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Long-term exposure to low-level NO_2 and mortality in the southeastern US

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Abstract

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By Yaoyao Qian

Background

Mounting evidence has shown that the long-term exposure to fine particulate matter and ozone can increase mortality. However, the health effects associated with long-term exposure to nitrogen dioxide (NO_2) are less clear, in particular the evidence is scarce for NO_2 at low levels below the current National Ambient Air Quality Standards (NAAQS).

Methods

We constructed a population-based full cohort comprised of all Medicare beneficiaries (aged ≥65, N=13,590,387) in the Southeastern US from 2000-2016, and then further defined the below-guideline cohort that only included those who were always exposed to low-level NO_2, with annual means below the current NAAQS (i.e., ≤53 ppb). We applied previously estimated spatially- and temporally-resolved NO_2 concentrations and assigned annual means to study subjects based on ZIP code of residence. Cox proportional hazards models were used to examine the association between long-term exposure to low-level NO_2 and all-cause mortality, adjusting for potential confounders.

Results

About 99.95% of the Medicare beneficiaries in the southeastern US were always exposed to low-level NO_2 over the study period. We observed a statistically significant association between long-term exposure to low-level NO_2 and all-cause mortality, with a hazard ratio (HR) of 1.040 (95% CI: 1.031, 1.050) in single-pollutant models and a HR of 1.042 (95% CI: 1.033, 1.052) in multi-pollutant models (adjusting for $PM_{2.5}$ and ozone), per 10 ppb increase in annual NO_2 concentrations. The penalized spline indicates a linear dose-response relationship across the entire NO_2 exposure range. Subjects who are white, female, and residing in urban areas are more vulnerable to long-term NO_2 exposure.

Conclusion

Using a large cohort, we provide epidemiological evidence that long-term exposure to NO_2, even below the NAAQS guideline, was significantly linearly associated with a higher risk of mortality, independent of $PM_{2.5}$ and ozone. Improving air quality by reducing NO_2 emissions may yield substantial health benefits.

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1. Introduction

Air pollution is one of the most important environmental and public health issues (Chen and Kan 2008). Mounting evidence has demonstrated that long-term $PM_{2.5}$ and ozone exposures increase the risk of death (Shi et al. 2016; Wei et al. 2020; Yu et al. 2020), cardiorespiratory diseases (Akintoye et al. 2016; Cole and Freeman 2009; Requia et al. 2018; Wang et al. 2015; Zu et al. 2017), and neurological disorders (Shi et al. 2020). The association between short-term exposure to NO_2 and mortality was also well documented (Chen et al. 2012; Chiusolo et al. 2011; Mills et al. 2016; Samoli et al. 2006). However, the effects of long-term exposure to NO_2 are less understood with limited epidemiology evidence.

In order to protect public health from adverse health outcomes induced by air pollution, the U.S. Environmental Protection Agency (EPA) set the National Ambient Air Quality Standards (NAAQS) based on evidence from multiple disciplines including epidemiological and toxicological studies, and periodically revised standard levels of various air pollutants. Current NAAQS for long-term exposure to NO_2 is set at an annual average of 53 ppb, and current near-road monitoring installed by EPA shows that air quality levels are well below the NAAQS for NO_2 in urban areas with large populations (EPA 2018). However, it is not clear whether the current NAAQS of 53 ppb is a safe threshold. Limited number of studies have investigated the relationship between long-term exposure to NO_2 and mortality, most of which assessed NO_2 exposure across the entire range and at relatively coarse resolution (Heinrich et al. 2013; Jerrett et al. 2013; Maheswaran et al. 2010). As a result, these studies provide limited insights on the health effects of long-term exposure to low levels of NO_2 with high resolution.

We recently estimated temporally and spatially resolved NO_2 concentrations in the US through an ensemble model that integrates multiple machine learning algorithms, including neural

network, random forest, and gradient boosting, with a variety of predictor variables (e.g., satellite remote sensing and chemical transport models) (Di et al. 2019b). This approach allows one to estimate daily NO_2 at a 1 km × 1 km resolution across the contiguous US from 2000 to 2016 with an excellent prediction model performance. Therefore, we were able to assess long-term exposures of NO_2 for population-based cohort studies, with residents living far from monitors and those potentially exposed to low-level NO_2.

To address these critical gaps in knowledge, we conducted a large population-based cohort study encompassing all Medicare beneficiaries (aged ≥65) from 2000-2016 in the southeastern US, using spatially and temporally resolved NO_2 concentrations from the ensemble model. The NO_2 annual mean levels in the study area were largely below the current NAAQS of 53 ppb. We examined the association between long-term exposure to the low-level NO_2 and mortality among the elderly, independent of $PM_{2.5}$ and ozone, and study the health effects associated with NO_2 at low level below the NAAQS. By doing a multi-state analysis, with both rural and urban locations included, we have the opportunity to explore the NO_2 effects in diverse exposure settings, where patterns of correlations between NO_2 and its co-pollutant may differ.

2. Materials and Methods

2.1 Study Population

The study population was comprised of all Medicare beneficiaries who were aged 65 or over from 2000-2016 in 7 southeastern US states (Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, and Tennessee). We constructed an open cohort from January 1, 2000 to December 31, 2016, with all-cause mortality as the outcome. We obtained the year and age of Medicare enrollment, date of death, age, sex, race, ZIP code of residence, and Medicaid eligibility (a proxy

for SES, socioeconomic status, i.e., an individual eligible for Medicaid usually has lower SES) at the individual level from the Medicare beneficiary denominator file, which was derived from the Center for Medicare and Medicaid Services. The ZIP code of residence and calendar year were used for further exposure assignment. This study was approved by the Institutional Review Board of Emory University and a waiver of informed consent was granted.

2.2 Exposure

We applied previously estimated daily NO_2 concentrations at 1 km × 1 km resolution in the US from 2000-2016 using an ensemble model, which integrated multiple machine learning algorithms and predictor variables (Di et al. 2019b). Briefly, we respectively fit a neural network, a random forest, and a gradient boosting model with input predictor variables (satellite remote sensing, chemical transport models, meteorological variables, and multiple land-cover terms) and monitored NO_2 measurements to generate daily NO_2 predictions. This ensemble learning approach yielded a cross-validated mean R^2 of 0.79 and an average root mean square error (RMSE) of 7.2 ppb. For each ZIP code, we averaged daily NO_2 concentrations across all covered 1 km × 1 km grid cells, and then the data were averaged annually for reference to long-term exposure.

2.3 Covariates

Daily $PM_{2.5}$ concentrations at 1 km × 1 km resolution in the US from 2000-2016 were previously estimated using the same ensemble model (Di et al. 2019a). This trained model produced a cross-validated mean R^2 of 0.84 and an average RMSE of 2.79 $\mu g/m^3$. Then we calculated daily $PM_{2.5}$ concentrations across all covered 1 km × 1 km grid cells according to the ZIP code, based on which we further calculated the annual averages.

Daily ozone concentrations at 1 km spatial resolution in the US from 2000-2016 were estimated using a neural network (Requia et al. 2020). Specifically, the modeling approach combined satellite-based ozone measurements, chemical transport model simulations, land-use

terms, and other auxiliary variables, and yielded a cross-validated mean R^2 of 0.90. For each ZIP code, we calculated daily average ozone across all covered 1 km × 1 km grid cells, and further calculated a warm-season mean in each year based on daily predictions from May through October.

We obtained eight ZIP code tabulation areas (ZCTA)-level variables from the 2000 US Census, 2010 US Census, and the American Community Survey for 2005-2012, and then we matched the ZCTA-level variables to ZIP codes. The eight variables included median home value, median household income, population density, Black percentage, Hispanic percentage, percentage of less-educated (i.e., with less than a high school degree), the percentage of those below the poverty level, and percentage of owner-occupied housing units. When any of the data were missing, we linearly interpolated or extrapolated them based on the available data (Junninen et al. 2004). Behavioral Risk Factor Surveillance System (BRFSS) provided information on county-level variables including body mass index (BMI) and percentage of the ever smokers from 2000 to 2016. These annual average data were assigned to individuals according to the ZIP code of residence. We assigned the county-level variables to ZIP codes whose centroids are located within the county boundary.

The data of daily air temperature, as well as relative humidity in southeastern US at 32 km × 32 km spatial resolution, were obtained from the North American Regional Reanalysis data (NARR) for 2000-2016. We linked the nearest 32 km grid cell for each ZIP code and assigned the daily meteorological data. We calculated the annual average of these two variables for each ZIP code and then assigned them to each subject residing in that ZIP code.

2.4 Statistical analysis

A counting process survival dataset was constructed using the individual-level data, which was based on the scheme presented by Andersen and Gill (Andersen and Gill 1982). We respectively fit single-, two-, and tri-pollutant Cox proportional hazards models to estimate the hazard ratio for

all-cause mortality from annual mean NO_2 exposure among the elderly population. All models were stratified by 5-year age categories, gender (female, male), race (white, black, and other), as well as Medicaid eligibility, adjusting for indicators for each year, annual average air temperature, relative humidity, median home value, median household income, population density, and the percentage of many variables including Hispanic, Black, population of less educated, population below the poverty level, owner-occupied housing units, BMI, and percentage of the ever smokers. To identify subgroups who are most vulnerable, we evaluated effect modification by sex (female vs male), race (black vs white vs other), age (over 80 vs below 80), Medicaid eligibility (dual vs non-dual), urbanicity (quartiles of population density), and area-level SES indicator (a measure showing socioeconomic status, high SES vs low SES) in tri-pollutant Cox models. Area-level low SES was defined as below the median and high SES as above the median of the distribution of percentage below the poverty level. To assess the potential non-linearity of the dose-response relationship, we fit a penalized spline for NO_2, which controlled for both individual- and area-level variables and co-pollutants.

To obtain more robust confidence intervals, we conducted the m-out-n bootstrapping method. We did this since the Cox model treats the observations as independent, yet the closer people live to each other, the more likely they were exposed to similar health risks. Therefore, we used the m-out-n bootstrapping method to break down the spatial dependence by randomly sampling ZIP codes for each bootstrap replicate and yield more robust standard errors and thus 95% confidence intervals. Namely, it is least likely that our findings are influenced by spatial correlation.

We performed some sensitivity analyses to test whether the results are robust. First, alternative models, with each excluding a different set of covariates including co-pollutant, time trends, SES, meteorology variables, behavioral risk factors, and stratification by individual-level

variables were fitted. We compared the results of these models to examine the influence of potential confounders. Second, we calculated the E-values, which measured the amount of unmeasured confounding that would be needed to explain away the effect estimates (VanderWeele and Ding 2017). A higher E-value indicates that the analyses are more robust to unmeasured confounding. Additionally, we evaluated the potential heterogeneity of associations by states.

The computations of the analyses were run on the Rollins High-Performance Computing (HPC) Cluster at Emory University. R software, version 4.0.2 (R projects for Statistical Computing) was used in this study.

3. Results

We included a total of 13,590,387 Medicare enrollees residing in 10,193 ZIP codes, with 107,291,652 person-years of follow-up in our full cohort study. A total of 4,898,015 (36.0%) participants died between 2000 to 2016. Among them, 13,583,802 (99.95%) Medicare enrollees living in 10,093 ZIP codes were exposed to annual mean NO_2 concentrations below NAAQS (53 ppb), with 4,894,351 (36.0%) deaths out of 107,264,499 person-years of follow-up. The median follow-up year for both the full cohort and the below-guideline cohort was 8 years. Table 1 shows the detailed characteristics of the study population.

The annual NO_2 concentrations across the southeastern US was 13.7 ppb with an interquartile range (IQR) of 9.3 ppb from 2000-2016 (Table 2). The spatial distribution of long-term NO_2 concentrations was displayed in Figure 1, which showed consistent patterns with major roads (supplemental Figure S1). The temporal trend of long-term NO_2 concentrations by state was shown in supplemental Figure S2. At the beginning of the study, Mississippi had the lowest NO_2 levels, and Florida had the highest levels. Overall, the NO_2 concentrations exhibited a declining trend over the study period, despite elevated levels between 2009 and 2011.

Broadly, long-term exposure to NO_2, even at low levels, was significantly and positively associated with mortality in all statistical analyses (Table 3). In single-pollutant models, we observed a HR of 1.040 (95% CI: 1.030, 1.050) per 10 ppb increase in NO_2 concentrations. After adjusting for $PM_{2.5}$ or/and ozone, the results were similar, i.e., the results were quite robust to inclusion of co-pollutants. The relationship between long-term NO_2 concentrations and mortality was almost linear across the exposure distribution, which may indicate the lack of an observed NO_2 threshold (Figure 2).

The results of effect modification analysis showed that being white (1.057, 95% CI: 1.046, 1.067), being female (1.072, 95% CI: 1.058, 1.087) and residing in urban areas (1.053, 95% CI: 1.034, 1.072) were at significantly higher risks than other subgroups (p-values<0.05). The relatively younger population (<80 years old) and the neighborhoods with a higher percentage below poverty were also at a higher risk, although not significant. Details can be found in the Figure 3.

In the sensitivity analyses, we examined the influence of potential confounders and the results showed that the time trend influenced the effect estimates of NO_2 most (Table S1). We obtained relatively large E-values (~1.20) for all hazard ratios, indicating that our results are robust to unmeasured confounding. All subgroup analyses stratified by state yielded consistently positive associations between long-term NO_2 exposure and mortality, with the highest HR among Medicare beneficiaries in North Carolina.

4. Discussion

This large-scale population-based cohort study showed that long-term exposure to NO_2, even at levels below current NAAQS, significantly linearly increases mortality, independent of $PM_{2.5}$ and ozone. Furthermore, the results of effect modification analysis showed that being white, female,

and urban residents had significantly higher risks of all-cause mortality associated with long-term NO_2 exposure than other subgroups. These findings collectively indicate that reducing NO_2 levels below current national standards, may yield substantial health benefits.

This study used spatio-temporally resolved exposure data to calculate hazard ratio for all-cause mortality and reported a hazard ratio of all-cause mortality at 1.042 (95% CI: 1.032,1.052) per 10 ppb increase of NO_2 concentrations. Our findings are consistent with previous studies (Crouse et al. 2015; Faustini et al. 2014; Hart et al. 2011; Hoek et al. 2013). In a meta-analysis, Faustini et al. (2014) reported a HR of 1.04 (95% CI: 1.02, 1.06) for all-cause mortality with an increase of 10 $\mu g/m^3$ (i.e., about 5.2 ppb) in annual NO_2 exposure (Faustini et al. 2014). Another recent meta-analysis estimated a pooled HR of 1.05 (95% CI: 1.03, 1.08) for all-cause mortality associated with per 10 $\mu g/m^3$ (i.e., about 5.2 ppb) increase in NO_2 (Hoek et al. 2013). Hart et al. (2011) examined the association between occupational NO_2 exposure with mortality in the US trunk industry, and observed an 8.2% (95% CI: 4.5, 12.1%) increased risk of all-cause mortality per 8 ppb increase in NO_2 (Hart et al. 2011). In a subset of the Canadian Census and Environment Cohort (CanCHEC), Crouse et al. (2015) reported a HR of 1.045 (95% CI: 1.037, 1.052) for non-accidental mortality per 5 ppb increase in NO_2, adjusting for $PM_{2.5}$ and ozone (Crouse et al. 2015).

The shape of the dose-response relationship between NO_2 and mortality has not been assessed in the literature. Our findings address this important gap and found that this relationship was linear across the exposure distribution, suggesting that there is no "safe" level of NO_2 pollution. Thus, our study further confirms the adverse health effects of NO_2 on human health, which lasts even at low levels far below the NAAQS. This is supported by emerging evidence in Oceania that also reported adverse health effects of low-level NO_2 (Dirgawati et al. 2019; Hanigan et al. 2019). Hanigan et al. (2019) found that in Sydney (mean NO_2 level = 17.75 $\mu g/m^3$), there was a 3%

increase in all-cause mortality per 5 μg/m^3 in NO_2. Dirgawati et al. (2019) also reported a significantly positive association between low-level NO_2 (mean = 13.4 μg/m^3) and all-cause mortality.

Our effect modification results show significant higher HR among white, female, and urban populations. The effect of modification of age was not apparent, which was similar to the study of NO_2 and mortality in three Canadian cities (Chen et al. 2013). People residing in urban areas had higher hazard ratio than those in rural areas, and similar results were found by other studies (Eum et al. 2019; Lewné et al. 2004). Huge motor vehicle emissions, industrial activities and other exposure sources (Neisi et al. 2018) could be possible explanations to the results. Besides, females have significant higher risk than males. Health disparities between genders could be explained by gender inequality and gender invisibility (Butter 2006). Women visit health-care professionals less often and have higher exposure to cooking fuels, which is an indoor source of NO_2 exposure (Duncan 2006).

NO_2 primarily forms from emissions from cars, buses, trucks, power plants, as well as off-road equipment, and therefore is often considered as a surrogate for traffic-related air pollutants (Alotaibi et al. 2019). Long-term exposure to NO_2 has been associated with both acute and chronic respiratory diseases, such as increased bronchial hyperresponsiveness, increased respiratory infection, and decreased lung function (Abbey et al. 1993; Faustini et al. 2014; Gan et al. 2012; Liang et al. 2020). Biological evidence has been reported for plausible mechanisms regarding the health effects of NO_2. A critical review suggested that NO_2 inhalation can induce lung function changes, accelerate pulmonary infections, and aggravate existing lung diseases by triggering proinflammatory response, which is an innate immune response (Hesterberg et al. 2009). NO_2 can also enhance oxidative stress, generate reactive oxygen and nitrogen species, and then deteriorate

the cardiovascular and immune system (Ayyagari et al. 2007; Bevelander et al. 2007; Liang et al. 2020).

To the best of our knowledge, this is the first of its kind to restrict exposure and to explore the dose-response relationship between NO_2 below the current U.S. EPA annual standards and mortality in a large-scale population-based study. All residents exposed to low-level NO_2 in the southeastern US, not just urban residents or those near monitors, were included in this analysis. The strengths of this study include the use of highly spatio-temporally resolved NO_2 exposure, which allows the inclusion of all Medicare beneficiaries in the Southeastern US. Our large sample size is representative of the nature of the cohort and our analysis of subgroups increases our statistical power in underrepresented populations. Our results are also robust in the ability to model the mortality effects exclusively of NO_2 exposure after we compared single-pollutant, two-pollutant, and multi-pollutant models. Moreover, our study obtained effect estimates along with statistically rigorous confidence intervals, using m-out-n bootstrapping. This approach allows one to account for the spatial dependence for covariates and derive standard errors that would not be impacted by the residual spatial dependence.

We acknowledge that our study has some limitations. First, a major limitation of the Medicare data is that only the ZIP code of residence for each Medicare beneficiary, but not home address, is available, therefore we were not able to assign our 1 km NO_2 exposure or calculate the distance to roads that can better capture the near-road environments to study subjects. As a result, the finest resolution for exposure we can get in our analysis is at the ZIP code level, limiting the ability to capture local or small area variation near roads and represent personal exposure to traffic-related air pollution. Despite this, the comparison of major roads (Figure S1) and NO_2 concentrations (Figure 1) suggests that ZIP code-level NO_2 may still serve as a good indicator of

traffic pollution. Second, the Medicare data do not provide the underlying cause of death that is necessary to understand possible pathways. Third, although we controlled for ozone and $PM_{2.5}$, we cannot rule out the possibility that NO_2 is an indicator of other traffic-related air pollutants, such as ultrafine particles, soot, and trace metals. Another limitation is that we only focused on the southeastern US, which may not represent the vast US where the air pollution profiling and demographic characters vary. In addition, unmeasured confounding is likely because individual-level risk factors for mortality such as smoking, alcohol consumption, and physical activity, were not considered in this study. However, Makar et al. (2017) reported that when using ZIP code-level exposure data, confounding by individual-level behavioral risk factors may not be much of an issue (Makar et al. 2017).

In conclusion, we found a statistically significant, positive, and linear association between long-term exposure to NO_2 and all-cause mortality, independent of $PM_{2.5}$ and ozone. Our results suggested an increased risk of mortality associated with traffic-related air pollution. Improving air quality by reducing NO_2 emissions may yield substantial health benefits.

5. References

Abbey D, Colome S, Mills P, Burchette R, Beeson W, Tian Y. 1993. Chronic disease associated with long-term concentrations of nitrogen dioxide. Journal of exposure analysis and environmental epidemiology 3:181-202.

Akintoye E, Shi L, Obaitan I, Olusunmade M, Wang Y, Newman JD, et al. 2016. Association between fine particulate matter exposure and subclinical atherosclerosis: A meta-analysis. European Journal of Preventive Cardiology 23:602-612.

Alotaibi R, Bechle M, Marshall JD, Ramani T, Zietsman J, Nieuwenhuijsen MJ, et al. 2019. Traffic related air pollution and the burden of childhood asthma in the contiguous united states in 2000 and 2010. Environment international 127:858-867.

Andersen PK, Gill RD. 1982. Cox's regression model for counting processes: A large sample study. The annals of statistics:1100-1120.

Ayyagari VN, Januszkiewicz A, Nath J. 2007. Effects of nitrogen dioxide on the expression of intercellular adhesion molecule-1, neutrophil adhesion, and cytotoxicity: Studies in human bronchial epithelial cells. Inhalation toxicology 19:181-194.

Bevelander M, Mayette J, Whittaker LA, Paveglio SA, Jones CC, Robbins J, et al. 2007. Nitrogen dioxide promotes allergic sensitization to inhaled antigen. The Journal of Immunology 179:3680-3688.

Butter ME. 2006. Are women more vulnerable to environmental pollution? Journal of Human Ecology 20:221-226.

Chen B, Kan H. 2008. Air pollution and population health: A global challenge. Environmental health and preventive medicine 13:94-101.

Chen H, Goldberg MS, Burnett RT, Jerrett M, Wheeler AJ, Villeneuve PJ. 2013. Long-term exposure to traffic-related air pollution and cardiovascular mortality. Epidemiology:35-43.

Chen R, Samoli E, Wong C-M, Huang W, Wang Z, Chen B, et al. 2012. Associations between short-term exposure to nitrogen dioxide and mortality in 17 chinese cities: The china air pollution and health effects study (capes). Environment international 45:32-38.

Chiusolo M, Cadum E, Stafoggia M, Galassi C, Berti G, Faustini A, et al. 2011. Short-term effects of nitrogen dioxide on mortality and susceptibility factors in 10 italian cities: The epiair study. Environmental health perspectives 119:1233-1238.

Cole MP, Freeman BA. 2009. Promotion of cardiovascular disease by exposure to the air pollutant ozone. American Journal of Physiology-Lung Cellular and Molecular Physiology 297:L205-L208.
Crouse DL, Peters PA, Hystad P, Brook JR, van Donkelaar A, Martin RV, et al. 2015. Ambient pm2. 5, o3, and no2 exposures and associations with mortality over 16 years of follow-up in the canadian census health and environment cohort (canchec). Environmental health perspectives 123:1180-1186.
Di Q, Amini H, Shi L, Kloog I, Silvern R, Kelly J, et al. 2019a. An ensemble-based model of pm2. 5 concentration across the contiguous united states with high spatiotemporal resolution. Environment international 130:104909.
Di Q, Amini H, Shi L, Kloog I, Silvern R, Kelly J, et al. 2019b. Assessing no2 concentration and model uncertainty with high spatiotemporal resolution across the contiguous united states using ensemble model averaging. Environmental Science & Technology.
Dirgawati M, Hinwood A, Nedkoff L, Hankey GJ, Yeap BB, Flicker L, et al. 2019. Long-term exposure to low air pollutant concentrations and the relationship with all-cause mortality and stroke in older men. Epidemiology 30:S82-S89.
Duncan K. 2006. Global climate change, air pollution, and women's health. WIT Transactions on Ecology and the Environment 99.
EPA. 2018. Nitrogen dioxide (no2) pollution

Available: https://www.epa.gov/no2-pollution/ambient-nitrogen-dioxide-monitoring-requirements [accessed January 6, 2021.
Eum K-D, Kazemiparkouhi F, Wang B, Manjourides J, Pun V, Pavlu V, et al. 2019. Long-term no2 exposures and cause-specific mortality in american older adults. Environment international 124:10-15.
Faustini A, Rapp R, Forastiere F. 2014. Nitrogen dioxide and mortality: Review and meta-analysis of long-term studies. European Respiratory Journal 44:744-753.
Gan WQ, Davies HW, Koehoorn M, Brauer M. 2012. Association of long-term exposure to community noise and traffic-related air pollution with coronary heart disease mortality. American journal of epidemiology 175:898-906.

Hanigan IC, Rolfe MI, Knibbs LD, Salimi F, Cowie CT, Heyworth J, et al. 2019. All-cause mortality and long-term exposure to low level air pollution in the '45 and up study'cohort, sydney, australia, 2006–2015. Environment international 126:762-770.
Hart JE, Garshick E, Dockery DW, Smith TJ, Ryan L, Laden F. 2011. Long-term ambient multipollutant exposures and mortality. American journal of respiratory and critical care medicine 183:73-78.
Heinrich J, Thiering E, Rzehak P, Krämer U, Hochadel M, Rauchfuss KM, et al. 2013. Long-term exposure to no2 and pm10 and all-cause and cause-specific mortality in a prospective cohort of women. Occupational and environmental medicine 70:179-186.
Hesterberg TW, Bunn WB, McClellan RO, Hamade AK, Long CM, Valberg PA. 2009. Critical review of the human data on short-term nitrogen dioxide (no2) exposures: Evidence for no2 no-effect levels. Critical reviews in toxicology 39:743-781.
Hoek G, Krishnan RM, Beelen R, Peters A, Ostro B, Brunekreef B, et al. 2013. Long-term air pollution exposure and cardio-respiratory mortality: A review. Environmental health 12:43.
Jerrett M, Burnett RT, Beckerman BS, Turner MC, Krewski D, Thurston G, et al. 2013. Spatial analysis of air pollution and mortality in california. American journal of respiratory and critical care medicine 188:593-599.
Junninen H, Niska H, Tuppurainen K, Ruuskanen J, Kolehmainen M. 2004. Methods for imputation of missing values in air quality data sets. Atmospheric Environment 38:2895-2907.
Lewné M, Cyrys J, Meliefste K, Hoek G, Brauer M, Fischer P, et al. 2004. Spatial variation in nitrogen dioxide in three european areas. Science of the Total Environment 332:217-230.
Liang D, Shi L, Zhao J, Liu P, Sarnat JA, Gao S, et al. 2020. Urban air pollution may enhance covid-19 case-fatality and mortality rates in the united states. The Innovation 1:100047.
Maheswaran R, Pearson T, Smeeton NC, Beevers SD, Campbell MJ, Wolfe CD. 2010. Impact of outdoor air pollution on survival after stroke: Population-based cohort study. Stroke 41:869-877.
Makar M, Antonelli J, Di Q, Cutler D, Schwartz J, Dominici F. 2017. Estimating the causal effect of fine particulate matter levels on death and hospitalization: Are levels below the safety standards harmful? Epidemiology (Cambridge, Mass) 28:627.
Mills I, Atkinson R, Anderson H, Maynard R, Strachan D. 2016. Distinguishing the associations between daily mortality and hospital admissions and nitrogen dioxide from those of particulate matter: A systematic review and meta-analysis. BMJ open 6.

Neisi A, Vosoughi M, Shirmardi M, Idani E, Goudarzi G, Hazrati S, et al. 2018. Concentration of air pollutants as toxic matter in urban and rural areas of ahvaz. Toxin reviews 37:243-250.
Requia WJ, Adams MD, Arain A, Papatheodorou S, Koutrakis P, Mahmoud M. 2018. Global association of air pollution and cardiorespiratory diseases: A systematic review, meta-analysis, and investigation of modifier variables. American journal of public health 108:S123-S130.
Requia WJ, Di Q, Silvern R, Kelly JT, Koutrakis P, Mickley LJ, et al. 2020. An ensemble learning approach for estimating high spatiotemporal resolution of ground-level ozone in the contiguous united states. Environmental Science & Technology 54:11037-11047.
Samoli E, Aga E, Touloumi G, Nisiotis K, Forsberg B, Lefranc A, et al. 2006. Short-term effects of nitrogen dioxide on mortality: An analysis within the aphea project. European Respiratory Journal 27:1129-1138.
Shi L, Zanobetti A, Kloog I, Coull BA, Koutrakis P, Melly SJ, et al. 2016. Low-concentration pm2. 5 and mortality: Estimating acute and chronic effects in a population-based study. Environmental health perspectives 124:46-52.
Shi L, Wu X, Yazdi MD, Braun D, Awad YA, Wei Y, et al. 2020. Long-term effects of pm2· 5 on neurological disorders in the american medicare population: A longitudinal cohort study. The Lancet Planetary Health.
VanderWeele TJ, Ding P. 2017. Sensitivity analysis in observational research: Introducing the e-value. Annals of internal medicine 167:268-274.
Wang C, Tu Y, Yu Z, Lu R. 2015. Pm2. 5 and cardiovascular diseases in the elderly: An overview. International journal of environmental research and public health 12:8187-8197.
Wei Y, Wang Y, Wu X, Di Q, Shi L, Koutrakis P, et al. 2020. Causal effects of air pollution on mortality rate in massachusetts. American journal of epidemiology 189:1316-1323.
Yu W, Guo Y, Shi L, Li S. 2020. The association between long-term exposure to low-level pm2. 5 and mortality in the state of queensland, australia: A modelling study with the difference-in-differences approach. PLoS medicine 17:e1003141.
Zu K, Liu X, Shi L, Tao G, Loftus CT, Lange S, et al. 2017. Concentration-response of short-term ozone exposure and hospital admissions for asthma in texas. Environment international 104:139-145.

6.Tables

6.1 Table 1. Descriptive statistics for the study population.

	Full cohort		Below-guideline cohort	
Full cohort	**Number**	**%**	**Number**	**%**
Number of Death	4,898,015	36.0	4,894,351	36.0
Number of Total Population	13,590,387	100	13,583,802	99.95
Total person-years	107,291,652	100	107,264,499	99.97
Median follow-up year	8		8	
Age at entry (years)				
65-74	13,527,082	99.5	13,520,643	99.5
75-84	53,181	0.4	53,062	0.4
85-94	9,523	0.07	9,498	0.07
95-114	599	0.004	597	0.004
Sex				
Male	5,943,391	43.7	5,940,441	43.7
Female	7,646,996	56.3	7,643,361	56.2
Race				
White	11,217,509	82.5	11,212,879	82.5
Black	1,745,096	12.8	1,744,111	12.8
Other	627,782	4.6	626,812	4.6
Medicaid Eligibility				
Dual-Eligible	1,718,169	12.6	1,715,867	12.6
Non-dual Eligible	11,872,218	87.4	11,867,935	87.3

6.2 Table 2. Spatial and Temporal Variability of Annual NO_2 levels (ppb) in years 2000-2016

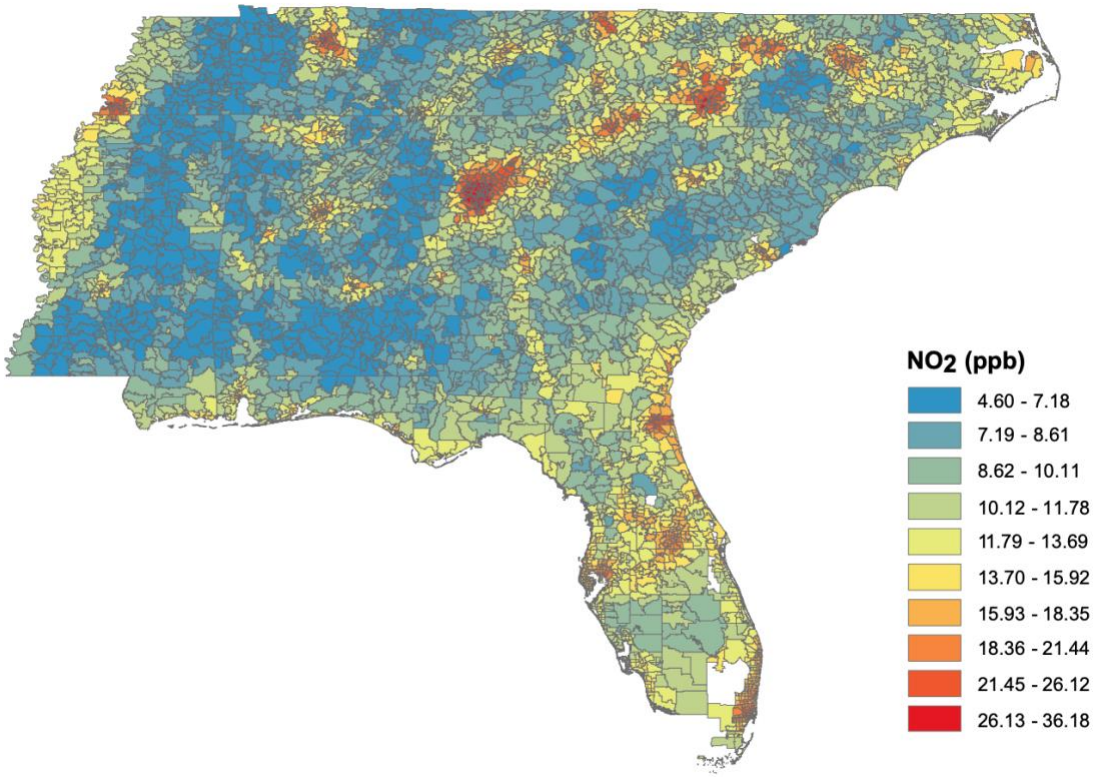
	Min	5th	25th	50th	75th	95th	Max	Mean
Overall								
	0.58	**5.25**	**8.36**	**12.09**	**17.68**	**27.10**	**56.95**	**13.65**
By Year								
2000	3.32	8.26	14.11	20.17	25.62	34.34	52.47	20.30
2001	4.06	7.51	11.65	17.39	23.74	33.56	49.62	18.45
2002	2.82	6.19	9.91	15.45	21.60	30.69	42.82	16.37
2003	2.71	5.28	8.97	14.06	20.60	29.67	52.11	15.32
2004	2.07	7.19	10.23	14.45	19.49	27.79	46.07	15.48
2005	3.33	6.07	9.04	13.49	19.50	27.43	44.91	14.72
2006	2.11	5.44	7.90	12.13	19.28	26.50	41.54	13.97
2007	1.93	4.70	6.57	9.97	17.11	27.09	42.75	12.41
2008	2.42	6.12	8.00	11.18	16.63	25.13	35.97	12.87
2009	0.93	4.74	6.33	9.30	14.56	21.00	30.62	10.78
2010	0.58	5.23	8.03	10.92	15.18	23.52	36.62	12.14
2011	3.98	7.37	10.25	12.58	15.64	22.95	41.41	13.49
2012	2.94	7.77	10.00	11.85	14.59	21.15	49.47	12.86
2013	2.37	4.67	7.01	9.51	12.82	19.18	56.95	10.44
2014	2.39	4.51	6.62	9.54	14.34	21.12	39.01	10.99
2015	0.97	4.78	7.78	10.48	13.83	20.05	32.08	11.16
2016	1.09	3.84	6.31	9.36	14.03	20.84	31.44	10.59
By State								
Alabama	2.21	4.71	6.96	9.77	14.45	21.50	37.55	11.17
Florida	1.98	7.28	11.35	14.87	19.32	25.50	45.19	15.54
Georgia	2.58	5.64	8.56	11.90	19.24	32.37	52.47	14.86
Mississippi	2.24	4.63	6.78	9.52	13.87	20.19	29.85	10.70
North Carolina	0.58	5.58	8.87	12.70	19.01	29.06	46.07	14.54
South Carolina	2.97	5.55	8.23	11.17	16.00	25.38	37.71	12.78
Tennessee	0.94	4.55	6.87	10.08	16.90	27.71	56.95	12.58

6.3 Table 3. Estimated Hazard Ratio of Mortality (95% CI) associated with an Increase of 10 ppb in NO_2 Concentration.

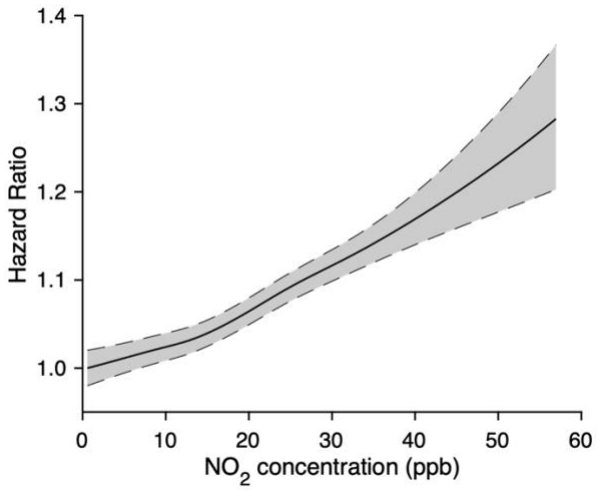
	Full cohort		Below-guideline cohort	
Models	**HR (95% CI)**	**E-value**	**HR (95% CI)**	**E-value**
Single-pollutant	1.040 (1.030, 1.050)	1.20 (1.17)	1.040 (1.031, 1.050)	1.20 (1.17)
Two-pollutant (+$PM_{2.5}$)	1.039 (1.030, 1.049)	1.19 (1.17)	1.040 (1.030, 1.049)	1.20 (1.17)
Two-pollutant (+Ozone)	1.042 (1.033, 1.052)	1.20 (1.18)	1.043 (1.033, 1.052)	1.20 (1.18)
Tri-pollutant	1.042 (1.032, 1.052)	1.20 (1.17)	1.042 (1.033, 1.052)	1.20 (1.18)

7. Figures

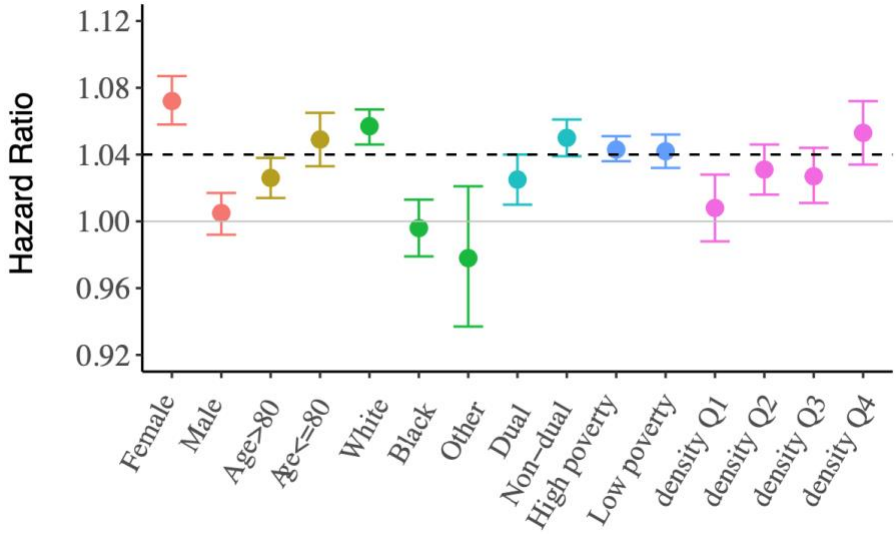
7.1 Figure 1. The spatial distribution of NO_2 concentrations (2000-2016) in the Southeastern US



7.2 Figure 2. The dose-response relationship between long-term exposure to NO2 and all-cause mortality. Shaded areas indicate the 95% confidence bands



7.3 Figure 3. The hazard ratios of mortality associated with a 10 ppb increase in NO2 concentrations for study subgroups. Density Q1-Q4 stand for low population density, low-medium population density, medium-high population density, and high population density, respectively



8. Supplementary Tables

8.1 Table S1. Estimated Hazard Ratio of Mortality (95% CI) associated with an Increase of 10 ppb in NO2 Concentration at different levels of confounding adjustment

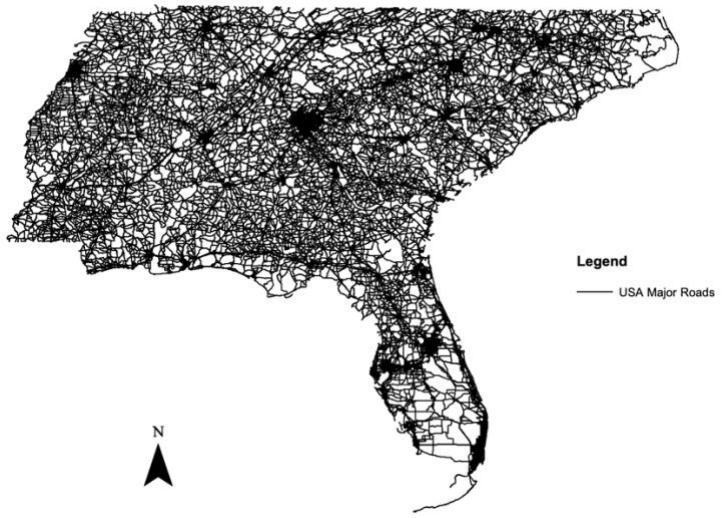
HR (95%CI)	Below-guideline cohort
Main Analysis	1.042 (1.033, 1.052)
Excluding co-pollutant	1.040 (1.031, 1.050)
Excluding time trends	1.247 (1.236, 1.259)
Excluding meteorological variables	1.043 (1.033, 1.052)
Excluding BRFSS	1.040 (1.031, 1.050)
Excluding US Census	1.053 (1.045, 1.061)
Excluding stratification by individual-level variables	1.018 (1.017, 1.019)

8.2 Table S2. Estimated Hazard Ratio of Mortality (95% CI) associated with an Increase of 10 ppb in NO2 Concentration by state

HR (95%CI)	Below-guideline cohort
Main Analysis	1.042 (1.033, 1.052)
Alabama	1.055 (1.043, 1.067)
Florida	1.035 (1.030, 1.040)
Mississippi	1.050 (1.033, 1.068)
North Carolina	1.062 (1.055, 1.069)
South Carolina	1.024 (1.013, 1.035)
Georgia	1.009 (1.002, 1.016)
Tennessee	1.013 (1.007, 1.020)

9. Supplementary Figures

9.1 Figure S1. The map of major roads in the Southeastern US



9.2 Figure S2. The temporal trend of NO_2 concentrations (2000-2016) in the Southeastern US