Ambient air pollution and primary liver cancer incidence in four European cohorts within the ESCAPE project

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ABSTRACT

Background: Tobacco smoke exposure increases the risk of cancer in the liver, but little is known about the possible risk associated with exposure to ambient air pollution.

Objectives: We evaluated the association between residential exposure to air pollution and primary liver cancer incidence.

Methods: We obtained data from four cohorts with enrolment during 1985–2005 in Denmark, Austria and Italy. Exposure to nitrogen oxides (NO_2 and NO_X), particulate matter (PM) with diameter of less than 10 μm (PM_{10}), less than 2.5 μm ($PM_{2.5}$), between 2.5 and 10 μm ($PM_{2.5-10}$) and $PM_{2.5\ absorbance}$ (soot) at baseline home addresses were estimated using land-use regression models from the ESCAPE project. We also investigated traffic density on the nearest road. We used Cox proportional-hazards models with adjustment for potential confounders for cohort-specific analyses and random-effects meta-analyses to estimate summary hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: Out of 174,770 included participants, 279 liver cancer cases were diagnosed during a mean follow-up of 17 years. In each cohort, HRs above one were observed for all exposures with exception of $PM_{2.5\ absorbance}$ and traffic density. In the meta-analysis, all exposures were associated with elevated HRs, but none of the associations reached statistical significance. The summary HR associated with a 10-μg/m^3 increase in NO_2 was

1.10 (95% confidence interval (CI): 0.93, 1.30) and 1.34 (95% CI: 0.76, 2.35) for a 5-µg/m³ increase in $PM_{2.5}$. *Conclusions:* The results provide suggestive evidence that ambient air pollution may increase the risk of liver cancer. Confidence intervals for associations with NO_2 and NO_X were narrower than for the other exposures.

1. Introduction

Primary liver cancer is the fifth most frequent cancer in men and the ninth in women (International Agency for Research on Cancer [IARC], World Cancer Report, 2014). Due to very poor prognosis it is a leading cause of cancer death, estimated to be responsible for nearly 746,000 deaths globally in 2012 (Ferlay et al., 2015). Hepatocellular carcinoma (HCC) accounts for 70–85% of the total liver cancer burden and HCC incidence has been rising in the United States and elsewhere in the past 30 years (Altekruse et al., 2009). Alcohol consumption and constituents of cigarette smoke are hepatic carcinogens in animals and humans (IARC, 2010, 2004). Current and former smokers have a relative risk for liver cancer of 1.5 and 1.1, respectively, compared with never smokers according to a meta-analysis of 38 studies (Lee et al., 2009). In the European Prospective Investigation into Cancer and nutrition (EPIC) cohort study smoking contributed to almost half of all HCC using the data collected from 1999 to 2006 (Trichopoulos et al., 2011). Also Hepatitis B and C virus infections are established risk factors (IARC, 1994).

Tobacco smoke and urban ambient air pollution are both complex mixtures of thousands of chemical components. Several carcinogenic combustion-related components such as polycyclic aromatic hydrocarbons (PAHs), heterocyclic hydrocarbons, volatile organic compounds, nickel, cadmium and other heavy metals are present both in tobacco smoke and urban ambient air (IARC, 2004, 2015). Diesel engine exhaust, which has been classified as being carcinogenic in humans, is another significant component of urban ambient air pollution (IARC, 2013). Recently, ambient air pollution with particulate matter (PM) has been classified as carcinogenic to humans (IARC, 2015). This classification was largely based on an increased risk for lung cancer, which has been linked to long-term exposure to ambient air pollution (IARC, 2015; Hamra et al., 2015; Raaschou-Nielsen et al., 2013).

Ambient air pollution exposure has been associated with increased serum levels of hepatic enzymes such as γ-glutamyltranspeptidase (GTP) and alanine transaminase (ALT), which are considered to be markers of liver damage usually caused by inflammation (Markevych et al., 2013; Kim et al., 2015; Pan et al., 2016). In humans and experimental studies exposure to fine particles have been reported to induce systemic oxidative stress and inflammation (Brook et al., 2003). Inhaled particles deposited in the airways are usually swallowed when removed from the airways by mucocilliary clearance resulting in gastrointestinal exposure with the largest internal dose and adverse effects observed in the liver (Conklin et al., 2013). Therefore, it is biologically plausible that exposure to ambient air pollution could contribute to the development of liver cancer. To date only a few epidemiological studies have investigated a possible association between exposure to ambient air pollution and liver cancer (Michelozzi et al., 1998; Pan et al., 2016; Raaschou-Nielsen et al., 2011; Soll-Johanning et al., 1998).

Most recently, a cohort study with 464 cases and a median follow-up of 17-years of participants living in two cities in Taiwan reported that elevated serum ALT levels mediated the association between exposure to PM with diameter of less than 2.5 µm ($PM_{2.5}$) and liver cancer risk (Pan et al., 2015). An association between $PM_{2.5}$ and liver cancer risk was reported in one of these areas, but the association did not reach statistical significance in analysis of both areas. This study relied on $PM_{2.5}$ concentrations averaged over a period of three years prior to the end of follow-up using data available from the nearest routine air monitoring station and lacked information on spatial variation in individual exposure. A prospective cohort study with 155 cases and a mean follow-up of 10 years of participants living in two cities in Denmark has reported an association between increased risk of liver cancer and higher traffic density within 200 m from the residential address at baseline (Raaschou-Nielsen et al., 2011). Increased risks were also suggested for residential proximity to a major road and modelled long-term residential exposure to nitrogen oxides (NO_x), an indicator of vehicle emissions, but these associations were not statistically significant. Among 15,249 urban male bus drivers and tramway employees from Denmark a 60% excess in liver cancer incidence rate was observed when compared with the general Danish male population (Soll-Johanning et al., 1998), based on only 40 cases and without adjustment for alcohol and smoking habits. A retrospective study including 310 cases from Rome, reported no increase in liver cancer mortality in association with residential proximity to industrial sources of air pollution (Michelozzi et al., 1998). Limitations of this study include an ecological exposure assessment based on the residence at the time of death, the inaccuracy of death certificates, and the lack of information on individual characteristics.

In the present study, our aim was to examine the associations between residential long-term exposures to ambient air pollution and liver cancer incidence in a large European population with fine-scale spatial exposure assessment and extensive control for potential confounders such as smoking and alcohol consumption.

2. Methods

2.1. Study population

The full ESCAPE (European Study of Cohorts for Air Pollution Effects) project included 36 European areas where cohort studies located and where air pollution measurements were performed and land-use regression (LUR) models were developed according to a common standardized protocol (Beelen et al., 2013). With these cohorts and methods of exposure assessment, it has previously been reported that ambient air pollution was associated with elevated risk for lung cancer and overall mortality (Beelen et al., 2014; Raaschou-Nielsen et al., 2013). The present study included participants from four of the ESCAPE cohorts with information on liver cancer incidence, with at least 20 incident liver cancer cases during follow-up period and where the time and personnel needed for the data analysis of the liver cancer study were available. Particularly the requirement of at least 20 cases during follow-up reduced the number of cohorts from 17 to four compared to the previous lung cancer analyses. We used this criterion to avoid unstable effect estimates that are commonly observed in particularly analyses based on a few cases, particularly when adjusted for several confounders.

The participants had baseline addresses in mostly large cities and surrounding suburban areas of Copenhagen, Denmark (Diet, Cancer and Health study (DCH)) (Tjonneland et al., 2007), both urban and rural areas in Austria (Vorarlberg Health Monitoring & Promotion Program (VHM & PP)) (Ulmer et al., 2007) and the Turin and Varese provinces in Italy (European Prospective Investigation into Cancer and Nutrition (EPIC))-Varese (Riboli et al., 2002) and EPIC-Turin (Cesaroni et al., 2008; Guarrera et al., 2012). A pooled analysis of all cohort data was not possible due to data-transfer and privacy issues, but data from the two Italian cohorts were pooled to achieve 20 cases. Participants with a cancer (except non-melanoma skin cancer) before enrolment or for whom information about exposure to air pollution or the most important potential confounders could not be obtained were excluded. We included 174,770 participants (91.0% of those enrolled, Table 1).

Table 1
Study population characteristics (N=174,770, n=279).

Characteristics Country	DCH Denmark	VHM & PP Austria	EPIC Varese Italy	EPIC Turin Italy
Years of enrolment	1993–1997	1985–2005	1993–1997	1993–1997
End of follow-up	2012	2011	2009	2010
Participants (n)	38,124	118,087	10,378	8181
Excluded from the original cohort (%)[a]	4.4	10.5	9.5	6.8[b]
Cases (n)	72	184	12	11
Persons years at risk	563,675	2,162,234	112,713	117,461
Years of follow-up [median (min-max)]	10.5 (0.1–19.1)	19.7 (0.0–27.0)	15.1 (0.0–17.7)	11.5 (0.0–13.3)
Age (years, mean $\pm$ SD)[b]	56.8 $\pm$ 4.4	41.9 $\pm$ 14.9	51.5 $\pm$ 8.2	50.4 $\pm$ 7.6
Men (%)	47.1	44.7	21.0	55.4
Smoking status at baseline				
Current smoking (%)	37.0	23.9	21.1	26.4
Former smoking (%)	27.9	6.1	21.8	32.2
Never smoking (%)	35.1	70.0	57.1	41.4
Alcohol consumption at baseline (g, mean $\pm$ SD)	21.9 $\pm$ 23.0	na	12.9 $\pm$ 17.3	18.9 $\pm$ 20.6
Abstainers (%)	2.4	na	15.4	8.5
< 7 drinks per week (%)	34.8	na	83.1	89.0
≥7 drinks per week (%)	62.8	na	1.5	2.6
In work at baseline (%)	79.4	69.7	na	na
Occupation with increased liver cancer risk (%)	4.6	na	na	na
Educational level				
Low (Primary school or less, %)	30.4	na	59.9	44.2
Medium (Secondary school, %)	47.1	na	33.2	42.4
High (University, %)	22.5	na	6.9	13.5
Non-movers from baseline to follow-up (%)	59.9	43.1	na	na
Urban residential area (%)	59.7	54.3	58.9	100.0

Abbreviations: DCH, Danish Diet, Cancer and Health cohort study; EPIC, European Prospective Investigation into Cancer and Nutrition; na, not available; VHM & PP, The Voralberg Health Monitoring and Prevention Program

[a] Excluded due to missing exposure, cancer before baseline, unsuccessful follow-up and/or missing information on covariates.

[b] Calculated on the basis of 8774 participants living in city areas. The 1830 participants outside city areas were never considered for this study.

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

2.2. Liver cancer outcome definition

Cases were identified by linkage to population-based cancer registries and we defined cases as those diagnosed with cancer in the liver, including intrahepatic bile ducts, recoded according to ICD-10 (*International Classification of Disease, 10th revision*) or ICD-O code C22.0, C22.1 C22.2, C22.3, C22.4, C22.7 and C22.9. Only primary cancer, not metastases, and only malignant tumors were included.

2.3. Exposure assessment

The annual average air pollution concentrations at the residential addresses of study participants at the time of enrolment in the cohort studies were estimated by area-specific LUR models (Beelen et al., 2013; Eeftens et al., 2012). In brief, air pollution was measured for 1 year between October 2008 and May 2011. PM with a diameter of less than 10 μm (PM_{10}), $PM_{2.5}$, and soot/blackness of the $PM_{2.5}$ exposed filter ($PM_{2.5\ absorbance}$) determined by measurement of light reflectance, were measured at 20 sites, and nitrogen dioxide (NO_2) and nitrogen oxides (NO_X), were measured at 40 sites selected to represent spatial variation of air pollution in the residential areas of the participants in each of the study 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 per site were averaged adjusting for temporal trends using continuous data from a reference site to estimate the annual mean at each position. $PM_{2.5-10}$ was calculated as the difference between PM_{10} and $PM_{2.5}$. Because of financial reasons, PM was not sampled in Varese (Fig. 1) and since we pooled EPIC Varese data with EPIC Turin data we could

not include the PM data from EPIC Turin in the present study.

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. The LUR models were applied to predict air pollution levels at residential addresses of the cohort participants.

We also collected information on traffic density (vehicles per day) on the nearest street, major or not as part of the original ESCAPE exposure assessment. Traffic intensity was obtained from existing GIS databases. In these databases, traffic intensity is typically obtained by direct counting for a few major roads supplemented with a traffic model.

We used similar methods to assess concentrations of eight PM elements in $PM_{2.5}$ and PM_{10} (de Hoogh et al., 2013; Tsai et al., 2015) to facilitate explorative analyses of associations with risk for liver cancer.

2.4. Statistical analysis

Cox proportional hazards models were used for the cohort specific analyses following the analysis protocol in our previous lung cancer incidence study (Raaschou-Nielsen et al., 2013). Age was used as the time scale. Follow-up started at enrolment into the cohort. Censoring was done at the time of death, emigration, a diagnosis of any other cancer (except non-melanoma skin cancer) or end of follow-up, whichever came first.

Air pollution exposure i.e. NO_2, NO_X, $PM_{2.5}$, $PM_{2.5\ absorbance}$, PM_{10}, $PM_{2.5-10}$ and traffic density were analyzed as continuous variables by introducing a linear term into the model using the following increments; 10 μg/m^3, 20 μg/m^3, 5 μg/m^3, 10^{-5}/m, 10 μg/m^3, 5 μg/m^3 and 5000 vehicle/day, respectively. Potential confounders were available from questionnaires at baseline. We specified *a priori* three confounder models with increasing levels of adjustment: Model 1 included only age (underlying time scale), sex and calendar year of enrolment. Model 2
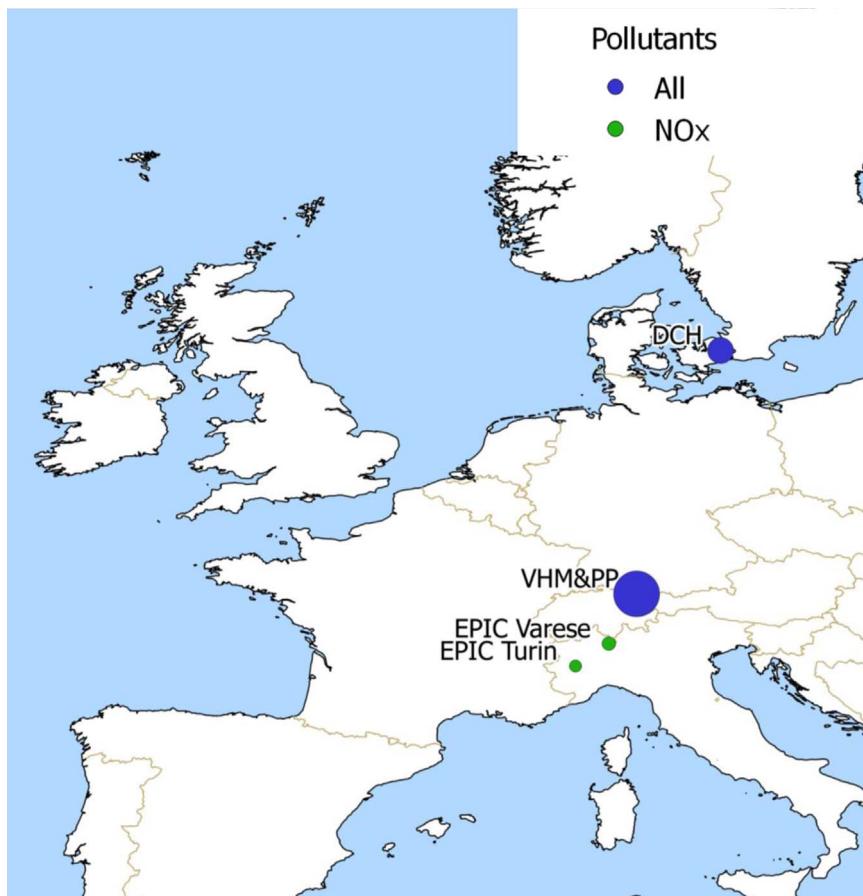
Fig. 1. Study areas.

was further adjusted for the following variables (as available for the individual cohorts): smoking status (never/former/current), alcohol consumption (none/ < 7 drinks per week/≥7 drinks per week), ever worked as waiter or cook (yes/no) which are occupations associated with higher liver cancer risk, employment status (not employed/ employed) and educational level (low/medium/high). Model 3, which furthermore included an area-level SES variable, was selected as the main model (Raaschou-Nielsen et al., 2013). For cohorts with missing information on covariates (Table 1), we used effect estimates from models adjusted for as many variables as possible.

We tested the assumption of a linear association between each air pollutant and liver cancer 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. We also assessed if there was a deviation from proportional hazards assumption in the Cox model by testing for a non-zero slope in a generalized linear regression of the scaled Schoenfeld residuals on functions of time (Grambsch, 1994).

In sensitivity analysis, we assessed effect modification by smoking status, alcohol use and sex as well as restricted analyses to participants who had lived at the baseline address throughout follow-up to minimize misclassification of long-term exposure. We added an indicator of extent of urbanization to model 3 to assess if the results are robust to adjustment of unmeasured differences related to degree of urbanization of the residential area (urban/rural). Finally, we fitted back-extrapolated NO_2 to take into account long-term trends in air pollution. Data from nearest routine monitoring stations were used to back-extrapolate the LUR estimates of NO_2 to the baseline year in all four study areas using the ratio-method (Beelen et al., 2013). Briefly, we calculated the ratio of the measured annual average concentration during the baseline period (e.g. 1995) and the ESCAPE monitoring

period (2009 or 2010) using routine monitoring data. We then multiplied the predictions of the (2009–2010) LUR models with this ratio. Back-extrapolation assumes that the spatial ranking remains the same, but the concentration levels may change.

A common script was used by all cohort-specific analysts. Cohort-specific effect estimates were combined by random-effects meta-analysis for each exposure (DerSimonian and Laird, 1986) at the Danish Cancer Society Research Centre. I^2 statistics and Q-test were used to assess the heterogeneity (Higgins and Thompson, 2002) among cohort-specific effect estimates when these were available in at least three cohorts.

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

3. Results

3.1. Study population

We included 174,770 participants of four cohorts from Denmark, Austria and Italy who contributed 2,956,083 person-years at risk; 279 liver cancer cases were diagnosed during the mean follow-up of 17 years. Most of the participants were recruited in the 1990s (Table 1). The VHM & PP cohort contributed most of the cases. The mean age of the participants at baseline was 46 ranging from 42 to 57 years. 44% of the participants were men and the proportion of men ranged from 21% (EPIC Varese) to 55% (EPIC Turin). At baseline, 27% of the participants were current smokers and the proportion of current smokers ranged from 21% (EPIC Varese) to 37% (DCH). Frequent alcohol drinking was most common in participants from Denmark (63%) and least common in Italy (< 3%). Although there was a large difference in the proportion of participants consuming 7 drinks per week between cohorts, the difference in population average alcohol consumption in

the three cohorts is modest (Beelen et al., 2013). Smoking status at individual level was available for all cohorts (Table 1). Information on alcohol and education was missing for the VHM & PP cohort and there was no information on occupational exposure and residential mobility in the two Italian cohorts.

Air pollution concentrations differed substantially between the four cohorts (Table 2), with lowest NO_2 concentrations in Denmark and highest in Italy. The Austrian study area had the lowest mean traffic density on nearest street (Table 2). The contrast of exposure was lower for $PM_{2.5}$ and PM_{10}. Table 2 summarizes the distribution by cohort for each exposure as the mean and the standard deviation. The standard deviations and the difference between the 5th and 95th percentile document that there is substantial contrast within each cohort especially for NO_2 and NO_X.

3.2. Ambient air pollution, traffic density and liver cancer risk

In each cohort, increased risk for liver cancer was associated with increased concentrations of all pollutants with exception of $PM_{2.5\ absorbance}$ in DCH (Table 3). However, none of the associations reached statistical significance and the 95% confidence intervals (CIs) were wide. Confidence intervals reflect number of cases, person-years and variability of exposure.

In the meta-analysis all exposures, including traffic density, were associated with elevated HRs, but none of the associations reached statistical significance (Table 4). The summary HR associated with a $10\ \mu g/m^3$ increase in NO_2 was 1.10 (95% CI: 0.93, 1.30), and 1.34 (95% CI: 0.76, 2.35) for $PM_{2.5}$, which was available in Denmark and Austria only. The estimates were slightly higher in the crude model adjusted for age and sex than those from models with more comprehensive adjustment (Table 4). Adjustment for alcohol, occupational exposure, employment status and education hardly affected the HRs in cohorts where these variables were available.

We did not pursue two-pollutant models as none of the associations in one-pollutant models reached statistical significance.

3.3. Sensitivity analyses

There was no evidence of deviation from linearity for any association with exception of the associations for $PM_{2.5-10}$ in the Austrian study area (see Supplemental Table S1). All cohorts met the assumptions for Cox proportional hazard models (see Supplemental Table S2).

We did not find significant differences in risk of liver cancer related to $PM_{2.5}$ and NO_2 in subgroups defined by smoking status, alcohol use and sex (Table S3).

Restriction to participants who had lived at the same home address throughout follow-up provided similar associations for NO_2 and $PM_{2.5}$ (see Supplemental Table S4). Additional adjustment for urbanization did not change the reported results (see Supplemental Table S4). Finally, we were able to back-extrapolate NO_2 and NO_X for all participants and the associations attenuated from 1.10 (95% CI:

0.93, 1.30) to 1.03 (95% CI: 0.93, 1.14) if the ratio-method was used for back-extrapolation and 1.07 (95% CI: 0.91, 1.25) if the absolute difference method was used for NO_2 and the patterns were similar for NO_X.

In DCH and VHM & PP, exposure to elemental components of PM was estimated (see Supplemental Table S5). In meta-analyses most elements were associated with elevated HRs with exception of Cu, K, V and Zn in $PM_{2.5}$ (see Supplemental Table S6). None of the associations reached statistical significance.

4. Discussion

We found that exposure to ambient air pollution at the residence was associated with a higher risk of liver cancer in both analyses of individual studies and the meta-analysis; however, none of our findings were statistically significant.

4.1. Previous studies

Our finding of higher HR in association with higher exposure is consistent with those of the two previous studies on ambient air pollution and liver cancer incidence (Pan et al., 2016; Raaschou-Nielsen et al., 2011), although the size of the estimated HRs differs for $PM_{2.5}$. In our study a 5-$\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 34% higher risk, and in the Taiwanese study a 13-$\mu g/m^3$ increase in $PM_{2.5}$ was associated with a 22% increased HCC risk of the population on the Penghu Islets (Pan et al., 2016). Differences in the composition of the PM could explain the variation in the magnitude of the associations or they may difference due to random error. In our study a 20-$\mu g/m^3$ increase in NO_x was associated with a 12% raised risk, which is similar to the estimated 66% raised risk in association with a 100-$\mu g/m^3$ increase in NO_X in the previous study conducted in the full DCH cohort (Copenhagen and Aarhus) with a different exposure assessment method (Raaschou-Nielsen et al., 2011).

4.2. Biological plausibility

Mechanistic evidence supports the idea that exposure to ambient air pollution can contribute to development of liver cancer in humans. In mice, exposure to concentrated ambient $PM_{2.5}$ induced a non-alcoholic steatohepatitis-like phenotype as well as liver fibrosis (Laing et al., 2010; Zheng et al., 2013). Non-alcoholic steatohepatitis represents an increasingly important etiology of liver cancer in particular with fibrosis present (Wong et al., 2016). In rats, intragastric exposure to diesel exhaust particles caused oxidative stress with DNA damage, bulky DNA adducts as well as apoptosis and upregulation of DNA repair in the liver (Danielsen et al., 2008; Dybdahl et al., 2003). Furthermore, it is hypothesized that the extrapulmonary effects of exposure to ambient air pollution are transmitted through pulmonary inflammatory and oxidative stress pathways, neuronal signaling and translocation of particles to the circulation (Newby et al., 2015). In

Table 2
Exposure distribution by cohort (annual mean ± sd (P5-P95)) for each study cohort.

Exposure/Cohort	DCH		VHM & PP		EPIC Varese		EPIC Turin	
NO_2 ($\mu g/m^3$)	16.4 ± 7.0	(8.2–30.1)	19.9 ± 5.5	(10.8–29.2)	43.5 ± 17.3	(13.9–59.9)	54.1 ± 11.7	(34.4–69.7)
NO_X ($\mu g/m^3$)	26.8 ± 18.4	(7.2–66.0)	40.0 ± 9.6	(25.8–57.6)	86.1 ± 41.8	(15.6–124.3)	99.5 ± 26.3	(62.9–140.9)
$PM_{2.5}$ ($\mu g/m^3$)	11.3 ± 0.9	(9.8–12.6)	13.6 ± 1.2	(11.3–15.4)	na	na	na	na
$PM_{2.5\ absorbance}$ ($10^{-5}/m$)	1.2 ± 0.2	(0.8–1.5)	1.7 ± 0.2	(1.3–2.0)	na	na	na	na
PM_{10} ($\mu g/m^3$)	17.2 ± 2.0	(14.0–20.4)	20.6 ± 2.4	(16.3–24.2)	na	na	na	na
$PM_{2.5-10}$ ($\mu g/m^3$)	5.7 ± 1.0	(4.1–7.4)	6.7 ± 0.9	(5.2–8.0)	na	na	na	na
Traffic density (vehicles/day)	3024 ± 7263	(200–16,251)	1699 ± 3620	(500–8992)	na	na	na	na

Na refers to not applicable as PM is not available for participants from Varese and since EPIC Varese data is pooled with EPIC Turin the PM EPIC Turin data is not used here; NO_2 refers to nitrogen dioxide; NO_X refers to nitrogen oxides; $PM_{2.5}$ refers to particulate matter with aerodynamic diameter < 2.5 μm; $PM_{2.5-10}$ refers to coarse particulate matter with aerodynamic diameter 2.5–10 μm; PM_{10} refers to particulate matter with aerodynamic diameter < 10 μm.

Table 3

Associations between air pollution, traffic density and risk for liver cancer in each cohort.

Exposure	Increase	DCH	VHM & PP	EPIC Varese & Turin
NO_2	10 µg/m³	1.12 (0.78, 1.61)	1.14 (0.88, 1.48)	1.03 (0.77, 1.37)
NO_x	20 µg/m³	1.02 (0.78, 1.32)	1.33 (1.00, 1.77)	1.07 (0.84, 1.37)
$PM_{2.5}$	5 µg/m³	2.21 (0.38, 12.91)	1.27 (0.70, 2.30)	na
$PM_{2.5\ absorbance}$	10^{-5}/m	0.74 (0.20, 2.66)	1.37 (0.71, 2.62)	na
PM_{10}	10 µg/m³	2.67 (0.64, 11.14)	1.29 (0.70, 2.37)	na
$PM_{2.5-10}$	5 µg/m³	1.86 (0.29, 11.83)	1.26 (0.57, 2.79)	na
Traffic density	5000 vehicle/day	0.96 (0.82, 1.13)	1.12 (0.95, 1.32)	na

Hazard ratio (HR) and 95% confidence intervals (CI) from cohort-specific Cox models adjusted for age (time scale), sex, calendar year, smoking status, alcohol, occupational exposure, employment status, education and area-specific SES.

NO_2 and NO_x are available for all cohorts.

PM and traffic density are not available for EPIC Varese and EPIC Turin.

The number of cases and person-years are summarized in Table 1.

humans, long-term exposure to ambient air pollution has been associated with liver damage biomarkers such as serum ALT levels (Markevych et al., 2013; Kim et al., 2015; Pan et al., 2016), which may mediate the effect of exposure to ambient air pollution on liver cancer risk as suggested in the study from Taiwan (Pan et al., 2016). ALT and other liver function and inflammatory biomarkers such as C-reactive protein (CRP) and interleukin-6 (IL-6) have been shown to predict liver cancer risk (Aleksandrova et al., 2014; Stepien et al., 2016). Accordingly, not only inflammation, but also well-known carcinogenic genotoxic effects as well as oxidative stress potentially driving carcinogenesis can be induced in the liver by exposure to ambient air pollution with particles providing biological plausibility of an association between ambient air pollution and liver cancer.

4.3. Strengths and limitations

Our study is the first multicenter prospective study of ambient air pollution and liver cancer. It relies on a 17-years mean follow-up of a large population from four European cohorts with very different air pollution levels and alcohol habits, which adds to the generalizability of the results. Another important strength is the standardized, extensive exposure assessment, which enable us to assess address-level spatial variation in concentrations of a more comprehensive number of air pollutants than any of the previous studies (Michelozzi et al., 1998; Pan et al., 2016; Raaschou-Nielsen et al., 2011; Soll-Johanning et al., 1998). We were able to adjust for individual habits of smoking and alcohol consumption and for area-levels of SES and degree of urbanization, which have not been considered in the previous studies. The HRs were only slightly affected by adjustment for individual risk factors such as smoking status and alcohol consumption and virtually not affected by additional adjustment for area-level SES. Adjustment

for alcohol, occupational exposures, employment status and education hardly affected the HRs in cohorts where these variables were available which makes marked confounding by these factors unlikely in cohorts where they were not available (such as alcohol in the large VHM & PP cohort). Hepatitis B and C infection are established causes of liver cancer and might be associated with educational level, area-level SES and degree of urbanization, and thereby with the level of air pollution; therefore, Hepatitis B and C are potential confounders. However, we believe that strong confounding is unlikely for several reasons. First, the prevalence of chronic Hepatitis B and C infection is low in European countries, ranging from close to zero in north-west European countries (e.g. 0.2–0.4% in Malmö, which we assume is comparable with the Copenhagen DCH cohort) to 1–3% in the Northern Italy (which we assume is comparable with the Varese and Turin cohorts) (Hahné et al. 2013). Secondly, although the prevalence of these infections might correlate with the general background air pollution concentration e.g. due to correlation with degree of urbanization, we doubt that the prevalence correlates with the fine-scale (address level) variation in air pollution, which was used for the exposure assessment in the present study. Thirdly, adjustment for degree of urbanization and area-level SES did not affect the HRs. We cannot exclude confounding from unaccounted factors such as obesity (Wang et al., 2012) and aflatoxin exposure (Ferlay et al., 2015).

We used air pollution measurements taken in 2009 to 2011 for development of the LUR models, which were used to assess exposure at the baseline home addresses between 4 and 26 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. Spatial contrasts in NO_2 have been shown to be stable with time (Cesaroni et al., 2012; Eeftens et al., 2011; Gulliver et al., 2013). NO_2 patterns are

Table 4

Meta-analysis of the associations between air pollution, traffic density and risk for liver cancer.

Exposure	Increase	Participants (n)	Cases (n)	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 3[c] HR (95% CI)	I² (%)[d]	P[d]
NO_2	10 µg/m³	174,770	279	1.12 (0.95, 1.33)	1.10 (0.93, 1.30)	1.10 (0.93, 1.30)	0.0	0.86
NO_x	20 µg/m³	174,770	279	1.14 (0.98, 1.33)	1.12 (0.96, 1.30)	1.12 (0.96, 1.30)	2.3	0.36
$PM_{2.5}$	5 µg/m³	156,211	256	1.44 (0.73, 2.87)	1.33 (0.76, 2.35)	1.34 (0.76, 2.35)		
$PM_{2.5\ absorbance}$	10^{-5}/m³	156,211	256	1.23 (0.69, 2.20)	1.17 (0.65, 2.08)	1.21 (0.68, 2.15)		
PM_{10}	10 µg/m³	156,211	256	1.72 (0.68, 4.36)	1.43 (0.80, 2.55)	1.44 (0.83, 2.52)		
$PM_{2.5-10}$	5 µg/m³	156,211	256	1.46 (0.68, 3.13)	1.33 (0.64, 2.73)	1.34 (0.65, 2.78)		
Traffic density	5,000 v/d	156,211	256	1.04 (0.90, 1.21)	1.04 (0.89, 1.21)	1.04 (0.89, 1.20)		

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

NO_2 and NO_x are available for all cohorts.

PM and traffic density are not available for EPIC Varese and EPIC Turin.

[a] Adjusted for age (time scale), sex and calendar time in Cox model.

[b] Additional adjusted for smoking status, alcohol, occupational exposure, employment status and education.

[c] Additional adjusted for area-level SES.

[d] I² and p refers to test for heterogeneity when data is available for at least three cohorts.

determined by combustion sources, including emissions by motorized vehicles. The NO_2 patterns may not reflect patterns for pollutants that have different major sources such as Ni or V.

We had no information about residential exposures at other addresses than that at baseline or about exposure elsewhere (at work, transport). Our exposure assessment might not have covered the time window most relevant for liver cancer development, which might range from few years before diagnosis (Pan et al., 2016) to 30 years earlier (Soll-Johanning et al., 1998). We cannot preclude that the lack of information on life-time residential history and life-time air pollution exposure may have attenuated our results. However, restriction to participants who lived at the same address throughout the follow-up period provided almost identical results.

Finally, primary liver cancer is a rare disease with 75–85% of cases being of the HCC type. The other primary liver cancer that occurs with a much lower, but still significant frequency is intrahepatic cholangio-carcinoma (ICC). More rare subtypes include hepatoblastoma, which is the most common childhood hepatic tumour, and angiosarcoma of liver (ASL). Since the etiology of these subtypes might differ (Schottenfeld and Fraumeni, 2006), our analysis based on all the subtypes combined might have caused an underestimation of the hazard ratio if air pollution is truly associated with risk for only one of the subtypes. Preferably, analyses of subtypes of primary liver cancer should be undertaken in a study including more cases than ours.

In conclusion, the results provide suggestive evidence that ambient air pollution can increase the risk of liver cancer.

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Conflict of interest

All authors declare that they have no actual or potential competing financial interests.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2017.01.006.

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