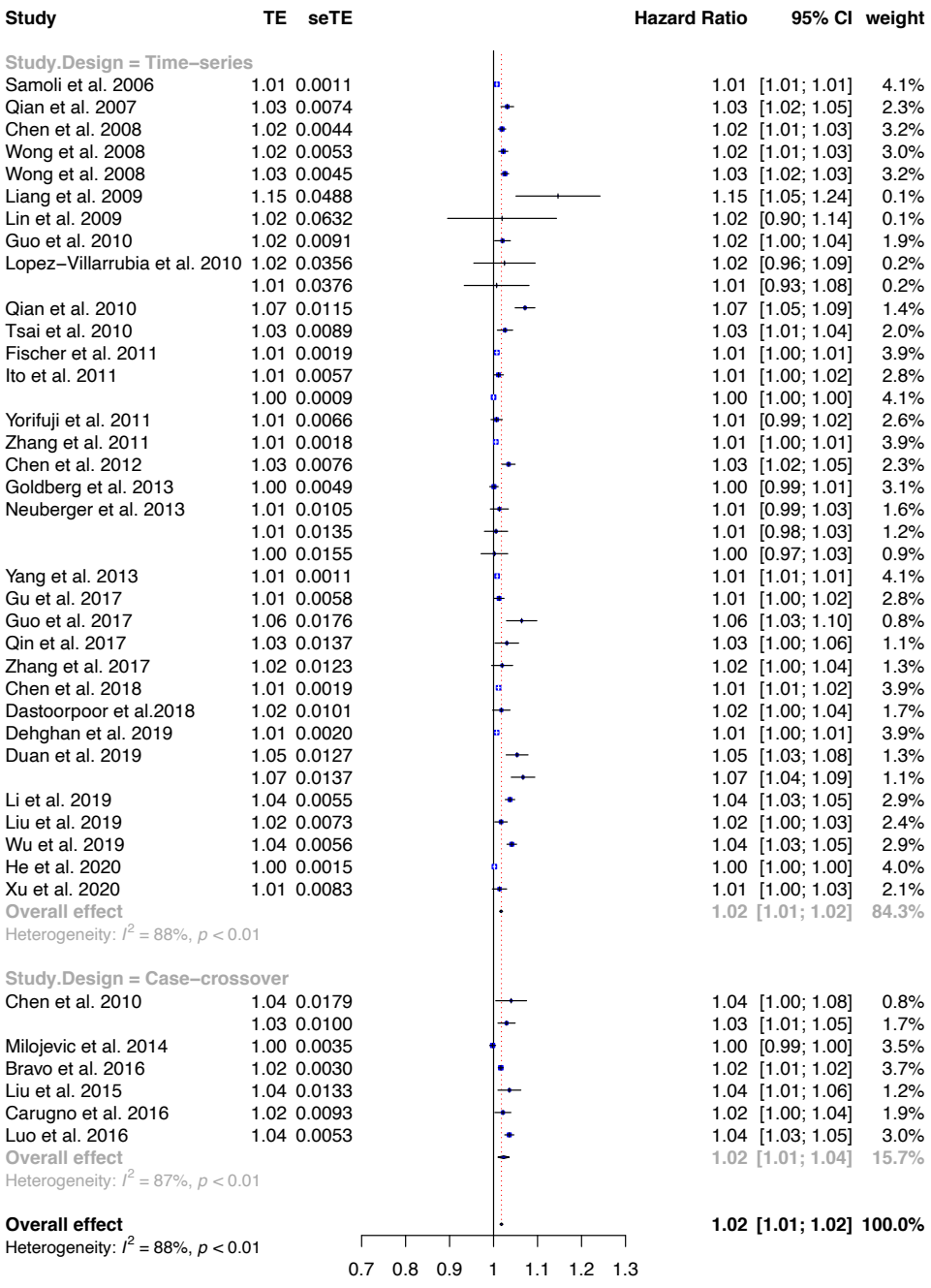


Figure 7. All available studies providing single-pollutant model estimates for meta-analysis for cardiovascular mortality in the study type stratification.

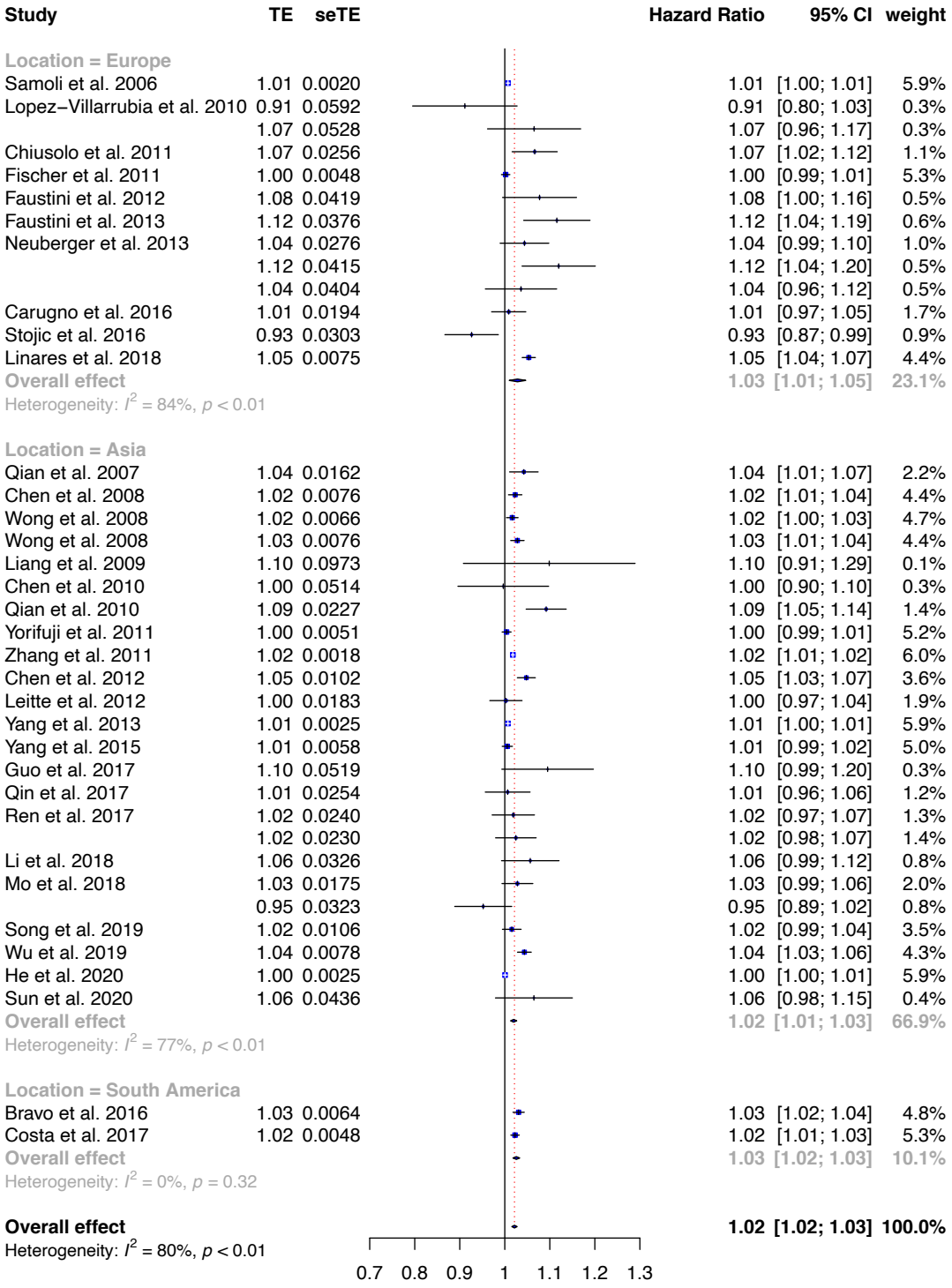


Figure 8. All available studies providing single-pollutant model estimates for meta-analysis for respiratory mortality in the regional stratification.

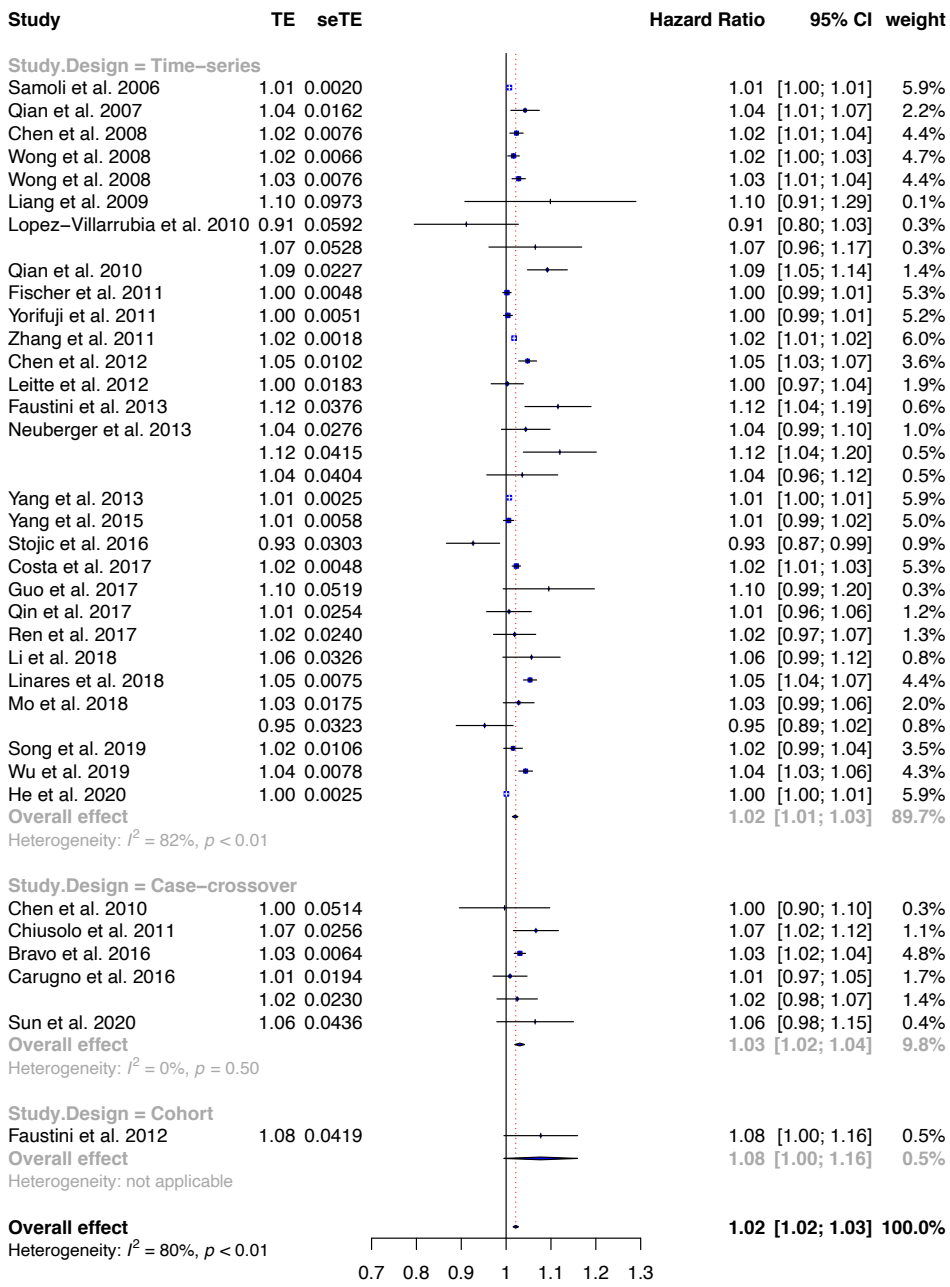
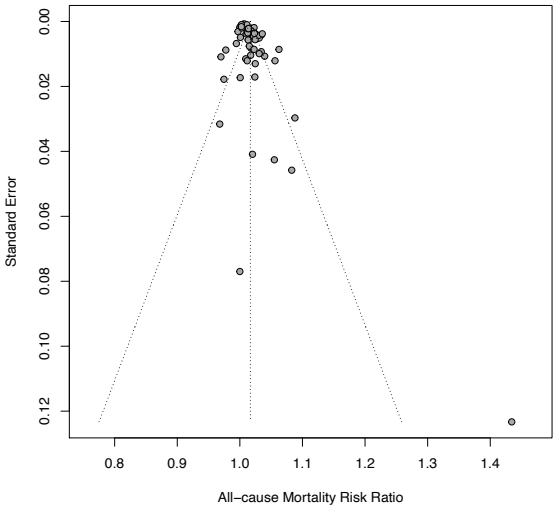


Figure 9. All available studies providing single-pollutant model estimates for meta-analysis for respiratory mortality in the study type stratification.

Figure 10. The funnel plots of all available studies providing single-pollutant model estimates for all-cause mortality, cardiovascular mortality and respiratory mortality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0.04
0.06
0.08
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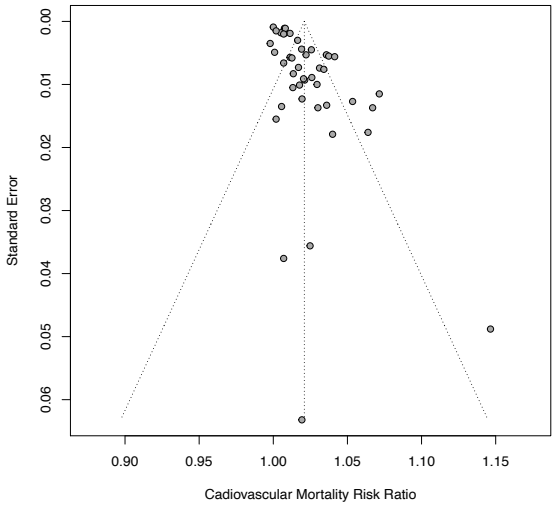
1.1
1.2
1.3
1.4
All−cause Mortality Risk Ratio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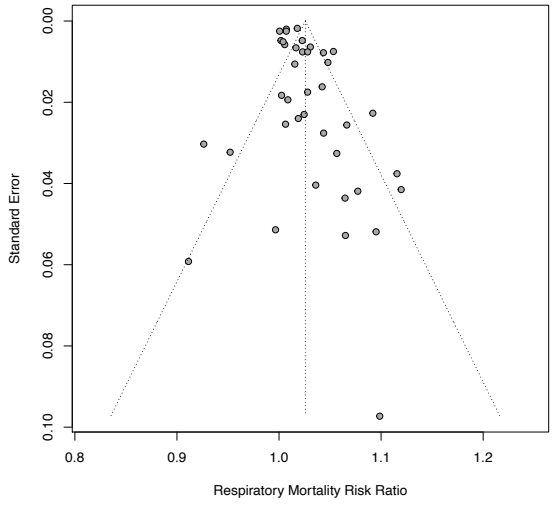
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1.1
1.2
Respiratory Mortality Risk Ratio

Table 1 Descriptive characteristics of the studies included

Country	Study	Study period	Number of events	Study type	Mean daily exposure (SD) or range	Lag pattern (Single-days/ Multiple-days/ Both)	Model(Mono-pollutant/Multi-pollutant/Both)
North America							
USA	Ito et al. (2011)	2000-2006	N/A	Time series	28.7 (8.8) ppb	Single-days	Mono-pollutant
	Schwartz et al. (2018)	1999-2010	7,277,274	Time series	14.2 (7.1) ppb	Multiple-days	Mono-pollutant
Canada	Brook et al. (2006)	1984-2000	N/A	Time series	N/A	Single days	Mono-pollutant
	Stieb et al. (2008)	1981-2000	N/A	Time series	33.6 ppb	Single days	Mono-pollutant
	Goldberg et al. (2013)	1990-2003	158,350	Time series	37.90(14.20) μg/m^3	Both	Mono-pollutant
Mexico	Reyna et al. (2012)	2003-2007	N/A	Time series	0.021(0.01) ppm	Single days	Mono-pollutant
South America							
Brazil	Bravo et al. (2015)	1996-2010	849,127	Case-crossover	28.6(12.75) μg/m^3	Both	Mono-pollutant
	Costa et al. (2017)	2000-2011	N/A	Time series	51.1μg/m^3	Both	Both
Chile	Cakmak et al. (2011)	1997-2007	N/A	Time series	40.2-51 ppb$^{\#}$	N/A	Mono-pollutant
Europe							
Spain	Lopez-Villarrubia et al. (2010)	2000-2004	N/A	Time series	30.3-45.8 μg/m$^{3\,\#}$	Both	Mono-pollutant
	Linares et al. (2018)	2000-2009	6,085	Time series	11.5-59.4 μg/m^3	Both	Mono-pollutant
Italy	Chiusolo et al. (2011)	2001-2005	276,205	Case-crossover	26-66 μg/m$^{3\#}$	Both	Both
	Zauli Sajani et al. (2011)	2002-2006	46,948	Case-crossover	20-72μg/m$^{3\#}$	Single-days	Mono-pollutant
	Faustini et al. (2012)	2005-2009	100,858	Cohort study	60.4(16.9) μg/m^3	Both	Mono-pollutant
	Faustini et al. (2013)	2001-2005	5,490	Time series	46.1-66.0μg/m$^{3\#}$	Both	Both
	Carugno et al. (2016)	2003-2006	N/A	Case-crossover	52.1 μg/m^3	Multiple-days	Mono-pollutant
	Renzi et al. (2017)	1998-2014	359,447	Time series	N/A	Multiple-days	Mono-pollutant
Netherlands	Fischer et al. (2011)	1992-2006	N/A	Time series	30.8 μg/m^3	Both	Mono-pollutant
France	Valari et al. (2011)	2001-2004	N/A	Time series	42.5 μg/m^3	N/A	Both
	Deguen et al. (2015)	2004-2009	79,107	Case-crossover	52.59(13.92) μg/m^3	Multiple-days	Mono-pollutant
Norway	Madsen et al. (2012)	1992-2001	51,226	Case-crossover	36.39(14.91) μg/m^3	Both	Mono-pollutant
Austria	Neuberger et al. (2013)	1990-2007	N/A	Time series	23.0-39.4μg/m$^{3\#}$	Both	Mono-pollutant
UK	Milojevic et al. (2014)	2003-2009	683,381	Case-crossover	24 μg/m^{3}*	Multiple-days	Mono-pollutant
	Williams et al. (2014)	2000-2005	N/A	Time series	20.4 ppb*	Single-days	Both
Serbia	Stojić et al. (2016)	2009-2014	113,615	Time series	15.52 μg/m^3	Both	Mono-pollutant

Sweden	Olstrup et al. (2019)	2000-2016	N/A	Time series	14.4 μg/m^3	Multiple-days	Mono-pollutant
Europe	Samoli et al. (2006)	1990-1997	2,893,430	Time-series	46-155μg/m$^{3\#}$	Multiple-days	Mono-pollutant
	Berglind et al. (2009)	1992-2002	N/A	Time series	21.1-68.1μg/m$^{3\#}$	Multiple-days	Mono-pollutant
Asia							
China	Tsai et al. (2006)	1994-2000	207	Case-crossover	28.61 ppb	Multiple-days	Multi-pollutant
	Yang et al. (2006)	1994-2000	471	Case-crossover	32.99 ppb	Multiple-days	Multi-pollutant
	Qian et al. (2007)	2000-2004	89,131	Time series	51.8(18.8) μg/m^3	Single-days	Both
	Chen et al. (2008)	2001-2004	173,911	Time series	66.6(24.9) μg/m^3	Multiple-days	Both
	Wong et al. (2008)	1996-2002	215,240	Time series	58.7(20.0) μg/m^3	Single-days	Mono-pollutant
	Liang et al. (2009)	1997-1999	N/A	Time series	28.30(7.83) ppb	Both	Both
	Lin et al. (2009)	1995-1999	33,818	Time series	29.0 ppb	Both	Mono-pollutant
	Chen et al. (2010)	2004-2006	31,847	Case-crossover	25.5(16.3) μg/m^3	Both	Both
	Guo et al. (2010)	2005-2007	N/A	Time series and Case-crossover	47(18) μg/m^3	N/A	Mono-pollutant
	Qian et al. (2010)	2000-2004	89,131	Time series	51.8 μg/m^3	Multiple-days	Mono-pollutant
	Tsai et al. (2010)	1993-2006	66,534	Time series	28.7(10.6)ppb	Single-days	Mono-pollutant
	Zhang et al. (2011)	2003-2008	N/A	Time series	64.8(24.2) μg/m^3	Multiple-days	Both
	Chen et al. (2012)	1996-2010	N/A	Time series	23-67μg/m$^{3\#}$	Multiple-days	Both
	Leitte et al. (2012)	2004-2005	3,528	Time series	67(24) μg/m^3	Both	Mono-pollutant
	Guo et al. (2013)	2004-2008	80,515	Time series	64.2(25.7) μg/m^3	Multiple-days	Both
	Yang et al. (2013)	2009-2010	152,714	Time series	55.02(24.04) μg/m^3	Both	Mono-pollutant
	Li et al. (2015)	2003-2011	205,549	Time series	45 (26) index?	Both	Mono-pollutant
	Liu et al. (2015)	2006-2009	8,955	Case-crossover	53.08(21.51) μg/m^3	Both	Mono-pollutant
	Lu et al. (2015)	2009-2013	147,956	Time series	51.5(19.8) μg/m^3	Both	Both
	Yang et al. (2015)	2009-2010	15,003	Time series	40.8 μg/m^{3*}	Both	Both
	Luo et al. (2016)	2009-2010	N/A	Case-crossover	55.02(24.04) μg/m^3	Both	Mono-pollutant
	Gu et al. (2017)	2006-2010	59,609	Time series	60.31(29.63) μg/m^3	Both	Mono-pollutant
	Guo et al. (2017)	2012-2014	35,261	Time series	44.93(17.12) μg/m^3	Both	Both
	Li et al. (2017)	2012-2015	179,356	Time series	47.29(18.33) μg/m^3	Single-days	Both
	Qin et al. (2017)	2008-2014	151,472	Time series	29.5 μg/m^3	Multiple-days	Mono-pollutant
	Ren et al. (2017)	2007-2009	2,120	Time series and Case-crossover	54.40(21.63) μg/m^3	Both	Both
	Zhang et al. (2017)	2012-2015	21,816	Time series	30.93(12.93) μg/m^3	Both	Both
	Zhu et al. (2017)	2012-2015	117,418	Time series	44(17) μg/m^3	Both	Both
	Chen et al. (2018)	2013-2015	N/A	Time series	31(11) μg/m^3	Both	Mono-pollutant
	Li et al. (2018)	2014-2015	9,365	Time series	41.46(17.15) μg/m^3	Both	Mono-pollutant

	Mo et al. (2018)	2014-2015	2,185 5,477[#]	Time series	49.538(16.739) μg/m^3- 22.930(13.085) μg/m$^{3\#}$	Single-days	Mono-pollutant
	Duan et al. (2019)	2013-2017	N/A	Time series	39.70(16.52) μg/m^3	Both	Mono-pollutant
	Li et al. (2019)	2009-2010	74,775	Time series	55.0(24.0) μg/m^3	Both	Mono-pollutant
	Liu et al. (2019)	2013-2016	62,159	Time series	42.97(17.34) μg/m^3	Both	Mono-pollutant
	Song et al. (2019)	2011-2017	18,952	Time series	53(21) μg/m^3	Both	Mono-pollutant
	Wang et al. (2019)	2014-2016	1,236	Time series	49.7(10.6) μg/m^3	Both	Mono-pollutant
	Wu et al. (2019)	2006-2016	N/A	Time series	32.5(16.1) μg/m^3	Multiple-days	Both
	He et al. (2020)	2013-2015	N/A	Time series	50.2 μg/m^3	Both	Mono-pollutant
	Liu et al. (2020)	2004-2009, 2014-2017	N/A	Time series	45.57(25.66) μg/m^3 54.65(21.65) μg/m^3	Both	Mono-pollutant
	Sun et al. (2020)	1998-2011	3,159	Case-crossover	57.7(20.1) μg/m^3	Multiple-days	Mono-pollutant
	Xu et al. (2020)	2007-2016	34,500	Time series	30.49(13.63) μg/m^3	Both	Mono-pollutant
	Yao et al. (2020)	2007.01-12	N/A	Time series	30.23(13.63) μg/m^3	N/A	Mono-pollutant
South Korea	Lee et al. (2007)	2000-2004	N/A	Time series	37.34(12.23)ppb 37.38(12.22)ppb[a]	Single-days	Mono-pollutant
	Son et al. (2008)	1999-2003	9,137	Time series and Case-crossover	35.60(12.03) ppb	Single-days	Mono-pollutant
	Son et al. (2012)	2000-2007	261,952	Case-crossover	36.91(12.36)ppb	Both	Mono-pollutant
	Lee et al. (2017)	2005-2013	1,899	Cohort study	0.022-0.028ppm[&]	N/A	Mono-pollutant
Japan	Yorifuji et al. (2011)	2003-2008	371,921	Time series	36.3(11.0)ppb 32.1(10.7)ppb[b]	Both	Mono-pollutant
India	Maji et al. (2017)	2008-2010	N/A	Time series	54(15) μg/m^3	Both	Mono-pollutant
Iran	Miri et al. (2016)	2014-2015	N/A	Time series	87.09μg/m^{3*}	N/A	Mono-pollutant
	Khaniabadi et al. (2017)	2014-2015	N/A	Time series	75.76μg/m^3	N/A	Mono-pollutant
	Dastoorpoor et al.(2018)	2008-2015	10,625	Time series	44.2(34.2) μg/m^3	Both	Mono-pollutant
	Dehghan et al. (2018)	2005-2014	37,967	Time series	53.47(16.52) μg/m^3	Both	Multi-pollutant
	Amini et al. (2019)	2011-2014	122,376	Time series	42.4(9.6)ppb	Both	Mono-pollutant
	Dehghan et al. (2019)	2005-2014	215,373	Time series	53.48(16.52)ppb	Single	Both
Thailand	Pothirat et al. (2019)	2016-2017	477	Time series	27.92μg/m^{3*}	Single	Mono-pollutant
Asia	Wong et al. (2008)	1996-2004	8,555	Time series	44.7-66.6 μg/m^3	Multiple-days	Mono-pollutant

Notes: [a] dust exclude; [b] after new regulation; * median; [#] mean annual exposure concentrations in multiple cities.

Model: mono-pollutant means only NO2 is included in the model as the pollutant; Multi-pollutant means several pollutants are included in the model; Both means both mono-pollutant model and multi-pollutant model are used in the study.

NA indicates Not Applicable, SD standard deviation

Table 2 Pooled effect estimates of NO_2 on all-cause, cardiovascular, and respiratory mortality

	All-cause mortality			Cardiovascular mortality			Respiratory mortality		
	Studies (n)	**HR (95%) CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**
Full meta-estimate	51	1.02 (1.01, 1.02)	91	38	1.02 (1.02, 1.03)	88	34	1.03 (1.01, 1.04)	80
Continent									
North America	5	1.01 (1.00, 1.03)	85	2	1.00 (0.99, 1.02)	48	0	n/a	n/a
South America	2	1.01 (0.95, 1.07)	92	1	1.02 (1.01, 1.02)	n/a	2	1.03 (0.98, 1.07)	0
Europe	16	1.01 (1.00, 1.02)	91	6	1.01 (1.00, 1.01)	21	10	1.03 (1.00, 1.07)	84
Asia	28	1.02 (1.01, 1.04)	91	29	1.03 (1.02, 1.03)	88	22	1.02 (1.01, 1.03)	77
Study Type									
Time-series	44	1.02 (1.01, 1.02)	91	32	1.02 (1.01, 1.03)	88	28	1.02 (1.01, 1.04)	82
Case-crossover	7*	1.01 (1.00, 1.03)	76	6	1.02 (1.01, 1.04)	87	5	1.03 (1.01, 1.05)	0
Cohort	1	1.03 (1.02, 1.05)	n/a	0	n/a	n/a	1	1.08 (1.00, 1.16)	n/a

Notes: One study (Son et al., 2008) contains both time-series study and case-crossover studies.

Table 3 The sensitivity analysis of NO_2 on all-cause, cardiovascular, and respiratory mortality (Add-back)

	All-cause mortality			Cardiovascular mortality			Respiratory mortality		
	Studies (n)	**HR (95%) CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**
Full meta-estimate	56	1.02 (1.01, 1.02)	91	40	1.02 (1.01, 1.02)	89	35	1.02 (1.02, 1.03)	82
Continent									
North America	5	1.01 (1.00, 1.02)	85	2	1.00 (1.00, 1.01)	48	0	n/a	n/a
South America	2	1.01 (1.00, 1.02)	92	1	1.02 (1.01, 1.02)	n/a	2	1.03 (1.02, 1.03)	0
Europe	17	1.01 (1.01, 1.02)	91	6	1.01 (1.00, 1.01)	21	10	1.03 (1.01, 1.05)	84
Asia	32	1.02 (1.02, 1.02)	92	31	1.02 (1.02, 1.03)	89	23	1.02 (1.01, 1.03)	80
Study Type									
Time-series	47	1.02 (1.01, 1.02)	92	34	1.02 (1.01, 1.02)	89	29	1.03 (1.01, 1.04)	84
Case-crossover	8*	1.01 (1.00, 1.02)	72	6	1.02 (1.01, 1.04)	87	5	1.02 (1.01, 1.03)	0
Cohort	2	1.06 (1.00, 1.13)	91	0	n/a	n/a	1	1.08 (1.00, 1.16)	n/a

Notes: One study (Son et al., 2008) contains both time-series study and case-crossover studies.

Table 4 The sensitivity analysis of NO_2 on all-cause, cardiovascular, and respiratory mortality (Multi-pollutant)

	All-cause mortality			Cardiovascular mortality			Respiratory mortality		
	Studies (n)	**HR (95%) CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**	**Studies (n)**	**HR (95% CI)**	**I^2 (%)**
Full meta-estimate	17	1.02 (1.01, 1.02)	85	10	1.01 (1.01, 1.02)	78	14	1.02 (1.01, 1.03)	48
Continent									
North America	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a
South America	1	1.00 (1.00, 1.01)	n/a	0	n/a	n/a	1	1.00 (0.99, 1.02)	n/a
Europe	3	1.02 (1.01, 1.03)	90	0	n/a	n/a	2	1.06 (1.02, 1.11)	0
Asia	13	1.02 (1.02, 1.03)	47	10	1.01 (1.01, 1.02)	78	11	1.02 (1.01, 1.03)	41
Study Type									
Time-series	13	1.02 (1.01, 1.02)	87	9	1.01 (1.01, 1.02)	80	29	1.02 (1.01, 1.03)	51
Case-crossover	4	1.03 (1.01, 1.05)	0	1	1.00 (0.94, 1.06)	n/a	5	1.03 (0.98, 1.08)	42
Cohort	0	n/a	n/a	0	n/a	n/a	0	n/a	n/a

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