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DNA Adducts and Lung Cancer Risk: A Prospective Study

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Abstract

Objectives were to investigate prospectively the ability of DNA adducts to predict cancer and to study the determinants of adducts, especially air pollutants. DNA adducts were measured in a case-control study nested in the European Prospective Investigation into Cancer and Nutrition (EPIC) investigation. Cases included newly diagnosed lung cancer (n = 115), upper respiratory cancers (pharynx and larynx; n = 82), bladder cancer (n = 124), leukemia (n = 166), and chronic obstructive pulmonary disease or emphysema deaths (n = 77) accrued after a median follow-up of 7 years among the EPIC former smokers and never-smokers. Three controls per case were matched for questionnaire analyses and two controls per case for laboratory analyses. Matching criteria were gender, age, smoking status, country of recruitment, and follow-up time. Individual exposure to air pollution was assessed using concentration data from monitoring stations in routine air quality monitoring networks. Leukocyte DNA adducts were analyzed blindly using ^{32}P postlabeling technique. Adducts were associated with the subsequent risk of lung cancer, with an odds ratio (OR) of 1.86 [95% confidence interval (95% CI), 0.88-3.93] when comparing detectable versus nondetectable adducts. The association with lung cancer was stronger in never-smokers (OR, 4.04; 95% CI, 1.06-15.42) and among the younger age groups. After exclusion of the cancers occurring in the first 36 months of follow-up, the OR was 4.16 (95% CI, 1.24-13.88). A positive association was found between DNA adducts and ozone (O_3) concentration. Our prospective study suggests that leukocyte DNA adducts may predict lung cancer risk of never-smokers. Besides, the association of DNA adduct levels with O_3 indicates a possible role for photochemical smog in determining DNA damage. (Cancer Res 2005; 65(17): 8042-8)

Introduction

Ambient air, particularly in densely populated areas, contains a variety of known carcinogens (1). The incomplete combustion of fossil fuels for power generation and transportation is the primary source of environmental carcinogens and contributes heavily to outdoor pollution in most urban settings. Particulate matters (PM) and gas phase contain hundreds of volatile pyrolysis products, including polycyclic aromatic hydrocarbons (PAH) and nitro-PAHs (2). In addition to those compounds released directly into the environment by combustion processes, others are created from primary combustion products via photochemical reactions in the outdoor environment (3). PAHs are one of the major classes of carcinogens present in atmosphere capable to form DNA adducts leading to DNA damage after metabolic activation (1, 2). When unrepaired, DNA adducts can cause mutations, including mutational hotspots in the *p53* tumor suppressor gene (4), which may ultimately induce cancer formation.

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Epidemiologic studies over the last years have suggested consistently that ambient air pollution may be responsible for increased rates of lung cancer (5). Relative to cigarette smoking, the excess lung cancer risk associated with air pollution is lower. However, given the ubiquity of outdoor pollution, the contribution of this exposure across the general population may be relevant. DNA adducts have been widely used to assess human exposures to genotoxic chemicals. Adducts tend to be higher among subjects heavily exposed to urban and occupational air pollutants (6, 7). In addition, high DNA adduct levels have been suggested to be predictive of lung cancer risk, reflecting both exposure to environmental carcinogens and individual susceptibility (8–11).

The current study was carried out to investigate prospectively the ability of DNA adducts to predict cancer and to study the determinants of DNA adduct levels, particularly air pollutants, in nonsmokers in 10 European countries (France, Denmark, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom). This was done with a nested case-control design in the European Prospective Investigation into Cancer and Nutrition (EPIC) investigation. Exposure assessment was made by experts based on the already available questionnaires plus objective information on air pollution in European cities. The main advantage of this longitudinal study was that the levels of DNA adducts were measured in blood samples collected several years before the onset of cancer. Thus, adducts were not influenced by early effects of cancer itself.

Subjects and Methods

Selection of subjects and collection of specimens: the European Prospective Investigation into Cancer and Nutrition/Gen-Air prospective study. EPIC is a multicenter European study, coordinated by the IARC (Lyon, France), in which >500,000 healthy volunteers were recruited in 10 European countries (France, Denmark, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom) corresponding to 23 recruitment centers. The cohort includes subjects of both genders, mostly ages 35 to 74 years at recruitment. Recruitment took place between 1993 and 1998. Detailed dietary and lifestyle histories collected mainly through self-administered questionnaires plus a 24-hour dietary recall through person-to-person interview (in a 10% sample), anthropometric measurements, and 30 to 40 mL blood sample are available. All questionnaire information is available in a computerized format. Signed informed consent forms were collected from all participants (except a subgroup of the Oxford cohort who gave consent on postal questionnaires).

The follow-up was based on population cancer registries in seven of the participating countries: Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom. In France, Germany, and Greece, a combination of methods was used, including health insurance records, cancer and pathology registries, and active follow-up through study participants and their next-of-kin. Mortality data were also obtained from either the cancer registry or mortality registries at the regional or national level. Follow-up was virtually 100% complete. We used the 10th Revision of the International Statistical Classification of Diseases, Injuries, and Causes of Death. All the information is centralized both at the national level and at IARC.

Gen-Air is a case-control study nested within the EPIC cohort, aiming at studying the relationship between some types of cancer and air pollution or environmental tobacco smoke (ETS). Cases are subjects with bladder, lung, oral, pharyngeal, or laryngeal cancers or leukemia, all newly diagnosed after recruitment. Also, deaths from respiratory diseases [i.e., chronic obstructive pulmonary disease (COPD) and emphysema] were identified and included. These diagnoses were chosen because they are suspected of being associated with air pollution or ETS exposure. Only nonsmokers or ex-smokers since at least 10 years have been included in Gen-Air. We have matched three controls per case for exposure assessment and the analysis of questionnaire data and two controls per case for laboratory analyses. Matching criteria were gender, age (±5 years), smoking status (never or former smoker), country of recruitment, and follow-up time. Matching was introduced to allow strict control of potentially confounding variables, considering that other risk factors may be stronger than ETS or air pollution. In addition, matching was needed for laboratory analyses to avoid differential sample degradation between cases and controls. Mean follow-up was 89 months (minimum, 51 months; maximum, 123 months).

Gen-Air has been approved by the Ethical Committee of the IARC, and by the local Ethical Committees of the 23 centers.

We have identified 1,074 cases, including 271 with lung cancer, who met the protocol criteria. The Malmö center has decided not to allow the use of their blood samples, but it participates in the rest of the project. After exclusion of the 231 Malmö cases, 843 cases were successfully matched (2:1) to 1,564 controls. DNA was available for 1,650 subjects (68% of those eligible, including 564 cases and 1,086 controls). In particular, we obtained DNA for only 30 subjects from France, of 306 eligible, and for 50% (494 of 982) of the eligible U.K. subjects. For the other countries, the proportion with DNA was ~90%. Blood samples of cases and controls for centers, which have released an ethical approval, have been sent to laboratories for investigation.

Air pollution exposure assessment. Exposure to air pollution was assessed using data from monitoring stations in routine air quality monitoring networks. We identified the study area of the individual EPIC cohorts and then obtained concentration data from available network stations relevant for the study area. Data were obtained through searching AIRBASE, the air pollution database from the European Topic Center on Air Quality in Bilthoven, the Netherlands (http://arch.rivm.nl/ieweb/ieweb/index.html?etc.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 to 1999 for all pollutants routinely monitored in Europe [ozone (O_3), sulfur oxide (SO_2), nitrous oxide (NO_2), NO, CO, benzene, and particulate (total suspended particulate, PM_{10}, $PM_{2.5}$, black smoke, and benzo(*a*)pyrene [B(a)P])]. Because we were interested in long-term health effects, we obtained annual average concentrations and winter/summer data (only for some cohorts). In addition, we obtained data on monitoring sites and monitoring methods to assess suitability of the methods.

The average concentration from background monitoring stations in the place of residence was assigned to each study subject. For each home address, we also 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 (12–14). For all homes, we used detailed Internet maps to evaluate whether the home was located in a major street (yes/no). The use of indicator variables follows a series of epidemiologic studies on chronic respiratory health effects of traffic-related air pollution (e.g., refs. 15, 16). Exposure assessment could not be done for the Greek cohort.

Detailed information on the methodology and levels of air pollutants in different countries is given in a separate article.[35]

DNA extraction and purification. DNA was extracted and purified from buffy coat using a method requiring enzymatic digestion of RNA and proteins followed by phenol/chloroform extractions (7), with the exception of the Danish samples that were extracted and purified from lymphocytes using a technique based on a salting out procedure (17). RNase treatment was not included in this protocol; thus, the Danish samples were treated with RNase A and T1 before ^{32}P postlabeling. Because DNA extraction and DNA source have been reported to significantly influence DNA adduct levels (6, 18), Danish adduct data were excluded from some statistical analyses. Coded DNA was stored at −80°C until laboratory analysis.

[^{32}P]DNA postlabeling technique. DNA adducts were analyzed blindly using the nuclease P1 technique (7, 19). DNA samples (1-5 μg) were digested

[35] P. Vineis et al. Air pollution and risk of lung cancer in a prospective study in Europe, submitted for publication.

with micrococcal nuclease (0.46 unit) and spleen phosphodiesterase (0.0174 unit). After treatment with 5 μg nuclease P1, samples were labeled by incubation with 25 to 50 μCi carrier-free [γ-^{32}P]ATP (3,000 Ci/mmol/L) and 10 units T4-polynucleotide kinase. Detection of DNA adducts was carried out by chromatography: plates were first developed using 1 mol/L sodium phosphate (pH 6.8); then, adduct resolution was achieved with a two-dimensional chromatography using 4 mol/L lithium formate, 7.5 mol/L urea (pH 3.5), 0.65 mol/L lithium chloride, 0.45 mol/L Tris, and 7.7 mol/L urea (pH 8.0). The plates were finally developed using 1.7 mol/L sodium phosphate (pH 5.0).

Detection and quantification of DNA adducts and total nucleotides was done by Cerenkov counting or storage phosphorimaging techniques. DNA adducts were expressed such as relative adduct labeling = cpm or screen response (volume) in adducted nucleotides / cpm or screen response (volume) in total nucleotides. A positive control sample [e.g., B(a)P DNA adducts] from liver of mice treated i.p. with 0.5 mg B(a)P was routinely processed.

As a validation analysis in Gen-Air, measurement of adducts has been repeated in 27% of the subjects (n = 311). An excellent correlation coefficient has been found (r = 0.93; P < 0.0001). Further validation data been have reported in Peluso et al. (19).

Chemicals. RNase A and T1, proteinase K, micrococcal nuclease, spleen phosphodiesterase, nuclease P1, and Kodak films were purchased from Sigma Chemical (St. Louis, MO/Steinheim, Germany). Carrier-free [γ-^{32}P]ATP (3,000 Ci/mmol) was from Amersham (Buckinghamshire, United Kingdom). T4-polynucleotide kinase was from Epicentre Technologies (Madison, WI). Poly(ethyleneimine)-cellulose TLC sheets were from Macherey-Nagel (Postfach, Germany) and Merck (Darmstadt, Germany). All other chemicals were of analytic grade and used without further purification.

Statistical analysis. Our analytic strategy was as follows: (*a*) We were interested primarily in the relationship between DNA adduct levels and the risk of lung cancer that we have explored with both crude estimates and conditional logistic models adjusting for relevant confounders. (*b*) The main goal of Gen-Air was the investigation of air pollution; therefore, we considered also air pollutants as potential determinants of adduct levels in controls. We have computed odds ratios (OR) and 95% confidence intervals (95% CI) in conditional logistic regression models. In addition to matching variables, the models included educational level, body mass index (BMI), physical activity, and intake of fruits, vegetables, meat, and energy (continuous variables) as further adjustment variables. These are variables that have been found associated with both adduct levels and the risk of lung cancer in previous investigations. More complex issues of the relationship among DNA adducts, the risk of lung cancer, and the role played by confounders and effect modifiers (e.g., diet) will be considered in a second article.[36]

We dichotomized DNA adducts into undetectable/detectable values (limit of detection of 0.1 DNA adducts per 10^9 normal nucleotides; refs. 7, 19) and, among the detectable, below and above the median in controls (0.6 DNA adducts per 10^8 normal nucleotides). We also used adducts as a continuous variable after log transformation.

Results

A diagonal radioactive zone (DRZ) or distinct adduct spots were detected in DNA adduct positive samples. Figure 1 reports the pattern of DNA adducts (e.g., the DRZ) that was more frequently found in the chromatograms of never-smoker lung cancer cases (A and B) and never-smoker controls (C and D). The average intensity of adduct patterns was stronger in the chromatograms of healthy nonsmokers who developed a lung cancer in the following years in comparison with the other samples. Conversely, lower or no chromatographic qualitative differences emerged between the DNA adduct patterns of the healthy subjects who developed a cancer at a different site and of controls.

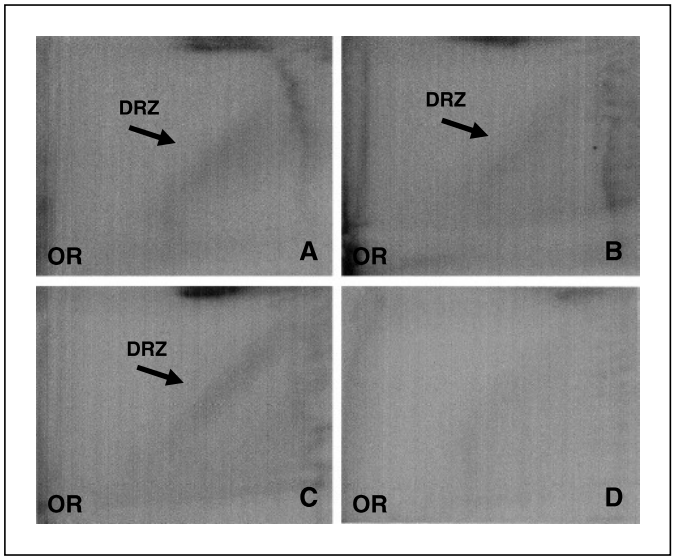

Figure 1. DNA adduct patterns found in the chromatograms of two never-smokers who developed a lung cancer in the following 2 to 12 years (*A* and *B*) and in those of two never-smoker controls (*C* and *D*).

Because DNA was available for only 68% of the eligible cases, we compared the distribution of relevant variables for subjects with and those without DNA. When France and United Kingdom were included, there was a statistically significant difference for the proportion of women according to DNA status (59% women among subjects without DNA versus 48% subjects with DNA; P = 0.0001) and for social class distribution (with a lower proportion of educated people among those with DNA; P < 0.0001). No differences were noted for smoking status and age. After the exclusion of France and United Kingdom, the distribution by sex (P = 0.9), age (P = 0.6), smoking status (P = 0.8), and education (P = 0.2) did not differ between subjects with DNA and subjects without DNA. Table 1 shows the distribution of detectable and undetectable adducts by demographic and exposure variables and by cases and controls. Of 115 lung cancer cases, 102 had detectable adduct levels versus 893 of 1,086 controls (crude OR, 1.69; 95% CI, 0.93-3.08).

The mean adduct levels were 0.83 DNA adducts per 10^8 normal nucleotides in never-smoker cases with lung cancer and 0.70 DNA adducts per 10^8 normal nucleotides in never-smoker controls (P = 0.12) and 0.62 adducts per 10^8 normal nucleotides in ex-smoker lung cancer cases and 0.68 adducts per 10^8 normal nucleotides in ex-smoker controls (P = 0.4).

Adducts were associated with the subsequent risk of lung cancer, with an OR of 1.86 (95% CI, 0.88-3.93; Table 2) when comparing detectable versus nondetectable adducts. We modeled risk estimates by excluding subjects whose cancer arose soon after blood collection; these estimates exceeded 1.86 [e.g., after exclusion of the first 36 months since recruitment, the OR was 4.16 (95% CI, 1.24-13.88)]. The association with lung cancer was stronger in never-smokers (OR, 4.04; 95% CI, 1.06-15.42) and among the younger age groups although not in a statistically significant manner (Table 2). However, such estimates are very unstable due to small numbers. The association with lung cancer was consistent across countries, except in the Netherlands and United Kingdom. ORs (95% CIs) were Italy 3.81 (0.48-30.09), Spain 1.82 (0.22-15.21),

[36] In preparation.

Table 1. Distribution of DNA adducts (detectable/-undetectable) by cancer cases and controls, by demographic variables, and by selected exposures

	DNA adducts	
	Undetectable	Detectable
Cancer cases (n = 564)		
Lung (n = 115)	13 (11.3)	102 (88.7)
Upper aerodigestive tract (n = 82)	14 (17.1)	68 (82.9)
Bladder (n = 124)	24 (19.4)	100 (80.6)
Leukemia (n = 166)	30 (18.0)	136 (82.0)
COPD (n = 77)	12 (15.6)	65 (84.4)
Controls (n = 1,086)	193 (17.8)	893 (82.2)
Gender		
Women	133 (16.7)	662 (83.3)
Men	152 (17.8)	701 (82.2)
P	0.77	
Age groups (y)		
<55	69 (19.2)	291 (80.8)
55-65	149 (18.0)	681 (82.0)
>65	67 (14.6)	391 (85.4)
P	0.40	
Smoking habits		
Never-smoker	160 (18.0)	731 (82.0)
Former smoker	125 (16.5)	632 (83.5)
P	0.58	
School completed*		
None	17 (17.2)	82 (82.8)
Primary	85 (15.5)	463 (84.5)
Secondary	116 (18.7)	503 (81.3)
University	51 (18.2)	229 (81.8)
P	0.86	
Country		
France	1	29
Italy	34	159
Spain	26	135
United Kingdom	66	428
The Netherlands	29	105
Greece	12	78
Germany	69	221
Denmark	48	208

*Unknown (n = 102).

United Kingdom 1.0 (0.32-2.93), the Netherlands 0.68 (0.19-2.40), and Germany 2.04 (0.58-7.19); in the remaining countries, there were no cases with undetectable adducts (i.e., all cases had levels greater than 0).

When we considered the trend from undetectable levels to levels below median and above median, an increase was seen only among never-smokers (Table 2). In general, there was not a clear-cut dose-response relationship with increasing DNA adducts. In ex-smokers, in particular, the ORs decreased from levels lower to levels higher than the median. When considering adducts as a continuous measurement, the OR for each unit increase of adducts was 1.25 (95% CI, 0.69-2.27). The logarithm of adducts was associated with cancer risk in a statistically significant way only in never-smokers (Table 2).

A nonstatistically significant association in never-smokers is also shown for upper aerodigestive cancers (mouth, pharynx, and larynx) that share some risk factors with lung cancer (particularly exposure to PAHs; Table 3). Such association was found in conditional regression and not in crude estimates. No association has been found with other cancers or COPD.

Role of exposures. We have examined the potential role of several exposures in influencing the levels of adducts in controls. Social class was not associated with adduct levels (P = 0.22), and information about occupational exposures was too scanty to allow an evaluation. In a multivariate model, including age, gender, country, and educational level, we found a weak association between the presence of adducts and smoking status (OR, 1.15; 95% CI, 0.87-1.54 for former versus never-smokers). The association with ETS was more complex: a negative association was found with the presence/absence of adducts (OR, 0.58; 95% CI, 0.34-1.00), whereas a weakly positive association was detected with the level of adducts (OR, 1.10; 95% CI, 0.67-1.80). We have reported the main associations between lung cancer and ETS or air pollution in separate articles.[36]

We calculated correlation coefficients between log(adducts) and the air pollutants that we have considered. A positive association was observed for O_3 and a statistically significant negative association for PM_{10} (data not shown). When all the pollutants were included in a multivariate model (Table 4), only the association with O_3 measured in 1990 to 1994 clearly persisted. Only adducts measured after the estimation of air pollutant levels were considered. In logistic models using detectable/undetectable levels of adducts as the dependent variable, only O_3 was associated in a statistically significant way to adducts after adjustment for confounders. A strong association is also shown for PM_{10}, but the estimate is very unstable.

Discussion

Air pollution has been reported to increase lung cancer risk (5, 20, 21). The risk of lung cancer death has been suggested to increase by 8% for every 10 μg of fine particles in a cubic meter of inhaled air (21). In the present case-control study nested in the EPIC investigation, we hypothesized that the levels of leukocyte DNA adducts could predict lung cancer in healthy never-smokers and former smokers. Never-smokers with detectable amounts of DNA damage were approximately twice more likely to be diagnosed with lung cancer years later than never-smokers with nondetectable adduct levels. Less convincing results were, instead, obtained with former smokers. Interestingly, DNA adducts were also suggested to be associated with cancers of the upper aerodigestive tract in never-smokers (excess not statistically significant and found only in conditional regression models). Nose, oral cavity, and larynx are primary sites of air entry and consequently can be a target of outdoor pollution exposures.

In a meta-analysis of cancer and DNA adducts (11), we have suggested that DNA adducts can be predictive of lung cancer, particularly in current smokers. In the meta-analysis, current smokers showed a significant difference between cases and controls, with cases having 83% higher levels of adducts than controls. Results for never-smokers were inconsistent, but two studies showed a significant positive difference between cases and controls (one based on the measurement of adducts in the lung tissue and one based on measurements in leukocytes of bladder cancer cases and controls). Overall, the weighed mean difference for never-smokers was 47%, not statistically significant and almost

Table 2. DNA adducts and lung cancer: crude estimates and conditional logistic regression models

	Cases/controls	Crude OR (95% CI)	Conditional OR (95% CI)
Detectable adducts	102/893		
Nondetectable adducts	13/193		
All subjects		1.69 (0.93-3.08)	1.86 (0.88-3.93)
Adjusted by center			2.05 (0.83-5.04)
Never-smokers		1.67 (0.69-4.01)	4.04 (1.06-15.42)
Ex-smokers		1.69 (0.74-3.84)	2.26 (0.78-6.53)
Age groups (y)			
<55		6.90 (0.91-52.1)	5.56 (0.75-41.23)
55-65		1.07 (0.62-1.84)	2.62 (0.53-13.00)
>65		1.51 (0.67-3.44)	0.81 (0.14-4.47)
Above versus below median in controls (0.6 DNA adducts per 10^8 normal nucleotides), excluding nondetectable levels			
All subjects			
Below median	42/322	1.00 (—)	1.00 (—)
Above median	60/571	0.98 (0.67-1.44)	0.83 (0.49-1.41)
Never-smokers			
Below median	11/167	1.00 (—)	1.00 (—)
Above median	34/318	1.73 (0.94-3.16)	2.35 (0.98-5.65)
Ex-smokers			
Below median	31/155	1.00 (—)	1.00 (—)
Above median	26/253	0.64 (0.38-1.10)	0.36 (0.16-0.78)
Nondetectable adducts, below and above median (median adduct value in controls, 0.6 DNA adducts per 10^8 normal nucleotides)			
All subjects			
Undetectable*		1.00 (—)	1.00 (—)
Below median		1.95 (1.01-3.70)	2.51 (0.96-6.55)
Above median		1.56 (0.84-2.90)	1.54 (0.58-4.07)
DNA adducts as continuous variable			1.25 (0.69-2.27)
P for trend		0.84†	
Never-smokers			
Undetectable		1.00 (—)	1.00 (—)
Below median		1.18 (0.42-3.30)	4.47 (0.73-27.24)
Above median		1.92 (0.78-4.71)	6.81 (1.11-41.64)
DNA adducts as continuous variable			2.17 (0.96-4.89)
P for trend		0.04†	
Ex-smokers			
Undetectable		1.00 (—)	1.00 (—)
Below median		2.43 (1.02-5.74)	4.93 (1.06-22.98)
Above median		1.25 (0.52-2.97)	0.80 (0.18-3.65)
DNA adducts as continuous variable			0.59 (0.27-1.33)
P for trend		0.17 †	

NOTE: In addition to matching variables, estimates are adjusted for education (years of school), BMI, physical activity, and intake of fruits, vegetables, meat, and energy.
*Reference category. Conditional ORs adjusted as above and by center.
†Log transformation of DNA adducts as continuous variable.

entirely attributable to the two studies mentioned above. The interpretation of the meta-analysis is limited by the fact that in case-control studies the biomarker may reflect the disease rather than the etiology. However, an exception is represented by the cohort study by Tang et al. (9), in which DNA adducts of smokers have been found to be prospectively predictive of lung cancer outcome (9, 10). The importance of that study, like the present one, rests on the measurement of adducts in blood samples that were collected years before cancer onset, thus ruling out the possibility that the higher adduct levels were due to metabolic changes associated with an already existing cancer. However, it should be noted that Tang et al. found an association with lung cancer among current smokers only, whereas we find it among never-smokers. The reasons for such a discrepancy, and for a lack of association in former smokers in our study, are unclear. Low statistical power could be one explanation. In addition, a survival effect might be invoked, because mortality in former smokers is very high, particularly at older ages and after few years since quitting. Those who are still alive after 10 years since quitting might have greater DNA repair ability than others.

We have to consider also the limitations of our study. First, we were not able to obtain DNA for all the eligible subjects but from

Table 3. DNA adducts (detectable versus nondetectable) and other cancers

	Crude OR (95% CI)	Conditional OR (95% CI)
Leukemia	0.98 (0.64-1.50)	1.03 (0.57-1.87)
Bladder	0.90 (0.56-1.44)	0.70 (0.36-1.33)
Upper aerodigestive tract, all	1.00 (0.43-2.27)	2.57 (0.78-8.45)
Nonsmokers	1.04 (0.29-3.67)	8.91 (0.71-112)
Ex-smokers	0.94 (0.31-2.84)	1.61 (0.24-10.95)

NOTE: Conditional logistic regression models. In addition to matching variables, estimates are adjusted for education (years of school), BMI, physical activity, and intake of fruits, vegetables, meat, and energy. For COPD, there were very few subjects to allow estimation.

68% on average. In particular, we obtained DNA for only 10% subjects from France and for 50% of the eligible U.K. subjects. After exclusion of these countries, the distribution by relevant variables did not differ between subjects with DNA and subjects without DNA. After exclusion of United Kingdom and France, the OR for detectable versus nondetectable adducts (same logistic model as in Table 2) was 1.87 (95% CI, 0.76-4.60; i.e., virtually unchanged).

In addition, the level of measurement error for DNA adducts is not well known but seems to be high (coefficient of variation, ~20-30%; ref. 22). However, the effect of measurement error is to attenuate a relationship if error is evenly distributed in the comparison groups. Therefore, measurement error is expected to blur existing associations rather than to reveal false associations. In the present investigation, the coefficient of variation was 19%. Measurement of DNA adducts in blood samples only indirectly refers to changes in the target tissues. Dosimetry studies should ideally be conducted at the level of the site of cancer. This is an obvious limitation in the interpretation of the present epidemiologic findings. However, a significant correlation between DNA adduct levels in the lung and blood samples has been reported (23, 24). In addition, the DRZ adduct profile we have observed has been described among subjects exposed to air pollution, such as police officers, bus drivers, and newspaper vendors (6, 7). This adduct profile broadly reflects exposures to PAHs and other aromatic compounds and suggests that such DNA adducts were primarily formed by complex mixtures of these pollutants.

DNA damage production reflects primarily carcinogen exposures, but it is also regulated from inherited and acquired susceptibilities. Indeed, age, gender, BMI, physical exercise, consumption of charcoal-broiled food, and intake of fresh fruits and vegetables have been reported to influence the levels of DNA adducts (6, 8, 25). DNA adducts have been found to be dependent on polymorphisms in metabolic genes (i.e., *Msp*I polymorphism of CYP1A1 and GSTM1 null genotype; refs. 26–30). DNA damage may be repaired, but the individual's ability to remove DNA adducts may vary from individual to individual (31, 32). Recently, specific dietary habits associated to polymorphisms in the detoxifying enzyme GSTM1 have been shown to modulate DNA damage (25, 33).

Our findings indicate that the average concentrations of O_3 may play a role in the modulation of DNA adducts of nonsmokers. This is consistent with a previous investigation showing a relationship between cumulative O_3 exposure and DNA adduct formation among nonsmokers in Florence, Italy (34). O_3 is a marker of photochemical smog, being produced by a complex series of reactions involving hydrocarbons and nitrogen dioxide, emitted primarily during combustion of fossil fuels by industry and transportation activities, and driven by UV radiation in sunlight (3). O_3 may have biological effects directly and/or via free radicals reacting with other air pollutants and has been reported to influence daily mortality and to increase lung cancer risk (35–38), but results need confirmation.

Transformation reactions occurring during O_3 episodes may induce the formation of reactive PAHs (3). Free radical may cause the formation of several oxidized degradation products, such as B(a)P-lactone and B(a)P-quinone, capable of binding to DNA and forming adducts without metabolic activation (39, 40). In addition, PAHs may be transformed by UV radiation, become directly mutagenic, and produce covalent adducts (41–43). An enhancement of the signature of mutations produced by B(a)P has been found after UV radiation (44).

Unfortunately, PAHs are not frequently monitored in Europe, and their contribution to DNA adducts could not be evaluated.

In conclusion, our prospective study suggests that leukocyte DNA adducts may predict lung cancer risk of never-smokers. Besides, our data indicate a possible role for photochemical smog in determining DNA damage possibly by a conversion of primary PAHs into more mutagenic species in the atmosphere during O_3 episodes.

Table 4.

Multivariate models with log (DNA adducts) as dependent variable and air pollutants, age, gender, education level, country, and batch as independent variables*

Pollutant	Time period	Slope†	*P*
O_3	1990-1994	0.066	0.0095
PM_{10}	1992-1994	0.028	0.09
NO_2	1990-1994	0.013	0.17
SO_2	1990-1994	−0.012	0.048
O_3	1995-1999	0.0025	0.26
PM_{10}	1995-1999	−0.002	0.35
NO_2	1995-1999	−0.002	0.32
SO_2	1995-1999	0.007	0.15

Model of logistic regression: DNA adducts (detectable/nondetectable) as dependent variable and air pollutants, age, gender, education level, country, and batch as independent variables‡

	OR§ (95% CI)	*P*
O_3	1.97 (1.08-3.58)	0.02
PM_{10}	4.62 (0.43-49.6)	0.21
NO_2	0.90 (0.50-1.61)	0.71
SO_2	1.12 (0.68-1.83)	0.66

*DNA adduct measurement preceding air pollution estimates have been deleted.
†Increase in log adduct level per unit increase of pollutant ($\mu g/m^3$).
‡Air pollution estimates refer to the same year of blood collection for adduct measurement. Controls only (n = 928).
§Adjusted OR comparing the upper tertile versus the lower two tertiles.

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