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2 Analysis of 15 European Cohorts?

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93 **Abstract**

94 *Background:* Ambient air pollution contains low concentrations of carcinogens implicated in the
 95 etiology of urinary bladder cancer. Little is known about whether exposure to air pollution
 96 influences bladder cancer in the general population.

97 *Objective:* We aimed to evaluate the association between long-term exposure to ambient air
 98 pollution and bladder cancer incidence.

99 *Design, setting, and participants:* We obtained data from 15 population-based cohorts enrolled
 100 between 1985 and 2005 in eight European countries (N=303,431; mean follow-up 14.1 years). We
 101 estimated exposure to nitrogen oxides (NO₂ and NO_x), particulate matter (PM) with diameter <10
 102 µm (PM₁₀), <2.5 µm (PM_{2.5}), between 2.5 and 10 µm (PM_{2.5-10}), PM_{2.5} absorbance (soot), elemental
 103 constituents of PM, organic carbon and traffic density at baseline home addresses using
 104 standardized land-use regression models from the ESCAPE (European Study of Cohorts for Air
 105 Pollution Effects) project.

106 *Outcome measurements and statistical analysis:* We used Cox proportional-hazards models with
 107 adjustment for potential confounders for cohort-specific analyses and meta-analyses to estimate
 108 summary hazard ratios (HRs) for bladder cancer incidence.

109 *Results and Limitations:* During follow-up, 943 incident bladder cancer cases were diagnosed. In
 110 the meta-analysis, none of the exposures were associated with bladder cancer risk. The summary
 111 HRs associated with a 10-µg/m³ increase in NO₂ and 5-µg/m³ increase in PM_{2.5} were 0.98 (95%
 112 confidence interval (CI): 0.89, 1.08) and 0.86 (95%CI: 0.63, 1.18), respectively. Limitations include

113 lack of information about lifetime exposure.

114 *Conclusion:* There was no evidence of an association between exposure to outdoor air pollution
115 levels at residence and risk of bladder cancer.

116 *Patient summary:* We assessed the link between outdoor air pollution at residence and bladder
117 cancer, using the largest study population to date, extensive assessment of exposure and
118 comprehensive data on personal risk factors such as smoking. We found no association between the
119 levels of outdoor air pollution at residence and bladder cancer risk.

1. Introduction

Urinary bladder cancer (BC) is the ninth most common cancer worldwide [1]. Smoking is the primary risk factor for BC with relative risks of 3 for current smoking and 2 for former smoking compared to never smoking [2]. Findings from the most recent studies suggest that the relative risk for current smoking has increased to four or five times the nonsmoker risk [3]. The relative risk for BC increases with duration and intensity of smoking [4]. BC occurs mainly in older people, is more frequent in men, and exhibits large geographical variation [5].

Ambient air pollution includes a mix of carcinogens such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds, transition metals, and diesel engine exhaust [6,7]. Ambient air pollution and particulate matter (PM) in ambient air have recently been classified as carcinogenic to humans [6]. This classification was largely based on an elevated risk for lung cancer [8-10]. However, there is suggestive evidence of an association between ambient air pollution and BC in humans [6,10].

Elevated risk for BC has been reported in some studies of taxi, bus, and/or truck drivers exposed to high levels of urban air pollution [6], including PAHs [11] and diesel engine exhaust [7], but no elevated risk was found among highly diesel exposed miners [12]. Some of these studies were incidence based and others were based on mortality, which also may contribute to the observed heterogeneity in results. Few studies have investigated a possible association between exposure to ambient air pollution and BC in the general population, and provided mixed results [13-17]. Limitations related to design, poor exposure assessment and lack of information on potential confounding complicate interpretation of the previous studies.

Our aim was to examine the associations between exposures to ambient air pollution at residence and BC incidence in a large European study population with fine-scale exposure assessment and extensive control for potential confounders such as smoking. We used the same study population, exposure assessment and data analysis methods as in our previous study documenting significant associations of air pollution and lung cancer [9].

2. Material and Methods

2.1. Study population

The ESCAPE (European Study of Cohorts for Air Pollution Effects) project included 36 European areas where air pollution measurements were performed, exposure models were developed, and cohort studies located [9,18]. The present study included 15 population-based prospective cohorts with information on incident BC cases with at least 20 incident BC cases during follow-up and where the resources needed for participation were available. The cohorts were in Sweden (European Prospective Investigation into Cancer and Nutrition[EPIC]-Umea, Swedish National Study on Aging and Care in Kungsholmen [SNAC-K], Stockholm Screening Across the Lifespan Twin Study and TwinGene [SALT], Stockholm 60 years old and IMPROVE study [Sixty], Stockholm Diabetes Prevention Program [SDPP]), Norway (Oslo Health Study [HUBRO]), Denmark (Diet, Cancer and Health Study [DCH]), the Netherlands (EPIC Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands [MORGEN], EPIC PROSPECT), the United Kingdom (EPIC Oxford), Austria (Vorarlberg Health Monitoring and Prevention Programme [VHM&PP]), Italy (EPIC Varese, EPIC Turin, Italian Studies of Respiratory Disorders in Childhood and Environment – Rome [SIDRIA] Rome) and Spain (EPIC San Sebastian) (Fig 1; Supplementary Table 1). A pooled analysis of all cohort data was not possible due to data-transfer and privacy issues, but data from the

four Stockholm cohorts (SNAC-K, SALT, Sixty and SALT) were pooled, analysed and denoted as one cohort (Cardiovascular Effects of Air pollution and Noise in Stockholm [CEANS]). Similarly, data from the two cohorts from the Netherlands (EPIC MORGEN and EPIC PROSPECT) were pooled, analysed and denoted as one cohort [EPIC NL] (Supplementary Table 2). Most of the participants were recruited in the 1990s (Table 1). Participants with a cancer (except non-melanoma skin cancer) before enrolment were excluded, as well as participants for whom information about exposure to air pollution and the most important potential confounders could not be obtained. We included 303,431 participants (81.7% of those enrolled).

Each cohort study followed the rules for ethics and data protection set up in the country in which it was based. All participants gave informed consent.

2.1. BC

In all cohorts, follow-up was based on linkage to cancer registries, with exception of SIDRIA Rome for which hospital discharge and mortality register data were used. Cases were defined as participants diagnosed with BC recoded according to ICD-9 (*International Classification of Disease, 9th revision*) code 1880-1889 and ICD-10 (10th revision) code C67. We did not include in situ carcinomas. Only primary cancers, i.e. not metastases, and only malignant tumors were included.

2.2. Exposure assessment

The annual average air pollution concentrations at the residential addresses at the time of enrolment in the cohort studies were estimated by area-specific land-use regression (LUR) models using

standardized methods developed within ESCAPE [19,20].

Air pollution was measured for 1 year in each study area between October 2008 and May 2011. PM with a diameter of less than 10 μm (PM_{10}), $\text{PM}_{2.5}$, and soot/blackness of the $\text{PM}_{2.5}$ exposed filter ($\text{PM}_{2.5}$ absorbance) determined by measurement of light reflectance, were measured at 20 sites.

Nitrogen dioxide (NO_2) and nitrogen oxides (NO_x) were measured at 40 sites in each of the areas.

Sites were selected to represent spatial variation of air pollution in the residential areas. Within each study area, measurements at each site were performed during three 2-week periods (during summer, winter, and an intermediate season) and the three measurements were averaged adjusting for temporal trends using continuous data from a reference site [9] to estimate the annual mean at each site. Because of financial reasons, sampling of PM was not done everywhere (Fig. 1).

Subsequently LUR models were developed for each pollutant in each study area, with the yearly mean concentration as the dependent variable and an extensive list of geographical attributes as possible predictors. Data from nearest routine monitoring stations were used to back-extrapolate the LUR estimates to the baseline year in fourteen of the fifteen study areas using the ratio-method [21].

We also collected information on traffic intensity (vehicles per day) on the nearest street for all cohorts except for two (Fig. 1, Table 2).

We used the same methods to assess concentrations of eight PM elements [22] and organic carbon in PM [23] to facilitate explorative analyses of associations with risk for BC.

2.3. Statistical analysis

Cox proportional hazards models were used for the cohort specific analyses following a standardized protocol [9]. Age was used as the time scale. Follow-up started at enrollment into the cohort. Censoring was done at the time of death or emigration, a diagnosis of any other cancer (except non-melanoma skin cancer) or end of follow-up, whichever came first.

Exposure was analyzed as a linear variable. Potential confounders were available from questionnaires at baseline (Supplemental Table 1). We specified *a priori* three confounder models with increasing levels of adjustment for both individual and contextual SES variables, following the methodology of our previous study of lung cancer [9]. Model 1 included only age (time axis), sex, and calendar time (year of enrollment, continuous). Model 2 added the following individual-level variables (as available for the individual cohorts; all referring to baseline): smoking status (never/former/current), smoking intensity (g tobacco/day linear and squared term), smoking duration (years), occupational class (ever worked in an industry/job associated with higher BC risk or white/blue collar classification), employment status and educational level (low, medium, high). Model 3 added area-level socioeconomic status (SES) variables, including mean income, percentage of people with a low income, unemployment rate, and educational level or deprivation index, which were defined for most of the cohorts at the neighborhood or municipality level. Model 3 was selected as the main confounder model. Detailed information on job associated with high BC risk was only available for DCH, while three cohorts had less detailed information on occupation and nine cohort had information on employment status.

We performed the following model checks and sensitivity analyses. Firstly, we tested the linearity assumption in the relation between each exposure and BC by replacing the linear term with a natural cubic spline with two equally spaced inner knots and compared the model fit of the linear and the spline models using a likelihood-ratio test. Secondly, we assessed if there was a deviation from the proportional hazards assumption in the Cox model. Thirdly, we assessed potential effect modification by sex, smoking status and level of education. Fourthly, we restricted analyses to participants who had lived at the baseline address throughout follow-up to minimize misclassification of long-term exposure relevant to the development of BC. Fifthly, we fitted back-extrapolated exposure to take into account long-term trends in air pollution. Finally, we added an indicator of extent of urbanization to the most comprehensively adjusted model.

Cohort-specific effect estimates were combined by random-effects meta-analysis for each exposure when it was available in at least three cohorts [24]. I^2 statistics and Q-test were used to assess the heterogeneity among cohort-specific effect estimates [25].

Stata software, version 11 (StataCorp) was used for all data analyses.

3. Results

3.1. Study Population

In total, the fifteen cohorts contributed 4,275,936 person-years at risk and 943 incident BC cases developed during a mean follow-up of 14.1 years (min-max: 0.0-27.0, Table 1). The mean age at baseline was 48 years ranging from 43 to 57 years across cohorts. 39% of the participants were men, 21% were current smokers and 18% were former smokers (Table 1).

The study areas represented a wide range of air pollution concentrations between and within each cohort. The modeled mean air pollution concentrations were lowest in the Swedish and highest in the Italian study areas (Table 2, Supplementary Table 3).

3.2 Air Pollution, Traffic Density and BC

In the meta-analysis, none of the exposures were significantly associated with BC incidence (Table 3, Fig. 2). All exposures investigated, including PM elements (Supplementary Table 4), were associated with HRs close to null. The summary HRs in model 3 associated with a 10- $\mu\text{g}/\text{m}^3$ increase in NO_2 and 5- $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ were 0.98 (95% confidence interval (CI): 0.89, 1.08) and 0.86 (95%CI: 0.63, 1.18), respectively.

For all exposures, the summary HRs were essentially 1 in models adjusted for age, sex and calendar year only. HRs were slightly reduced in models with comprehensive adjustment (Table 3).

The meta-analyses showed significant heterogeneity between cohorts for PM_{10} , $\text{PM}_{2.5-10}$ and a few

271 elemental components of PM, while no substantial heterogeneity were observed for most of the
272 exposures assessed (Table 3, Fig. 2, Supplementary Table 4).

274 3.3 *Model Check, Sensitivity Analysis and Effect Modification*

275 In most cohorts, there was no evidence of deviation from linearity for any of the pollutants
276 (Supplementary Table 5 and 6). All cohorts met the proportional hazards assumptions for Cox
277 models (Supplementary Table 7). Higher risk of BC among men was suggested for NO₂, but the
278 effect modification by sex was borderline statistically significant, not evident for PM_{2.5} and there
279 was no effect modification by smoking habits and education for any of the pollutants
280 (Supplementary Table 8). Results reported in Table 3 were similar to those in sensitivity analysis
281 restricted to non-movers, models fitted with back-extrapolated exposure to NO₂ and models
282 additionally adjusted for degree of urbanization (Supplementary Table 9).

4. Discussion

In this prospective study of 15 European cohorts, long-term exposure to ambient air pollution was not associated with risk of BC.

Our study is the largest study with detailed individual level confounder variables to date on the relationship between ambient air pollution and BC in the general population. The lack of an association between air pollution with NO_x, traffic density, PAH and BC risk observed in our study is consistent with some of the previous studies from Denmark, Spain and the Netherlands [13,14,16] and inconsistent with others from Spain and Taiwan [14,15]. Inconsistency among studies may partly be explained by the differences in design, exposure assessment and the ability to adjust for smoking. Only two of the five previous studies adjusted for smoking [13,14]. Three case-control studies reporting elevated risk of BC in associations with crude indicators of exposure to outdoor air pollution such as residence in urban area of 22 to 46% [17], residence in urban area of 4 to 63% [14] or in municipalities characterized by high air pollution exposure of 36 to 188% [15]. However, residential exposure to specific air pollutants was not assessed at fine-scale individual levels in four of the five previous studies [14-17], the results were not consistent within these studies and non-significant results for other indicators such as residential proximity to PAH emissions or diesel engine exhaust from industries [14] and urban residence [15] were reported too. The previous studies relying on crude area-specific indicators [14,15,17] may have captured differences related to area of living, rather than fine-scale, individual-level differences in air pollution.

Similar to tobacco smoke, it is evident that ambient air pollution can induce DNA damage in the

general population [6]. Several studies have linked exposure to ambient air pollution with biomarkers of exposure to genotoxic carcinogens and cancer-related early effect biomarkers, such as bulky DNA adducts, chromosome aberrations, micronuclei and DNA methylation [6, 26]. Accordingly, carcinogenic genotoxic effects may be induced in the urinary bladder by exposure to ambient air pollution with particles providing biological plausibility of an association between ambient air pollution and BC.

A major strength of our study is that it relies on 8 to 18-years mean follow-up of large European population-based cohorts spread throughout a wide geographic area with very different air pollution levels, which adds to the generalizability of the results. Another important strength is the state of the art assessment of quantitative exposure of key air pollutants, instead of crude indicators of exposure. We used standardized, extensive exposure assessment, which enabled us to assess fine-scale, address-specific, spatial variation in concentrations of a more comprehensive number of air pollutants than previous studies. In contrast to any prior studies, we were able to assess $PM_{2.5}$, PM_{10} , $PM_{2.5-10}$, $PM_{2.5}$ absorbance and components of PM. Only a case-control study from Taiwan previously evaluated exposure to PM_{10} [15], but this study relied on routine air pollution monitoring stations, which do not adequately capture within city exposure variability. Assessment of PM is important because PM is considered to be the most relevant for carcinogenesis [8,10]. Indeed, our previous analysis showed that elevated HRs for lung cancer were associated with long-term exposure to PM, but not with NO_2 and NO_x [9]. Furthermore, detailed information about individual baseline characteristics such as smoking habits were available for adjustment. Three of the previous five studies [15-17] could not control for smoking.

329 This study has some limitations. Our LUR models were developed based on measurements and data
330 from 2008 to 2011, whereas they were applied to baseline home addresses typically 10-15 years
331 earlier (Table 1). To address this discrepancy, we back-extrapolated exposure to the baseline period
332 using long-term routine monitoring data which was available in all cohorts except HUBRO, which
333 contributed only 2% of the cases. In our study, the HRs of the meta-analyses were not sensitive to
334 back-extrapolation of NO₂. This approach relied on the assumption that the spatial distribution of
335 the determinants of air pollution (e.g. traffic, land use, and household density) had not changed
336 substantially. We believe that the correlations between the levels of air pollution at baseline
337 residence and concentrations in earlier periods would be high as spatial contrasts of NO₂ have been
338 shown to be stable over time [27,28], but we could not back-extrapolate all exposures and we
339 recognize the potential for exposure misclassification. Furthermore, the exposure at the baseline
340 address does not necessarily cover the entire exposure time window of relevance for BC
341 development, which seems to range from a few years [2] to many decades [13, 29] before diagnosis,
342 and we might have overlooked an association between exposure many decades ago and the risk for
343 BC. Restriction of the study population to those who lived at the baseline address throughout
344 follow-up provided similar null-results (Supplementary Table 9). The lack of information on
345 exposure prior to baseline and elsewhere, e.g. at work and during transport is a potential source of
346 exposure misclassification that may have biased our results towards null. We have previously
347 reported an association between ambient air pollution and lung cancer using the same methodology
348 [9], which supports that we do capture exposures relevant for cancer development with this method.
349 However, we cannot rule out that an inadequate follow-up period coupled with some unavoidable
350 misclassification of exposure could have masked any low level BC risk associated with ambient air
351 pollution exposure in the general population. It is not possible in this study to estimate latency since

exposure, which would have begun prior to baseline, was not estimated in our study. Nevertheless, subjects in our study were all over 40 years of age at baseline, and thus would have been exposed to air pollution for an adequate period of time to demonstrate an increased risk. Furthermore, we did find increased BC risk in association with active smoking recorded at baseline in all cohorts (adjusted HR ranging from 1.01 to 1.20 per g/day), which suggests that there was adequate follow-up time for these cohorts.

If our study participants had been older at baseline or had been followed up for a longer time, more BC cases could be included to increase the precision of the risk estimates. Nonetheless, our study included 941 BC cases and already provided results with very narrow confidence intervals. We adjusted the analyses for a number of potential confounding factors. The small change in HR after adjustment was mainly due to smoking. The potential for residual confounding by smoking seems limited since adjustment only affected HRs moderately and because similar null results were observed among never-smokers (Supplementary Table 8). Information on education was not available in the Austrian cohort, which contributed a third of the cases in our study. However, in all the other cohorts, the HRs associated with PM_{2.5} and NO₂ exposure were similar with and without adjustment for education. Therefore, it seems unlikely that this may have caused a substantial bias. Furthermore, we did not assess effect modification by age at diagnosis and stage of disease so we cannot exclude that the null effect of air pollution was impacted by age and/or restriction of the cases series to non-muscle invasive BC. Finally, we cannot exclude confounding from unaccounted for potential risk factors for BC such as disinfection by-products [30] or arsenic [31] in drinking water. Future studies should consider to evaluated polycyclic aromatic amines which was not available in our study.

375 In conclusion, this large prospective study does not provide evidence of an association between
 376 ambient air pollution at the residence and BC incidence.

377

378 **Author contribution:**

379 M Pedersen had full access to the cohort-specific results and takes responsibility for the integrity of
 380 data and accuracy of the meta-analysis.

381 *Study concept and design:* B Brunekreef, G Hoek, O Raaschou-Nielsen, P Vineis, R Vermeulen.

382 *Statistical analysis:* M Pedersen, M Stafoggia, G Weinmayr, Z J Andersen, L T Stayner, G Hoek.

383 *Cohort-specific data analysis:* J Sommar, A Pyko, B Oftedal, M Sørensen, M Plusquin, A Jaensch,
 384 R Beelen, G Hoek, M Stafoggia, S Grioni, F Ricceri, I Tamayo.

385 *Exposure assessment:* B Brunekreef, R Beelen, K de Hoogh, G Hoek, D Olsson, M Korek, K T

386 Eriksen, A Marcon, M Eeftens, M-Y Tsai, A Ranzi, G Cesaroni, P Amiano, M J Nieuwenhuijsen, R
 387 Sokhi, G Aamodt, M Wang.

388 *Obtaining funding, cohort maintenance and outcome data:* B Brunekreef, B Forsberg, G Pershagen,
 389 U De Faire, N L Pedersen, C-G Östenson, L Fratiglioni, A Tjønneland, P H Peeters, B Bueno-de-
 390 Mesquita, T J Key, G Nagel, H Concin, P Vineis, S Grioni, V Krogh, C Sacerdote, F Forastiere, C
 391 Galassi, P Amiano, M Dorronsoro.

392 *Drafting of the manuscript:* M Pedersen.

393 *Interpretation of data:* M Pedersen, O Raaschou-Nielsen.

394 *Critical revision of the manuscript for intellectual content:* All authors.

395

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 397 interests and relationships and affiliations relevant to the subject matter or materials discussed in the

manuscript (e.g., employment/affiliation, grants or funding, consultancies, honoraria, stock ownership or options, expert testimony, royalties, or patents filed, received, or pending), are the following: None.

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Appendix A. Supplementary data Supplementary data associated with this article can be found, in the online version, at xxx

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Reply to the reviewers' comments received October the 19th, 2016:

Reviewer #1: *The paper by Pederson et al. describes an analysis of the relationship between various measures of ambient air pollution (eg, PM2.5 and NO2) and bladder cancer incidence in 15 European cohorts, which were part of the ESCAPE project. A previous paper from the ESCAPE project found a significant association between air pollution and lung cancer. In contrast to lung cancer, the association between air pollution and bladder cancer has not been an important public health question and little data exist supporting such an association. Thus, the null findings of this paper are not unexpected. The manuscript is well written and the analysis is thorough with extensive supplementary material. My main concern is that this null finding may be misleading because of inadequate latency. The latent period for bladder cancer can be extremely long, with reports of a 40- to 50-year latent period for bladder cancer among truck drivers and those heavily exposed to arsenic in drinking water. Because no data are available on the residential history of the cohort participants prior to baseline enrollment, the average latent period of this analysis is about 14 years, which may be inadequate to detect an increased bladder cancer risk. The investigators acknowledge this point in the discussion, but the seriousness of this limitation is not fully comprehended. Pederson et al. take solace in the fact that an association for lung cancer was identified in this cohort. They imply that they would have also identified an association for bladder cancer and air pollution, if one existed. The latent period for lung cancer appears to be considerably shorter than that for bladder cancer, raising some doubt about the validity of the paper's null findings. An inadequate latent period coupled with some unavoidable misclassification of exposure could have masked any low level bladder cancer risk associated with air pollution exposure in the general population.*

RI.1). *An in-depth discussion of these points would be an important addition to the paper.*

We thank the reviewer for this suggestion and have added the following text (highlighted with bold) to the discussion, p.16:

‘We believe that the correlations between the levels of air pollution at baseline residence and concentrations in earlier periods would be high as spatial contrasts of NO₂ have been shown to be stable over time [26,27], but we could not back-extrapolate all exposures and we recognize the potential for exposure misclassification. Furthermore, the exposure at the baseline address does not necessarily cover the entire exposure time window of relevance for BC development, which seems to range from a few years [2] to many decades [13, 28] before diagnosis, and we might have overlooked an association between exposure many decades ago and the risk for BC. Restriction of the study population to those who lived at the baseline address throughout follow-up provided similar null-results (Supplementary Table 9). The lack of information on exposure **prior to baseline and elsewhere, e.g. at work and during transport** is a potential source of exposure misclassification that may have biased our results towards null. We have previously reported an association between ambient air pollution and lung cancer using the same methodology [9], which supports that we do capture exposures relevant for cancer development with this method. **However, we cannot rule out that an inadequate follow-up period coupled with some unavoidable misclassification of exposure could have masked any low level BC risk associated with ambient air pollution exposure in the general population. It is not possible in this study to estimate latency since exposure, which would have begun prior to baseline, was not estimated in our study. Nevertheless, subjects in our study were all over 40 years of age at baseline, and thus would have been exposed to air pollution for an adequate period of time to demonstrate**

an increased risk.

***Reviewer #3:** This is a study of environmental exposure to pollutants and impact on bladder cancer diagnosis. The study is novel and includes a large population with a significant number of incident cases. The study did not find an impact on pollution and likelihood of bladder cancer. There are several acknowledged limitations to the study.*

***R3.1).** The population studied is really suboptimal for bladder cancer. Overall the patients are too young. The average age of bladder cancer is in the late 60s and early 70s which this population is aged 43-57. It may be too young to detect a difference in incidence.*

We understand the reviewers concern. The situation concerning age might not be too bad since the ages given for each cohort in Table 1 are the mean values. For example, for one of the largest cohorts, DCH, participants were 50-64 (mean=57) years at enrolment and these participants were followed up for up to 19 years (15 years on average), such that a large proportion of cohort members would have reached the late 60s and early 70s at the end of follow-up. An older study population and/or a longer follow-up would have been an advantage due to more cases and, thereby, better statistical power. However, our study included almost a thousand BC cases with sufficient power to detect associations.

We have added the following to the discussion, p. 17:

‘If our study **participants had been older at baseline or had been** followed up for longer time, more BC cases could be included to increase the precision of the risk estimates. Nonetheless, our study included 941 BC cases **and already provided results with very narrow** confidence intervals.’

Please also see the reply above for R1.1) and the reply for the comment below.

***R3.2).** Most of the population is female and rates of smoking are low. This is contrary to risk factors for bladder cancer. The authors do not report on the impact of known risk factors such as age, gender and smoking. Did these impact bladder cancer incidence? This would add some internal validity to the data.*

Both sex and smoking did indeed impact the BC incidence as would be expected (see reviewer table 1 below). We did not evaluate age as a risk factor because age was the underlying time scale of the Cox models. As mentioned by the reviewer, the results for smoking and sex add to the confidence in the internal validity. We have now mentioned the results for smoking results in the discussion of the manuscript, p. 17:

‘...We have previously reported an association between ambient air pollution and lung cancer using the same methodology [9], which supports that we do capture exposures relevant for cancer development with this method. However, we cannot rule out that an inadequate follow-up period coupled with some unavoidable misclassification of exposure could have masked any low level BC risk associated with ambient air pollution exposure in the general population. It is not possible in this study to estimate latency since exposure, which would have begun prior to baseline, was not estimated in our study. Nevertheless, subjects in our study were all over 40 years of age at baseline, and thus would have been exposed to air pollution for an adequate period of time to demonstrate an increased risk. **Furthermore, we did find increased BC risk in association with active smoking recorded at baseline in all cohorts (adjusted HR ranging from 1.01 to 1.20 per g/day), which suggests that there was adequate follow-up time for these cohorts.**’

Reviewer table 1. Impact of sex and smoking on the BC incidence¹.

Study cohort (country)	Sex (women=ref vs. men)	Smoking intensity (g/day)
EPIC Umea (Sweden)	2.82 (1.65, 4.83)	1.09 (0.82, 1.44)
HUBRO (Norway)	5.09 (1.77, 14.61)	1.03 (0.85, 1.25)
CEANS (Sweden)	3.49 (1.72, 7.07)	1.01 (0.91, 1.13)
DCH (Denmark)	2.72 (1.88, 3.93)	1.07 (1.00, 1.14)
EPIC NL (The Netherlands)	3.96 (1.89, 8.31)	1.06 (0.96, 1.16)
EPIC Oxford (England)	2.32 (1.46, 3.69)	1.10 (0.99, 1.21)
VHM&PP (Austria) ²	4.10 (3.13, 5.38)	2.35 (1.04, 5.35)
		3.29 (1.47, 7.40)
EPIC Varese (Italy)	9.05 (1.41, 57.94)	1.20 (0.90, 1.60)
EPIC Turin (Italy)	2.15 (1.02, 4.53)	1.07 (0.94, 1.23)
SINDRA Rome (Italy)	8.08 (2.53, 25.78)	1.10 (1.00, 1.20)
EPIC San Sebastian (Spain)	6.76 (1.46, 31.17)	1.04 (0.93, 1.17)

¹Adjusted hazard ratios and 95% confidence intervals from the Cox model adjusted for age (time-scale), sex, calendar time, smoking (status, intensity and duration), occupation, employment, education and area-level SES.

Age was fitted in the Cox proportional hazards models as the time scale in each analyzing center and I do not have the results for the impact of age on the BC incidence.

R3.3). *The authors correctly point out that the measurements were from 2008-2011 so there is little time for the true effects of this exposure to have caused bladder cancer and they would need to back extrapolate up to 20-30 years to determine if a carcinogen can cause cancer. It is impossible to determine if these extrapolations are accurate.*

As the reviewer correctly point out we used air pollution measurements taken in 2008 to 2011 for development of the LUR models, which were used to assess exposure at the baseline home addresses between 10-15 years earlier, depending on the cohort. This assessment relied on the assumption that the spatial distribution of the determinants of air pollution (e.g. traffic, land use, and household density) had not changed substantially. Indeed, spatial contrasts in NO₂ have been shown to be stable over time (Cesaroni et al. 2012; Eeftens et al. 2011; Gulliver et al. 2013).

Cesaroni G, Porta D, Badaloni C, Stafoggia M, Eeftens M, Meliefste K, et al. 2012. Nitrogen dioxide levels estimated from land use regression models several years apart and association with mortality in a large cohort study. *Environ Health* 11:48.

Eeftens M, Beelen R, Fischer P, Brunekreef B, Meliefste K, Hoek G. 2011. Stability of measured and modelled spatial contrasts in NO₂ over time. *Occup Environ Med* 68:765–770.

Gulliver J, de Hoogh K, Hansell A, Vienneau D. 2013. Development and back-extrapolation of NO₂ land use regression models for historic exposure assessment in Great Britain. *Environ Sci Technol* 47:7804–7811.

R3.4). *For bladder cancer, CIS should be included and this was not done.*

Carcinoma in situ of (CIS) the bladder is not registered consistently across the European cancer registries. For consistency reasons, we only included invasive BC, which is registered in all cancer registries. Further, there is evidence that among non-invasive bladder tumours, some do not develop into manifest BC, indicating that the etiology of such CIS differ from that of invasive BC.

Reviewer #4: *Comments to the Authors: Pedersen et al. evaluate an interesting topic, which is the association between long-term exposure to ambient air pollution and bladder cancer incidence. The authors should consider the following points: Major comments:*

R4.1). *Title: In my opinion authors should change the current title, highlighting the lack of*

association between air pollution and bladder cancer risk.

We have changed the title as suggested by the reviewer to: **‘Is there an Association Between Ambient Air Pollution and Bladder Cancer Incidence - Analysis of 15 European Cohorts?’**

We prefer to use this title or the original title as we cannot really justify a very firm statement of no association in the title given the limitations pointed out by reviewer #1, which is now acknowledged in the discussion.

***R4.2).** Discussion is scarce: authors should expand discussion section by extending comment and comparison with previous reports in this field.*

We have expanded the discussion and hope that the editors permit that we then exceed the word limit by adding the text highlighted with bold, p. 14:

‘Our study is the largest study with detailed individual level confounder variables to date on the relationship between **ambient** air pollution and BC **in the general population**. The lack of an association between air pollution **with NO_x, traffic density, PAH** and BC risk observed in our study is consistent with some of the previous studies **from Denmark, Spain and the Netherlands** [13,14,16] and inconsistent with others **from Spain and Taiwan** [14,15]. Inconsistency among studies may partly be explained by the differences in design, exposure assessment and the ability to adjust for smoking. Only two of the five previous studies adjusted for smoking [13,14]. Three case-control studies reported elevated risk of BC in associations with crude indicators of exposure to outdoor air pollution such as residence in urban area **of 22 to 46%** [17], **residence in urban area of 4 to 63%** [14] or in municipalities characterized by high air pollution exposure **of 36 to 188%** [15]. **However, residential exposure to specific air pollutants was not assessed at fine-scale individual levels in four of the five previous studies [14-17], the results were not consistent within these studies and non-significant results for other indicators such as residential proximity to PAH emissions or diesel engine exhaust from industries [14] and urban residence [15] were reported too. The previous studies relying on crude area-specific indicators [14,15,17] may have captured differences related to area of living, rather than fine-scale, individual-level differences in air pollution.**’

***R4.3).** Secondly, they should briefly explain biological bases of the association they investigated.*

We have added the following text and reference to the discussion, p. 14: **‘Similar to tobacco smoke, it is evident that ambient air pollution can induce DNA damage in the general population [6]. Several studies have linked exposure to ambient air pollution with biomarkers of exposure to genotoxic carcinogens and cancer-related early effect biomarkers, such as bulky DNA adducts, chromosome aberrations, micronuclei and DNA methylation [6, 26]. Accordingly, carcinogenic genotoxic effects may be induced in the urinary bladder by exposure to ambient air pollution with particles providing biological plausibility of an association between ambient air pollution and BC.’**

Demetriou CA, Vineis P. Carcinogenicity of ambient air pollution: use of biomarkers, lessons learnt and future directions. J Thorac Dis. 2015;7:67-95.

We already mentioned in the introduction that: ‘Ambient air pollution includes a mix of

carcinogens such as polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds, transition metals, and diesel engine exhaust [6,7]. Ambient air pollution and particulate matter (PM) in ambient air have recently been classified as carcinogenic to humans [6].’

R4.4). Tables and figures:

Authors in my opinion present too many supplemental tables and figures: Supplementary Table 1 should be used and discussed in discussion only but not reported as supplementary table.

As suggested we have now removed the supplementary table 1, which summarized the findings of the results of the previous five studies and added more text to the discussion, see also reply for comment R4.2.

R4.5). All the supplementary table 2 should be summarized in one single supplementary table reporting characteristics of the cohorts included in the study.

This has been changed as suggested.

Table 1. Study population characteristics (N=303,431, n=943).

Study cohort (country)	Enrolment	N ³	Persons years at risk	n ⁴	Age (years) ⁵	Follow-up (years) ⁶	Men (%)	Smoking (%) ⁷
EPIC Umea (Sweden)	1992-1996	21,901	294,493	69	45.9 ± 10.9	13.4 (0.0-16.9)	48	19
HUBRO (Norway)	2000-2001	17,958	152,973	21	47.9 ± 15.2	8.5 (0.0-9.7)	44	26
CEANS (Sweden) ¹	1992-2004	17,534	182,429	60	55.9 ± 11.7	10.4 (0.0-17.8)	37	22
DCH (Denmark)	1993-1997	37,676	556,904	179	56.8 ± 4.3	14.8 (0.0-19.1)	47	37
EPIC NL (The Netherlands) ²	1993-1997	30,134	355,933	88	50.4 ± 11.3	11.8 (0.0-15.0)	24	29
EPIC Oxford (England)	1993-1998	38,567	423,542	81	45.5 ± 13.7	11.0 (0.0-14.8)	24	11
VHM&PP (Austria)	1985-2005	104,714	1,899,063	306	42.9 ± 14.9	18.1 (0.0-27.0)	44	13
EPIC Varese (Italy)	1993-1997	10,310	111,212	20	51.6 ± 8.2	10.8 (0.0-13.3)	21	21
EPIC Turin (Italy)	1993-1997	7,946	104,461	54	50.4 ± 7.5	13.1 (0.0-16.6)	55	24
SIDRIA Rome (Italy)	1999	9,105	102,130	38	44.3 ± 6.0	11.2 (0.0-12.0)	47	42
EPIC San Sebastian (Spain)	1992-1995	7,586	92,796	27	49.4 ± 7.7	12.2 (0.0-14.7)	46	27

¹ Pooled data from the SNAC-K, SALT, Sixty and SDPP cohorts.

² Pooled data from the EPIC MORGEN and EPIC PROSPECT cohorts.

³ Total number of included participants.

⁴ Number of bladder cancer incidence cases

⁵ Mean ± SD.

⁶ Mean (min-max).

⁷ Current smoking.

Table 2. Exposure distribution by cohort (annual mean \pm SD for each study cohort) at the baseline addresses.

Study cohort	NO ₂ ($\mu\text{g}/\text{m}^3$)	NO _x ($\mu\text{g}/\text{m}^3$)	PM _{2.5} ($\mu\text{g}/\text{m}^3$)	PM _{2.5} absorbance ($10^{-5}/\text{m}^3$)	PM ₁₀ ($\mu\text{g}/\text{m}^3$)	PM _{2.5-10} ($\mu\text{g}/\text{m}^3$)	Traffic density (vehicles/day)	Organic Carbon ($\mu\text{g}/\text{m}^3$)
EPIC Umea	5.2 \pm 2.5	8.7 \pm 5.8	na	na	na	na	846 \pm 1,532	na
HUBRO	20.9 \pm 8.0	38.3 \pm 15.4	8.9 \pm 1.3	1.2 \pm 0.3	13.5 \pm 3.1	4.0 \pm 2.0	2,501 \pm 5,100	na
CEANS	10.8 \pm 4.6	19.0 \pm 10.2	7.1 \pm 1.3	0.6 \pm 0.2	14.6 \pm 4.1	7.1 \pm 3.2	1,556 \pm 4,572	na
DCH	16.4 \pm 7.0	26.8 \pm 18.4	11.3 \pm 0.9	1.2 \pm 0.2	17.2 \pm 2.0	5.7 \pm 1.0	3,022 \pm 7,249	1.6 \pm 0.2
EPIC NL	25.2 \pm 6.2	37.9 \pm 11.3	16.8 \pm 0.6	1.4 \pm 0.2	25.4 \pm 1.5	8.5 \pm 0.9	1,291 \pm 3,804	1.5 \pm 0.4
EPIC Oxford	24.5 \pm 8.0	40.9 \pm 15.6	9.8 \pm 1.1	1.1 \pm 0.3	16.1 \pm 2.0	6.4 \pm 0.9	1,383 \pm 4,353	na
VHM&PP	19.9 \pm 5.5	39.9 \pm 9.5	13.6 \pm 1.2	1.7 \pm 0.2	20.6 \pm 2.4	6.7 \pm 0.9	1,684 \pm 3,584	na
EPIC Varese	43.5 \pm 17.3	86.1 \pm 41.8	na	na	na	na	na	na
EPIC Turin	53.2 \pm 10.8	96.4 \pm 21.0	30.1 \pm 1.7	3.1 \pm 0.4	46.4 \pm 4.2	16.5 \pm 2.7	3,981 \pm 9,272	na
SIDRIA Rome	39.1 \pm 9.1	82.0 \pm 23.9	19.4 \pm 1.8	2.7 \pm 0.5	36.5 \pm 5.0	16.7 \pm 3.4	2,955 \pm 6,728	3.5 \pm 0.3
EPIC San Sebastian	23.8 \pm 6.6	47.2 \pm 12.5	na	na	na	na	na	na

Na refers to not available; NO₂ refers to nitrogen dioxide; NO_x refers to nitrogen oxides; PM_{2.5} refers to particulate matter with aerodynamic diameter $<2.5 \mu\text{m}$; PM_{2.5-10} refers to coarse particulate matter with aerodynamic diameter $2.5\text{--}10 \mu\text{m}$; PM₁₀ refers to particulate matter with aerodynamic diameter $<10 \mu\text{m}$.

Table 3. Hazard ratios of the associations between air pollution, traffic density and bladder cancer.

Exposure	Increase	Cohorts (N)	Participants (N)	Cases (N)	Model 1 ¹ HR (95% CI)	Model 2 ² HR (95% CI)	Model 3 ³ HR (95% CI)	I ² (%) ⁴	P ⁴
NO ₂	10 µg/m ³	15	303,431	943	1.01 (0.92, 1.11)	0.99 (0.90, 1.09)	0.98 (0.89, 1.08)	0.0	0.71
NO _x	20 µg/m ³	15	303,431	943	1.03 (0.93, 1.13)	1.00 (0.91, 1.11)	0.99 (0.91, 1.09)	0.0	0.45
PM _{2.5} ⁵	5 µg/m ³	12	263,634	827	0.96 (0.71, 1.31)	0.94 (0.69, 1.27)	0.86 (0.63, 1.18)	0.0	0.44
PM _{2.5} absorbance ⁵	10 ⁻⁵ /m ³	12	263,634	827	0.97 (0.74, 1.28)	0.92 (0.70, 1.22)	0.87 (0.66, 1.16)	0.0	0.50
PM ₁₀ ⁵	10 µg/m ³	12	263,634	827	0.95 (0.62, 1.45)	0.93 (0.60, 1.43)	0.92 (0.58, 1.48)	59.3	0.02
PM _{2.5-10} ⁵	5 µg/m ³	12	263,634	827	1.15 (0.71, 1.84)	1.10 (0.71, 1.69)	1.08 (0.70, 1.68)	63.7	0.01
Traffic density ⁶	5,000 v/d	13	285,535	896	0.98 (0.90, 1.07)	0.97 (0.90, 1.06)	0.98 (0.90, 1.06)	7.2	0.38
Organic carbon ⁷	1 µg/m ³	3	76,915	305	1.00 (0.54, 1.83)	0.91 (0.55, 1.53)	0.86 (0.49, 1.51)	28.2	0.25

Summary hazard ratio (HR) and 95% confidence intervals (CI) from random-effect meta-analysis.

¹Adjusted for age (time scale), sex and calendar time in Cox model.

²Additional adjusted for smoking (status, intensity and duration), occupation, employment and education.

³Additional adjusted for area-level SES.

⁴I² and p refers to assessment of heterogeneity.

⁵PM are not available for EPIC Umea, EPIC Varese and EPIC San Sebastian.

⁶Traffic density is not available for EPIC Varese and EPIC San Sebastian.

⁷Organic carbon is only available in DCH, EPIC NL and SIDRIA Rome.

No information on occupation or employment is available from EPIC NL, EPIC Turin and EPIC San Sebastian.

Figure 1. Study areas.

Circle indicates that NO_2 , NO_x and PM are available. Triangle indicates that only NO_2 , NO_x and traffic density are available. Square indicates that only NO_2 and NO_x are available. The size of the symbol indicates the size of the study cohort (N=303,431).

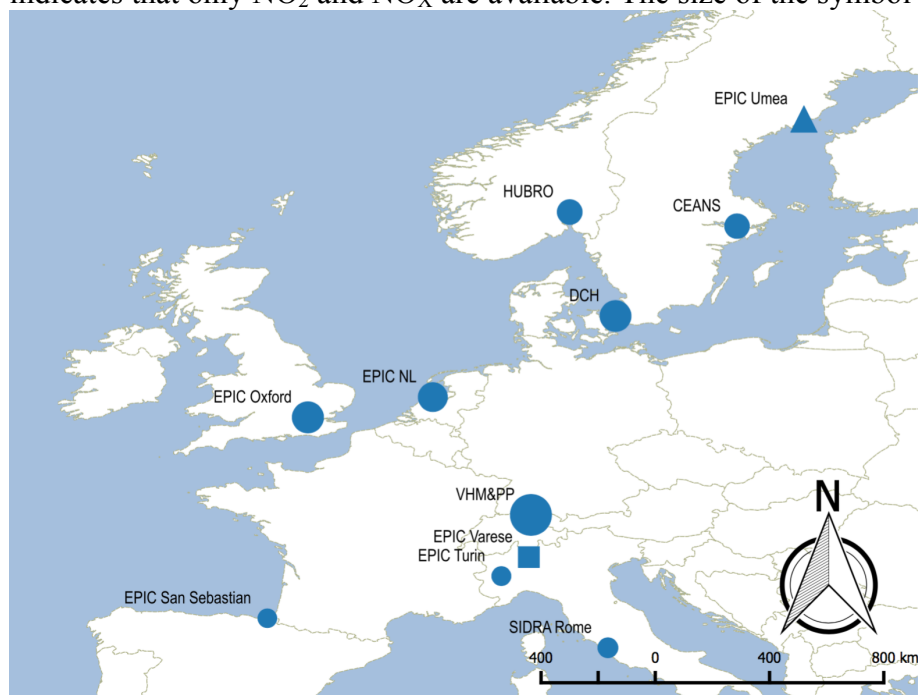
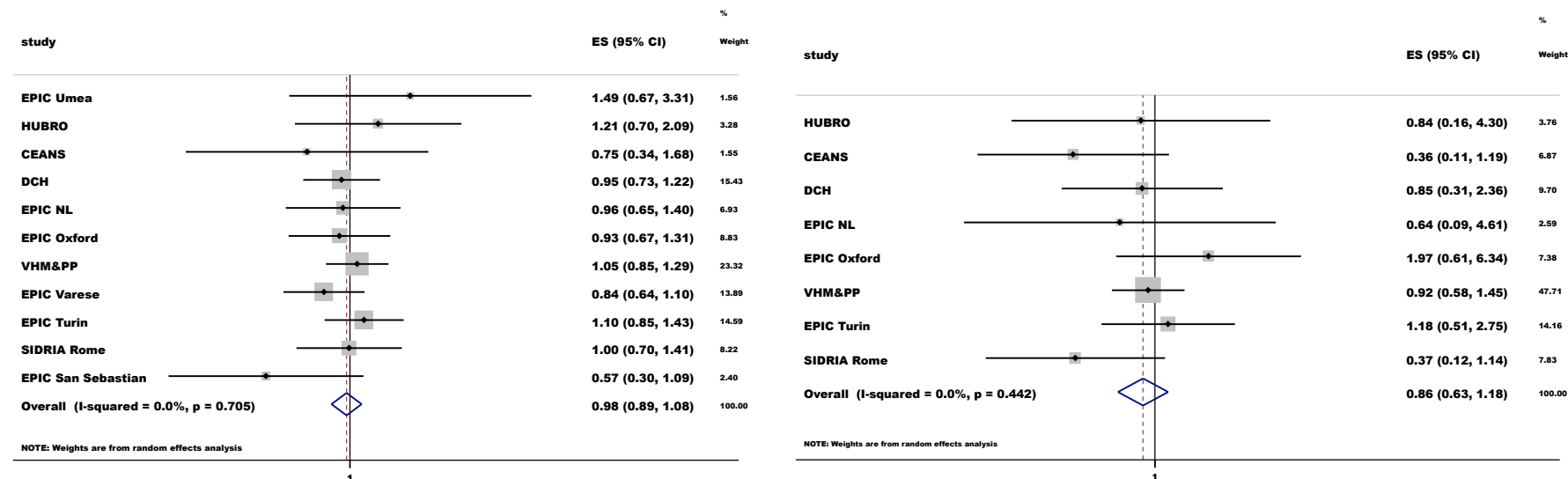


Figure 2. Risk for bladder cancer associated with ambient air pollution levels in each cohort study and overall.



Hazard ratios (HRs) and 95% confidence intervals (CI) for bladder cancer per 10 $\mu\text{g}/\text{m}^3$ increment in NO_2 (left) and per 5 $\mu\text{g}/\text{m}^3$ increment in $\text{PM}_{2.5}$ (right) from models adjusted for age, sex, calendar time, smoking (status, intensity and duration), occupation, employment, education and area-level SES. Data points show HR; lines show 95% CI; grey boxes show the weight with which each cohort contributed to the summary HR; vertical dashed line shows summary HR.

Supplementary Materials

Tables

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6. Test of linearity of the association between elemental component of PM and bladder cancer.
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Figure

1. Bladder cancer in associations with air pollution and traffic density by cohort.

Table 1. Study population characteristics (N=303,431, n=943).

Study cohort	Excluded (%) ¹	Former (%)	Smoking Intensity (cig/day)	Duration (yrs)	Employed (%)	High BC risk occupation (%) ²	Low (%)	Education Middle (%)	High (%)	Changed address (%) ³
EPIC Umea ⁴	15	19	2.4 ± 5.6	8.8 ± 13.0	86	na	28	51	21	na
HUBRO ⁵	16	27	6.7 ± 8.4	11.4 ± 14.3	74	na	18	36	46	52
CEANS										
SNACK-K ⁶	29	37	3.7 ± 5.9	14.9 ± 19.1	20	na	26	39	76	0
SALT ⁷	22	33	7.5 ± 9.8	14.6 ± 16.9	67	28 ⁸	26	37	37	14
Sixty	9	39	8.0 ± 9.3	15.3 ± 16.2	69	30 ⁸	39	33	28	52
SDPP	12	36	8.5 ± 8.9	12.3 ± 12.4	91	36	31	39	30	57
DCH ¹¹	6	27	10.4 ± 11.0	19.3 ± 17.2	79	16	30	47	23	40
EPIC NL										
EPIC MORGEN ¹²	26	29	10.3 ± 11.0	14.2 ± 13.6	na	na	12	66	23	na
EPIC PROSPECT ¹³	10	37	5.6 ± 7.3	15.0 ± 16.5	na	na	22	62	16	na
EPIC Oxford ¹⁴	28	29	4.6 ± 8.2	6.4 ± 11.0	73	na	36	24	40	48
VHM&PP ¹⁵	21	7	Categorical	Categorical	68	na	na	na	na	11
EPIC Varese ¹⁶	10	21	4.5 ± 6.8	10.0 ± 13.5	50	na	60	33	7	13
EPIC Turin ¹⁷	9	32	7.3 ± 8.3	13.6 ± 13.9	na	na	46	45	14	na
SIDRIA Rome ¹⁸	14	23	10.1 ± 10.5	11.7 ± 10.4	71	na	45	40	15	18
EPIC San Sebastian ¹⁹	9	19	6.9 ± 10.0	11.5 ± 14.2	na	na	71	29	0	na

Na: not available.

¹Excluded due to missing on exposure, covariates and or cancer before baseline.

²Chemical industry (dyeworks), rubber industry, textile industry (dyeworks), metal processing (painting, coating), glass industry, truck, bus or taxi driver, painter, hairdresser, waiter or cook.

³Changed address from baseline to follow-up.

⁴The European Prospective Investigation into Cancer and Nutrition (EPIC) Umea cohort study design is described here: <http://epic.iarc.fr/centers/sweden.php> (Assessed July 4, 2016). Definition of area-SES: Unemployment rate in neighborhood (continuous scale), 1992, Statistics Sweden.

⁵The population-based Oslo Health Study (HUBRO) cohort design is described here: Sogaard AJ, Selmer R, Bjertness E, Thelle D. The Oslo Health Study: The Impact of self-selection in a large, population-based survey. Int J Equity Health 2004; 3:3. Date of end of follow-up: 31/12/2009. Definition of area-SES: Unemployment rate in municipality (continuous scale), 2002, Statistics Norway.

⁶The Swedish National Study of Aging and Care in Kungsholmen (SNAC-K) cohort study design is described here: Lagergren M, Fratiglioni L, Hallberg IR et al. A longitudinal study integrating population, care and social services data. The Swedish National study on Aging and Care (SNAC). Aging Clin Exp Res 2004;16:158-68. Date of end of follow-up: 31/12/2009 Definition of area-SES: Mean income of neighborhood (categories), 2009, Statistics Sweden

⁷The Stockholm Screening Across the Lifespan Twin Study & TwinGene (SALT) cohort study design is described here: Lichtenstein P, De Faire U, Floderus B, et al. The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. J Internal Med 2002; 252: 184- 205. Date of end of follow-up: 31/12/2009. Definition of area-SES: Mean income of municipality (categories), 2009, Statistics Sweden.

⁸Blue collar worker.

⁹The Stockholm 60 Years Old & IMPROVE (Sixty) cohort study design is described here: Wandell PE, Wajngot A, de Faire U, Hellenius ML. Increased prevalence of diabetes among immigrants from non-European countries in 60-year-old men and women in Sweden. Diabetes Metab 2007; 33: 30-6. Date of end of follow-up: 31/12/2009. Definition of area-SES: Mean income of municipality (categories), 2009, Statistics Sweden.

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- ¹¹ The Diet, Cancer and Health (DCH) cohort study design is described here: Tjønneland A, Olsen A, Boll K et al. Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark. *Scand J Public Health* 2007; 35: 432-41. Date of end of follow-up: 31/12/2012. Definition of area-SES: Mean income of municipality (cont.), 1995, Statistics Denmark.
- ¹² The EPIC Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN) cohort study design is described here: Beulens JWJ, Monninkhof EM, Verschuren WMM et al. Cohort Profile: The EPIC-NL study. *International Journal of Epidemiology* 2010; 39: 1170–78. Definition of area-SES: Percentage of people with low income in the neighborhood (cont.), 2001, Statistics Netherlands.
- ¹³ The EPIC PROSPECT cohort study design is described here: Boker LK, van Noord PA, van der Schouw YT, Koot NV, Bueno de Mesquita HB, Riboli E, Grobbee DE, Peeters PH. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. *European Prospective Investigation into Cancer and Nutrition. Eur J Epidemiol.* 2001;17:1047-53. Definition of area-SES: Percentage of people with low income in the neighborhood (cont.), 2001, Statistics Netherlands.
- ¹⁴ The EPIC Oxford cohort study design is described here: Davey GK, Spencer EA, Appleby PN, et al. EPIC-Oxford: lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr* 2003; 6: 259-69. Date of end of follow-up: 31/12/2007. Definition of area-SES: Carstairs index 2001 (cont.) For details see: Morgan O. Measuring deprivation in England and Wales using 2001 Carstairs scores. *Health Statistics Quarterly* 31, autumn 2006. National Statistics, UK.
- ¹⁵ The Voralberg Health Monitoring and Prevention Program (VHM&PP) cohort study design is described here: Ulmer H, Kelleher CC, Fitz-Simon N, Diem G, Concin H. Secular trends in cardiovascular risk factors: an age-period cohort analysis of 6 98 954 health examinations in 1 81 350 Austrian men and women. *J Intern Med* 2007; 261: 566-76. Date of end of follow-up: 31/12/2011. Definition of area-SES: Average income of municipality (cont.), 2008, Bundesanstalt Statistik Österreich
- ¹⁶ The EPIC Varese cohort study design is described here: Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002; 5(6B): 1113-24. Date of end of follow-up: 19/12/1997. Definition of area-SES: Unemployment rate of municipality (cont.), 2001, Italian National Institute of Statistics.
- ¹⁷ The EPIC Turin cohort study design is described here: Guarrera S, Ricceri F, Polidoro S, Sacerdote C, Allione A, Rosa F, Voglino F, Critelli R, Russo A, Vineis P, Matullo G. Association between total number of deaths, diabetes mellitus, incident cancers, and haplotypes in chromosomal region 8q24 in a prospective study. *Am J Epidemiol.* 2012;175:479-87. Date of end of follow-up: 31/12/2008. Definition of area-SES: Deprivation index of census block (5 cat.), 2001. See details: Caranci N, Biggeri A, Grisotto L, et al. The Italian deprivation index at census block level: definition, description and association with general mortality. *Epidemiol Prev* 2010; 34: 167-7.
- ¹⁸ The Italian Studies of Respiratory Disorders in Childhood and Environment - Rome (SIDRIA) Rome cohort study design is described here: Cesaroni G, Badaloni C, Porta D, Forastiere F, Perucci CA. Comparison between various indices of exposure to traffic-related air pollution and their impact on respiratory health in adults. *Occup Environ Med* 2008; 65: 683-90. Date of end of follow-up: 31/12/2010. Definition of area-SES: Index of socio-economic position in census block (categorical), 2001. See details Cesaroni G, Badaloni C, Romano V, Donato E, Perucci CA, Forastiere F. Socioeconomic position and health status of people who live near busy roads: the Rome Longitudinal Study (RoLS). *Environ Health.* 2010; 9:41.
- ¹⁹ The EPIC San Sebastian cohort study design is described here: Amiano P, Dorronsoro M, de Renobales M, et al. Very-long-chain omega-3 fatty acids as markers for habitual fish intake in a population consuming mainly lean fish: the EPIC cohort of Gipuzkoa. *European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr* 2001; 55: 827-32. Date of end of follow-up: 31/12/2008. Definition of area-SES: Mean income per person of municipality (cont.), 2001, Eustat.

Table 2. Distribution of bladder cancer cases and exposures in the individual cohorts which were analyzed as pooled cohorts.

Study Cohort	n	NO ₂ (µg/m ³)	NO _x (µg/m ³)	PM _{2.5} (µg/m ³)	PM _{2.5} absorbance (10 ⁻⁵ /m)	PM ₁₀ (µg/m ³)	PM _{2.5-10} (µg/m ³)	Traffic density (vehicles/day)	Organic carbon (µg/m ³)
CEANS									
SNAC-K	9	17.5 ± 4.9	33.4 ± 12.6	8.0 ± 1.3	0.8 ± 0.2	16.4 ± 6.0	8.6 ± 4.8	3,883 ± 9,900	na
SALT	19	11.0 ± 4.3	19.0 ± 9.5	7.3 ± 1.3	0.6 ± 0.2	15.0 ± 3.9	7.3 ± 3.0	1,491 ± 3,458	na
Sixty	17	10.6 ± 4.2	18.4 ± 9.3	7.2 ± 1.3	0.6 ± 0.2	14.9 ± 3.8	7.3 ± 2.9	1,403 ± 3,388	na
SDPP	15	8.4 ± 1.7	14.4 ± 3.3	6.6 ± 1.2	0.5 ± 0.1	13.6 ± 3.2	6.3 ± 2.4	865 ± 1,634	na
EPIC NL									
EPIC MORGEN	47	23.8 ± 7.0	36.5 ± 11.8	16.9 ± 0.6	1.4 ± 0.2	25.4 ± 1.7	8.6 ± 1.1	1,535 ± 4,086	1.5 ± 0.4
EPIC PROSPECT	41	26.7 ± 4.6	39.6 ± 10.5	16.8 ± 0.5	1.4 ± 0.2	25.3 ± 1.2	8.5 ± 0.7	1,023 ± 3,448	1.5 ± 0.4

Na: not available; NO₂: nitrogen dioxide; NO_x: nitrogen oxides; PM_{2.5}: particulate matter with aerodynamic diameter <2.5 µm; PM_{2.5-10}: PM 2.5–10 µm; PM₁₀: PM<10 µm.

Table 3. Distribution of exposure to elemental component of PM (ng/m³).

Exposure	Cu	Fe	K	Ni	S	Si	V	Zn
PM _{2.5}								
HUBRO	3.2 ± 1.8	48.0 ± 33.1	90.2 ± 18.5	0.6 ± 0.3	376.6 ± 28.6	na	na	14.5 ± 2.7
CEANS	1.8 ± 1.7	90.2 ± 64.3	102.6 ± 18.4	na	507.9 ± 14.6	172.6 ± 66.3	1.3 ± 0.2	10.5 ± 0.9
DCH	2.9 ± 1.4	97.1 ± 52.8	112.1 ± 5.0	1.1 ± 0.1	716.7 ± 26.1	50.6 ± 7.2	2.4 ± 0.3	12.7 ± 0.8
EPIC NL	3.7 ± 1.1	95.5 ± 28.4	125.6 ± 11.0	1.7 ± 0.7	926.6 ± 54.6	86.9 ± 12.9	2.8 ± 1.2	39.9 ± 16.8
VHM&PP	4.0 ± 1.1	66.3 ± 7.9	331.2 ± 31.3	0.1 ± 0.1	618.6 ± 31.5	64.2 ± 7.1	na	22.3 ± 3.3
EPIC Turin	10.1 ± 2.5	300.4 ± 61.3	378.5 ± 21.7	2.7 ± 0.5	1,081.4 ± 41.7	194.8 ± 32.8	2.2 ± 0.3	37.3 ± 4.4
SIDRA Rome	12.7 ± 4.0	253.0 ± 83.8	294.9 ± 41.8	1.5 ± 0.1	972.3 ± 38.5	233.5 ± 38.4	2.9 ± 0.2	22.5 ± 5.3
PM ₁₀								
HUBRO	10.8 ± 7.2	359.2 ± 194.9	na	na	428.6 ± 32.0	532.6 ± 243.1	2.5 ± 0.8	18.0 ± 5.7
CEANS	9.3 ± 9.3	461.9 ± 264.5	297.1 ± 101.0	na	547.6 ± 23.8	1,020.2 ± 490.8	2.1 ± 0.5	15.7 ± 7.6
DCH	12.0 ± 9.5	302.7 ± 189.1	189.0 ± 10.7	1.0 ± 0.2	793.6 ± 32.5	301.2 ± 82.1	2.8 ± 0.5	19.7 ± 4.0
EPIC NL	14.0 ± 5.5	445.8 ± 175.4	210.7 ± 18.9	2.1 ± 1.0	1,055.8 ± 44.6	416.0 ± 131.1	3.4 ± 1.4	57.7 ± 20.9
VHM&PP	9.0 ± 2.6	319.5 ± 44.1	398.0 ± 35.0	0.2 ± 0.2	658.5 ± 46.5	379.5 ± 59.4	0.6 ± 0.1	29.1 ± 5.6
EPIC Turin	66.7 ± 18.5	1,679.8 ± 425.7	604.1 ± 54.9	6.7 ± 1.3	1,186.8 ± 82.4	1,452.8 ± 260.8	3.8 ± 0.6	68.1 ± 11.0
SIDRA Rome	45.9 ± 25.1	1,083.8 ± 518.7	519.6 ± 143.5	2.8 ± 1.1	1,103.3 ± 48.2	1,040.5 ± 265.8	4.4 ± 0.8	40.6 ± 12.3

Na: not available; PM is not available for EPIC Umea, EPIC Oxford, EPIC Varese and EPIC San Sebastian.

Table 4. Hazard ratios of the associations between elemental component of PM and bladder cancer.

Exposure	Increase	Cohorts (N)	Participants (N)	Cases (n)	Model ¹ HR (95% CI)	Model ² HR (95% CI)	Model ³ HR (95% CI)	I ² (%) ⁴	P ⁴
PM _{2.5} Cu	5 ng/m ³	11 ⁵	225,067	746	0.98 (0.74, 1.29)	0.95 (0.69, 1.31)	0.94 (0.61, 1.44)	48.0	0.07
PM _{2.5} Fe	100 ng/m ³	11	225,067	746	1.09 (0.91, 1.31)	1.05 (0.87, 1.26)	1.04 (0.84, 1.30)	9.7	0.35
PM _{2.5} K	50 ng/m ³	11	225,067	746	0.93 (0.80, 1.08)	0.93 (0.80, 1.08)	0.93 (0.80, 1.08)	0.0	0.59
PM _{2.5} Ni	1 ng/m ³	11	225,067	746	1.04 (0.69, 1.57)	1.06 (0.67, 1.66)	1.12 (0.68, 1.85)	37.9	0.15
PM _{2.5} S	200 ng/m ³	11	225,067	746	1.34 (0.52, 3.41)	1.24 (0.51, 2.98)	1.21 (0.47, 3.11)	57.4	0.29
PM _{2.5} Si	100 ng/m ³	10 ⁶	207,109	725	1.08 (0.49, 2.40)	1.04 (0.47, 2.30)	1.03 (0.45, 2.33)	66.2	0.01
PM _{2.5} V	2 ng/m ³	9 ⁷	102,395	419	1.01 (0.29, 3.47)	1.04 (0.31, 3.46)	1.01 (0.31, 3.32)	67.9	0.01
PM _{2.5} Zn	10 ng/m ³	11	225,067	746	1.08 (0.93, 1.25)	1.07 (0.92, 1.24)	1.06 (0.92, 1.24)	0.0	0.82
PM ₁₀ Cu	20 ng/m ³	11	225,067	746	1.01 (0.86, 1.18)	0.99 (0.84, 1.16)	1.00 (0.85, 1.18)	0.0	0.48
PM ₁₀ Fe	500 ng/m ³	11	225,067	746	1.10 (0.84, 1.43)	1.06 (0.80, 1.42)	1.06 (0.76, 1.49)	54.9	0.04
PM ₁₀ K	100 ng/m ³	10	207,109	725	0.92 (0.79, 1.07)	0.91 (0.79, 1.04)	0.90 (0.77, 1.05)	4.2	0.39
PM ₁₀ Ni	2 ng/m ³	10	207,109	725	1.27 (0.52, 3.08)	1.20 (0.53, 2.75)	1.22 (0.54, 2.73)	69.7	0.10
PM ₁₀ S	200 ng/m ³	11	225,067	746	0.95 (0.65, 1.40)	0.89 (0.63, 1.26)	0.87 (0.61, 1.23)	0.0	0.43
PM ₁₀ Si	500 ng/m ³	11	225,067	746	1.15 (0.80, 1.64)	1.13 (0.79, 1.63)	1.14 (0.77, 1.68)	58.6	0.02
PM ₁₀ V	3 ng/m ³	11	225,067	746	1.39 (0.64, 3.02)	1.38 (0.63, 3.02)	1.39 (0.63, 3.09)	59.2	0.02
PM ₁₀ Zn	20 ng/m ³	11	225,067	746	1.07 (0.89, 1.28)	1.05 (0.87, 1.25)	1.02 (0.79, 1.31)	20.3	0.27

Summary hazard ratio (HR) and 95% confidence intervals (CI) from random-effect meta-analysis.

¹Adjusted for age (time scale), sex and calendar time in Cox model.

²Additional adjusted for smoking (status, intensity and duration), occupation, employment and education.

³Additional adjusted for area-level SES.

⁴I² and p refers to test for heterogeneity.

⁵Elemental components in PM is not available for EPIC Umea, EPIC Oxford, EPIC Varese and EPIC San Sebastian.

⁶PM_{2.5} Si, PM₁₀ K and PM₁₀ Ni is not available for HUBRO.

⁷PM_{2.5} V is not available for HUBRO and VHM&PP.

Table 5. P-values from the test of linearity of the association between air pollutants and bladder cancer.

Study cohort	NO ₂	NO _x	PM _{2.5}	PM _{2.5} absorbance	PM ₁₀	PM _{2.5-10}
EPIC Umea	0.57	0.62	na	na	na	na
HUBRO	0.65	0.66	0.15	0.14	0.43	0.99
CEANS	0.44	0.23	0.97	0.71	0.23	0.25
DCH	0.94	0.99	0.38	0.96	0.40	0.58
EPIC NL	0.95	0.76	0.07	0.19	0.78	0.76
EPIC Oxford	0.64	0.41	0.95	0.70	0.27	0.18
VHM&PP	0.74	0.24	0.08	0.53	0.36	0.49
EPIC Varese	0.18	0.19	na	na	na	na
EPIC Turin	0.98	0.68	0.64	0.99	0.01	0.01
SIDRA Rome	0.06	0.13	0.48	0.21	0.72	0.15
EPIC San Sebastian	0.76	0.76	na	na	na	na

Na: not available; NO₂: nitrogen dioxide; NO_x: nitrogen oxides; PM_{2.5}: particulate matter with aerodynamic diameter <2.5 µm; PM_{2.5-10}: PM 2.5–10 µm; PM₁₀: PM<10 µm.

Table 6. P-values from the test of linearity of the association between elemental components of PM and bladder cancer.

PM fraction Study cohort		Cu	Fe	K	Ni	S	Si	V	Zn
PM _{2.5}	HUBRO	0.99	0.37	0.16	0.26	0.26	na	na	0.07
	CEANS	0.15	0.15	0.25	na	na	na	0.32	na
	DCH	0.31	0.64	0.98	0.07	0.53	0.69	na	0.16
	EPIC NL	0.15	0.54	0.16	0.86	0.93	0.58	0.86	0.26
	VHM&PP	0.05	0.82	0.21	0.72	0.65	0.99	na	0.60
	EPIC Turin	0.05	0.13	0.73	0.45	0.33	0.08	0.16	0.51
	SIDRA Rome	0.15	0.02	0.01	0.53	0.02	0.06	0.68	0.20
PM ₁₀	HUBRO	0.37	0.98	na	na	0.16	0.40	0.05	0.03
	CEANS	0.09	0.79	0.22	na	na	0.25	0.95	0.82
	DCH	0.70	0.64	na	0.35	0.26	0.20	na	0.54
	EPIC NL	0.29	0.96	0.29	0.76	0.88	0.94	0.86	0.32
	VHM&PP	0.86	0.74	0.23	0.81	0.17	0.76	0.59	0.57
	EPIC Turin	0.04	0.00	0.22	0.06	0.05	0.28	0.83	0.77
	SIDRA Rome	0.25	0.12	0.10	0.18	0.56	0.64	0.51	0.04

Na: not available; elements in PM are not available for EPIC Umea, EPIC Oxford, EPIC Varese and EPIC San Sebastian.

Table 7. P-value for deviation from Cox proportional hazard assumption.

Study cohort	P
Epic Umea	0.54
HUBRO	0.71
CEANS	0.88
DCH	0.94
EPIC NL	0.92
EPIC Oxford	0.83
VHM&PP	0.92
EPIC Varese	0.56
EPIC Turin	0.29
SIDRIA Rome	0.29
EPIC San Sebastian	0.13

The p-values are a global test of model 3 in each study cohort using the “estat phtest” function in Stata, which implements the test of the non-zero slope in a generalized linear regression of the scaled Schoenfeld residuals on functions of time, by the Therneau and Grambsch method, where a non-zero slope is an indication of violation of the proportional hazard assumption.

Table 8. Modification of the associations between NO₂, PM_{2.5} and bladder cancer.

Modifier	Stratum	Cohorts (N)	Participants (N)	NO ₂ Cases (N)	HR (95% CI)	P	Cohort s (N)	Participants (N)	PM _{2.5} Cases (N)	HR (95% CI)	P
Sex	Women	15	184,655	271	0.82 (0.69, 0.99)	0.046	12	161,044	244	0.65 (0.36, 1.16)	0.30
	Men	15	121,234	672	1.04 (0.93, 1.17)		12	105,048	583	0.95 (0.58, 1.55)	
Smoking	Never	15	64,616	347	0.98 (0.84, 1.15)	0.40	12	46,967	306	0.88 (0.49, 1.60)	0.71
	Former	15	84,640	254	1.07 (0.87, 1.33)		12	67,896	223	0.96 (0.53, 1.74)	
	Current	15	53,171	342	0.88 (0.73, 1.05)		12	47,767	298	0.78 (0.48, 1.26)	
Education	Low	14	177,605	282	1.00 (0.83, 1.21)	0.98	11	154,031	218	1.39 (0.71, 2.71)	0.18
	Medium	14	62,968	248	0.86 (0.70, 1.04)		11	55,124	206	0.48 (0.24, 0.95)	
	High	13	63,513	108	1.07 (0.81, 1.40)		10	55,125	98	0.74 (0.32, 1.74)	

HRs and 95% CIs from model 3 per 10 µg/m³ increases in NO₂ and per 5 µg/m³ increases in PM_{2.5}, respectively.
PM_{2.5} is not available for EPIC Umea, EPIC Varese and EPIC San Sebastian.

Table 9. Sensitivity analyses for NO₂ and PM_{2.5} in association with risk of bladder cancer.

Analyses	Cohorts (N)	Participants (N)	Cases (N)	Model 3 HR (95% CI)	I ²	P
NO₂						
Full study population	15	303,431	943	0.98 (0.89, 1.08)	0.0	0.71
Restricted to cohorts with information on mobility ¹	10	235,864	705	0.97 (0.86, 1.08)	0.0	0.84
Restricted to non-moving participants	10	126,039	455	0.94 (0.79, 1.11)	0.0	0.44
Restricted to cohorts with information needed for back-extrapolation ²	14	285,473	922	0.97 (0.87, 1.08)	0.0	0.58
Back-extrapolation ratio-method	14	285,473	922	0.99 (0.93, 1.06)	0.0	0.54
Restricted to cohorts with information on urbanization ³	13	277,887	868	0.95 (0.83, 1.06)	0.0	0.69
Further adjusted for urbanization	13	277,887	868	0.97 (0.86, 1.09)	0.0	0.50
PM_{2.5}						
Full study population ⁴	12	263,634	827	0.86 (0.63, 1.18)	0.0	0.45
Restricted to cohorts with information on mobility	9	225,554	685	0.60 (0.37, 0.96)	0.0	0.41
Restricted to non-moving participants	9	117,060	435	0.74 (0.48, 1.15)	0.0	0.80
Restricted to cohorts with information on urbanization	10	245,676	752	0.78 (0.50, 1.22)	19.9	0.28
Further adjusted for urbanization	10	245,676	752	0.82 (0.45, 1.47)	40.0	0.14

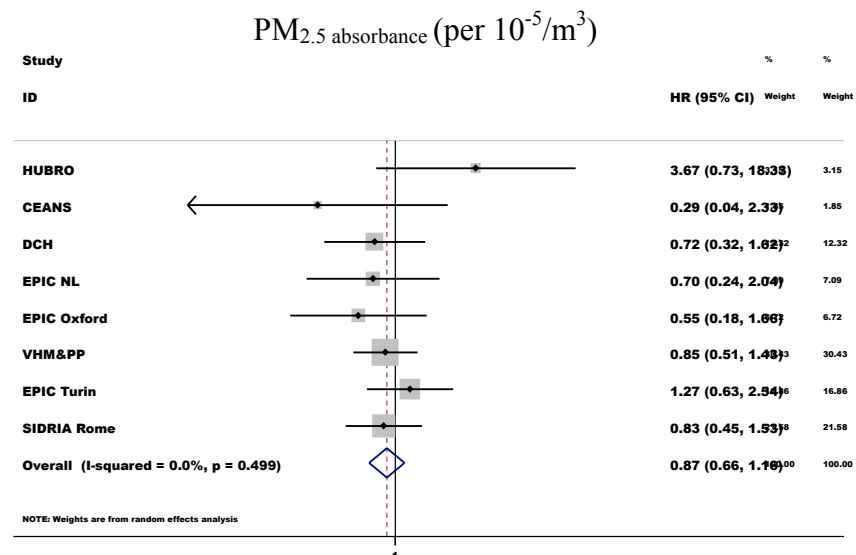
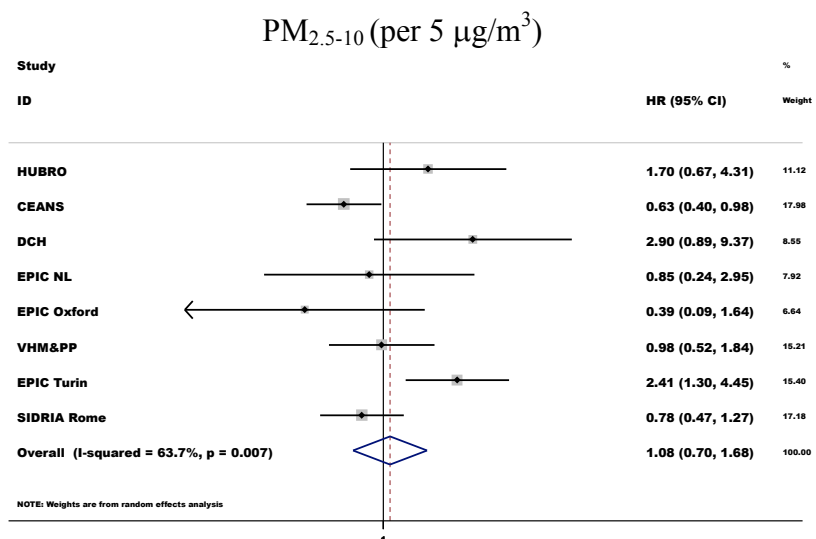
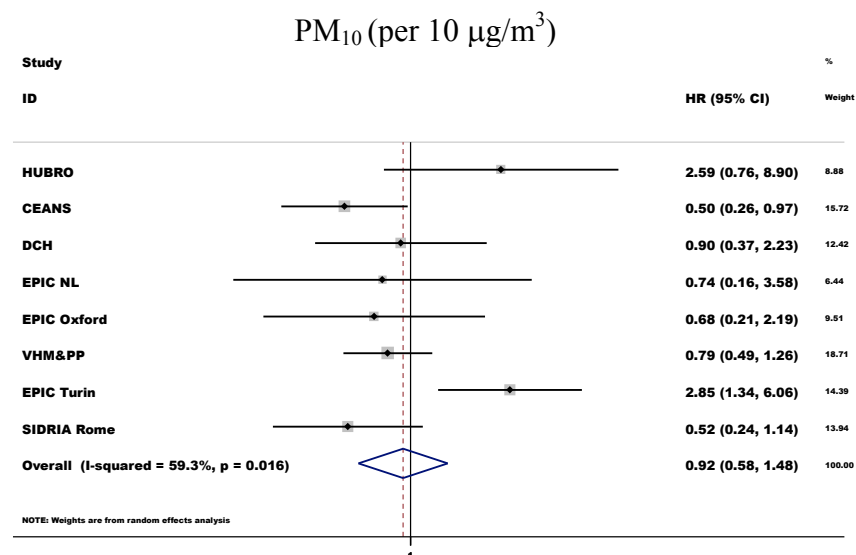
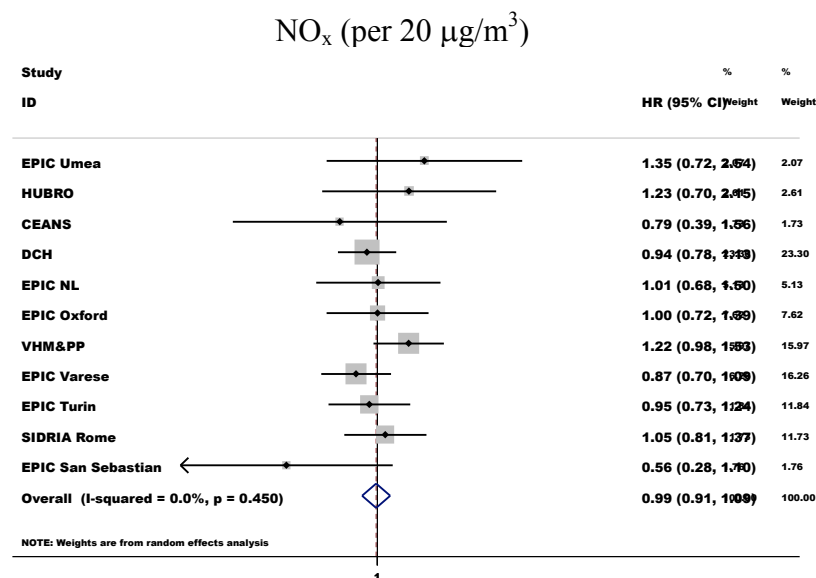
¹Information on mobility from baseline to follow-up is not available for EPIC Umea, EPIC NL, EPIC Turin and EPIC San Sebastian.

²Information on back-extrapolation is not available for HUBRO.

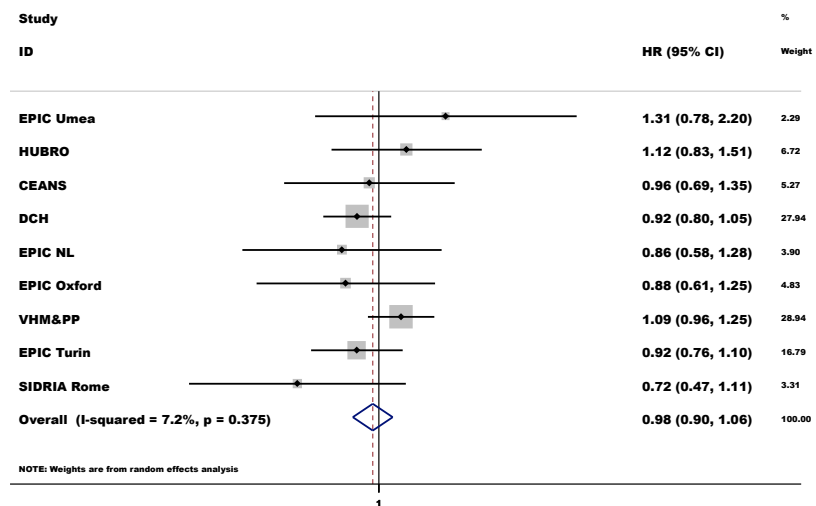
³Information on urbanization is not available for HUBRO and EPIC Turin.

⁴PM_{2.5} is not available for EPIC Umea, EPIC Varese and EPIC San Sebastian.

Figure 1. Bladder cancer in associations with air pollution and traffic density by cohort.



Traffic density (per 5,000 vehicles/day)



***Take Home Message**

We assessed the link between outdoor air pollution at residence and bladder cancer, using the largest study population to date, extensive assessment of exposure and comprehensive data on personal risk factors. Exposure to outdoor air pollution was not associated with bladder cancer incidence.