

## Air pollution and risk of lung cancer in a prospective study in Europe

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To estimate the relationship between air pollution and lung cancer, a nested case-control study was set up within EPIC (European Prospective Investigation on Cancer and Nutrition). Cases had newly diagnosed lung cancer, accrued after a median follow-up of 7 years among the EPIC exsmokers (since at least 10 years) and never smokers. Three controls per case were matched. Matching criteria were gender, age ( $\pm 5$  years), smoking status, country of recruitment and time elapsed between recruitment and diagnosis.

We studied residence in proximity of heavy traffic roads as an indicator of exposure to air pollution. In addition, exposure to air pollutants (NO<sub>2</sub>, PM10, SO<sub>2</sub>) was assessed using concentration data from monitoring stations in routine air quality monitoring networks. Cotinine was measured in plasma. We found a nonsignificant association between lung cancer and residence nearby heavy traffic roads (odds ratio = 1.46, 95% confidence interval, CI, 0.89–2.40). Exposure data for single pollutants were available for

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197 cases and 556 matched controls. For NO<sub>2</sub> we found an odds ratio of 1.14 (95% CI, 0.78–1.67) for each increment of 10 µg/m<sup>3</sup>, and an odds ratio of 1.30 (1.02–1.66) for concentrations greater than 30 µg/m<sup>3</sup>. The association with NO<sub>2</sub> did not change after adjustment by cotinine and additional potential confounders, including occupational exposures. No clear association was found with other pollutants.

**Key words:** air pollution; lung cancer; prospective study

Air pollution is a mixture of a large number of chemical compounds, mainly due to vehicle traffic, heating systems and industrial plants. Prior to the publication of cohort studies, the evidence for a relationship between air pollution and lung cancer was somewhat equivocal, having been based on geographical comparisons, case-control studies and occupational studies of workers exposed to PAHs or diesel exhaust.<sup>1,2</sup> Estimation of the effects of air pollution on lung cancer incidence is difficult for several reasons: (i) clinically detectable lung cancer takes many years to develop; (ii) long-term records of air pollution are required; (iii) information on other risk factors (potential confounders) is needed; (iv) measurements of both air pollution and confounding factors are often difficult and subject to errors of classification and changes over time and (v) individual exposure is difficult to estimate. Air pollution has been related to lung cancer in 5 previous prospective studies,<sup>3–7</sup> although their results are consistent, most of them were conducted in countries with relatively low pollution levels (United States and Norway).

We report here on a case-control study nested within a large European cohort, in which exposure to air pollution was assessed on the basis of residence.

## Methods

### *The EPIC cohort*

EPIC (European Prospective Investigation into Cancer and Nutrition) is a multicentre European study, coordinated by the International Agency for Research on Cancer (Lyon), in which more than 500,000 healthy volunteers have been recruited in 10 European countries (Sweden, Denmark, Netherlands, UK, France, Germany, Spain, Italy, Greece and Norway).<sup>8</sup> The cohort includes subjects of both genders, in the age range of 35–74 at recruitment.

Recruitment took place in 1993–1998. Dietary information on the frequency of consumption of more than 120 foods and drinks has been obtained by dietary questionnaires developed and validated in a pilot phase in each participating country. At enrolment, weight, height, waist and hip circumferences have been measured for each participant. Detailed information has been collected on reproductive history, physical activity, smoking and alcohol drinking history, medical history, occupation, education level and other socioeconomic variables; the questionnaire was printed in 2 separate versions for men and women. A computerized central database has been developed after checking, coding and quality control procedures. We also collected information on high-risk occupations (main job held in life), in particular (as relevant for lung cancer) welding, shipyard working, asbestos production and use, working with asphalt, metal working, construction and demolition work.

The EPIC cohort has been followed-up since inception through Cancer Registries, vital statistics (mortality), active follow-up (France and Germany) and—in some areas—hospital discharge data. Whenever available, diagnosis is based on histologic confirmation. All incident cancers and all causes of death are registered and checked centrally. All follow-up procedures are coordinated by an Endpoint Committee that includes pathologists. The median follow-up time when the present cases and controls were identified was 7 years.

### *Design of nested case-control study (GenAir)*

GenAir is a case-control study nested within the EPIC cohort, aiming at studying the relationship between some types of cancer and air pollution or ETS. Cases are all subjects with bladder, lung, oral, pharyngeal, laryngeal cancer or leukemia, all newly diagnosed after recruitment. Also deaths from respiratory diseases (COPD, emphysema) were identified and included. These diagnoses were chosen because they are suspected of being associated with air pollution or ETS exposure. Only never smokers or exsmokers since at least 10 years have been included in GenAir. We have matched 3 controls per case. Matching criteria were gender, age ( $\pm 5$  years), smoking status, country of recruitment and time elapsed between recruitment and diagnosis. Matching was introduced to allow strict control of potentially confounding variables, considering that the risk factors above may be stronger than air pollution. In addition, matching was needed for laboratory analyses to avoid differential sample degradation between cases and controls. For the analysis of biological samples matching was 2:1.

GenAir has been approved by the Ethical Committee of the International Agency for Research on Cancer, and by all the local Ethical Committees at the participating centres.

### *Air pollution exposure assessment*

The areas involved were (i) metropolitan areas around big cities or towns, with study areas of about 10–25 km of diameter (Heidelberg, Potsdam, Copenhagen, Utrecht, Florence, Varese, Turin), or (ii) larger territories including one or more cities/towns, with diameters of about 100 km (Amsterdam, Oviedo, Pamplona, San Sebastian, Granada, Umea) or (iii) entire countries (UK, France).

We used 2 methods for the assessment of exposure to traffic-related air pollution. First, for each home address we assessed whether the home was located in a major street (yes/no). Several studies have documented substantial differences in concentration of traffic-related pollutants between traffic and background locations.<sup>9,10</sup> For all homes, we used detailed Internet maps ([www.streetmap.co.uk](http://www.streetmap.co.uk) and [www.mappy.com](http://www.mappy.com)) to evaluate whether the home was located in a major street. The map-based classification of streets was validated using traffic count data obtained from Municipalities and local environmental agencies, or were downloaded from Internet sites.

Second, exposure to air pollution was assessed using concentration data from monitoring stations in routine air quality monitoring networks. We identified the study areas of the individual EPIC cohorts and then obtained concentration data from available network stations relevant for those areas. We excluded traffic and industrial network sites and instead focused on urban or rural background locations, *i.e.* the site should be at least 50 m away from any major road and at least 100 m from a freeway and not located in an industrial area (preferably in a residential area). Traffic sites are hotspots that do not provide a representative exposure situation for most homes. Data were obtained through searching AIRBASE, the air pollution database from the European Topic Centre on Air Quality in Bilthoven, the Netherlands (<http://www.bettie.rivm.nl/etc-acc/appletstart.html>). In addition we contacted national/local monitoring agencies using a questionnaire and used Internet sites from national agencies. We aimed at obtaining data from 1980–1999 for all pollutants, routinely monitored on a reasonable scale in Europe (O<sub>3</sub>, SO<sub>2</sub>, NO<sub>2</sub>, NO, CO, benzene and Particulate: TSP, PM<sub>10</sub>, PM<sub>2.5</sub>, Black Smoke, Benzo(a)pyrene). Because we were interested in long-term health effects, we obtained annual average concentrations and winter/summer data (only for some cohorts) from the network agencies. In addition to concentration data, we obtained data on monitoring sites and monitoring methods to assess suitability of the methods. Exposure was assessed based on the residence address at the time of enrolment and the average concentration of pollutants from the nearest background monitoring stations. If no monitoring station existed in the city of residence, data from the nearest monitoring

**TABLE I** – MEAN LEVELS OF DIFFERENT POLLUTANTS FOR ALL GENAIR CASES AND CONTROLS (SEE TEXT), IN EPIC CENTRES WITH AT LEAST 15 SUBJECTS WITH EXPOSURE ASSESSMENT AVAILABLE

Center	Years of measurement	NO <sub>2</sub>	O <sub>3</sub>	PM10	SO <sub>2</sub>
France					
Ile-de-France	1990–94	47.5 (31)	25.8 (37)	22.3 (52)	14.3 (47)
	1995–99	40.9 (75)	34.6 (75)	19.9 (60)	11.4 (69)
Northeast of France	1990–94	22.6 (19)	40.2 (20)	30.2 (45)	11.0 (23)
	1995–99	26.0 (83)	43.1 (89)	29.5 (70)	8.5 (85)
Italy					
Turin	1990–94	64.7 (62)	42.0 (62)	73.4 (61)	28.5 (62)
	1995–99	44.3 (78)	42.5 (78)	61.1 (61)	12.2 (78)
Florence	1990–94	49.7 (85)	–	40.4 (85)	11.5 (73)
	1995–99	42.1 (90)	45.7 (90)	33.3 (85)	6.6 (73)
Varese	1990–94	46.9 (19)	66.6 (19)	–	10.2 (33)
	1995–99	41.8 (19)	55.5 (19)	–	5.7 (33)
United Kingdom					
Oxford	1990–94	31.3 (78)	49.0 (277)	29.0 (38)	16.1 (85)
	1995–99	27.2 (360)	45.6 (412)	25.6 (171)	8.2 (356)
Cambridge	1990–94	–	52.3 (520)	–	–
	1995–99	25.2 (822)	45.8 (824)	25.4 (821)	10.8 (822)
The Netherlands					
Utrecht	1990–94	39.7 (130)	34.2 (130)	42.8 (130)	10.2 (130)
	1995–99	35.1 (130)	35.0 (130)	40.0 (130)	5.5 (130)
Bilthoven	1990–94	24.0 (18)	36.5 (18)	39.0 (24)	8.1 (18)
	1995–99	25.8 (24)	37.4 (18)	37.2 (24)	4.8 (24)
Germany					
Heidelberg	1990–94	42.0 (60)	39.8 (60)	–	15.8 (60)
	1995–99	32.3 (188)	42.6 (188)	27.0 (60)	9.6 (188)
Potsdam	1990–94	21.8 (226)	53.3 (200)	32.0 (226)	30.6 (226)
	1995–99	21.0 (226)	47.3 (200)	28.9 (226)	10.5 (226)
Sweden					
Umea	1990–94	12.0 (195)	53.3 (195)	–	1.5 (195)
	1995–99	12.3 (195)	56.4 (195)	–	1.1 (195)
Denmark					
Copenhagen	1990–94	12.0 (63)	52.6 (238)	–	7.3 (238)
	1995–99	22.4 (238)	49.1 (238)	–	4.9 (238)

Values given are in  $\mu\text{g}/\text{m}^3$ . Values in parentheses indicate the numbers of subjects.

station was assigned taking into account the nature of the site: rural stations for smaller towns and urban stations for the larger cities. Altitude was taken into account, as well as mountain ranges and seaside winds, to obtain more accurate assessments.

Assessment of single pollutants was limited by the relatively small number of monitoring stations and the ensuing misclassification (Appendix). We had no access to methods such as geographic information system and air pollution dispersion modelling across all cohorts.

#### *Cotinine measurements and metabolic genetic polymorphisms*

Cotinine is a short-term marker of recent exposure to tobacco smoke (24 hr), and has been measured to adjust more accurately for such exposure (both active and passive). Cotinine was extracted from plasma by ion exchange chromatography and analyzed by liquid chromatography-atmospheric pressure ionization-tandem mass spectrometry (API LC-MS/MS) at the Mario Negri Institute (Milano) in 1,574 subjects, *i.e.* the cases included in GenAir and their matched controls (2:1) with plasma samples available. Cotinine was measured in 100 lung cancer cases and 343 controls with air pollution assessment available. Subjects with values greater than 10 ng/ml were excluded because they were likely to be active smokers or sniffers/chewers.

Analyses of genotypes for genetic polymorphisms were performed in white blood cells (WBC) DNA. The metabolic genes we considered were *N*-acetyltransferase 1 and 2 (NAT1, NAT2), Glutathione-S-Transferase (GST) M1 and GSTM3, GSTT1, GSTP1 (genes involved in carcinogen detoxifying); CYP1A1, CYP1B1 (involved in activation); and MnSOD, MPO, NQO1 (involved in oxidative damage scavenging). Metabolic polymorphisms have been investigated at IARC (C. Malaveille), at the Aarhus University (H. Autrup) and at the Genetics Research Institute, Milan (S. Garte). A paper on the main effect of metabolic

polymorphisms is in preparation. Here we consider only the interaction with air pollution in modulating the risk of lung cancer.

#### *Statistical analysis*

We have computed odds ratios (OR) and 95% confidence intervals (CI) in conditional logistic regression models. In addition to matching variables (age, sex, country, smoking status, time since recruitment), we also fit models including educational level, BMI, physical activity, intake of fruit, vegetables, meat, alcohol, and energy (continuous) as further adjustment variables. Also age (continuous) was added to models. These variables were potential confounders, being risk factors or protective factors for lung cancer and correlates of air pollution. Cotinine was added in further logistic regression models as an additional marker for exposure to active and passive smoking. We also fit models adjusted by centre; results were virtually identical to unadjusted estimates and are not shown here.

We computed odds ratios for (i) residence nearby roads with different traffic loads; (ii) increases of pollutants by  $10 \mu\text{g}/\text{m}^3$  (as a frequent metric used by most previous researchers); (iii) categories  $<30$  and  $\geq 30 \mu\text{g}/\text{m}^3$  for NO<sub>2</sub> (as used by *e.g.* ref. 6),  $<27$  and  $\geq 27 \mu\text{g}/\text{m}^3$  for PM10, and  $<11$  and  $\geq 11 \mu\text{g}/\text{m}^3$  for SO<sub>2</sub>. These categories correspond to the upper tertile of the distribution with the lower 2 tertiles as the reference category.

#### **Results**

Table I shows average exposure levels by single pollutants in the EPIC centres, for all Genair cases (all cancer types,  $N = 1074$ ) and controls ( $N = 2977$ ). Information is shown for different pollutants and different years of measurement, and only for centres with at least 15 subjects with exposure assessment available. The numbers of subjects change from one pollutant to another because the

TABLE II – DISTRIBUTION OF CASES ( $N = 271$ ) AND CONTROLS ( $N = 737$ ) BY RELEVANT VARIABLES

	No. Lung cancers	No. Matched controls
Gender		
Men	91 (33.6) <sup>1</sup>	241 (33)
Women	180 (66.4)	496 (67)
Age (average and SD)	60.4 (8.8)	60.0 (8.8)
School level		
None/primary	91 (33)	234 (32)
Secondary	105 (39)	320 (43)
University	44 (16)	146 (19)
Missing values	31 (11)	37 (5)
Smoking		
Never smokers	143 (53)	403 (55)
Exsmokers	128 (47)	334 (45)
Country		
France	79 (29)	227 (31)
Italy	18 (6.6)	54 (7)
Spain	10 (3.7)	30 (4)
UK	60 (22)	149 (20)
The Netherlands	16 (6)	42 (6)
Greece	7 (2.6)	21 (3)
Germany	24 (8.9)	70 (9)
Sweden	43 (16.9)	102 (14)
Denmark	14 (5.2)	42 (6)

<sup>1</sup>Values in parentheses are in percentages.

information was not always available for each pollutant in each site, and for each period of measurement. Large intercentre differences can be appreciated, with much higher levels of exposure in Southern Europe. A positive correlation was observed between NO<sub>2</sub>, PM10 and SO<sub>2</sub> (NO<sub>2</sub> and PM10,  $R = 0.59$ ; SO<sub>2</sub> and PM10,  $R = 0.33$ ) and a strong negative correlation with O<sub>3</sub> (NO<sub>2</sub> and O<sub>3</sub>,  $R = -0.58$ ). O<sub>3</sub> was in fact strongly correlated with residence in generally less polluted areas (*e.g.* Copenhagen, Umea, Potsdam and Cambridge) and for this reason was not considered in the following analyses.

Table II shows the distribution of lung cancer cases and matched controls, by demographic variables, country, and smoking. Overall 271 cases and 737 matched controls were available for analysis (Table I), but exposure data (type of road or single pollutants) were obtained for 197 cases, and 556 matched controls, with numbers also varying depending on each pollutant and with incomplete overlapping between pollutants. Exsmokers had cotinine levels (0.33 ng/ml, standard error 0.04) slightly higher than that of never smokers (0.25, SE 0.04); the difference is not statistically significant ( $p = 0.26$ , Wilcoxon test), and is too small to have any impact on cancer risk.

Table III shows an increase in risk associated with residence nearby different types of road (low and heavy traffic load; the information was available only for 186 cases and 508 controls), but odds ratios are not significant. A significant odds ratio was observed in conditional regression only after adjustment by cotinine (OR = 2.87, 95% CI, 1.13–7.35), but this result was likely to be due to the sparse and unstable data. Light traffic corresponds to a traffic flow of about less than 10,000 cars per day, while heavy traffic was more than 10,000 and usually more than 20,000.

There was some indication of effect modification by smoking status. Among never smokers, residence near heavy traffic roads, compared to that near light traffic, had an OR of 1.02; among ex-smokers, the OR was 2.09 (based on regression models for matched pairs without additional adjustment). However, the test for heterogeneity was not statistically significant (Breslow–Day test,  $p = 0.32$ ).

Table IV shows data for single pollutants. Numbers of cases and controls differ among pollutants and with Table III because information about exposure assessment was incomplete and did not overlap completely for the different exposure variables. Table IV shows that nonsignificant elevated risks were associated with increases of 10 µg/m<sup>3</sup> of exposure to NO<sub>2</sub> and SO<sub>2</sub>, but not PM10.

TABLE III – ASSOCIATION BETWEEN LUNG CANCER AND RESIDENCE NEARBY DIFFERENT TYPES OF ROAD. ODDS RATIOS FOR RESIDENCE NEARBY HEAVY TRAFFIC ROADS, FROM CONDITIONAL LOGISTIC REGRESSION FOR MATCHED PAIRS (3 CONTROLS PER CASE)

Exposure category (residence near heavy traffic road)	
Exposed cases	34
Exposed controls	76
Reference category (light traffic)	
Cases	152
Controls	432
OR (95% CI) <sup>1</sup>	1.38 (0.87–2.19)
OR (95% CI) <sup>2</sup>	1.46 (0.89–2.40)
OR (95% CI) <sup>3</sup>	2.87 (1.13–7.35)
OR (95% CI) <sup>4</sup>	1.31 (0.82–2.09)

Matching variables are gender, age ( $\pm 5$  yrs), smoking habits (former or never smoker), time since recruitment, country. Light traffic is the reference category and corresponds to a traffic flow of about <10,000 cars per day, heavy traffic is usually more than 20,000.

<sup>1</sup>Regression model for matched pairs. <sup>2</sup>As in footnote 1 but additionally adjusted by BMI, education, intake of fruit and vegetables, intake of meat, intake of alcohol, physical activity. <sup>3</sup>As in footnote 1 but additionally adjusted by cotinine. <sup>4</sup>As in footnote 1 but additionally adjusted for the occupational index (see text).

An odds ratio of 1.30, with a confidence interval excluding unity, was found for exposures to NO<sub>2</sub> greater than 30 µg/m<sup>3</sup>, while no clear association was observed with PM10 and the association with SO<sub>2</sub> (exposure greater than 11 µg/m<sup>3</sup>) was not significant. The association with NO<sub>2</sub> was not affected by adjustment by cotinine, and persisted also after adjustment by further potential confounders (Table IV). The large OR associated with PM10 after adjustment by cotinine likely reflects instability due to small numbers; there is no independent evidence of a negative confounding effect by cotinine. The association with NO<sub>2</sub> (highest tertile) differed according to smoking status (among exsmokers: OR = 1.59, 95% CI: 1.10–2.30; among never smokers OR = 1.09, 95% CI: 0.78–1.52) but the difference was not significant (Breslow–Day test for heterogeneity  $p = 0.48$ ).

We have also considered the association with high-risk jobs (main job held in life) by creating an occupational index based on welding, shipyard working, asbestos production and use, working with asphalt, metal working, construction and demolition work. The odds ratio for such index in conditional logistic regression was 1.86 (95% CI, 1.02–3.39). When we adjusted the estimates for air pollution by the occupational index the estimates were virtually unchanged (OR = 1.18, 0.80–1.72 for NO<sub>2</sub>, increment by 10 µg/m<sup>3</sup>; 1.31, 0.82–2.09 for heavy-traffic roads) (Tables III and IV).

#### Genetic polymorphisms

We analyzed 3 pathways for genes involved in carcinogen metabolism: a detoxifying pathway, one that activates carcinogens, and one involved in oxidative damage scavenging. Detailed results are provided elsewhere (paper in preparation). Among the different genes we have considered, only a polymorphism for NQO1 (involved in oxidative damage scavenging) was strongly associated with lung cancer, with an odds ratio of 8.06 (95% CI, 1.74–37.41) for the homozygous variant. In a conditional regression model including NO<sub>2</sub> the odds ratio for the NQO1 polymorphism (homozygous) was 9.75 (1.16–82.21), and the OR for each 10 µg/m<sup>3</sup> increment in NO<sub>2</sub> was 1.21 (0.53–2.72). The interactive term between exposure to NO<sub>2</sub> and the NQO1 polymorphism was not significant ( $p = 0.84$ ).

#### Discussion

Conducting studies on air pollution in Europe is valuable because ambient levels of several key pollutants are more variable within Europe than in the United States, and are usually higher. Results from previous prospective studies suggest that air pollution is likely to increase the risk of lung cancer. The Adventist

TABLE IV – ASSOCIATION BETWEEN LUNG CANCER AND AIR POLLUTANTS. ODDS RATIOS (OR) ARE FROM CONDITIONAL LOGISTIC REGRESSION FOR MATCHED PAIRS (3 CONTROLS PER CASE)

	Pollutant		SO <sub>2</sub>
	NO <sub>2</sub>	PM10	
No. Cases/controls with exposure assessment	122/352	113/312	135/397
Increments by 10 µg/m <sup>3</sup> OR (95% CI)	1.14 (0.78–1.67)	0.91 (0.70–1.18)	1.08 (0.89–1.30)
Analysis by tertiles:			
Reference category (lowest + intermediate tertiles) <sup>1</sup>			
OR	1.0	1.0	1.0
Exposure category (upper tertile) <sup>1</sup>			
Exposed cases	46	53	43
OR (95% CI)	1.30 (1.02–1.66)	0.98 (0.66–1.45)	1.01 (0.84–1.22)
OR (95% CI) <sup>2</sup>	1.56 (1.13–2.16)	1.05 (0.65–1.69)	1.15 (0.92–1.43)
OR (95% CI) <sup>3</sup>	1.62 (0.93–2.83)	2.85 (0.97–8.33)	1.15 (0.85–1.56)
OR (95% CI) <sup>4</sup>	1.37 (1.06–1.75)	1.02 (0.68–1.51)	1.00 (0.83–1.21)

Matching variables are gender, age (65 yrs), smoking habits (former or never smoker), time since recruitment and country.

<sup>1</sup>comparisons are  $\geq 30$  vs  $< 30$  µg/m<sup>3</sup> for NO<sub>2</sub>,  $\geq 27$  vs  $< 27$  µg/m<sup>3</sup> for PM10, and  $\geq 11$  vs  $< 11$  µg/m<sup>3</sup> for SO<sub>2</sub> (see text). –<sup>2</sup>As in footnote 1 but additionally adjusted by BMI, education, intake of fruit and vegetables, intake of meat, intake of alcohol, physical activity. –<sup>3</sup>As in footnote 1 but additionally adjusted by cotinine. –<sup>4</sup>As in footnote 1 but additionally adjusted by occupational index (see text).

Health Study on SMOG (AHSMOG)<sup>3</sup> was based on 6,338 California Seventh Day Adventists followed from 1977 through 1992. The investigators reported substantial increases in relative risks of lung cancer among men in relation to long-term ambient concentrations of PM10 (RR = 5.21, 95% CI: 1.94–13.99, associated with an interquartile range of 24 µg/m<sup>3</sup>) and sulfur dioxide (RR = 2.66, 95% CI: 1.62–4.39, associated with an interquartile range of 3.7 ppb); however, as suggested by the wide confidence intervals, these results were based on very few cases ( $n = 16$  histologically confirmed cases). The Harvard Six Cities Study<sup>4</sup> was based on 8,111 residents of 6 US cities, followed from 1974 through 1989. Exposure was estimated on the basis of average levels of pollution over the risk period, assuming residential stability. Relative risk estimates were adjusted for age, gender, smoking habits, body mass index and education. The total number of lung cancer deaths was reported as 8.4% of 1,429 (or 120). The difference in the long-term average PM concentrations between the most and least polluted cities was approximately 20 µg/m<sup>3</sup>, corresponding to a relative risk of 1.37, *i.e.* an approximately 18.5% increase in risk per 10 µg/m<sup>3</sup>. The third and largest US investigation is the American Cancer Society Study<sup>5,11</sup> (ACS-II), based on the mortality experience of approximately 500,000 adult men and women who were followed from 1982 through 1998. Participants were assigned to metropolitan areas of residence, and mean PM2.5 concentrations were compiled for each metropolitan area from several data sources. Personal information on risk factors (confounders or effect modifiers) was collected by questionnaire at enrolment. The study indicated a significantly increased mortality risk ratio for lung cancer (RR = 1.14, 95% CI: 1.04–1.23) for a difference of 10 µg/m<sup>3</sup> of PM2.5, controlling for age, gender, race, smoking, education, marital status, body mass, alcohol consumption, occupational exposure and diet.

The first published European cohort study examining long-term exposure to air pollution was conducted in the Netherlands.<sup>6</sup> Enrolment in the Netherlands Cohort study on Diet and Cancer (NLCS) started in 1986. At baseline there were 120,852 adults (age, 55–59 years) living in 204 small towns and large cities throughout the Netherlands. A baseline questionnaire requesting information on active and passive smoking, occupation, education, nutrition and up to 4 residential addresses was administered. For the air pollution study, a subcohort of 5,000 subjects was used to estimate person-time within a case-cohort design. Mortality between 1986 and 1994 was studied in the sub-cohort. Exposure assessment was based on the estimation of long-term exposure at the baseline home address, and included assessment of NO<sub>2</sub> and black smoke. The risk of lung cancer was slightly elevated, but the

estimate was based on relatively few cases ( $n = 60$ ; RR = 1.06, 95% CI: 0.43–2.63, for a 10 µg/m<sup>3</sup> increment in exposure to black smoke, and 1.25, 95% CI: 0.42–3.72, for a 30 µg/m<sup>3</sup> increment in exposure to nitrogen dioxide). A second European study has been reported from Norway.<sup>7</sup> Nafstad and co-workers studied lung cancer incidence among 16,209 (40–49 years–old) men living in Oslo, who were recruited in 1972–73. Exposure assessment was based on measured concentrations of two gaseous air pollutants (NO<sub>2</sub> and SO<sub>2</sub>) available from 1974 to 1995. The population was followed through the Cancer Registry. Information on several potential confounders (smoking, social class, occupation, physical exercise) was available. The authors found a risk ratio of 1.08 (1.02–1.15) for an increment of 10 µg/m<sup>3</sup> of NO<sub>2</sub>, with an exposure–response relationship.

In our large prospective study, we have found that residence in proximity to heavy-traffic roads, or exposure to NO<sub>2</sub> (particularly when considering levels of exposure greater than 30 µg/m<sup>3</sup>), can increase the risk of lung cancer. The choice of 30 µg/m<sup>3</sup> corresponds to the highest tertile in the distribution of the enrolled subjects. Major strengths of the study include: (i) the longitudinal study design that rules out major information bias; (ii) exposure assessment based on monitoring stations; (iii) inclusion of a large range of European populations; (iv) accurate information on potential confounders; (v) the availability of cotinine measurements to allow for residual confounding from smoking, including environmental tobacco smoke. Limitations include the following: (i) the availability of only one address of residence for the study subjects (however, only 5–10% of the subjects had changed their residence during follow-up, depending on country); (ii) relatively limited statistical power due to small numbers of subjects; (iii) potential confounding from unknown variables, including indoor pollution related to cooking practices; (iv) reciprocal correlation and potential mutual confounding among different pollutants; (v) a short follow-up period; (f) a possibly poor representativeness of monitoring stations in time and place; and (g) a relatively limited number of air pollutant measurements in each centre, which might imply confounding by other centre-related characteristics; however, centre-adjusted estimates were virtually identical to unadjusted estimates.

The reciprocal correlation among pollutants is a general problem of this kind of studies. It is likely that PM10, NO<sub>2</sub> and O<sub>3</sub> act by a similar mechanism, *i.e.* oxidative stress.<sup>12</sup> Therefore, it is not necessarily relevant, at least in causal assessment, to distinguish among single pollutants; rather, they might interact in unpredictable ways. O<sub>3</sub> has been found to damage DNA in 2 epidemiological studies, including the present one,<sup>13,14</sup> but its role is more difficult to assess because of strong negative association with other

pollutants. The sources of pollutants are different: NO<sub>2</sub> and PM10 mainly reflect traffic (but the latter includes particles originating from remote emissions, while NO<sub>2</sub> mainly reflects local combustion sources); SO<sub>2</sub> has mainly industrial sources, and O<sub>3</sub> is strongly influenced by sun irradiation and tends to have higher concentrations in the countryside. The issue of disentangling the effects of single pollutants is still open and cannot be resolved by the present study.

Interestingly, among the different genes we have considered in our study of gene–environment interactions, a polymorphism for NQO1 was strongly associated with lung cancer, with an odds ratio of 8.06 (95% CI, 1.74–37.41) for the homozygous variant. The association of lung cancer with this metabolic polymorphism involved in oxidative damage scavenging reinforces previous evidence that air pollutants might act through oxidative damage to DNA.

The observation of an elevated risk for living on a major road is consistent with studies in Stockholm<sup>15</sup> and Oslo,<sup>7</sup> where traffic related NO<sub>2</sub> at the residential address was associated with increased

risk of lung cancer. In those studies dispersion models were used to quantify the exposure at the home address. In the current study, covering a much wider region, we had to rely on a simpler characterization of the residential address. Various studies have however documented substantial contrasts related to the function of a road. In a study in Amsterdam, we observed that roads that were part of the main road network had approximately twice higher concentrations of soot, benzene and PAHs than those simultaneously measured in minor streets.<sup>16</sup> Also Nafstad *et al.*<sup>7</sup> and Nyberg *et al.*<sup>15</sup> found that the risk of lung cancer was mainly associated with levels of exposure greater than 30 µg/m<sup>3</sup>. This is lower than the guideline level of 40 µg/m<sup>3</sup> set by the World Health Organization for annual average NO<sub>2</sub> concentration<sup>17</sup> (see also [http://www.euro.who.int/document/aiaq/71nitrogen\\_dioxide.pdf](http://www.euro.who.int/document/aiaq/71nitrogen_dioxide.pdf)).

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APPENDIX – NUMBER OF RELEVANT AVAILABLE BACKGROUND NETWORK SITES<sup>1</sup>

Country	Center	N sites	N urban sites	N rural sites
France	Lyon + Paris	365	326 <sup>2</sup>	39
Italy	Florence	3	2	1
	Turin	8	3	5
	Naples	3	3	0
	Varese	7	2	5
Spain	Oviedo	3	2	1
	Pamplona	1	1	0
	San Sebastian	3	0	3
	Murcia	0	0	0
United Kingdom	Cambridge	3	1	2
	Oxford	112	93	19
The Netherlands	Utrecht	3	1	2
	Bilthoven	6	3	3
Germany	Heidelberg	3	1	2
	Potsdam	4	1	3
Sweden	Umeå	5	2	3
	Malmö	3	3	0
Denmark	Aarhus	0	0	0
	Copenhagen	2	1	1

<sup>1</sup>In 1999 or year close to 1999; not all pollutants measured at all sites.—<sup>2</sup>Urban ( $n = 225$ ) and periurban ( $n = 101$ ) added automatic network sites only (there are additional Smoke and SO<sub>2</sub> and NO<sub>2</sub> diffusion tube survey sites).