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REVIEW

Smoking-related DNA and protein adducts in human tissues

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Tobacco smoking causes not only lung cancer but also cancer of the oral and nasal cavities, oesophagus, larynx, pharynx, pancreas, liver, kidney, stomach, urinary tract and cervix. Tobacco smoke contains many carcinogens that exert their biological effects through interaction of reactive intermediates with DNA to form DNA adducts. The same electrophilic species also react with cellular proteins. The effects of smoking are evident by the detection of elevated levels of carcinogen–DNA adducts in many human tissues and of carcinogen–protein adducts in blood. Components of tobacco smoke also induce oxidative DNA damage. Systemic exposure to tobacco-derived carcinogens is demonstrated by the observation of elevated levels of DNA adducts in tissues not directly exposed to tobacco smoke. For many of these tissues there is epidemiological evidence, varying from comprehensive to preliminary, that smoking is a causative factor in cancer of that site. The effects of passive smoking, which also causes lung cancer in non-smokers, is also evident in elevated levels of protein adducts in exposed non-smokers so exposed, relative to non-exposed non-smokers. This paper reviews the literature on smoking-related DNA and protein adducts in human tissues and shows how such studies have provided mechanistic insight into the epidemiological associations between smoking and cancer.

Introduction

Tobacco is a uniquely dangerous consumer product that is addictive and harmful to human health when used as intended. Tobacco smoking is the greatest single cause of preventable illness and premature death, killing half of all people who continue to smoke for most of their lives (1). Half of these will die before the age of 69. Thirty per cent of all cancer deaths, including nearly 90% of deaths from lung cancer are caused by smoking. The cancers for which epidemiological studies have established a causal association with tobacco smoking are lung, urinary tract, kidney, oral cavity, sinonasal, nasopharynx, hypopharynx and oropharynx, oesophagus, larynx, pancreas, stomach, liver, colorectal, cervix and myeloid leukaemia (2).

Tobacco smoke contains many thousands of chemicals including a plethora of carcinogens (3). Many carcinogens undergo metabolic activation in mammalian tissues to reactive intermediates that interact with and modify informational macromolecules, such as DNA (4,5), with potentially mutagenic consequences. The exposure of human tissues and organs to these carcinogens, and their metabolic activation therein, is the mechanism by which smoking-related cancer is initiated. Over the past 20 or so years the systemic nature of the exposure of the human body to carcinogens inhaled in tobacco smoke has become apparent. This is evident from the widespread formation of DNA and protein adducts in human tissues, and the detection of tobacco-related carcinogens and their metabolites in various bodily fluids. In a recent article Hecht (6) reviewed the detection of human urinary carcinogen metabolites as biomarkers for investigating the relationship between tobacco smoking and cancer. The current article reviews the literature on smoking-related DNA and protein adducts and their uses in monitoring human exposure to, and assessing cancer risk from, tobacco smoking.

Abbreviations: 3-ABP, 3-aminobiphenyl; 4-ABP, 4-aminobiphenyl; BAL, bronchoalveolar lavage; BPDE, benzo[*a*]pyrene diol-epoxide; CEVal, *N*-(2-cyanoethyl)valine; DRZ, diagonal radioactive zone; ECD, electrochemical detection; ELISA, enzyme-linked immunosorbent assay; ETS, environmental tobacco smoke; GC-MS, gas chromatography-mass spectrometry; HPB, 4-hydroxy-1-(3-pyridyl)-1-butanone; HOEtVal, *N*-(2-hydroxyethyl)valine; 7-MedG, 7-methyldeoxyguanosine; 8-oxo-dG, 8-oxo-deoxyguanosine; PAH, polycyclic aromatic hydrocarbon; WBC, white blood cell.

Smoking-related DNA adducts in human tissues

Because DNA damage, frequently in the form of chemically stable adduct formation, is an early and essential step in the process by which genotoxic carcinogens initiate the carcinogenic process, the detection of DNA adducts in human tissues is a useful and appropriate means to assess human exposure to such agents. A number of different procedures have been used for this. ^{32}P-Postlabelling analysis, in which DNA is digested and the resultant carcinogen-modified nucleotides are radiolabelled enzymatically with [^{32}P]orthophosphate, constitutes a sensitive method for which prior knowledge of structures of the adducts formed is not required. A common feature of postlabelled DNA from cells or tissues exposed to complex mixtures of carcinogens, such as tobacco smoke, is the appearance of a diagonal radioactive zone (DRZ) when the material is resolved by 2-dimensional thin-layer chromatography. Although this material has not been fully characterized, its properties are compatible with those of a complex mixture of aromatic and/or hydrophobic DNA adducts. Another method used to detect DNA adducts in human tissues employs antibodies raised against various carcinogen-modified DNA or nucleotides. Immunohistochemistry allows localization of adducts within a tissue specimen and measurement of fluorescent staining intensity permits a semi-quantitative estimate of adduct levels. Thirdly, fluorescence detection of adducts in DNA, or of products released from DNA by hydrolysis, has been used for such carcinogens that have strongly fluorescent properties, for example polycyclic aromatic hydrocarbons (PAHs). Fourthly, mass spectrometry has been used for the chemical-specific detection of certain adducts in DNA samples and finally, electrochemical detection (ECD) has been used for some smaller DNA lesions, for example 8-oxo-deoxyguanosine (8-oxo-dG), formed in DNA by oxidative processes. The uses, strengths and limitations of these techniques have been

widely described (7–13). Earlier studies of tobacco-related DNA adducts in human tissues have been reviewed (14).

Respiratory tract

Lung and bronchus

A large number of studies have been published in which the levels and characteristics of DNA adducts in the lung and bronchus of smokers and non-smokers have been compared. These are summarized in Table I.

In most of these studies, significantly elevated levels of DNA adducts were detected in the peripheral lung, bronchial epithelium or bronchioalveolar lavage (BAL) cells of the smokers. This is the case for total bulky DNA adducts (as detected by the ^{32}P-postlabelling method) and for more chemical-specific methods including HPLC/fluorescence and gas chromatography-mass spectrometry (GC-MS). However, 4-aminobiphenyl(4-ABP)–DNA adduct levels in lung did not correlate with smoking status (15). In some cases where smoking status was not known but inferred from plasma cotinine levels (indicating smoking habit at the time of death of the subjects) there was generally a poor correlation with the detection of one specific type of adduct, such as 7-methyldeoxyguanosine (7-MedG) (16) or those formed by benzo[*a*]pyrene (17).

Some studies have found a linear correlation between adduct levels and daily or lifetime consumption of cigarettes (18–21), but in other studies this relationship was not found (22,23). Among a group of male smokers all with lung cancer, there was a linear relationship between adduct levels and the inverse of years of smoking (24); this result implies that individuals with higher levels of adducts may get cancer from smoking in a shorter time than smokers with lower levels. Mean levels of adducts in ex-smokers (usually with at least a 1 year interval since smoking cessation) are found generally to be intermediate between the levels of smokers and life-long non-smokers. From these comparisons estimations of the half-life of adducts in lung tissue are put at ~1–2 years. This is somewhat longer than would be estimated from a consideration of the biochemical processes of DNA repair and the rate of cell turnover, and may be a consequence of the slow clearance of carcinogen-containing tar and particulate deposits in the lung, resulting in continued metabolic activation of tobacco carcinogens after smoking cessation.

In a Norwegian cohort, adduct levels were higher in the lungs of women smokers than in men's, a difference that was greater after adjustment of the lower smoking exposure of the women (24,25). However, in a Hungarian cohort, adduct levels in male and female smokers with lung cancer were the same (22).

Larynx

^{32}P-Postlabelling analysis of DNA from larynx mucosa revealed the presence of hydrophobic adducts (the DRZ) in smokers (n = 21) but not non-smokers (n = 4). The ability of both the nuclease P1 digestion and the butanol extraction procedures to detect these adducts suggested that they were formed primarily by PAHs rather than aromatic amines. Adduct levels correlated with levels of CYP1A, 2C and 3A4, but not with levels of expression of CYP2E1 and 2A6 (26).

In another study, adduct levels in laryngeal tumours were compared with those in non-tumour tissue from 43 patients, most of them smokers. Adduct levels, detected by ^{32}P-postlabelling, were higher in tumour tissue than in normal, and the few non- or ex-smokers (n = 5) had lower levels than the smokers (not significant) (27). Subsequently, ^{32}P-postlabelling analysis was used to measure levels of *N*7-alkylguanine in DNA from laryngeal biopsies. Levels in heavy smokers (>40 cigarettes/day) were 2.5-fold higher than in moderate smokers (~20 cigarettes/day) and 6-fold higher than in non- and ex-smokers, and there was a significant correlation between *N*7-alkylguanine levels and aromatic/hydrophobic adduct levels in these tissue samples (28).

An immunohistochemical study of laryngeal biopsies from 38 patients, using antibodies raised against 4-ABP–DNA adducts, included analysis of tumours, polyps and surrounding tissue. The study showed significant differences in staining intensity of polyps and surrounding tissue from smokers compared with that from non-smokers. Differences between smokers and non-smokers in tumour tissue were not significant (limited number of samples). The study shows that 4-ABP–DNA adducts, related to smoking exposure, are formed in the larynx (29).

Analysis of laryngeal biopsies from 33 Polish patients, all of whom had tumours, was carried out by ^{32}P-postlabelling. DNA from tumour, non-tumour and the interarytenoid area was analysed separately. Large inter-individual differences in adduct levels were seen, with highest levels in the interarytenoid area. Adduct levels were reported to be correlated with age, sex (28 of the patients were men, five were women; adduct levels were higher in the males), cigarette smoking (all patients were smokers, 19 smoked 20 cigarettes/day, 14 smoked 30–40) and stage of tumour progression (P values not reported) (30).

Sputum

DNA isolated from sputum, induced by inhalation of nebulized saline solution, was compared in a group of smokers (n = 20) and non-smokers (n = 24). Using the nuclease P1 digestion method of ^{32}P-postlabelling analysis, all smokers and only one non-smoker showed a DRZ in their adduct patterns, and adduct levels were significantly higher in smokers than in non-smokers (3.1 ± 1.4 versus $0.6 \pm 0.8/10^8$ nt; P = 0.0007) (31). In another report, the same authors found that the adduct levels in the induced sputum of smokers (n = 9, monitored on three occasions), were significantly higher than the levels in non-smokers (n = 9, monitored once) (32). When immunohistochemical analysis was carried out on induced sputum, the cells of smokers (n = 20) had significantly greater staining using 4-ABP–DNA antibodies than cells of non-smokers (n = 24) (P = 0.001), but not using benzo[*a*]pyrene diol-epoxide(BPDE)–DNA antibodies (P = 0.07) (33).

DNA damage in human lung cells in vitro

In cultured human lung cells, the effect of bubbling cigarette smoke through the medium (phosphate-buffered saline) is to induce DNA single strand breaks and formation of 8-oxo-dG in DNA, and evidence was presented that this was mediated, in part, through the formation of reactive oxygen species (34). It was shown that cigarette tar promotes neutrophil-induced DNA damage in human lung cells and this activity is further enhanced by iron and inhibited by catalase (35).

Treatment of human fetal lung cells with the tobacco-specific nitrosamines *N*′-nitrosonornicotine (NNN) and 4-(*N*-methyl-*N*-nitrosamino)-1-butanone (NNK) caused strand breaks in DNA. Inhibition by oxygen radical scavengers suggested that the hydroxyl radical was an important intermediate in the process (36).

DNA damage, as measured by the single-cell gel electrophoresis (comet) assay, has also been shown to result in human embryo lung cells and in human lymphoid cells treated with water-soluble compounds from cigarette smoke (37,38).

In another study, treatment of tracheobronchial epithelial cells with gas-phase cigarette smoke caused DNA strand breakage accompanied by increases in the levels of a number of DNA lesions, including 8-oxo-dG, xanthine and hypoxanthine. These latter lesions can arise from the deamination of guanine and adenine, by a mechanism involving reactive nitrogen species. Thus, DNA damage induced by cigarette smoking may be mediated by both reactive oxygen species and reactive nitrogen species (39).

Oral and nasal cavities

In two early ^{32}P-postlabelling studies of DNA from oral mucosal cells, a variety of aromatic and/or hydrophobic adducts were seen at varying levels in smokers and non-smokers, but none of them were specific to tobacco smoke exposure (40,41).

DNA from clinically normal oral tissue (mucosa) from patients undergoing surgery for intraoral squamous cell carcinoma was analysed by ^{32}P-postlabelling using both nuclease P1 digestion and butanol extraction enhancement methods. Both the range and levels of adducts were greater with the latter method, and there was a statistically significantly higher mean level of adducts in the smokers (n = 20) than in the ex-smokers (n = 4) and non-smokers (n = 9) combined (42). A comparison between DNA from oral biopsies and from buccal mucosa from the same individuals showed that there was a good correlation between adduct levels in the two sources, with a significantly higher level in DNA from smokers (n = 20) than from non-smokers (n = 10) (43).

In a small study of nasal mucosal DNA from smokers (n = 9), ex-smokers (n = 2) and non-smokers (n = 10), ^{32}P-postlabelling analysis detected the DRZ in eight smokers, one ex-smoker and one non-smoker. The mean adduct level in smokers was significantly higher than that in the non-smokers ($P < 0.01$; 4.8 versus 1.4/10^8 nucleotides) and a linear relationship between adduct levels and daily cigarette consumption was observed among the smokers (44). In another study, the levels of DNA adducts detected in smokers' nasal cells (n = 6) by ^{32}P-postlabelling analysis was also significantly higher than the levels in non-smokers' cells (n = 14) (mean value 17.0 versus 6.8/10^8 nucleotides) (45).

Using a ^{32}P-postlabelling method developed to detect cyclic adducts such as 1,N^2-propanodeoxyguanosine, possible products of the reaction of tobacco smoke constituents such as acrolein and crotonaldehyde with DNA, significantly higher levels of each of three such adducts were detected in gingival tissue DNA of smokers (n = 11) than of non-smokers (n = 12); the total levels of the adducts were 4.4-fold higher in the smokers than in the non-smokers (46).

Using the comet assay to detect DNA strand breaks in exfoliated buccal cells, significantly increased DNA migration was found with the cells of smokers (n = 11) than with those of non-smokers (n = 9) (47).

A study of exfoliated oral cells from healthy volunteers used an immunohistochemical approach to stain cells with antibodies raised against BPDE–DNA adducts. Staining intensity was significantly greater with cells from smokers (n = 33) than from non-smokers (n = 64), and increasing staining was observed with increasing numbers of cigarettes smoked per day among the former group (48). Similar results were obtained in another study using this technique, in which both buccal cavity and mouth floor cells were investigated; staining intensity in both cell types was significantly higher in smokers (n = 26) than in non-smokers (n = 22) (49). When antibodies to 4-ABP–DNA adducts were used, staining intensity was also significantly greater in exfoliated oral cells of smokers (n = 12) compared with non-smokers (n = 12) (50).

Immunohistochemical staining of human oral mucosal cells, using antibodies to BPDE–DNA adducts, revealed significantly higher levels of staining in smokers (n = 16) than in matched non-smokers (n = 16) (51). A second study, using both these antibodies and ones derived from 4-ABP-modified DNA, showed significantly higher levels of DNA damage in smokers (n = 20) than in matched non-smokers (n = 20) in both circumstances (52). Subsequently, an analogous analysis using antibodies to malondialdehyde–DNA adducts has demonstrated elevated levels of this adduct, derived from lipid peroxidation, in the oral mucosal cells of smokers (n = 25) compared with matched non-smokers (n = 25) (53).

Urogenital tissues

Bladder

Investigations of the presence of smoking-related DNA adducts in bladder cells have made use of autopsy and biopsy specimens and also exfoliated epithelial cells excreted in urine.

When DNA adduct levels were determined in bladder biopsy samples using ^{32}P-postlabelling with both butanol extraction and nuclease P1 enhancement procedures, the mean levels of several specific adducts were elevated in smokers (n = 13) compared with ex-smokers (n = 20) and non-smokers (n = 9); some adducts were present at similar levels in all groups and thus were not smoking-related. One of the smoking-related adducts was chromatographically identical to the major adduct formed in DNA by 4-ABP (54).

Exfoliated urothelial cells recovered from urine of 73 individuals yielded sufficient DNA for ^{32}P-postlabelling in 40 of the samples. The DNA was found to contain multiple DNA adducts, of which four were present in smokers' DNA at two to 20 times higher levels than in non-smokers' DNA. One of these corresponded chromatographically to *N*-(deoxyguanosin-8-yl)–4-ABP (55). In a further study, two adducts detected in exfoliated uroepithelial cells from healthy male subjects were specifically associated with smoking and with the levels of 4-ABP–haemoglobin adducts (50 smokers, 50 non-smokers) (56).

In a study of bladder biopsies of 20 bladder cancer patients and exfoliated bladder cells of 36 healthy individuals, a dose–response relationship between smoking and adduct levels was found for both groups (statistics not given; note many groups are small). Adduct levels were not higher in the cases than in the controls (57).

Analysis by ^{32}P-postlabelling of DNA of bladder tissue samples taken at autopsy from 56 individuals revealed a positive but weak association (P = 0.09) between adduct levels and tobacco smoking, and a correlation between adduct levels in lung and bladder ($P < 0.01$, Spearman rank correlation test) (58).

Comparison of the adducts detected by ^{32}P-postlabelling in bladder biopsies from 30 smokers with those from 24 non-smokers found that, overall, adduct levels were not significantly different in the two groups; 3–5-fold higher levels of adducts

Table I. Studies of smoking-related DNA adducts in human lung and bronchus

Study	Tissue	Method of analysis	Number of subjects	Outcome and comments
Randerath *et al.* (186)	Bronchus	^{32}P-Postlabelling	1 smoker 1 non-smoker	Detection of DRZ in smoker only
Phillips *et al.* (18)	Lung	^{32}P-Postlabelling	17 smokers 7 ex-smokers 5 non-smokers	Adduct levels significantly higher in smokers. Linear relationship between levels and cigarettes/day
Randerath *et al.* (106)	Lung, bronchus	^{32}P-Postlabelling	7 smokers 3 ex-smokers 1 non-smoker	DRZ detected in smokers' lungs in a dose-dependent fashion
Weston *et al.* (187)	Lung (tumour and normal tissue)	Synchronous fluorescence spectroscopy	5 lung tumours and 4 matching normal tissues	Only one of four normal tissues positive for BPDE-DNA
Phillips *et al.* (19)	Bronchus	^{32}P-Postlabelling	37 smokers 8 ex-smokers 8 non-smokers	Adduct levels in smokers significantly higher than in ex- and non-smokers. Among smokers, correlation between adduct levels and smoke exposure
Van Schooten *et al.* (188)	Lung (tumour and normal tissue)	^{32}P-Postlabelling, ELISA	13 smokers 8 ex-smokers	DRZ detected in all non-tumourous tissue, but in only 4/21 tumours. ELISA positive for five non-tumour, three tumours. Poor correlation between adduct levels and smoking among current smokers (n = 13)
Dunn *et al.* (20)	Bronchus	^{32}P-Postlabelling	28 smokers 40 ex-smokers 10 non-smokers	DRZ observed in all smokers, 24/40 ex-smokers and 4/10 non-smokers. Among smokers, levels correlated with smoke exposure
Geneste *et al.* (189)	Lung	^{32}P-Postlabelling	19 smokers 4 ex-smokers	Smokers had significantly higher adduct levels, which correlated with AHH inducibility
Izzotti *et al.* (190)	Alveolar macrophages	Synchronous fluorescence spectroscopy	16 smokers 4 recent smokers 6 ex-smokers 13 non-smokers	BPDE-DNA detected in 84.6% smokers and two of four recent smokers, but not in ex- or non-smokers
Foiles *et al.* (191)	Lung, tracheobronchus	GC-MS for HPB after acid hydrolysis	9 smokers, 8 non-smokers (lung); 4 smokers, 4 non-smokers (bronchus)	Adduct levels higher in smokers for both tissues (lung, 11 ± 16 versus 0.9 ± 2.3 fmol/mg DNA, seven of nine positive versus one of seven; tracheobronchus, 16 ± 18 versus 0.9 ± 1.7, two of four positive versus one of four).
Weston and Bowman (17)	Lung	Synchronous fluorescence spectroscopy	20 adults 5 infants	BPDE–DNA detected in 6/25 samples; levels did not correlate with serum cotinine levels
Alexandrov *et al.* (192)	Lung	HPLC/fluorescence, ^{32}P-postlabelling	13 lung cancer patients	BPDE–DNA detected in 9/11 smokers and two of two ex-smokers. Linear correlation between the two adduct methods
Routledge *et al.* (58)	Lung	^{32}P-Postlabelling	16 smokers 7 ex-smokers 14 non-smokers	Adduct levels significantly higher in smokers and ex-smokers than in non-smokers

Table I. Continued

Study	Tissue	Method of analysis	Number of subjects	Outcome and comments
Mustonen *et al.* (193)	Bronchus	^{32}P-Postlabelling for 7-MedG	13 smokers 7 non-smokers	Adduct levels significantly higher in smokers than in non-smokers. For five smokers lung adduct levels correlated with lymphocyte adduct levels
Schoket *et al.* (194)	Bronchus	^{32}P-Postlabelling	45 smokers 37 ex-smokers 16 non-smokers	Levels in smokers significantly greater than in non-smokers. Weak association between adduct levels and daily cigarette consumption.
Gallagher *et al.* (92)	Lung	^{32}P-Postlabelling	5 smokers 11 non-smokers	Adduct levels 1.6-fold higher in smokers
Ryberg *et al.* (24)	Lung	^{32}P-Postlabelling	49 smokers (38 male) 14 non-smokers (7 male)	Significantly higher levels of adducts in smokers than non-smokers. Women had higher levels than men after adjusting for smoke exposure. Linear relationship between adducts and inverse of years of smoking among male lung cancer patients
Wiencke *et al.* (117)	Lung	^{32}P-Postlabelling	6 smokers 10 ex-smokers (<1 year) 15 ex-smokers (>1 year) 3 non-smokers	Correlation with adduct levels in blood mononuclear cells (mean value 2.5-fold higher in lung)
Sherman *et al.* (195)	BAL cells	DNA strand breaks by fluorescence analysis of DNA unwinding	11 smokers 11 non-smokers	Alveolar macrophages from smokers had 35 ± 3% double stranded DNA, compared with 41 ± 5% for non-smokers
Andreassen *et al.* (196)	Lung	^{32}P-Postlabelling, fluorescence	26 smokers 11 ex-smokers 2 non-smokers	Heavy smokers (>20 cigarettes/day) had significantly higher levels of adducts than light smokers. 33/39 samples had detectable adducts by ^{32}P-postlabelling
Blomeke *et al.* (16)	Lung	^{32}P-Postlabelling for 7-MedG	10 autopsies	Levels of adduct did not correlate with smoking exposure at time of death (plasma cotinine levels)
Ryberg *et al.* (197)	Lung	^{32}P-Postlabelling	70 smokers	Mean adduct levels significantly different according to *GSTP1* genotype AA>AG>GG (n = 25, 35, 10, respectively; P = 0.01, AA versus AG; P = 0.02, AA versus GG)
Culp *et al.* (15)	Lung	^{32}P-Postlabelling, ELISA, GC-MS	14 smokers 11 ex-smokers	Levels of 4-ABP–DNA adducts in lung tissue did not correlate with smoking status
Asami *et al.* (21)	Lung	HPLC/ECD detection of 8-oxo-dG	14 smokers 7 ex-smokers 9 non-smokers	Levels significantly higher in smokers than non-smokers; linear correlation with cigarettes smoked (daily and cumulative)

Table I. Continued

Study	Tissue	Method of analysis	Number of subjects	Outcome and comments
Schoket *et al.* (22)	Bronchus	^{32}P-Postlabelling	82 smokers 25 short-term ex-smokers (<1 year) 20 long-term ex-smokers (>1 year) 23 non-smokers	Adduct levels significantly higher in current and short-term ex-smokers compared with long-term ex- and non-smokers. Adduct levels the same in males (n = 66) and females (n = 16). Apparent half-life of adducts ~1.7 years. Adduct levels did not correlate with daily cigarette dose or with *GSTM1* or *CYP1A1 Msp*1 genotypes
Godshalk *et al.* (23)	BAL cells	^{32}P-Postlabelling	78 smokers 23 non-smokers	Adduct levels in BAL cells did not correlate with smoking (pack-years) after correcting for age
Rojas *et al.* (198)	Lung	HPLC/fluorescence	20 smokers	Adduct levels higher in two individuals with *GSTM1* null and *CYP1A1 Msp*1 variant genotypes
Wiencke *et al.* (118)	Lung	^{32}P-Postlabelling	57 smokers 79 ex-smokers 7 non-smokers	Smokers had significantly higher adduct levels than ex-smokers. Early age of commencing smoking associated with higher adduct levels in ex-smokers, but not in current smokers
Butkiewicz *et al.* (199)	Lung	^{32}P-Postlabelling	120 smokers 22 ex-smokers 23 non-smokers	Adduct levels significantly higher in smokers compared with ex- and non-smokers. High adduct levels (upper quartile) significantly associated with *CYP1A1 Val* allele carriers among individuals who were *GSTM1 null* (n = 86)
Mollerup *et al.* (25)	Lung	^{32}P-Postlabelling	122 smokers (29 female) 37 non-smokers (13 female)	Adduct levels significantly higher in females than in males (P = 0.047 before adjustment for pack-years, P = 0.0004 after adjustment). Lung expression of *CYP1A1* (15 females, 12 males) was significantly higher in females (P = 0.016) and in both sexes correlation between *CYP1A1* expression and adduct levels was significant (P = 0.009)
Piipari *et al.* (200)	BAL cells	^{32}P-Postlabelling	31 smokers 16 non-smokers	Adduct levels 3-fold higher in smokers than in non-smokers (P < 0.001) and correlated with cigarettes smoked daily. Smokers with high levels of *CYP3A5* expression had higher adduct levels (P < 0.002)
Cheng *et al.* (201)	Lung	^{32}P-Postlabelling	73 cancer cases (32 smokers, 38 non-smokers) 33 non-cancer controls (11 smokers, 22 non-smokers)	Adduct levels were significantly higher in cases than in controls, but not higher in smokers than in non-smokers. Adduct levels not influenced by *CYP1A1 Msp*1 or *GSTM1* genotypes
Schoket *et al.* (202)	Bronchus	^{32}P-Postlabelling	82 smokers 25 short-term ex-smokers (<1 year) 20 long-term ex-smokers (>1 year) 23 non-smokers [as in ref. (22)]	Evidence for a weak influence of some combinations of *CYP* and *GST* genotypes on adduct levels

Non-tumourous tissue was analysed except where indicated.

were detected by the butanol extraction procedure than by the nuclease P1 digestion procedure, implying a preponderance of aromatic amine-like adducts over those formed by PAHs. However, one minor adduct was found to be present at a 2-fold higher level in the smokers' samples analysed ($n = 17$) than in non-smokers ($n = 8$) ($P < 0.005$, one-tailed) (59).

Human bladder biopsy samples were analysed by immunohistochemical analysis with antibodies to 4-ABP–DNA adducts and the levels of staining compared with smoking history. Adduct levels were significantly higher in current smokers ($n = 24$) than in non-smokers ($n = 22$) ($P < 0.0001$). There was also a linear relationship between mean levels of relative adduct staining and numbers of cigarettes smoked per day (60). In another study using the same technique, higher levels of staining were also observed with bladder specimens from smokers ($n = 30$) than from ex-smokers ($n = 41$) and non-smokers ($n = 24$), but significance was borderline ($P = 0.07$) (61).

Immunohistochemical staining of human exfoliated urothelial cells, using antibodies to BPDE–DNA adducts or to 4-ABP–DNA adducts, revealed significantly higher levels of staining in smokers ($n = 20$) than in matched non-smokers ($n = 20$) in both cases (52).

In a more recent study, DNA adducts of 4-ABP were measured in bladder biopsies from cancer patients by GC-MS. Detectable levels were found in 37/75 samples, which included smokers (24/46), ex-smokers (9/17) and non-smokers (5/8). However, among the patients with higher grade (i.e. grade 3) tumours, there was a significantly greater probability of the smokers having adducts than the ex- or non-smokers (10/11 versus 5/11) (62).

The presence of 3-alkyladenines in human urine was determined in two smokers who underwent a period of voluntary abstinence from smoking. In one of them, the excretion of 3-ethyladenine was significantly lower on non-smoking days ($P < 0.01$). Smoking-dependent differences in levels of 3-methyladenine were only apparent when volunteers consumed a diet low in 3-alkyladenines during the study period. Thus, both adducts are produced by alkylating agents in tobacco smoke, but natural dietary levels of 3-methyladenine make this an insensitive biomarker of smoking (63).

In a study of depurinated adducts of benzo[*a*]pyrene in urine, detectable levels were found in the urine of 3/7 women smokers (0.1–0.6 fmol/mg creatinine), but not in any of 13 non-smokers (64).

Analysis of bladder cells for DNA damage, using the single-cell gel electrophoresis (comet) assay, revealed significantly increased comet tail moments for smokers and ex-smokers ($n = 18$) compared with non-smokers ($n = 12$) ($P < 0.03$) (65).

In a study of oxidative damage measured by means of 8-oxo-dG excretion in urine, smokers ($n = 30$) were reported to have 50% higher levels of the nucleoside (corrected for body weight) than non-smokers ($n = 53$) (66).

Taken together, the studies analysing DNA adducts in bladder tissue or exfoliated urothelial cells demonstrate that smoking-related DNA adduct formation is detectable in them, but that some of the adducts present derive from other sources of environmental carcinogens.

Cervix

A pilot study of DNA isolated from cervical scrapes of 22 women and analysed by the butanol extraction enrichment procedure of ^{32}P-postlabelling showed the presence of the diagonal radioactive zone and elevated levels of adducts in 3/6 smokers compared with the levels in 13 non-smokers (67). In a subsequent study, sufficient DNA was isolated from 33/38 cervical smear samples and subjected to ^{32}P-postlabelling analysis. Adduct levels were significantly higher in DNA from smokers ($n = 18$) than from non-smokers ($n = 15$) (68).

Using DNA isolated from cervical biopsies, ^{32}P-postlabelling analysis with butanol extraction was carried out on samples from 39 women (11 smokers, seven ex-smokers and 21 never smokers). Adduct levels were significantly higher in the smokers and ex-smokers than in the non-smokers ($P = 0.048$). Exclusion of those women whose urinary cotinine levels did not confirm their self-reported non-smoking status ($n = 7$) increased the differences between smokers and non-smokers ($P = 0.03$) (69).

A study of cervical biopsy samples from 35 women (19 smokers, five ex-smokers and 11 non-smokers) found that DNA adduct levels, determined by ^{32}P-postlabelling analysis with the butanol extraction procedure, were significantly higher in the smokers than in the non-smokers, with an intermediate mean value obtained for the ex-smokers (70). This study population was subsequently increased to 22 smokers, four ex-smokers and 14 non-smokers and analysed by both butanol extraction and nuclease P1 digestion procedures of ^{32}P-postlabelling. Adduct levels were significantly higher in smokers when the former procedure was used, but not with the latter (71). The lack of a difference between smokers and non-smokers using nuclease P1 digestion was also reported in another small study (16 smokers, eight non-smokers) (72). However, in another larger study, ^{32}P-postlabelling analysis using nuclease P1 digestion enrichment showed significantly higher levels in DNA from histologically normal cervical biopsies from current smokers ($n = 48$) than from non-smokers ($n = 48$) (73).

Immunohistochemical analysis of human cervical cells, using antibodies to BPDE–DNA adducts, demonstrated significantly increased staining intensity in smears from smokers ($n = 16$) compared with smears from non-smokers ($n = 16$) (74).

GC-MS analysis of BPDE–DNA adducts in cervical epithelial cells showed an ~2-fold higher level in smokers ($n = 7$) than in non-smokers ($n = 7$) and this difference was statistically significant ($P = 0.02$) (75).

Other tissues

Breast

In a pilot study, a total of 31 human breast tissue samples were analysed by ^{32}P-postlabelling using nuclease P1 digestion. These included tumour and tumour-adjacent samples from 15 women and four samples from reduction mammoplasties. The characteristic DRZ was detected in 5/15 cancer patients, all of whom were tobacco smokers. None of the eight ex-smokers and non-smokers displayed this pattern ($P < 0.01$) (76).

In another ^{32}P-postlabelling study of DNA from breast tissue from breast cancer patients and non-cancer patients undergoing reduction mammoplasty, the characteristic DRZ was detected in 17/17 smokers, 4/8 ex-smokers and 4/52 non-smokers. In addition, a particular adduct spot was observed in 36 normal tissue samples (27 of them from non-smokers) but was not significantly associated with smoking (77).

Putative malondialdehyde-derived DNA adducts, detected by ^{32}P-postlabelling, were detected in tumour-adjacent tissue

from breast cancer patients (n = 51) at higher levels (P = 0.0001) than in tissue from controls (reduction mammoplasty, n = 28). The levels of these adducts were also associated with the previously detected major DNA adduct (see above), but significantly lower in smokers than in ex-smokers and non-smokers (78).

Immunohistochemical staining of breast tumour tissue using antibodies raised against BPDE–DNA adducts was not significantly different between smokers (n = 35), ex-smokers (n = 72) and non-smokers (n = 75). However, among smokers and ex-smokers, there was a trend towards higher levels of staining with greater levels of exposure or earlier age of starting (79). Positive staining was also obtained with breast tumour tissue (n = 48) and benign disease tissue (n = 30) from inhabitants of Upper Silesia, a region of Poland that has received high levels of environmental pollution. However, in neither group of patients were staining levels elevated in smokers, relative to non-smokers (80).

In a study of PAH–DNA adducts in breast tissue of cancer patients (n = 119) and of those with benign breast disease (n = 108), immunohistochemical analysis indicated a significant association between adduct levels and breast cancer, but there was no relationship between smoking status and adduct levels or disease status (81).

Pancreas

^{32}P-Postlabelling analysis of 20 pancreatic tumours and 13 normal tissue samples, using nuclease P1 digestion, led to the detection of significantly higher levels of adducts in these normal tissues than in either the tumour material or in normal pancreatic tissue from 19 previously healthy organ donors. A number of qualitative and quantitative differences between the adduct profiles of smokers and non-smokers was observed, including some that were statistically significant (82).

In another study, fluorescence spectral analysis for the presence of BPDE–DNA adducts in pancreatic DNA did not reveal detectable levels in any of 11 samples (six smokers, five non-smokers) (83).

Colon

Fluorescence spectral analysis of colon DNA for the presence of BPDE–DNA adducts resulted in the detection of adducts in three of four samples from smokers and one of three samples from non-smokers. Adduct levels were estimated to be in the range 0.2–1.0 adducts/10^8 nucleotides (83).

Stomach

Tumour DNA from 26 patients with gastric cancer was analysed for DNA adducts using ^{32}P-postlabelling with nuclease P1 digestion. For males only, DNA adducts were significantly higher in the material from smokers (n =14) than in that of non-smokers (n = 4) (84), although it should be noted that the number of non-smokers analysed was small.

Anal epithelium

Samples of anal epithelium from haemorrhoidectomy specimens from current smokers (n = 20) and age-matched life-long non-smokers (n = 16) were analysed for DNA adducts by ^{32}P-postlabelling. Qualitative and quantitative differences were observed in the adduct profiles of the two groups. The mean adduct level was significantly higher in the smokers than in the non-smokers (1.88 ± 0.71 versus 1.36 ± 0.60 adducts/10^8 nucleotides, P = 0.02); furthermore, the adduct pattern revealed the smoking-related DRZ in 17/20 smokers, but not in any of the non-smokers ($P < 0.00001$) (85).

Placenta and fetal tissue

In the first study of placental DNA for the presence of DNA adducts, ^{32}P-postlabelling analysis and a competitive enzyme-linked immunosorbent assay (ELISA) with antibodies to BPDE–DNA adducts were used. The immunological method showed a small but non-significant increase in adducts in material from smokers compared with non-smokers. Using ^{32}P-postlabelling analysis, one particular adduct was found to be present in the DNA from 16/17 smokers, but in only 3/14 non-smokers (86). In a subsequent study, up to seven different adducts were detected by ^{32}P-postlabelling in 53 specimens of human placental tissue, three of which were found 'almost exclusively' in smokers (87).

The combined use of immunological, fluorescence spectral and mass spectrometric methods has led to the detection and characterization of BPDE–DNA adducts in human placental DNA and also provided evidence for the presence of adducts formed by other PAHs (88,89). Synchronous fluorescence spectroscopy for the detection of BPDE-modified DNA gave positive results with 10/28 human placental samples, but there was no correlation with smoking status (90). In a subsequent study, 5/7 placentas from smokers and three/nine from non-smokers were positive for these adducts (91).

In another small study, placental DNA from smokers (n = 5) was found to contain adducts, detected as a DRZ by ^{32}P-postlabelling, at a 1.8-fold higher mean level than that in placental DNA from non-smokers (n = 5) (92) (4.3 ± 1.7 versus 2.3 ± 0.4 adducts/10^8 nucleotides, statistical significance not stated).

Using ^{32}P-postlabelling with nuclease P1 digestion, DNA adducts were detected in placental and umbilical cord DNA regardless of whether the mothers were smokers or not. Adduct levels were significantly higher in maternal than in fetal tissue and, combining data for all tissues, total DNA adduct levels were significantly higher in smokers (n = 8) than in non-smokers (n = 11). Although individual tissues showed a trend towards increased adduct levels in smokers, the differences were not statistically significant (93,94). DNA adducts derived from PAH were detected by ELISA in 6/14 placentas and in 5/12 matched fetal lung samples from spontaneous abortions. None of the samples were from women who reported smoking during pregnancy, suggesting that smoking was not a likely source of the adducts (95). However, in another study using ELISA analysis using antibodies to BPDE–DNA adducts, there was a linear correlation between adduct levels and urinary cotinine levels for both placental DNA and umbilical cord DNA, the former tissue having the higher adduct levels. Overall, adducts were detected in 13/15 placental samples from smokers and three/10 from non-smokers (96).

When placental DNA was analysed both for bulky DNA adducts by ^{32}P-postlabelling and for 8-oxo-dG by ECD, neither method showed a difference between 11 smokers, 10 non-smokers and nine non-smokers exposed to passive smoking (97).

Thus, while some studies indicate the presence of smoking-related DNA adducts in human placenta, overall the association between smoking status and adduct levels, determined by a variety of different methods, is weak. The results would seem to indicate that there are significant sources of environmental carcinogens other than tobacco smoke that result in DNA adducts being formed in this tissue.

Sperm

In a study in which sperm DNA from 12 heavy smokers (>20 cigarettes/day), 12 light smokers (1–19 cigarettes/day) and 12 non-smokers was subjected to ^{32}P-postlabelling analysis, no discernible differences were observed between the patterns or levels of DNA adducts between the three groups of subjects (98).

However, in a study in which immunohistochemical staining of sperm, using antibodies to BPDE–DNA adducts, was carried out, staining intensity was significantly higher in the sperm of smokers ($n = 11$) than in the sperm of non-smokers ($n = 12$) (99). Furthermore, *in vitro* fertilization experiments using smokers' sperm indicated the transmission of BPDE-modified DNA to the embryos of non-smoking partners (100). In related studies, granulosa-lutein cells of women undergoing *in vitro* fertilization were analysed. Immunostaining for BPDE–DNA adducts was confined to the nucleus and significantly greater in cells from smokers ($n = 14$) than in those of passive smokers ($n = 7$) and non-smokers ($n = 11$) (101). (See also section on passive smoking.)

It was found that the sperm DNA of smokers ($n = 28$) contained about 1.5 times the level of 8-oxo-dG found in the sperm DNA of age-matched non-smokers ($n = 32$), a difference that was statistically significant (6.19 ± 1.71 versus 3.93 ± 1.33 8-oxo-dG/10^5 dG, $P < 0.001$) (102). The same authors have reported a correlation between sperm defects and levels of 8-oxo-dG in sperm DNA, indicating that this lesion is useful for assessing sperm quality and male fertility (103). In a study of sperm from heavy smokers (>20 cigarettes/day, $n = 10$), light smokers (<20 cigarettes/day, $n = 11$) and non-smokers ($n = 10$), the frequency of disomy was increased among the smokers for some chromosomes (e.g. 13) but not others (21, X, Y) (104).

In another study, the sperm DNA of smokers ($n = 35$) was found to be more sensitive to acid-induced denaturation ($P < 0.02$) and to possess higher levels of DNA strand breaks ($P < 0.05$) than the DNA of non-smokers ($n = 35$) (105).

Cardiovascular tissues

In a pilot study investigating the presence of DNA adducts by ^{32}P-postlabelling in a number of human tissues at autopsy, the diagonal radioactive zone characteristic of smoking-related DNA damage was observed in heart tissue of two smokers, but not in that of a non-smoker. Moreover, the levels of adducts were highest in heart of all the tissues examined, which included lung and bronchus (106). A study comparing ^{32}P-postlabelling, HPLC-fluorescence detection of BPDE–DNA adduct hydrolysis products, and synchronous fluorescence spectroscopy detection of the same products demonstrated that all three methods were capable of detecting DNA adducts in smooth muscle of human atherosclerotic lesions. However, due to the limited number of samples investigated (four from smokers, three from ex-smokers) no conclusion could be drawn regarding the origins of the adducts detected (107). Another study measured, using ^{32}P-postlabelling, DNA adducts in the thoracic aorta of 133 victims of sudden or accidental death. Those with significant atherosclerotic changes and where atherosclerosis was classified as the main cause of death were designated as cases ($n = 76$), those without as controls ($n = 57$). Smoking status was determined by measuring cotinine levels in plasma. DNA adduct levels were significantly higher in the cases than in the controls, but when smokers and non-smokers were compared, significant differences were observed among the controls, but not among the cases (108).

Immunohistochemical staining of endothelial and smooth muscle cells of blood vessels using antibodies to BPDE–DNA adducts resulted in higher staining of the endothelial cells, although the correlation with smoking habits among the 33 subjects studied (9/11 smokers gave positive staining, compared with 12/21 non-smokers) did not reach statistical significance (109).

In a study of DNA from the right atrial appendage of 41 patients undergoing open heart surgery, the levels of adducts detected by ^{32}P-postlabelling were significantly higher in the smokers ($n = 15$) than in the ex-smokers ($n = 15$) and in the non-smokers ($n = 11$) ($P < 0.01$ and 0.001, respectively). Among the smokers, a significant linear relationship between adduct levels and daily cigarette smoking was observed (110).

Blood cells

Although nucleated peripheral blood cells are not generally considered target cells for tobacco-induced tumourigenesis, the greater ease with which they can be obtained from human subjects, relative to many target organs, has led to a large number of studies exploring the differences between cells from smokers and non-smokers. Studies in which smoking-related DNA adducts have been determined are summarized in Table II.

The picture that emerges from these many studies is an inconsistent one. Measurements made in the longer-lived cells (lymphocytes and monocytes) are more likely to reveal significant differences between smokers and non-smokers than measurements in whole white blood cell (WBC) preparations (see Table II). However, a study of adduct levels in smokers following cessation of the habit did show a decline over time (111). It is clear from studies of populations occupationally exposed to environmental agents such as PAHs that there are multiple sources of exposure resulting in DNA adducts in blood cells and whether or not the influence of tobacco smoking is discernible probably depends on the contribution of other occupational or environmental sources in particular study populations. There are also reports of the influence of nutritional factors on DNA adduct levels in blood cells in some studies (112–114) but not others (115). The modulating effects of genetic polymorphisms in xenobiotic metabolizing genes and DNA repair genes have been implicated in several studies. In general, these observations have been made on the basis of rather small numbers of subjects, the magnitude of the effects are not large, and confirmation of the findings is required in larger studies.

There have been a limited number of reports to suggest that adduct levels in the blood of smokers correlate with adduct levels in the lung (116–118). However, there are also conflicting findings in which no association was found between blood DNA adduct levels and adducts in lung (119) or BAL cells (120).

In the case of oxidative damage in lymphocytes, a number of studies report an increase in oxidative DNA damage in smokers (121–123). The magnitude of this increase, although significant, is generally small (<2-fold) and its biological significance is unclear. In contrast, there are two reports in which smokers had *lower* levels of this lesion in their WBC or lymphocyte DNA (124,125).

Several studies that have compared smokers with cancer with smokers who are free of the disease report an ~2-fold higher adduct level in the cases. This has been observed

Table II. Studies of smoking-related DNA adducts in human blood cells

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Perera *et al.* (140)	WBC	ELISA	22 smokers 24 non-smokers	5/22 samples from smokers positive for adducts, 7/24 non-smokers positive. Among positive samples only, adduct levels higher in smokers (borderline significance)
Perera *et al.* (126)	WBC	ELISA	81 lung cancer cases (38 smokers, 43 ex- and non-smokers) 67 controls (19 smokers, 48 ex- and non-smokers)	For cases, 19/48 smokers and 21/43 ex/non-smokers were positive for adducts; for controls, 9/19 smokers and 27/48 ex/non-smokers were positive. Current smokers who were cases had higher adduct levels than smoking controls. But adduct levels in smokers not different from levels in ex/non-smokers
Holz *et al.* (134)	Monocytes	^{32}P-Postlabelling	5 smokers	In a controlled experiment, smoking caused the formation of DNA adducts in blood monocytes. Not all adducts observed were smoking-related
Jahnke *et al.* (203)	Lymphocytes	^{32}P-Postlabelling	11 smokers 15 non-smokers	Differences between smokers and non-smokers not significant
Phillips *et al.* (19)	WBC	^{32}P-Postlabelling	31 smokers 20 non-smokers	Adduct levels not significantly different in smokers and non-smokers
van Schooten *et al.* (188)	WBC	ELISA	56 coke oven workers 44 controls	47% of workers and 30% of controls had detectable adducts. In the total population smokers had significantly higher adduct levels ($P = 0.017$)
Kiyosawa *et al.* (121)	WBC	ECD of 8-oxo-dG	10 smokers	10 min after smoking two cigarettes, adduct levels in volunteers were increased 1.5-fold ($P < 0.05$)
Savela and Hemminki (204)	Lymphocytes, granulocytes	^{32}P-Postlabelling	11 smokers 10 non-smokers	Adducts in smokers' lymphocytes significantly higher ($P < 0.05$, two-tailed) than in non-smokers'. Differences in granulocytes not significant
van Schooten *et al.* (119)	WBC	^{32}P-Postlabelling, ELISA	39 lung cancer patients	Adduct levels not significantly associated with smoking status, or with adduct levels in lung tissue (matching tissue for 20 subjects)
Mustonen and Hemminki (205)	WBC, lymphocytes, granulocytes	^{32}P-Postlabelling	10 smokers 10 non-smokers	Adduct levels significantly higher in smokers in all three cell populations. Adduct levels highest in lymphocytes and lowest in granulocytes
Santella *et al.* (206)	Mononuclear cells	ELISA	63 smokers 27 non-smokers	Subjects were all males. Adduct levels were elevated in smokers compared with non-smokers, and 70% of smokers' DNA scored positive for adducts, 22% of non-smokers. DNA adduct levels in smokers did not correlate with cigarettes/day or pack-years
Mustonen *et al.* (193)	Lymphocytes	^{32}P-Postlabelling for 7-MedG	13 smokers 7 non-smokers	Adduct levels significantly higher than in non-smokers. For five smokers lung adduct levels correlated with lung adduct levels
Gallagher *et al.* (98)	WBC, lymphocytes	^{32}P-Postlabelling	23 smokers 16 non-smokers	Adduct levels 2.5-fold higher in smokers for both cell types ($P < 0.01$)

Table II. Continued

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Popp *et al.* (207)	Mononuclear cells	^{32}P-Postlabelling and alkaline elution	23 oral cancer patients 15 hospital controls 21 healthy non-smokers	Significant correlation between smoking and DNA elution rates, but not DNA adduct levels
Holz *et al.* (135)	Lymphocytes	Nick translation to detect DNA single strand breaks	5 smokers	Under controlled conditions, single strand breaks increased in four of five smokers who smoked 24 cigarettes in 8 h, having refrained from smoking for 12 h prior to the test
Rojas *et al.* (208)	WBC	HPLC/fluorescence, ^{32}P-postlabelling and ELISA	7 lung cancer patients (6 smokers); 3 controls (2 non-smokers)	Adducts detected in the six smoking cases at substantially higher levels than in the non-smoking controls. A good correlation obtained with results from ^{32}P-postlabelling but not from ELISA
Ichiba *et al.* (209)	WBC	^{32}P-Postlabelling	26 chimney sweeps 14 controls	Smokers had 48% higher adduct levels than non-smokers (statistically significant)
Grinberg-Funes *et al.* (112)	Mononuclear cells	ELISA	63 male smokers	No relationship between smoking and adduct levels observed previously (206), but significant influence of serum vitamin E and C observed among *GSTM1 null* individuals on adduct levels observed in this study
Rojas *et al.* (210)	Mononuclear cells	HPLC/fluorescence	39 coke oven workers 39 controls	Workers had higher levels of adduct than controls. In both groups mean level in smokers was higher but differences not statistically significant (large inter-individual variation)
Binkova *et al.* (211)	WBC	^{32}P-Postlabelling	9 smokers 21 non-smokers	In a study designed to investigate exposure of women working outdoors to PAHs, no effect of smoking on adduct levels was observed
Tang *et al.* (116)	WBC	ELISA	119 lung cancer cases (52 smokers, 58 ex-smokers, 9 non-smokers) 98 controls (25 smokers, 34 ex-smokers, 39 non-smokers)	Adduct levels were significantly higher in cases than in controls ($P < 0.01$) and higher in smokers in both groups ($P < 0.05$). Adducts increased with number of cigarettes smoked in cases who were current smokers ($n = 51$) but not in control smokers. Adducts in WBC correlated with adducts in lung tumour tissue ($P < 0.05$, $n = 34$)
Schell *et al.* (212)	WBC, lymphocytes	^{32}P-Postlabelling	103 smokers 107 non-smokers	In a study of several populations with occupational exposures to PAHs and controls, no influence of smoking on adduct levels in WBC was evident, and only a weak trend in lymphocytes
Wienke *et al.* (117)	Mononuclear cells	^{32}P-Postlabelling	6 smokers 10 ex-smokers (<1 year) 15 ex-smokers (>1 year)	Correlation with adduct levels in lung (mean value 2.5-fold higher in lung)
Mooney *et al.* (111)	WBC	ELISA	40 smokers	In a smoking cessation study, a 50–75% reduction in adduct levels was observed 8 months after cessation. Similar reductions in 4-ABP–haemoglobin adduct levels

Table II. Continued

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Asami *et al.* (122)	WBC	HPLC/ECD	10 smokers 10 ex-smokers 10 non-smokers	Levels of 8-oxo-dG 1.88-fold higher in smokers than in non-smokers ($P = 0.013$)
Zhao *et al.* (45)	WBC	^{32}P-Postlabelling	6 smokers 14 non-smokers	Difference in adduct levels between smokers and non-smokers of borderline significance ($P = 0.051$). No correlation between adducts and daily cigarette consumption
Wang *et al.* (115)	Lymphocytes	^{32}P-Postlabelling	94 smokers 98 non-smokers	Adduct levels in smokers not significantly different from levels in non-smokers. Plasma levels of beta-carotene and alpha-tocopherol did not influence adduct levels significantly, nor did *CYP1A1 (*exon 7 Val allele) or *GSTM1* genotype
Van Schooten *et al.* (120)	Lymphocytes	^{32}P-Postlabelling	54 smokers 9 non-smokers	Adduct levels significantly related to daily cigarette tar exposure, with evidence of saturation at highest exposures. No significant correlation with adduct levels in BAL cells
Mooney *et al.* (113)	WBC	ELISA	159 smokers	DNA adduct levels 2-fold higher ($P < 0.03$) in individuals with *CYP1A1* exon 7 Val allele ($n = 10$). Association between plasma levels of beta-carotene and adducts in *GSTM1 null* subjects
Godshalk *et al.* (23)	Lymphocytes, monocytes, granulocytes	^{32}P-Postlabelling	86 smokers 23 non-smokers	Adduct levels significantly higher in smokers' lymphocytes and monocytes, but not granulocytes. Adduct levels in smokers' monocytes + lymphocytes ($n = 78$) linearly related to daily exposure to 'cigarette tar' but not cigarette consumption or pack-years
Rojas *et al.* (198)	WBC	HPLC/fluorescence	20 smokers (coke oven workers)	Adduct levels higher in 1 individual with *GSTM1* null and *CYP1A1 Msp*1 variant genotypes
Dallinga *et al.* (155)	Lymphocytes	^{32}P-Postlabelling	55 smokers 4 non-smokers	DNA adduct levels correlated with cigarette and tar consumption, with evidence of saturation at higher smoking levels. No effect of *GSTM1* genotype. Slow acetylators (*NAT2*) had significantly higher adduct levels ($P < 0.05$)
Arnould *et al.* (213)	WBC	ELISA	58 smokers 20 non-smokers	Adducts not detected in non-smokers. Among smokers, adduct levels correlated with tobacco consumption ($P < 0.001$)
Pastorelli *et al.* (160)	Lymphocytes	GC-MS	44 male lung cancer patients	Significant correlation between levels of BPDE-DNA adducts and numbers of cigarettes smoked daily ($P = 0.02$) and pack-years ($P = 0.05$). No effect of *CYP1A1, mEH* or *GSTM1* genotype on levels of adducts
van Zeeland *et al.* (124)	WBC	HPLC/ECD for 8-oxo-dG	57 smokers 16 ex-smokers 29 non-smokers	Levels of 8-oxo-dG significantly lower in smokers compared with non-smokers

Table II. Continued

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Wiencke *et al.* (118)	Mononuclear cells	^{32}P-Postlabelling	54 cancer patients	A significant correlation was observed between adduct levels in lung and in blood mononuclear cells ($r = 0.77$, $P < 0.001$)
Shinozaki *et al.* (133)	Lymphocytes	Flow cytometry for BPDE–DNA adducts	40 smokers 35 non-smokers	Mean level of adducts in smokers significantly higher than in non-smokers. In smokers, adduct levels correlated with age, years of smoking and pack-years, but not with cigarettes/day
Pavanello *et al.* (214)	Mononuclear cells	HPLC/fluorescence	130 subjects	Subjects were exposed to PAHs from a variety of occupational and iatrogenic sources. Smoking did not influence BPDE–DNA adduct levels
Poli *et al.* (215)	WBC	Comet assay	50 smokers 50 non-smokers	Differences in DNA migration between smokers and non-smokers were significant ($P < 0.001$)
Hou *et al.* (129)	Lymphocytes	^{32}P-Postlabelling	179 lung cancer cases (36 smokers, 29 recent ex-smokers, 27 long-term ex-smokers, 87 non-smokers) 161 controls (46 smokers, 12 recent ex-smokers, 24 long-term ex-smokers, 79 non-smokers)	Adduct levels significantly higher in smokers (cases + controls) than in long-term ex-smokers (>2 year) and non-smokers ($P = 0.0003$). No significant differences between cases and controls for any of the smoking categories
Peluso *et al.* (130)	WBC	^{32}P-Postlabelling	162 bladder cancer cases 104 hospital based controls	No relationship between smoking and adduct levels found, although there was a strong association with case/control status. Adduct levels significantly reduced in subjects with higher fruit and vegetable consumption. Adduct levels influenced by *NAT2* genotype, but not by *GSTM1, GSTT1, GSTP1, COMT* or *NQO1* genotypes
Lodovici *et al.* (123)	WBC	HPLC/ECD for 8-oxo-dG	16 smokers 9 ex-smokers 31 non-smokers	Adduct levels significantly higher in smokers than in non-smokers (mean level 2-fold higher, $P < 0.01$). Values for ex-smokers similar to those of non-smokers
Georgiadis *et al.* (216)	Peripheral and cord WBC	Competitive repair assay for O^6-MedG	28 smokers 5 smokers	Adduct levels not influenced by smoking status
Rojas *et al.* (217)	WBC	HPLC/fluorescence	89 coke oven workers (35 smokers, 36 ex-smokers, 18 non-smokers) 44 controls (all smokers)	Adducts increased with cigarette dose and smoking duration. *GSTM1* genotype also influenced adduct levels, as did combinations of *GSTM1* and CYP1A1 genotypes, but *GSTT1* genotype did not
Duell *et al.* (218)	Mononuclear cells	^{32}P-Postlabelling	11 smokers 38 ex-smokers 11 non-smokers	DNA repair *XRCC1* allele 399Gln associated with higher levels of adducts, but not statistically significant. No effect of *ERCC2* polymorphism observed

Table II. Continued

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Vulimiri *et al.* (127)	Lymphocytes	^{32}P-Postlabelling for bulky adducts and 8-oxo-dG	55 lung cancer cases (46 smokers, 6 ex-smokers, 3 non-smokers) 58 controls (39 smokers, 6 ex-smokers, 13 non-smokers)	Lung cancer cases had higher levels of bulky adducts and 8-oxo-dG than controls regardless of smoking status and also for smokers only (bulky adducts). No correlation between levels of the two adduct types
Nia *et al.* (32)	Lymphocytes	^{32}P-Postlabelling	9 smokers 9 non-smokers	Adduct levels significantly higher in smokers than in non-smokers ($P = 0.0001$). Study compared adducts in lymphocytes with adducts in induced sputum
Jacobson *et al.* (114)	Mononuclear cells	ELISA for PAH–DNA and 8-oxo-dG	121 smokers	DNA adduct levels in smokers reduced by vitamin supplements, but differences from placebo group were not significant
Godshalk *et al.* (156)	Lymphocytes	^{32}P-Postlabelling	67 smokers	A positive correlation was found between self-reported numbers of cigarettes smoked daily and adduct levels ($P = 0.04$). DNA adduct levels correlated with 4-ABP–haemoglobin adduct levels. Individuals with some combinations of *GSTT1* or *GSTM1* genotypes with *NAT1* and *NAT2* genotypes had significantly different adduct levels
Matullo *et al.* (219)	WBC	^{32}P-Postlabelling	81 smokers 92 ex-smokers 131 non-smokers	No effect of smoking on adduct levels apparent, but within each group, polymorphisms in *XRCC3* associated with higher adduct levels. *XRCC1* and *XPD* genotype affected adduct levels in non-smokers only
Tang *et al.* (128)	WBC	^{32}P-Postlabelling	86 lung cancer cases (36 smokers, 35 ex-smokers, 15 non-smokers) 158 controls (64 smokers, 64 ex-smokers, 30 non-smokers)	In this prospective study, among current smokers the mean level of adducts among cases was double that among the controls ($P = 0.03$). Smokers had higher levels of adducts than ex- and non-smokers, but differences not significant
van Delft *et al.* (220)	Lymphocytes	^{32}P-Postlabelling	35 coke oven workers (15 smokers, 20 non-smokers) 37 controls (18 smokers, 19 non-smokers)	Adduct levels significantly higher in smokers of control group and control + workers ($P < 0.05$), but not in workers only
Whyatt *et al.* (221)	WBC	^{32}P-Postlabelling, ELISA	160 mother/newborn infant pairs (16 smokers, 38 ex-smokers, 106 non-smokers)	Cord blood of infants had higher DNA adduct levels than maternal WBC. When analysed by ELISA, adduct levels were higher in 7/10 smoking mothers than in their infants ($P = 0.05$). Among pairs where blood samples were obtained concurrently ($n = 60$), adducts were higher in the newborn than the parental WBC in all groups except current smokers
Nia *et al.* (125)	Lymphocytes	HPLC/ECD for 8-oxo-dG; comet assay for oxidized pyrimidines	21 smokers 24 non-smokers	Smokers had lower levels of adduct than smokers (38.6 ± 5.2 versus $50.9 \pm 4.6/10^6$ dG, $P = 0.05$). Levels of oxidized pyrimidines were lower (not statistically significant) than in non-smokers

Table II. Continued

Study	Blood cells[a]	Method of analysis	Number of subjects	Outcome and comments
Hou *et al.* (222)	Lymphocytes	^{32}P-Postlabelling	170 lung cancer cases 144 controls (113 smokers and recent ex-smokers, 201 long-term ex-smokers and non-smokers)	Adduct levels higher in smokers than in non-smokers, especially in controls. Controls with *NAT2 slow* genotype had higher adduct levels than *rapid*
Hou *et al.* (223)	Lymphocytes	^{32}P-Postlabelling	185 lung cancer cases (97 smokers, 88 non-smokers) 162 controls (83 smokers, 79 non-smokers)	Adduct levels similar in cases and controls, but increased in smokers and recent ex-smokers (statistically significant). Adduct levels significantly increased in individuals possessing *XPD* exon 10 or exon 23 variant alleles
Teixeira *et al.* (224)	Lymphocytes	^{32}P-Postlabelling	18 coke oven workers (8 smokers, 10 non-smokers) 21 controls (10 smokers, 11 non-smokers)	Smokers in the control group had significantly higher adduct levels than non-smokers ($P = 0.003$), with a significant correlation between adduct levels and number of cigarettes smoked/day ($P < 0.0005$). Among smokers in control group, adduct levels were dependent on *CYP1A1 Msp*1 genotype, but not *GSTP1, GSTM1* or *GSTT1* genotypes

[a]Mononuclear cells: lymphocytes plus monocytes.

in two lung cancer case-control studies (126,127) and one prospective study (128), but not in another case-control study (129). The effect was also observed in a bladder cancer case-control study (130). This suggests that DNA adducts can be considered as biomarkers of risk in some populations, although the magnitude is small when seen against a background of wide inter-individual variation, making assessment of risk on an individual basis problematic.

Another approach to monitoring human exposure to carcinogens has been to detect the antigenic response to BPDE–DNA adducts in blood cells. Serum antibodies to these adducts have been detected in a number of groups of occupationally exposed workers, but in a study of smokers only a small percentage (8%) of heavy smokers ($n = 50$) initially tested positive for the antibodies. Paradoxically, the frequency of positivity increased during a cessation programme in which 28 subjects quit smoking and 22 reduced consumption by 75% (131). Nevertheless, in a larger population study of 1345 individuals for the presence of detectable BPDE–DNA antibodies in serum, there was a positive association with smoking (17.8% of non-smokers, 21.5% of ex-smokers and 25.7% of smokers tested positive) and there was a synergism between smoking and family history of lung cancer in determining the prevalence of antibodies ($P = 0.02$) (132).

Smoking-related DNA adducts in non-smokers

In comparison to the number of studies that have investigated the levels of DNA adducts, or other measures of DNA damage, in the tissues of smokers, ex-smokers and non-smokers (see preceding section), relatively few studies have addressed the issue of using these biomarkers to monitor exposure of non-smokers to environmental tobacco smoke (ETS). Considering that the magnitude of the difference between smokers and non-smokers is often quite small (for example, <2-fold in the case of DNA adducts in WBC), it is probable that in many circumstances this biomarker may lack the sensitivity to distinguish the effects of exposure to ETS from exposure to other sources of environmental carcinogens. Studies in which a distinction has been made between non-smokers exposed to ETS and those unexposed are described below.

In a study in which declining DNA adduct levels in the WBC of smokers enrolled in a smoking cessation programme were measured by ELISA using antibodies to BPDE–DNA adducts, levels of adducts both at baseline and 10 weeks after cessation were significantly associated with hours of exposure to ETS at home and were also higher if the subject lived with another smoker ($P = 0.009$, 0.02 and 0.02, respectively). However, there was no observable influence of exposure to ETS in the workplace (111).

Using the prevalence of serum antibodies to BPDE–DNA adducts in an Italian population as a biomarker of exposure to environmental PAHs, investigators reported an association between per cent positivity and smoking status (25.7% of smokers, 21.5% of ex-smokers and 17.8% of non-smokers) and living environment (26.0% urban dwellers and 17.9% suburban dwellers), but no association with passive smoking was found (132).

In a study in which significant differences were observed between the levels of adducts, determined by a flow cytometric method using BPDE–DNA antibodies, in peripheral lymphocytes of smokers ($n = 40$) and non-smokers ($n = 35$), the mean value for non-smokers with no or low exposure to ETS

(n = 17) was lower than that of non-smokers who were exposed (n = 18), but the difference was not statistically significant (133).

A group of five non-smokers were exposed to ETS under controlled conditions (exposure to gas-phase only for 8 h, followed by exposure to whole ETS for 8 h, 40 h later). When their monocyte DNA was analysed by ^{32}P-postlabelling, no changes in the adduct patterns were seen after either exposure period, compared with the samples obtained before exposure (134). Similarly, the frequency of DNA single strand breaks in mononuclear cells of non-smokers was not affected by controlled exposure to ETS for 8 h (135).

In a study of biomarkers of air pollution exposure in two Greek populations, one from an urban location, the other rural, ^{32}P-postlabelling analysis of lymphocyte DNA revealed the presence of DNA adducts that were more abundant in the rural subjects than in the urban ones. The variation in adduct levels was found to significantly ($P < 0.001$) parallel exposure to ETS, as judged by self-reporting, plasma cotinine concentrations and profiles of personal exposure to PAHs characteristic of ETS rather than other environmental sources (136).

When a group of 15 non-smokers (11 female, five male), who provided blood and sputum samples before, and 1–2 h after, spending 3 h in a smoky environment were studied, their plasma cotinine and nicotine levels were both significantly increased by the exposure to ETS, but exposure did not result in the induction of DNA adducts in peripheral blood lymphocytes. However, changes in the adduct profiles of sputum DNA of some individuals were observed, including the formation of an adduct characteristic of the major BPDE–DNA adduct in three individuals (137).

When placental DNA was analysed both for bulky DNA adducts by ^{32}P-postlabelling and for 8-oxo-dG by ECD, neither method showed a difference between 11 smokers, 10 non-smokers and nine non-smokers exposed to passive smoking (97).

Immunohistochemical staining, using antibodies to BPDE–DNA adducts, of ovarian granulosa-lutein cells of women undergoing IVF was intermediate in intensity for non-smoking women exposed to passive smoking (n = 7) to the values for smokers (n = 14) and unexposed non-smokers (n = 11) and statistically significantly different from both these groups ($P < 0.0001$) (101).

Smoking-related protein adducts in human tissues

Even though proteins are not the target molecules for mutagenic events, their use as biomarkers can be advantageous because of their greater abundance than DNA and the fact that they are not subject to enzymatic repair. The rate of turnover of the proteins and/or of the cells that harbour them means that carcinogen–protein adducts can provide evidence of exposure on a time-scale of up to several months. In theory, haemoglobin, serum albumin, histones and collagen are suitable proteins for study (138), but in practice only haemoglobin and albumin have been extensively studied as biomarkers of human exposure to environmental carcinogens.

Protein adducts are generally detected in one of three ways: mass spectrometric detection of the carcinogen moiety after its release from protein by mild acid or base hydrolysis, or release and detection of the modified N-terminal valine of haemoglobin; immunochemical analysis using antibodies raised against protein adducts; and HPLC with fluorescence detection of the released carcinogen (139). The use of protein adducts as biomarkers of human exposure to carcinogens has been extensively reviewed elsewhere (9,10,12,138).

Adducts formed by aromatic amines

In an early study of smokers and non-smokers, 4-ABP–haemoglobin adducts were measured by GC-MS. The smokers (n = 22) had adduct levels significantly higher than the non-smokers (n = 24) and there was a significant correlation with average packs/day, pack-years and cumulative lifetime tar exposure. It is noteworthy that the range of values for smokers (75–256 pg/g haemoglobin) did not overlap with that for non-smokers (7–51 pg/g) (140).

Non-overlap in values was also observed in another study involving 19 smokers (range 70–260 pg/g; mean 154) and 26 non-smokers (range 10–50 pg/g; mean 28) and the difference between the two groups was highly significant ($P < 0.001$). The finding of detectable levels of adducts in all the non-smokers is consistent with other environmental sources of 4-ABP besides tobacco smoking (141).

In a group of male volunteers arising from a case-control study of bladder cancer in Turin, Italy, haemoglobin adducts of 15 different aromatic amines were determined in non-smokers and smokers of blond- or black-tobacco cigarettes. The smokers of the blond tobacco (n = 40) and the black tobacco (n = 18; a further three subjects smoked both types) had significantly higher levels of ABP adducts than non-smokers (n = 25). Furthermore, adduct levels were significantly higher (40–50%) for black tobacco smoking than for blond tobacco. Adduct levels correlated with amount smoked per day for all smokers (P = 0.0015) and this was also significant for blond tobacco smoking only (P = 0.0074). For other aromatic amines, the levels of 3-ABP adducts were also significantly elevated in smokers ($P < 0.0001$) and associated with numbers of cigarettes smoked by the blond tobacco users (P = 0.02). Adducts derived from a further five aromatic amines, 2-naphthylamine, *o*-toluidine, *p*-toluidine, 2-ethylaniline and 2,4-dimethylaniline were significantly higher in the smokers, while those of *m*-toluidine, 2,5-, 2,6-, 2,3-, 3,5- and 3,4-dimethylaniline and 3- and 4-ethylaniline were not (142). In a subsequent analysis, the authors sought explanations for the inter-individual variability observed. There appeared to be a distinct difference between the values obtained with the binuclear compounds and those obtained with the mononuclear compounds. Correlations between the levels of the three binuclear amines (2-naphthylamine, 4-ABP and 3-ABP) were significant ($P < 0.05$) and 49/54 of the correlations between different mononuclear amines were significant, but only 2/33 correlations between a binuclear and a mononuclear amine were significant. These results suggest the existence of two distinct pathways of metabolic activation of aromatic amines and explain a part, but not all, of the inter-individual variation in adduct levels observed (143).

As part of a validation exercise in a study of 4- and 3-ABP–haemoglobin adducts in non-smokers exposed to ETS (see following section), seven quitting smokers were monitored on the day they stopped smoking and again at least 2 months later. The mean levels of 4-ABP adducts fell by 75% and the mean level of 3-ABP fell by at least 80% (in four cases of the latter, to levels below the limit of sensitivity of the assay) (144). A further study of smokers enrolled at a cessation clinic found that the 34 subjects at the start had a mean level of 4-ABP–haemoglobin of 120 ± 7 (SE) pg/g which declined to

82 ± 6 pg/g after 3 weeks. In the 15 smokers who remained abstainers after 2 months the level was 34 ± 5 pg/g; the rate of decline was slightly more rapid than would be expected from an assumption that erythrocytes have a life-span of 120 days. At the start of the study there was no correlation with plasma cotinine levels or with the tar content of the cigarette brands that had been smoked (145). Similar findings were reported in another study of smoking cessation involving 40 smokers. Mean levels for 16 subjects were <50 and 25% of those at the start, at 10 weeks and 8 months, respectively, both reductions highly significant. Depending on the model used, the half-life of the adducts was estimated at between 7 and 12 weeks (111).

In a study of 50 non-smokers, 31 smokers of blond tobacco, 16 smokers of black tobacco and three pipe smokers, the relationship between consumption of blond and black tobacco and 4-ABP–haemoglobin adduct levels was confirmed ($P = 0.0001$ in each case). A linear relationship between adduct levels and the numbers of cigarettes smoked in the preceding 24 h was also observed ($P = 0.0001$). Furthermore, when the subjects were divided into slow and rapid acetylators (by measuring urinary excretion of caffeine after administration of a test dose), it was found that the ratio of adduct levels slow:rapid was 1.6 for non-smokers, 1.3 for blond tobacco and 1.5 for black tobacco. This approximates to the estimated relative risk of the slow acetylator phenotype for bladder cancer of 1.32 (95% CI 1.02–1.71) (146). A further study of this cohort, while confirming these findings, also found that urinary mutagenicity was associated with the numbers of cigarettes smoked but not with the acetylator phenotype (147). Subsequently, 4-ABP–haemoglobin adduct levels in 39 individuals (21 non-smokers, 11 blond-tobacco smokers and seven black-tobacco smokers) were found to correlate with total DNA adduct levels in exfoliated urothelial cells ($P = 0.03$) (55).

In a case-control study of lung cancer, there were 53 cases and 56 controls (33 with non-cancer pulmonary disease and 23 with non-pulmonary cancer). Adduct levels were higher in smokers than non-smokers and reflected recent smoking exposure. However, there was no association between adduct levels and lung cancer diagnosis (148). However, in a case-control study of bladder cancer, the mean adduct level in the cases ($n = 13$, all smokers) was significantly higher than in the controls ($n = 13$, all smokers) [103 ± 47 (SD) pg/g haemoglobin versus 65 ± 44 pg/g, $P = 0.04$] (149).

4-ABP–haemoglobin adduct levels were also significantly higher in pregnant women who smoked ($n = 15$) than in those who did not ($n = 40$) (183 ± 108 pg/g haemoglobin versus 22 ± 8 pg/g, $P<0.001$), in a study in which levels of the adduct in fetal blood were also measured (see following section) (150). Similarly, two studies of non-smokers and an equal number of smokers measured paired maternal–fetal blood samples. Levels of 4-ABP–haemoglobin adducts were significantly higher in the smokers, and similar ratios of adduct levels in maternal and cord blood were found to those reported in the previous study (151,152). There was also a correlation between adduct levels in maternal blood and cigarettes smoked per day (152).

Analysis of smokers and non-smokers for haemoglobin adducts of several aromatic amines revealed significantly higher levels in smokers of those formed by 4-ABP ($P < 0.001$), 3-ABP ($P < 0.001$) and 2,4-dimethylaniline ($P < 0.05$), but not by aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, 2-ethylaniline and *o*-anisidine. For many of these comparisons the numbers of subjects was small (four to 22 smokers, four to 16 non-smokers) (153).

Comparisons of 3- and 4-ABP–haemoglobin adducts in three different racial groups (white, black and Asian) led to the expected detection of higher levels of both adducts in smokers ($n = 61$) than in non-smokers ($n = 72$), and of a correlation with the numbers of cigarettes smoked per day ($P < 0.0005$). Those with the slow acetylator phenotype had higher adduct levels in all three ethnic groups (2.5-fold higher for 3-ABP, $P < 0.0005$; 1.2 fold higher for 4-ABP, $P = 0.19$) (154).

In a cohort of 55 smokers and four non-smokers, 4-ABP–haemoglobin adduct levels were related to numbers of cigarettes smoked, although saturation of adduct formation was apparent at >30 cigarettes/day. A relationship between aromatic DNA adducts in lymphocytes and daily cigarette consumption was not found. In the same study, *GSTM1* and *NAT2* polymorphisms did not affect protein adduct levels (155). The same investigators went on to investigate additional genetic polymorphisms in 67 smokers and found no overall effects on 4-ABP–haemoglobin adduct levels of polymorphisms in *NAT1*, *NAT2*, *GSTM1* or *GSTT1*, except that in smokers of <25 cigarettes/day, *NAT2* slow acetylators had significantly higher adduct levels than fast acetylators ($P = 0.03$) (156).

In another study NAT2 slow acetylators also had significantly higher levels of 4-ABP–haemoglobin adducts than rapid acetylators, but there was no association with possession of the *NAT1*10* allele. As before, smokers had significantly higher levels of 3- and 4-ABP–haemoglobin adducts and the levels increased with increasing cigarettes smoked per day ($P < 0.0001$, $n = 68$ smokers, 288 non-smokers) (157).

Adducts formed by polycyclic aromatic hydrocarbons

In a study involving 87 mothers and their 87 children in which PAH–albumin adducts were investigated as a biomarker of the children's exposure to ETS from their smoking mothers (see following section) it was also noted that adduct levels were significantly higher in the smoking mothers than in the non-smokers (158).

A GC-MS method was used to measure BPDE–globin adduct in 10 smokers and 10 non-smokers. Subjects were also monitored for formation of globin adducts of another PAH, namely chrysene. In both cases the procedure involved measurement of the respective PAH tetrols released from globin by acid hydrolysis. Levels of BPDE adducts in the smokers were 2.7-fold higher than in the non-smokers ($P < 0.01$), and although the chrysene diol-epoxide adducts were 25% higher in smokers, this was not statistically significant ($P = 0.06$) (159).

BPDE adducts with both haemoglobin and with serum albumin were determined by GC-MS of tetrols released by acid hydrolysis in a study of 44 male lung cancer patients. Individuals who were positive for haemoglobin adducts ($n = 6$) all had detectable albumin adducts, but not vice versa (24 subjects were positive for albumin adducts). Those who were carriers of a *CYP1A1* variant allele were more frequently positive for albumin adducts ($P = 0.03$) and those with two 'slow' *mEH* alleles had a lower frequency of these adducts (160).

In another study, tobacco smokers ($n = 27$) had significantly higher levels of BPDE–albumin adducts and non-significantly higher levels of BPDE–haemoglobin than non-smokers ($n = 42$) (161) (see also following section).

Adducts formed by tobacco-specific nitrosamines

Treatment of haemoglobin adducted with NNK or NNN releases 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), which is amenable to detection by GC-MS after purification and derivatization. In the first determination of HPB levels in smokers, a group of 40 smokers had a mean value of 79.6 ± 189 fmol/g haemoglobin (undetectable in 11 individuals; limit of detection was 5 fmol/g) while among 21 non-smokers the mean value was 29.3 ± 25.9 fmol/g (undetectable in four individuals). (Statistical significance not stated, although among 22 snuff dippers HPB levels were 517 ± 538 fmol/g, significantly higher than in smokers, $P < 0.0001$.) No relationship was apparent between HPB levels in smokers and levels of plasma cotinine or numbers of cigarettes smoked (162).

In a comparison of 20 smokers and 15 non-smokers, HPB levels in haemoglobin were significantly higher in the smokers (69.2 ± 43.9 versus 34.4 ± 16.0 fmol/g) ($P < 0.005$) (153).

In another study of 18 smokers and ex-smokers and 52 non-smokers, levels of HPB in haemoglobin were significantly higher in the smokers [26 ± 13 (SD) fmol/g haemoglobin] than in the non-smokers (20 ± 8 fmol/g) ($P = 0.02$). No differences were found between the sexes (14 males, 56 females) or between the smokers ($n = 10$) and ex-smokers ($n = 8$) (duration of smoking cessation not stated) (163).

Adducts formed by other compounds

Ethylene is a major gaseous constituent of tobacco smoke that converts, via ethylene oxide, the N-terminal valine of haemoglobin to *N*-(2-hydroxyethyl)valine (HOEtVal). Eleven smokers of >20 cigarettes/day had HOEtVal in haemoglobin in the range 217–690 pmol/g (mean ± SD, 389 ± 138) while for 14 non-smokers the range was 27–106 pmol/g (58 ± 25). It is noteworthy that there was no overlap in values between the two groups (164). In a controlled experiment, two volunteers who were regular smokers (29 and 18 cigarettes/day, respectively) abstained from smoking for 7 days and then resumed. From the levels of HOEtVal before stopping, 7 days after stopping and 7 days after resuming, it was calculated that in the 29-a-day smoker each cigarette smoked increased the level of HOEtVal by 0.12 pmol/g haemoglobin. For the other smoker the increment was 0.08 pmol/g (165).

In a study of smokers ($n = 26$) and non-smokers ($n = 24$), background levels of HOEtVal in the non-smokers averaged 49.9 pmol/g haemoglobin (range 22–106). In the smokers the levels were significantly higher by an estimated 71 pmol/10 cigarettes/day, and the correlation with cigarettes smoked per day was significant ($P < 0.01$) (166).

As part of a maternal–fetal comparison of HOEtVal in haemoglobin (see following section), samples from 10 non-smoking pregnant women and 13 pregnant women smoking 15 or more cigarettes per day were analysed. The non-smokers had 63 ± 20 (mean ± SD) pmol/g haemoglobin, while the smokers had 361 ± 107 pmol/g ($P < 0.01$) (167).

In a study of 146 adults (82 males and 64 females), smokers ($n = 44$) had significantly higher levels of HOEtVal in haemoglobin than non-smokers ($n = 74$) and self-reported passive smokers ($n = 28$) (mean ± SD: 59 ± 52, 17 ± 21 and 17 ± 23, respectively). Linear regression analysis of adduct levels and cigarettes smoked per day showed a highly significant correlation coefficient ($r = 0.63$, $P < 0.0001$). Adduct levels were also significantly higher in males than in females (mean value 2.6-fold higher, $P = 0.00001$) (168).

Ethylene oxide is a substrate for GSTT1. To determine whether polymorphisms in *GSTT1* modulate HOEtVal formation, blood samples from 10 women and 17 men were analysed. The median level of the adduct in smokers was 5-fold higher in the smokers (five males, one female) than the non-smokers (12 males, nine females) but no correlation with daily cigarette consumption was found. *GSTM1* and *GSTT1* genotype did not influence adduct levels in the smokers, although there was an effect of *GSTT1* genotype on levels in non-smokers (169).

The effect of *GSTT1* was also investigated in another study involving 14 non-smokers, 16 one-pack-a-day smokers and 13 two-pack-a-day smokers. HOEtVal levels increased with increasing smoking, and differences between the three groups were statistically significant. In addition, levels of *N*-(2-cyanoethyl)valine (CEVal), which is formed from acrylonitrile, were also significantly correlated with smoking in these subjects. HOEtVal and CEVal levels were found to be significantly correlated in smokers ($P = 0.003$). There was no effect of *GSTM1* or *GSTT1* genotypes on CEVal levels in any of the groups or of *GSTM1* on HOEtVal levels; however, *GSTT1 null* individuals had higher levels of HOEtVal (slope of regression line 50% higher) (170).

A study of acrylamide and acrylonitrile exposure in laboratory workers, smokers and non-smokers included also measurement of ethylene oxide haemoglobin adducts. The mean levels of adducts from acrylamide, acrylonitrile and ethylene oxide in 10 smokers were 116, 106 and 126 pmol/g, respectively, significantly higher than in eight non-smokers (31, <2 and 17 pmol/g, respectively). In smokers, both acrylamide and acrylonitrile adducts correlated with the number of cigarettes smoked per day (171).

Two other studies have reported acrylonitrile–haemoglobin adducts in smokers. In the first, four smokers had 75–106 pmol CEVal/g haemoglobin (mean 88) while in four non-smokers the levels were all below the limit of detection of 20 pmol/g (172). In the second study, 13 smoking mothers had adduct levels in the range 92.5–373 pmol/g (mean ± SD: 217 ± 85.1) but adduct levels were below the limit of detection (1 pmol/g) in 10 non-smoking mothers. Among the smokers, there was a linear correlation with cigarettes smoked per day ($P = 0.02$) and also with the levels of the adducts in their newborn babies (see following section) (173).

In a study of 43 Chinese workers exposed to benzene and 44 unexposed controls, in which haemoglobin and albumin adducts of two metabolites, benzene oxide and 1,4-benzoquinone were measured, tobacco smoking was found to have an additive effect on 1,4-benzoquinone–albumin formation ($P = 0.034$) but not on benzene oxide–albumin formation ($P = 0.23$) (174).

Human haemoglobin contains relatively high background levels of *N*-methylvaline, which could limit its sensitivity as a biomarker of exposure to environmental carcinogens. However, in a study of 11 pairs of monozygotic twins discordant for tobacco smoking, both HOEtVal and *N*-methylvaline were found to be higher in the smokers than the non-smokers. For the smokers the levels of HOEtVal and *N*-methylvaline were 143 ± 26 (mean ± SE) and 268 ± 13 pmol/g, respectively and for the non-smokers 15.6 ± 1.9 and 225 ± 11 pmol/g, respectively. Thus, *N*-methylvaline adduct levels were significantly different between smokers and non-smokers ($P = 0.006$) and there was a highly significant correlation with number of cigarettes per day ($P < 0.001$) (175).

Despite the relatively high background levels of *N*-methylvaline in human haemoglobin, levels of the adduct were found

to be elevated in a group of 32 smokers compared with 37 non-smokers (mean ± SD 1546 ± 432 versus 1175 ± 432 pmol/g, $P < 0.001$); for the smokers there was also a linear correlation between adduct levels and cigarettes smoked per day ($P < 0.001$). A significant difference between smokers and non-smokers for HOEtVal was also observed in this study (176).

Since cigarette smoke contains reactive species that can cause oxidative and nitrative damage in cellular macromolecules, plasma protein from 52 lung cancer patients (24 smokers, 28 non-smokers) and 43 control subjects (18 smokers, 25 non-smokers) was analysed for nitrotyrosine and carbonyl groups as markers of nitration and oxidation, respectively. Nitrated proteins were significantly higher in the patients than in the controls ($P = 0.003$) but not related to smoking status. In contrast, oxidized proteins were higher in smokers ($P < 0.001$) but not related to disease status (177).

Smoking-related protein adducts in non-smokers

In order to determine whether involuntary smoking increased the levels of aromatic amine–haemoglobin adducts, a group of 14 volunteers who reported negligible exposure to ETS was compared with 21 non-smokers who reported exposure to at least one pack of cigarettes per day smoked by others and with 15 non-smokers of unknown ETS exposure. All three groups had no measurable cotinine in their blood. A further 13 non-smokers, including six bartenders who were heavily exposed to ETS, had measurable levels of cotinine in their blood. Background levels of adducts from 4-ABP and 3-ABP were detected in all subjects, but higher levels were found in subjects with detectable cotinine levels ($P = 0.05$ and 0.027, respectively) (144).

In another study of 4-ABP–haemoglobin adducts in smokers and passive smokers (see also preceding section) adduct levels among 15 non-smokers who reported exposure to ETS were not significantly higher than in 35 unexposed non-smokers [87.9 ± 19 (SE) pg/g haemoglobin versus 69.5 ± 7 pg/g]. It should be noted that only 4/15 of those reporting exposure to ETS, and 4/35 of those reporting no exposure, had measurable levels of urinary cotinine (147).

Exposure of young (pre-school) children to environmental tobacco smoke from their mothers' smoking was investigated by measuring plasma cotinine levels and PAH–albumin adducts in peripheral blood, the latter detected by ELISA. The study involved 87 mother–child pairs; 31 mothers smoked and 56 did not. Not only did the mothers who smoked have higher levels of adducts (see preceding section) but the levels in the children of the smoking mothers were also significantly higher ($P < 0.05$). There was also a significant correlation between the adduct levels in the mothers and the levels in their children ($P = 0.014$) (158). In a subsequent study, PAH–albumin adducts were measured by ELISA and 4-ABP–haemoglobin adducts were measured by GC-MS. In 109 pre-school children, PAH–albumin adducts were significantly higher in the children whose mothers smoked ($P = 0.001$) or who lived with other smokers ($P = 0.017$), compared with those in non-smoking households; levels of 4-ABP–haemoglobin adducts were also higher in these children, but not significantly ($P = 0.073$ and 0.066, respectively) (178).

Measurements of maternal–fetal exchange of 4-ABP in smoking ($n = 14$) and non-smoking ($n = 38$) pregnant women (see also preceding section) found that levels of the haemoglobin adduct in cord blood were consistently lower than in maternal blood. A significant correlation was found by linear regression between maternal and fetal levels; this was true for all subjects ($P < 0.001$) and for smokers only ($P = 0.002$) but not for non-smokers only ($P = 0.06$) (150). A further report on this same sample set explored the relationship between adduct levels and ETS exposure to the mothers. The level of ETS exposure was determined by questionnaire, diary and personal air monitor and reported as <0.5, 0.5–1.9 and ⩾2.0 μg/m^3 weekly average nicotine concentration. Among 40 non-smoking women there was a significant correlation between 4-ABP–haemoglobin and exposure category ($P = 0.009$) (179). In two other reports, lower levels of adducts were also found in fetal blood, relative to maternal blood, at levels that correlated with the smoking status of the mothers (151,152).

Blood samples from smoking and non-smoking mothers and cord blood from their newborns were analysed for HOEtVal in haemoglobin. Adduct levels were significantly higher in the smoking women than in the non-smokers (see preceding section), and the concentrations in the newborns were significantly higher ($P < 0.01$) in those with smoking mothers [$n = 13$, 147 ± 68 (mean ± SD) pmol/g] than in those with non-smoking mothers ($n = 10$, 42 ± 18 pmol/g). There was also a significant correlation ($P < 0.01$) between adduct levels in the infants and levels in their mothers (167).

The same maternal–fetal samples were subsequently analysed for CEVal formed by acrylonitrile. Adduct levels were significantly higher in the smoking mothers than in the non-smoking ones (see preceding section) and there was a significant correlation between the adduct levels in the smoking mothers and their newborns ($P < 0.001$). The levels were lower in the babies than in their mothers (99.5 ± 53.8 pmol/g versus 217 ± 85.1 pmol/g for the smokers) and there was a significant correlation between the levels in the babies and the numbers of cigarettes smoked per day by the mothers ($P = 0.009$). For the babies of non-smoking mothers, CEVal levels were below the limit of detection of the assay (1 pmol/g) (173).

In a study of 69 adults, smokers comprised 27 of the group and of the 42 non-smokers, 19 were classified as passive smokers by self-reporting and cotinine levels. Although the levels of BPDE adducts with albumin and haemoglobin were higher in the smokers (differences in the former were statistically significant, but in the latter were not; see preceding section), there was no difference found between non-smokers exposed to ETS and those not or rarely exposed to ETS (161).

Levels of HOEtVal in haemoglobin, while higher in smokers than in non-smokers (see preceding section) were found to be present at the same levels in non-smokers who did not live or work with a smoker ($n = 74$) as those who did ($n = 28$) (168).

Conclusions

Smoking induces DNA adducts and oxidative DNA damage in human tissues. Inter-individual variations in adducts levels have been widely documented and evidence points both to the influence of genetic factors and environmental factors (e.g. levels of vitamins and anti-oxidants). Formation of carcinogen–DNA adducts in critical genes can lead to mutations that alter protein function and that cause the carcinogenic progression of cells from normality to neoplasia. This is apparent from studies of the genes mutated in human tumours

and in the analysis of their mutation spectra, e.g. *TP53* (180–182).

Measuring adduct levels provides an integrated measure of exposure, metabolism and metabolic activation of carcinogens. Adducts are subject to DNA repair and loss through cell turnover, but with the constant replenishment that chronic exposure to tobacco smoke elicits, it seems probable that an approximate steady-state level may be reached in the tissues of habitual smokers. However, investigators are seldom, if ever, presented with the opportunity of making multiple measurements, over time, of DNA adduct levels in target tissues. There is much current effort being put into determining whether genetic polymorphisms in xenobiotic metabolizing genes or DNA repair genes can determine either cancer risk or adduct levels, but such research should never be viewed as a means to identify individuals who may smoke with impunity, as possession of a certain polymorphism might lower the risk of cancer at one site, but not at others. It should also be remembered that more smokers die of smoking-induced heart disease than of lung cancer, and there are many other diseases attributable to tobacco smoking.

Protein adducts are a useful surrogate biomarker for DNA adducts. Although 4-ABP is not a tobacco-specific compound, its protein adduct levels are significantly higher in smokers than non-smokers, and the lack of overlap between smokers' levels and non-smokers' levels means this biomarker can distinguish smokers from non-smokers. This is frequently not possible on an individual basis for DNA adducts, even though mean levels of adducts are significantly elevated in groups of smokers relative to non-smokers.

There is now abundant evidence for the formation of DNA adducts in tissues of the body that are directly exposed to tobacco smoke. Furthermore, the formation of smoking-related DNA adducts is not restricted to these organs; there is clear evidence of the systemic distribution of tobacco carcinogens to many other organs of the body, including the cardiovascular system, peripheral blood, bladder, cervix and anal epithelium. For some of these sites, there is sufficient epidemiological evidence that smoking is causally involved in cancer of that site, although in some cases this has become apparent relatively recently. Although smoking has been a suspected risk factor for cervical cancer for some time, the belief that this was a confounding factor also persisted for some time. Nevertheless, the biomarker evidence that the tissue is exposed to tobacco carcinogens and that adducts are formed there, existed before cervical cancer joined the list of those cancer sites with which tobacco smoking is causally associated (2). There are other sites for which there is evidence of DNA adduct formation but for which the epidemiological evidence is not extensive, e.g. anal epithelium (183,184). Also, the scientific literature frequently cites tobacco smoking as a risk factor for several relatively uncommon cancers of the urogenital tract (184,185). Analysis of these tissues for smoking-related adducts is therefore warranted. In addition, there are several cancer sites for which smoking is a causative agent (stomach, liver, colon, pancreas and kidney) where the formation of smoking-related DNA adducts has either not been studied, or studied only to a very limited extent. In all target tissues, the limited opportunities for obtaining more than one sample from each human subject has meant that adduct persistence has been inferred, rather than observed. Correlations between adduct levels in target tissues and a surrogate tissue (blood) have been sought in only a few studies, and results have been mixed.

With the risks to the health of smokers well established, attention has turned in recent years to the health effects on non-smokers exposed to ETS. There is now sufficient evidence to conclude that passive smoking causes lung cancer (2). For other cancers the risk may be real, but too small to detect by epidemiological methods. Protein adduct measurement can distinguish non-smokers exposed to ETS from those unexposed. DNA adduct measurement appears to be a less sensitive biomarker of exposure of passive smoking, although the numbers of studies conducted so far is small.

Overall, methods to detect protein and DNA adducts have proved their usefulness in monitoring the deleterious effects of smoking on human cells, revealing events that are relevant to the mechanism of action of the many carcinogens that are present in tobacco smoke. Continued exploration of these phenomena will increase our understanding of why tobacco smoking is the cause of so many different cancers, and serve to educate the public, regulatory authorities and governments on the need to curb tobacco marketing and consumption, for the sake of human health.

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References

1. Doll,R., Peto,R., Wheatley,K., Gray,R. and Sutherland,I. (1994) Mortality in relation to smoking: 40 years' observations on male British doctors. *Br. Med. J.*, **309**, 901–911.
2. IARC (2003) Monographs on the Evaluation of Carcinogenic Risks to Humans. *Tobacco Smoke and Involuntary Smoking, Vol. 83*. IARC Scientific Publications, IARC, Lyon.
3. Hoffmann,D. and Hecht,S.S. (1990) Advances in tobacco carcinogenesis. In Cooper,C.S. and Grover,P.L. (eds.), *Chemical Carcinogenesis and Mutagenesis I*. Springer-Verlag, Berlin, pp. 63–102.
4. Miller,E.C. and Miller,J.A. (1981) Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer*, **47**, 2327–2345.
5. Phillips,D.H. (2002) The formation of DNA adducts. In Allison,M.R. (ed.), *The Cancer Handbook*. Macmillan, London, pp. 293–306.
6. Hecht,S.S. (2002) Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis*, **23**, 907–922.
7. Beach,A.C. and Gupta,R.C. (1992) Human biomonitoring and the ^{32}P-postlabeling assay. *Carcinogenesis*, **13**, 1053–1074.
8. Phillips,D.H. (1997) Detection of DNA modifications by the ^{32}P-postlabelling assay. *Mutat. Res.*, **378**, 1–12.
9. Poirier,M.C., Santella,R.M. and Weston,A. (2000) Carcinogen macromolecular adducts and their measurement. *Carcinogenesis*, **21**, 353–359.
10. Strickland,P.T., Routledge,M.N. and Dipple,A. (1993) Methodologies for measuring carcinogen adducts in humans. *Cancer Epidemiol. Biomarkers Prev.*, **2**, 607–619.
11. Weston,A. (1993) Physical methods for the detection of carcinogen-DNA adducts in humans. *Mutat. Res.*, **288**, 19–29.
12. Wild,C.P. and Pisani,P. (1998) Carcinogen DNA and protein adducts as biomarkers of human exposure in environmental cancer epidemiology. *Cancer Detect. Prev.*, **22**, 273–283.
13. Kriek,E., Rojas,M., Alexandrov,K. and Bartsch,H. (1998) Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. *Mutat. Res.*, **400**, 215–231.
14. Phillips,D.H. (1996) DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke. *Environ Health Perspect.*, **104** (suppl. 3), 453–458.
15. Culp,S.J., Roberts,D.W., Talaska,G., Lang,N.P., Fu,P.P., Lay,J.O. Jr, Teitel,C.H., Snawder,J.E., Von Tungeln,L.S. and Kadlubar,F.F. (1997) Immunochemical, ^{32}P-postlabeling and GC/MS detection of 4-aminobiphenyl-DNA adducts in human peripheral lung in relation to metabolic activation pathways involving pulmonary N-oxidation, conjugation and peroxidation. *Mutat. Res.*, **378**, 97–112.

16. Blomeke,B., Greenblatt,M.J., Doan,V.D., Bowman,E.D., Murphy,S.E., Chen,C.C., Kato,S. and Shields,P.G. (1996) Distribution of 7-alkyl-2′-deoxyguanosine adduct levels in human lung. *Carcinogenesis*, **17**, 741–748.
17. Weston,A. and Bowman,E.D. (1991) Fluorescence detection of benzo[*a*]pyrene–DNA adducts in human lung. *Carcinogenesis*, **12**, 1445–1449.
18. Phillips,D.H., Hewer,A., Martin,C.N., Garner,R.C. and King,M.M. (1988) Correlation of DNA adduct levels in human lung with cigarette smoking. *Nature*, **336**, 790–792.
19. Phillips,D.H., Schoket,B., Hewer,A., Bailey,E., Kostic,S. and Vincze,I. (1990) Influence of cigarette smoking on the levels of DNA adducts in human bronchial epithelium and white blood cells. *Int. J. Cancer*, **46**, 569–575.
20. Dunn,B.P., Vedal,S., San,R.H., Kwan,W.F., Nelems,B., Enarson,D.A. and Stich,H.F. (1991) DNA adducts in bronchial biopsies. *Int. J. Cancer*, **48**, 485–492.
21. Asami,S., Manabe,H., Miyake,J., Tsurudome,Y., Hirano,T., Yamaguchi,R., Itoh,H. and Kasai,H. (1997) Cigarette smoking induces an increase in oxidative DNA damage, 8-hydroxydeoxyguanosine, in a central site of the human lung. *Carcinogenesis*, **18**, 1763–1766.
22. Schoket,B., Phillips,D.H., Kostic,S. and Vincze,I. (1998) Smoking-associated bulky DNA adducts in bronchial tissue related to CYP1A1 MspI and GSTM1 genotypes in lung patients. *Carcinogenesis*, **19**, 841–846.
23. Godschalk,R.W., Maas,L.M., Van Zandwijk,N., van't Veer,L.J., Breedijk,A., Borm,P.J., Verhaert,J., Kleinjans,J.C. and van Schooten,F.J. (1998) Differences in aromatic-DNA adduct levels between alveolar macrophages and subpopulations of white blood cells from smokers. *Carcinogenesis*, **19**, 819–825.
24. Ryberg,D., Hewer,A., Phillips,D.H. and Haugen,A. (1994) Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Res.*, **54**, 5801–5803.
25. Mollerup,S., Ryberg,D., Hewer,A., Phillips,D.H. and Haugen,A. (1999) Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. *Cancer Res.*, **59**, 3317–3320.
26. Degawa,M., Stern,S.J., Martin,M.V., Guengerich,F.P., Fu,P.P., Ilett,K.F., Kaderlik,R.K. and Kadlubar,F.F. (1994) Metabolic activation and carcinogen-DNA adduct detection in human larynx. *Cancer Res.*, **54**, 4915–4919.
27. Szyfter,K., Hemminki,K., Szyfter,W., Szmeja,Z., Banaszewski,J. and Yang,K. (1994) Aromatic DNA adducts in larynx biopsies and leukocytes. *Carcinogenesis*, **15**, 2195–2199.
28. Szyfter,K., Hemminki,K., Szyfter,W., Szmeja,Z., Banaszewski,J. and Pabiszczak,M. (1996) Tobacco smoke-associated N7-alkylguanine in DNA of larynx tissue and leucocytes. *Carcinogenesis*, **17**, 501–506.
29. Flamini,G., Romano,G., Curigliano,G., Chiominto,A., Capelli,G., Boninsegna,A., Signorelli,C., Ventura,L., Santella,R.M., Sgambato,A. and Cittadini,A. (1998) 4-Aminobiphenyl-DNA adducts in laryngeal tissue and smoking habits: an immunohistochemical study. *Carcinogenesis*, **19**, 353–357.
30. Banaszewski,J., Szmeja,Z., Szyfter,W., Szyfter,K., Baranczewski,P. and Moller,L. (2000) Analysis of aromatic DNA adducts in laryngeal biopsies. *Eur. Arch. Otorhinolaryngol.*, **257**, 149–153.
31. Nia,A.B., Maas,L.M., Van Breda,S.G., Curfs,D.M., Kleinjans,J.C., Wouters,E.F. and Van Schooten,F.J. (2000) Applicability of induced sputum for molecular dosimetry of exposure to inhalatory carcinogens: ^{32}P-postlabeling of lipophilic DNA adducts in smokers and nonsmokers. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 367–372.
32. Nia,A.B., Maas,L.M., Brouwer,E.M., Kleinjans,J.C. and Van Schooten,F.J. (2000) Comparison between smoking-related DNA adduct analysis in induced sputum and peripheral blood lymphocytes. *Carcinogenesis*, **21**, 1335–1340.
33. Besarati Nia,A., Van Straaten,H.W., Kleinjans,J.C. and Van Schooten,F.J. (2000) Immunoperoxidase detection of 4-aminobiphenyl- and polycyclic aromatic hydrocarbons-DNA adducts in induced sputum of smokers and non-smokers. *Mutat. Res.*, **468**, 125–135.
34. Leanderson,P. (1993) Cigarette smoke-induced DNA damage in cultured human lung cells. *Ann. N. Y. Acad. Sci.*, **686**, 249–259; discussion 259–261.
35. Leanderson,P. and Tagesson,C. (1994) Cigarette tar promotes neutrophil-induced DNA damage in cultured lung cells. *Environ. Res.*, **64**, 103–111.
36. Weitberg,A.B. and Corvese,D. (1993) Oxygen radicals potentiate the genetic toxicity of tobacco-specific nitrosamines. *Clin. Genet.*, **43**, 88–91.
37. Yang,Q., Hergenhahn,M., Weninger,A. and Bartsch,H. (1999) Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. *Carcinogenesis*, **20**, 1769–1775.
38. Wang,Q., Fan,J., Wang,H. and Liu,S. (2000) DNA damage and activation of c-ras in human embryo lung cells exposed to chrysotile and cigarette smoking solution. *J. Environ. Pathol. Toxicol. Oncol.*, **19**, 13–19.
39. Spencer,J.P., Jenner,A., Chimel,K., Aruoma,O.I., Cross,C.E., Wu,R. and Halliwell,B. (1995) DNA damage in human respiratory tract epithelial cells: damage by gas phase cigarette smoke apparently involves attack by reactive nitrogen species in addition to oxygen radicals. *FEBS Lett.*, **375**, 179–182.
40. Chacko,M. and Gupta,R.C. (1988) Evaluation of DNA damage in the oral mucosa of tobacco users and non-users by ^{32}P-adduct assay. *Carcinogenesis*, **9**, 2309–2313.
41. Dunn,B.P. and Stich,H.F. (1986) ^{32}P-postlabelling analysis of aromatic DNA adducts in human oral mucosal cells. *Carcinogenesis*, **7**, 1115–1120.
42. Jones,N.J., McGregor,A.D. and Waters,R. (1993) Detection of DNA adducts in human oral tissue: correlation of adduct levels with tobacco smoking and differential enhancement of adducts using the butanol extraction and nuclease P1 versions of ^{32}P postlabeling. *Cancer Res.*, **53**, 1522–1528.
43. Stone,J.G., Jones,N.J., McGregor,A.D. and Waters,R. (1995) Development of a human biomonitoring assay using buccal mucosa: comparison of smoking-related DNA adducts in mucosa versus biopsies. *Cancer Res.*, **55**, 1267–1270.
44. Peluso,M., Amasio,E., Bonassi,S., Munnia,A., Altrupa,F. and Parodi,S. (1997) Detection of DNA adducts in human nasal mucosa tissue by ^{32}P-postlabeling analysis. *Carcinogenesis*, **18**, 339–344.
45. Zhao,C., Georgellis,A., Flato,S., Palmberg,L., Thunberg,E. and Hemminki,K. (1997) DNA adducts in human nasal mucosa and white blood cells from smokers and non-smokers. *Carcinogenesis*, **18**, 2205–2208.
46. Nath,R.G., Ocando,J.E., Guttenplan,J.B. and Chung,F.L. (1998) 1,N^2-propanodeoxyguanosine adducts: potential new biomarkers of smoking-induced DNA damage in human oral tissue. *Cancer Res.*, **58**, 581–584.
47. Rojas,E., Valverde,M., Sordo,M. and Ostrosky-Wegman,P. (1996) DNA damage in exfoliated buccal cells of smokers assessed by the single cell gel electrophoresis assay. *Mutat. Res.*, **370**, 115–120.
48. Romano,G., Sgambato,A. and Boninsegna,A. (1999) Evaluation of polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral cells by an immunohistochemical assay. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 91–96.
49. Besarati Nia,A., Van Straaten,H.W., Godschalk,R.W., Van Zandwijk,N., Balm,A.J., Kleinjans,J.C. and Van Schooten,F.J. (2000) Immuno-peroxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in mouth floor and buccal mucosa cells of smokers and nonsmokers. *Environ. Mol. Mutagen.*, **36**, 127–133.
50. Romano,G., Mancini,R., Fedele,P., Curigliano,G., Flamini,G., Giovagnoli,M.R., Malara,N., Boninsegna,A., Vecchione,A., Santella,R.M. and Cittadini,A. (1997) Immunohistochemical analysis of 4-aminobiphenyl-DNA adducts in oral mucosal cells of smokers and nonsmokers. *Anticancer Res.*, **17**, 2827–2830.
51. Zhang,Y.J., Hsu,T.M. and Santella,R.M. (1995) Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in oral mucosa cells of smokers and nonsmokers. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 133–138.
52. Hsu,T.M., Zhang,Y.J. and Santella,R.M. (1997) Immunoperoxidase quantitation of 4-aminobiphenyl- and polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral and urothelial cells of smokers and nonsmokers. *Cancer Epidemiol. Biomarkers Prev.*, **6**, 193–199.
53. Zhang,Y., Chen,S.Y., Hsu,T. and Santella,R.M. (2002) Immunohistochemical detection of malondialdehyde-DNA adducts in human oral mucosa cells. *Carcinogenesis*, **23**, 207–211.
54. Talaska,G., al-Juburi,A.Z. and Kadlubar,F.F. (1991) Smoking related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. *Proc. Natl Acad. Sci. USA*, **88**, 5350–5354.
55. Talaska,G., Schamer,M., Skipper,P., Tannenbaum,S., Caporaso,N., Unruh,L., Kadlubar,F.F., Bartsch,H., Malaveille,C. and Vineis,P. (1991) Detection of carcinogen-DNA adducts in exfoliated urothelial cells of cigarette smokers: association with smoking, hemoglobin adducts and urinary mutagenicity. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 61–66.
56. Vineis,P., Talaska,G., Malaveille,C., Bartsch,H., Martone,T., Sithisarankul,P. and Strickland,P. (1996) DNA adducts in urothelial cells: relationship with biomarkers of exposure to arylamines and polycyclic aromatic hydrocarbons from tobacco smoke. *Int. J. Cancer*, **65**, 314–316.
57. Talaska,G., Schamer,M., Casetta,G., Tizzani,A. and Vineis,P. (1994) Carcinogen-DNA adducts in bladder biopsies and urothelial cells: a risk assessment exercise. *Cancer Lett.*, **84**, 93–97.

58. Routledge,M.N., Garner,R.C., Jenkins,D. and Cuzick,J. (1992) ^{32}P-Postlabelling analysis of DNA from human tissues. *Mutat. Res.*, **282**, 139–145.
59. Phillips,D.H. and Hewer,A. (1993) DNA adducts in human urinary bladder and other tissues. *Environ. Health Perspect.*, **99**, 45–49.
60. Curigliano,G., Zhang,Y.J., Wang,L.Y., Flamini,G., Alcini,A., Ratto,C., Giustacchini,M., Alcini,E., Cittadini,A. and Santella,R.M. (1996) Immunohistochemical quantitation of 4-aminobiphenyl-DNA adducts and p53 nuclear overexpression in T1 bladder cancer of smokers and nonsmokers. *Carcinogenesis*, **17**, 911–916.
61. Romano,G., Garagnani,L., Boninsegna,A. *et al*. (1999) Analysis of 4-ABP-DNA adducts and p53 alterations in urinary bladder carcinoma. *Anticancer Res.*, **19**, 4571–4576.
62. Airoldi,L., Orsi,F., Magagnotti,C., Coda,R., Randone,D., Casetta,G., Peluso,M., Hautefeuille,A., Malaveille,C. and Vineis,P. (2002) Determinants of 4-aminobiphenyl-DNA adducts in bladder cancer biopsies. *Carcinogenesis*, **23**, 861–866.
63. Prevost,V. and Shuker,D.E. (1996) Cigarette smoking and urinary 3-alkyladenine excretion in man. *Chem. Res. Toxicol.*, **9**, 439–444.
64. Casale,G.P., Singhal,M., Bhattacharya,S. *et al*. (2001) Detection and quantification of depurinated benzo[a]pyrene-adducted DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem. Res. Toxicol.*, **14**, 192–201.
65. Gontijo,A.M., Elias,F.N., Salvadori,D.M., de Oliveira,M.L., Correa,L.A., Goldberg,J., Trindade,J.C. and de Camargo,J.L. (2001) Single-cell gel (comet) assay detects primary DNA damage in nonneoplastic urothelial cells of smokers and ex-smokers. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 987–993.
66. Loft,S., Vistisen,K., Ewertz,M., Tjonneland,A., Overvad,K. and Poulsen,H.E. (1992) Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, **13**, 2241–2247.
67. Phillips,D.H., Hewer,A., Malcolm,A.D., Ward,P. and Coleman,D.V. (1990) Smoking and DNA damage in cervical cells. *Lancet*, **335**, 417.
68. Simons,A.M., Mugica van Herckenrode,C., Rodriguez,J.A., Maitland,N., Anderson,M., Phillips,D.H. and Coleman,D.V. (1995) Demonstration of smoking-related DNA damage in cervical epithelium and correlation with human papillomavirus type 16, using exfoliated cervical cells. *Br. J. Cancer*, **71**, 246–249.
69. Simons,A.M., Phillips,D.H. and Coleman,D.V. (1993) Damage to DNA in cervical epithelium related to smoking tobacco. *Br. Med. J.*, **306**, 1444–1448.
70. Phillips,D.H. and Ni She,M. (1993) *Smoking-Related DNA Adducts in Human Cervical Biopsies*. IARC Scientific Publications, IARC, Lyon, pp. 327–330.
71. Phillips,D.H. and Ni She,M. (1994) DNA adducts in cervical tissue of smokers and non-smokers. *Mutat. Res.*, **313**, 277–284.
72. King,M.M., Hollingsworth,A., Cuzick,J. and Garner,R.C. (1994) The detection of adducts in human cervix tissue DNA using ^{32}P-postlabelling: a study of the relationship with smoking history and oral contraceptive use. *Carcinogenesis*, **15**, 1097–1100.
73. Ali,S., Astley,S.B., Sheldon,T.A., Peel,K.R. and Wells,M. (1994) Detection and measurement of DNA adducts in the cervix of smokers and non-smokers. *Int. J. Gynecol. Cancer*, **4**, 188–193.
74. Mancini,R., Romano,G., Sgambato,A., Flamini,G., Giovagnoli,M.R., Boninsegna,A., Carraro,C., Vecchione,A. and Cittadini,A. (1999) Polycyclic aromatic hydrocarbon-DNA adducts in cervical smears of smokers and nonsmokers. *Gynecol. Oncol.*, **75**, 68–71.
75. Melikian,A.A., Sun,P., Prokopczyk,B., El-Bayoumy,K., Hoffmann,D., Wang,X. and Waggoner,S. (1999) Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. *Cancer Lett.*, **146**, 127–134.
76. Perera,F.P., Estabrook,A., Hewer,A., Channing,K., Rundle,A., Mooney,L.A., Whyatt,R. and Phillips,D.H. (1995) Carcinogen-DNA adducts in human breast tissue. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 233–238.
77. Li,D., Wang,M., Dhingra,K. and Hittelman,W.N. (1996) Aromatic DNA adducts in adjacent tissues of breast cancer patients: clues to breast cancer etiology. *Cancer Res.*, **56**, 287–293.
78. Wang,M., Dhingra,K., Hittelman,W.N., Liehr,J.G., de Andrade,M. and Li,D. (1996) Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 705–710.
79. Santella,R.M., Gammon,M.D., Zhang,Y.J. *et al*. (2000) Immunohistochemical analysis of polycyclic aromatic hydrocarbon-DNA adducts in breast tumor tissue. *Cancer Lett.*, **154**, 143–149.
80. Motykiewicz,G., Malusecka,E., Michalska,J., Kalinowska,E., Wloch,J., Butkiewicz,D., Mazurek,A., Lange,D., Perera,F.P. and Santella,R.M. (2001) Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in breast tissue sections. *Cancer Detect. Prev.*, **25**, 328–335.
81. Rundle,A., Tang,D., Hibshoosh,H., Estabrook,A., Schnabel,F., Cao,W., Grumet,S. and Perera,F.P. (2000) The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. *Carcinogenesis*, **21**, 1281–1289.
82. Wang,M., Abbruzzese,J.L., Friess,H., Hittelman,W.N., Evans,D.B., Abbruzzese,M.C., Chiao,P. and Li,D. (1998) DNA adducts in human pancreatic tissues and their potential role in carcinogenesis. *Cancer Res.*, **58**, 38–41.
83. Alexandrov,K., Rojas,M., Kadlubar,F.F., Lang,N.P. and Bartsch,H. (1996) Evidence of anti-benzo[*a*]pyrene diolepoxide-DNA adduct formation in human colon mucosa. *Carcinogenesis*, **17**, 2081–2083.
84. Dyke,G.W., Craven,J.L., Hall,R. and Garner,R.C. (1992) Smoking-related DNA adducts in human gastric cancers. *Int. J. Cancer*, **52**, 847–850.
85. Phillips,D.H., Hewer,A., Scholefield,J.H. and Skinner,P. (2002) Smoking-related DNA adducts in anal epithelium. *Cancer Epidemiol. Biomarkers Prev.*, submitted for publication.
86. Everson,R.B., Randerath,E., Santella,R.M., Cefalo,R.C., Avitts,T.A. and Randerath,K. (1986) Detection of smoking-related covalent DNA adducts in human placenta. *Science*, **231**, 54–57.
87. Everson,R.B., Randerath,E., Santella,R.M., Avitts,T.A., Weinstein,I.B. and Randerath,K. (1988) Quantitative associations between DNA damage in human placenta and maternal smoking and birth weight. *J. Natl Cancer Inst.*, **80**, 567–576.
88. Weston,A., Manchester,D.K., Poirier,M.C., Choi,J.S., Trivers,G.E., Mann,D.L. and Harris,C.C. (1989) Derivative fluorescence spectral analysis of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. *Chem. Res. Toxicol.*, **2**, 104–108.
89. Manchester,D.K., Wilson,V.L., Hsu,I.C., Choi,J.S., Parker,N.B., Mann,D.L., Weston,A. and Harris,C.C. (1990) Synchronous fluorescence spectroscopic, immunoaffinity chromatographic and ^{32}P-postlabeling analysis of human placental DNA known to contain benzo[*a*]pyrene diol epoxide adducts. *Carcinogenesis*, **11**, 553–559.
90. Manchester,D.K., Weston,A., Choi,J.S., Trivers,G.E., Fennessey,P.V., Quintana,E., Farmer,P.B., Mann,D.L. and Harris,C.C. (1988) Detection of benzo[a]pyrene diol epoxide-DNA adducts in human placenta. *Proc. Natl Acad. Sci. USA*, **85**, 9243–9247.
91. Manchester,D.K., Bowman,E.D., Parker,N.B., Caporaso,N.E. and Weston,A. (1992) Determinants of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. *Cancer Res.*, **52**, 1499–1503.
92. Gallagher,J., Mumford,J., Li,X., Shank,T., Manchester,D. and Lewtas,J. (1993) *DNA Adduct Profiles and Levels in Placenta, Blood and Lung in Relation to Cigarette Smoking and Smoky Coal Emissions*. IARC Scientific Publications, IARC, Lyon, pp. 283–292.
93. Hansen,C., Sorensen,L.D., Asmussen,I. and Autrup,H. (1992) Transplacental exposure to tobacco smoke in human-adduct formation in placenta and umbilical cord blood vessels. *Teratog. Carcinogen. Mutagen.*, **12**, 51–60.
94. Hansen,C., Asmussen,I. and Autrup,H. (1993) Detection of carcinogen-DNA adducts in human fetal tissues by the ^{32}P-postlabeling procedure. *Environ. Health Perspect.*, **99**, 229–231.
95. Hatch,M.C., Warburton,D. and Santella,R.M. (1990) Polycyclic aromatic hydrocarbon-DNA adducts in spontaneously aborted fetal tissue. *Carcinogenesis*, **11**, 1673–1675.
96. Arnould,J.P., Verhoest,P., Bach,V., Libert,J.P. and Belegaud,J. (1997) Detection of benzo[a]pyrene-DNA adducts in human placenta and umbilical cord blood. *Hum. Exp. Toxicol.*, **16**, 716–721.
97. Daube,H., Scherer,G., Riedel,K., Ruppert,T., Tricker,A.R., Rosenbaum,P. and Adlkofer,F. (1997) DNA adducts in human placenta in relation to tobacco smoke exposure and plasma antioxidant status. *J. Cancer Res. Clin. Oncol.*, **123**, 141–151.
98. Gallagher,J.E., Vine,M.F., Schramm,M.M., Lewtas,J., George,M.H., Hulka,B.S. and Everson,R.B. (1993) ^{32}P-postlabeling analysis of DNA adducts in human sperm cells from smokers and nonsmokers. *Cancer Epidemiol. Biomarkers Prev.*, **2**, 581–585.
99. Zenzes,M.T., Bielecki,R. and Reed,T.E. (1999) Detection of benzo (a)pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. *Fertil. Steril.*, **72**, 330–335.
100. Zenzes,M.T., Puy,L.A., Bielecki,R. and Reed,T.E. (1999) Detection of benzo[a]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. *Mol. Hum. Reprod.*, **5**, 125–131.

101. Zenzes,M.T., Puy,L.A. and Bielecki,R. (1998) Immunodetection of benzo[a]pyrene adducts in ovarian cells of women exposed to cigarette smoke. *Mol. Hum. Reprod.*, **4**, 159–165.
102. Shen,H.M., Chia,S.E., Ni,Z.Y., New,A.L., Lee,B.L. and Ong,C.N. (1997) Detection of oxidative DNA damage in human sperm and the association with cigarette smoking. *Reprod. Toxicol.*, **11**, 675–680.
103. Shen,H.M., Chia,S.E. and Ong,C.N. (1999) Evaluation of oxidative DNA damage in human sperm and its association with male infertility. *J. Androl.*, **20**, 718–723.
104. Shi,Q., Ko,E., Barclay,L., Hoang,T., Rademaker,A. and Martin,R. (2001) Cigarette smoking and aneuploidy in human sperm. *Mol. Reprod. Dev.*, **59**, 417–421.
105. Potts,R.J., Newbury,C.J., Smith,G., Notarianni,L.J. and Jefferies,T.M. (1999) Sperm chromatin damage associated with male smoking. *Mutat. Res.*, **423**, 103–111.
106. Randerath,E., Miller,R.H., Mittal,D., Avitts,T.A., Dunsford,H.A. and Randerath,K. (1989) Covalent DNA damage in tissues of cigarette smokers as determined by ^{32}P-postlabeling assay. *J. Natl Cancer Inst.*, **81**, 341–347.
107. Izzotti,A., De Flora,S., Petrilli,G.L., Gallagher,J., Rojas,M., Alexandrov,K., Bartsch,H. and Lewtas,J. (1995) Cancer biomarkers in human atherosclerotic lesions: detection of DNA adducts. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 105–110.
108. Binkova,B., Strejc,P., Boubelik,O., Stavkova,Z., Chvatalova,I. and Sram,R.J. (2001) DNA adducts and human atherosclerotic lesions. *Int. J. Hyg. Environ. Health*, **204**, 49–54.
109. Zhang,Y.J., Weksler,B.B., Wang,L., Schwartz,J. and Santella,R.M. (1998) Immunohistochemical detection of polycyclic aromatic hydrocarbon-DNA damage in human blood vessels of smokers and non-smokers. *Atherosclerosis*, **140**, 325–331.
110. Van Schooten,F.J., Hirvonen,A., Maas,L.M., De Mol,B.A., Kleinjans,J.C., Bell,D.A. and Durrer,J.D. (1998) Putative susceptibility markers of coronary artery disease: association between VDR genotype, smoking and aromatic DNA adduct levels in human right atrial tissue. *FASEB J.*, **12**, 1409–1417.
111. Mooney,L.A., Santella,R.M., Covey,L. *et al*. (1995) Decline of DNA damage and other biomarkers in peripheral blood following smoking cessation. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 627–634.
112. Grinberg-Funes,R.A., Singh,V.N., Perera,F.P., Bell,D.A., Young,T.L., Dickey,C., Wang,L.W. and Santella,R.M. (1994) Polycyclic aromatic hydrocarbon-DNA adducts in smokers and their relationship to micronutrient levels and the glutathione-S-transferase M1 genotype. *Carcinogenesis*, **15**, 2449–2454.
113. Mooney,L.A., Bell,D.A., Santella,R.M. *et al.* (1997) Contribution of genetic and nutritional factors to DNA damage in heavy smokers. *Carcinogenesis*, **18**, 503–509.
114. Jacobson,J.S., Begg,M.D., Wang,L.W., Wang,Q., Agarwal,M., Norkus,E., Singh,V.N., Young,T.L., Yang,D. and Santella,R.M. (2000) Effects of a 6-month vitamin intervention on DNA damage in heavy smokers. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1303–1311.
115. Wang,Y., Ichiba,M., Oishi,H., Iyadomi,M., Shono,N. and Tomokuni,K. (1997) Relationship between plasma concentrations of beta-carotene and alpha-tocopherol and life-style factors and levels of DNA adducts in lymphocytes. *Nutr. Cancer*, **27**, 69–73.
116. Tang,D., Santella,R.M., Blackwood,A.M., Young,T.L., Mayer,J., Jaretzki,A., Grantham,S., Tsai,W.Y. and Perera,F.P. (1995) A molecular epidemiological case-control study of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 341–346.
117. Wiencke,J.K., Kelsey,K.T., Varkonyi,A., Semey,K., Wain,J.C., Mark,E. and Christiani,D.C. (1995) Correlation of DNA adducts in blood mononuclear cells with tobacco carcinogen-induced damage in human lung. *Cancer Res.*, **55**, 4910–4914.
118. Wiencke,J.K., Thurston,S.W., Kelsey,K.T., Varkonyi,A., Wain,J.C., Mark,E.J. and Christiani,D.C. (1999) Early age at smoking initiation and tobacco carcinogen DNA damage in the lung. *J. Natl Cancer Inst.*, **91**, 614–619.
119. van Schooten,F.J., Hillebrand,M.J., van Leeuwen,F.E., van Zandwijk,N., Jansen,H.M., den Engelse,L. and Kriek,E. (1992) Polycyclic aromatic hydrocarbon–DNA adducts in white blood cells from lung cancer patients: no correlation with adduct levels in lung. *Carcinogenesis*, **13**, 987–993.
120. Van Schooten,F.J., Godschalk,R.W., Breedijk,A., Maas,L.M., Kriek,E., Sakai,H., Wigbout,G., Baas,P., Van't Veer,L. and Van Zandwijk,N. (1997) ^{32}P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures. *Mutat. Res.*, **378**, 65–75.
121. Kiyosawa,H., Suko,M., Okudaira,H., Murata,K., Miyamoto,T., Chung,M.H., Kasai,H. and Nishimura,S. (1990) Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes. *Free Radic. Res Commun.*, **11**, 23–27.
122. Asami,S., Hirano,T., Yamaguchi,R., Tomioka,Y., Itoh,H. and Kasai,H. (1996) Increase of a type of oxidative DNA damage, 8-hydroxyguanine and its repair activity in human leukocytes by cigarette smoking. *Cancer Res.*, **56**, 2546–2549.
123. Lodovici,M., Casalini,C., Cariaggi,R., Michelucci,L. and Dolara,P. (2000) Levels of 8-hydroxydeoxyguanosine as a marker of DNA damage in human leukocytes. *Free Radic. Biol. Med.*, **28**, 13–17.
124. van Zeeland,A.A., de Groot,A.J., Hall,J. and Donato,F. (1999) 8-Hydroxydeoxyguanosine in DNA from leukocytes of healthy adults: relationship with cigarette smoking, environmental tobacco smoke, alcohol and coffee consumption. *Mutat. Res.*, **439**, 249–257.
125. Nia,A.B., Van Schooten,F.J., Schilderman,P.A., De Kok,T.M., Haenen,G.R., Van Herwijnen,M.H., Van Agen,E., Pachen,D. and Kleinjans,J.C. (2001) A multi-biomarker approach to study the effects of smoking on oxidative DNA damage and repair and antioxidative defense mechanisms. *Carcinogenesis*, **22**, 395–401.
126. Perera,F., Mayer,J., Jaretzki,A. *et al*. (1989) Comparison of DNA adducts and sister chromatid exchange in lung cancer cases and controls. *Cancer Res.*, **49**, 4446–4451.
127. Vulimiri,S.V., Wu,X., Baer-Dubowska,W., de Andrade,M., Detry,M., Spitz,M.R. and DiGiovanni,J. (2000) Analysis of aromatic DNA adducts and 7,8-dihydro-8-oxo-2′-deoxyguanosine in lymphocyte DNA from a case-control study of lung cancer involving minority populations. *Mol. Carcinogen.*, **27**, 34–46.
128. Tang,D., Phillips,D.H., Stampfer,M. *et al.* (2001) Association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the physicians health study. *Cancer Res.*, **61**, 6708–6712.
129. Hou,S.M., Yang,K., Nyberg,F., Hemminki,K., Pershagen,G. and Lambert,B. (1999) Hprt mutant frequency and aromatic DNA adduct level in non-smoking and smoking lung cancer patients and population controls. *Carcinogenesis*, **20**, 437–444.
130. Peluso,M., Airoldi,L., Magagnotti,C., Fiorini,L., Munnia,A., Hautefeuille,A., Malaveille,C. and Vineis,P. (2000) White blood cell DNA adducts and fruit and vegetable consumption in bladder cancer. *Carcinogenesis*, **21**, 183–187.
131. Pulera,N., Petruzzelli,S., Celi,A., Puntoni,R., Fornai,E., Sawe,U., Paoletti,P. and Giuntini,C. (1997) Presence and persistence of serum anti-benzo[a]pyrene diolepoxide-DNA adduct antibodies in smokers: effects of smoking reduction and cessation. *Int. J. Cancer*, **70**, 145–149.
132. Petruzzelli,S., Celi,A., Pulera,N., Baliva,F., Viegi,G., Carrozzi,L., Ciacchini,G., Bottai,M., Di Pede,F., Paoletti,P. and Giuntini,C. (1998) Serum antibodies to benzo[*a*]pyrene diol epoxide-DNA adducts in the general population: effects of air pollution, tobacco smoking and family history of lung diseases. *Cancer Res.*, **58**, 4122–4126.
133. Shinozaki,R., Inoue,S., Choi,K.S. and Tatsuno,T. (1999) Association of benzo[a]pyrene-diol-epoxide-deoxyribonucleic acid (BPDE-DNA) adduct level with aging in male smokers and nonsmokers. *Arch. Environ. Health*, **54**, 79–85.
134. Holz,O., Krause,T., Scherer,G., Schmidt-Preuss,U. and Rudiger,H.W. (1990) ^{32}P-postlabelling analysis of DNA adducts in monocytes of smokers and passive smokers. *Int. Arch. Occup. Environ. Health*, **62**, 299–303.
135. Holz,O., Meissner,R., Einhaus,M., Koops,F., Warncke,K., Scherer,G., Adlkofer,F., Baumgartner,E. and Rudiger,H.W. (1993) Detection of DNA single-strand breaks in lymphocytes of smokers. *Int. Arch. Occup. Environ. Health*, **65**, 83–88.
136. Georgiadis,P., Topinka,J., Stoikidou,M., Kaila,S., Gioka,M., Katsouyanni,K., Sram,R., Autrup,H. and Kyrtopoulos,S.A. (2001) Biomarkers of genotoxicity of air pollution (the AULIS project): bulky DNA adducts in subjects with moderate to low exposures to airborne polycyclic aromatic hydrocarbons and their relationship to environmental tobacco smoke and other parameters. *Carcinogenesis*, **22**, 1447–1457.
137. Besaratinia,A., Maas,L.M., Brouwer,E.M., Moonen,E.J., De Kok,T.M., Wesseling,G.J., Loft,S., Kleinjans,J.C. and Van Schooten,F.J. (2002) A molecular dosimetry approach to assess human exposure to environmental tobacco smoke in pubs. *Carcinogenesis*, **23**, 1171–1176.
138. Skipper,P.L., Peng,X., Soohoo,C.K. and Tannenbaum,S.R. (1994) Protein adducts as biomarkers of human carcinogen exposure. *Drug Metab. Rev.*, **26**, 111–124.
139. Phillips,D.H. and Farmer,P.B. (1995) Protein and DNA adducts as biomarkers of exposure to environmental mutagens. In Phillips,D.H. and Venitt,S. (eds.), *Environmental Mutagenesis*. Bios, Oxford, pp. 367–395.
140. Perera,F.P., Santella,R.M., Brenner,D., Poirier,M.C., Munshi,A.A., Fischman,H.K. and Van Ryzin,J. (1987) DNA adducts, protein adducts

and sister chromatid exchange in cigarette smokers and nonsmokers. *J. Natl Cancer Inst.*, **79**, 449–456.
141. Bryant,M.S., Skipper,P.L., Tannenbaum,S.R. and Maclure,M. (1987) Hemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res.*, **47**, 602–608.
142. Bryant,M.S., Vineis,P., Skipper,P.L. and Tannenbaum,S.R. (1988) Hemoglobin adducts of aromatic amines: associations with smoking status and type of tobacco. *Proc. Natl Acad. Sci. USA*, **85**, 9788–9791.
143. Ronco,G., Vineis,P., Bryant,M.S., Skipper,P.L. and Tannenbaum,S.R. (1990) Haemoglobin adducts formed by aromatic amines in smokers: sources of inter-individual variability. *Br. J. Cancer*, **61**, 534–537.
144. Maclure,M., Katz,R.B., Bryant,M.S., Skipper,P.L. and Tannenbaum,S.R. (1989) Elevated blood levels of carcinogens in passive smokers. *Am. J. Public Health*, **79**, 1381–1384.
145. Maclure,M., Bryant,M.S., Skipper,P.L. and Tannenbaum,S.R. (1990) Decline of the hemoglobin adduct of 4-aminobiphenyl during withdrawal from smoking. *Cancer Res.*, **50**, 181–184.
146. Vineis,P., Caporaso,N., Tannenbaum,S.R., Skipper,P.L., Glogowski,J., Bartsch,H., Coda,M., Talaska,G. and Kadlubar,F. (1990) Acetylation phenotype, carcinogen-hemoglobin adducts and cigarette smoking. *Cancer Res.*, **50**, 3002–3004.
147. Bartsch,H., Caporaso,N., Coda,M., Kadlubar,F., Malaveille,C., Skipper,P., Talaska,G., Tannenbaum,S.R. and Vineis,P. (1990) Carcinogen hemoglobin adducts, urinary mutagenicity and metabolic phenotype in active and passive cigarette smokers. *J. Natl Cancer Inst.*, **82**, 1826–1831.
148. Weston,A., Caporaso,N.E., Taghizadeh,K., Hoover,R.N., Tannenbaum,S.R., Skipper,P.L., Resau,J.H., Trump,B.F. and Harris,C.C. (1991) Measurement of 4-aminobiphenyl-hemoglobin adducts in lung cancer cases and controls. *Cancer Res.*, **51**, 5219–5223.
149. Del Santo,P., Moneti,G., Salvadori,M., Saltutti,C., Delle Rose,A. and Dolara,P. (1991) Levels of the adducts of 4-aminobiphenyl to hemoglobin in control subjects and bladder carcinoma patients. *Cancer Lett.*, **60**, 245–251.
150. Coghlin,J., Gann,P.H., Hammond,S.K., Skipper,P.L., Taghizadeh,K., Paul,M. and Tannenbaum,S.R. (1991) 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen *in utero*. *J. Natl Cancer Inst.*, **83**, 274–280.
151. Pinorini-Godly,M.T. and Myers,S.R. (1996) HPLC and GC/MS determination of 4-aminobiphenyl haemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen *in utero*. *Toxicology*, **107**, 209–217.
152. Myers,S.R., Spinnato,J.A., Pinorini-Godly,M.T., Cook,C., Boles,B. and Rodgers,G.C. (1996) Characterization of 4-aminobiphenyl-hemoglobin adducts in maternal and fetal blood-samples. *J. Toxicol. Environ. Health*, **47**, 553–566.
153. Falter,B., Kutzer,C. and Richter,E. (1994) Biomonitoring of hemoglobin adducts: aromatic amines and tobacco-specific nitrosamines. *Clin. Invest.*, **72**, 364–371.
154. Yu,M.C., Skipper,P.L., Taghizadeh,K., Tannenbaum,S.R., Chan,K.K., Henderson,B.E. and Ross,R.K. (1994) Acetylator phenotype, aminobiphenyl-hemoglobin adduct levels and bladder cancer risk in white, black and Asian men in Los Angeles, California. *J. Natl Cancer Inst.*, **86**, 712–716.
155. Dallinga,J.W., Pachen,D.M., Wijnhoven,S.W., Breedijk,A., van't Veer,L., Wigbout,G., van Zandwijk,N., Maas,L.M., van Agen,E., Kleinjans,J.C. and van Schooten,F.J. (1998) The use of 4-aminobiphenyl hemoglobin adducts and aromatic DNA adducts in lymphocytes of smokers as biomarkers of exposure. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 571–577.
156. Godschalk,R.W., Dallinga,J.W., Wikman,H., Risch,A., Kleinjans,J.C., Bartsch,H. and Van Schooten,F.J. (2001) Modulation of DNA and protein adducts in smokers by genetic polymorphisms in GSTM1,GSTT1, NAT1 and NAT2. *Pharmacogenetics*, **11**, 389–398.
157. Probst-Hensch,N.M., Bell,D.A., Watson,M.A., Skipper,P.L., Tannenbaum,S.R., Chan,K.K., Ross,R.K. and Yu,M.C. (2000) N-acetyltransferase 2 phenotype but not NAT1*10 genotype affects aminobiphenyl-hemoglobin adduct levels. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 619–623.
158. Crawford,F.G., Mayer,J., Santella,R.M., Cooper,T.B., Ottman,R., Tsai,W.Y., Simon-Cereijido,G., Wang,M., Tang,D. and Perera,F.P. (1994) Biomarkers of environmental tobacco smoke in preschool children and their mothers. *J. Natl Cancer Inst.*, **86**, 1398–1402.
159. Melikian,A.A., Sun,P., Pierpont,C., Coleman,S. and Hecht,S.S. (1997) Gas chromatographic-mass spectrometric determination of benzo[a]pyrene and chrysene diol epoxide globin adducts in humans. *Cancer Epidemiol. Biomarkers Prev.*, **6**, 833–839.
160. Pastorelli,R., Guanci,M., Cerri,A. *et al.* (1998) Impact of inherited polymorphisms in glutathione S-transferase M1, microsomal epoxide hydrolase, cytochrome P450 enzymes on DNA and blood protein adducts of benzo[*a*]pyrene-diolepoxide. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 703–709.
161. Scherer,G., Frank,S., Riedel,K., Meger-Kossien,I. and Renner,T. (2000) Biomonitoring of exposure to polycyclic aromatic hydrocarbons of nonoccupationally exposed persons. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 373–380.
162. Carmella,S.G., Kagan,S.S., Kagan,M., Foiles,P.G., Palladino,G., Quart,A.M., Quart,E. and Hecht,S.S. (1990) Mass spectrometric analysis of tobacco-specific nitrosamine hemoglobin adducts in snuff dippers, smokers and nonsmokers. *Cancer Res.*, **50**, 5438–5445.
163. Atawodi,S.E., Lea,S., Nyberg,F., Mukeria,A., Constantinescu,V., Ahrens,W., Brueske-Hohlfeld,I., Fortes,C., Boffetta,P. and Friesen,M.D. (1998) 4-Hydroxy-1-(3-pyridyl)-1-butanone-hemoglobin adducts as biomarkers of exposure to tobacco smoke: validation of a method to be used in multicenter studies. *Cancer Epidemiol. Biomarkers Prev.*, **7**, 817–821.
164. Tornqvist,M., Osterman-Golkar,S., Kautiainen,A., Jensen,S., Farmer,P.B. and Ehrenberg,L. (1986) Tissue doses of ethylene oxide in cigarette smokers determined from adduct levels in hemoglobin. *Carcinogenesis*, **7**, 1519–1521.
165. Granath,F., Westerholm,R., Peterson,A., Tornqvist,M. and Ehrenberg,L. (1994) Uptake and metabolism of ethene studied in a smoke-stop experiment. *Mutat. Res.*, **313**, 285–291.
166. Bailey,E., Brooks,A.G., Dollery,C.T., Farmer,P.B., Passingham,B.J., Sleightholm,M.A. and Yates,D.W. (1988) Hydroxyethylvaline adduct formation in haemoglobin as a biological monitor of cigarette smoke intake. *Arch. Toxicol.*, **62**, 247–253.
167. Tavares,R., Ramos,P., Palminha,J., Bispo,M.A., Paz,I., Bras,A., Rueff,J., Farmer,P.B. and Bailey,E. (1994) Transplacental exposure to genotoxins. Evaluation in haemoglobin of hydroxyethylvaline adduct levels in smoking and non-smoking mothers and their newborns. *Carcinogenesis*, **15**, 1271–1274.
168. Bono,R., Vincenti,M., Meineri,V., Pignata,C., Saglia,U., Giachino,O. and Scursatone,E. (1999) Formation of N-(2-hydroxyethyl)valine due to exposure to ethylene oxide via tobacco smoke: a risk factor for onset of cancer. *Environ. Res.*, **81**, 62–71.
169. Muller,M., Kramer,A., Angerer,J. and Hallier,E. (1998) Ethylene oxide-protein adduct formation in humans: influence of glutathione-S-transferase polymorphisms. *Int. Arch. Occup. Environ. Health*, **71**, 499–502.
170. Fennell,T.R., MacNeela,J.P., Morris,R.W., Watson,M., Thompson,C.L. and Bell,D.A. (2000) Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of glutathione S-transferase T1-null and M1-null genotypes. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 705–712.
171. Bergmark,E. (1997) Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem. Res. Toxicol.*, **10**, 78–84.
172. Osterman-Golkar,S.M., MacNeela,J.P., Turner,M.J., Walker,V.E., Swenberg,J.A., Sumner,S.J., Youtsey,N. and Fennell,T.R. (1994) Monitoring exposure to acrylonitrile using adducts with N-terminal valine in hemoglobin. *Carcinogenesis*, **15**, 2701–2707.
173. Tavares,R., Borba,H., Monteiro,M., Proenca,M.J., Lynce,N., Rueff,J., Bailey,E., Sweetman,G.M., Lawrence,R.M. and Farmer,P.B. (1996) Monitoring of exposure to acrylonitrile by determination of N-(2-cyanoethyl)valine at the N-terminal position of haemoglobin. *Carcinogenesis*, **17**, 2655–2660.
174. Yeowell-O'Connell,K., Rothman,N., Waidyanatha,S. *et al.* (2001) Protein adducts of 1,4-benzoquinone and benzene oxide among smokers and nonsmokers exposed to benzene in China. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 831–838.
175. Tornqvist,M., Svartengren,M. and Ericsson,C.H. (1992) Methylations in hemoglobin from monozygotic twins discordant for cigarette smoking: hereditary and tobacco-related factors. *Chem. Biol. Interact.*, **82**, 91–98.
176. Bader,M., Lewalter,J. and Angerer,J. (1995) Analysis of N-alkylated amino acids in human hemoglobin: evidence for elevated N-methylvaline levels in smokers. *Int. Arch. Occup. Environ. Health*, **67**, 237–242.
177. Pignatelli,B., Li,C.Q., Boffetta,P. *et al.* (2001) Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer Res.*, **61**, 778–784.
178. Tang,D., Warburton,D., Tannenbaum,S.R., Skipper,P., Santella,R.M., Cereijido,G.S., Crawford,F.G. and Perera,F.P. (1999) Molecular and genetic damage from environmental tobacco smoke in young children. *Cancer Epidemiol. Biomarkers Prev.*, **8**, 427–431.
179. Hammond,S.K., Coghlin,J., Gann,P.H., Paul,M., Taghizadeh,K., Skipper,P.L. and Tannenbaum,S.R. (1993) Relationship between environmental tobacco smoke exposure and carcinogen-hemoglobin adduct levels in nonsmokers. *J. Natl Cancer Inst.*, **85**, 474–478.

180. Hainaut,P. and Pfeifer,G.P. (2001) Patterns of p53 G→T transversions in lung cancers reflect the primary mutagenic signature of DNA-damage by tobacco smoke. *Carcinogenesis*, **22**, 367–374.
181. Hainaut,P., Olivier,M. and Pfeifer,G.P. (2001) TP53 mutation spectrum in lung cancers and mutagenic signature of components of tobacco smoke: lessons from the IARC TP53 mutation database. *Mutagenesis*, **16**, 551–553, 555–556.
182. Cooper,C.S. (2002) Smoking, lung cancers and their TP53 mutations. *Mutagenesis*, **17**, 279–280.
183. Daling,J.R., Weiss,N.S., Hislop,T.G., Maden,C., Coates,R.J., Sherman,K.J., Ashley,R.L., Beagrie,M., Ryan,J.A. and Corey,L. (1987) Sexual practices, sexually transmitted diseases and the incidence of anal cancer. *N. Engl J. Med.*, **317**, 973–977.
184. Daling,J.R., Sherman,K.J., Hislop,T.G., Maden,C., Mandelson,M.T., Beckmann,A.M. and Weiss,N.S. (1992) Cigarette smoking and the risk of anogenital cancer. *Am. J. Epidemiol.*, **135**, 180–189.
185. Zarcone,R., Mainini,G., Carfora,E. and Cardone,A. (1997) Current etiopathogenetic views in vulvar cancer. *Panminerva Med.*, **39**, 30–34.
186. Randerath,E., Avitts,T.A., Reddy,M.V., Miller,R.H., Everson,R.B. and Randerath,K. (1986) Comparative ^{32}P-analysis of cigarette smoke-induced DNA damage in human tissues and mouse skin. *Cancer Res.*, **46**, 5869–5877.
187. Weston,A., Rowe,M.L., Manchester,D.K., Farmer,P.B., Mann,D.L. and Harris,C.C. (1989) Fluorescence and mass spectral evidence for the formation of benzo[*a*]pyrene anti-diol-epoxide-DNA and -hemoglobin adducts in humans. *Carcinogenesis*, **10**, 251–257.
188. van Schooten,F.J., van Leeuwen,F.E., Hillebrand,M.J., de Rijke,M.E., Hart,A.A., van Veen,H.G., Oosterink,S. and Kriek,E. (1990) Determination of benzo[*a*]pyrene diol epoxide-DNA adducts in white blood cell DNA from coke-oven workers: the impact of smoking. *J. Natl Cancer Inst.*, **82**, 927–933.
189. Geneste,O., Camus,A.M., Castegnaro,M., Petruzzelli,S., Macchiarini,P., Angeletti,C.A., Giuntini,C. and Bartsch,H. (1991) Comparison of pulmonary DNA adduct levels, measured by ^{32}P-postlabelling and aryl hydrocarbon hydroxylase activity in lung parenchyma of smokers and ex-smokers. *Carcinogenesis*, **12**, 1301–1305.
190. Izzotti,A., Rossi,G.A., Bagnasco,M. and De Flora,S. (1991) Benzo[*a*]pyrene diolepoxide-DNA adducts in alveolar macrophages of smokers. *Carcinogenesis*, **12**, 1281–1285.
191. Foiles,P.G., Akerkar,S.A., Carmella,S.G., Kagan,M., Stoner,G.D., Resau,J.H. and Hecht,S.S. (1991) Mass spectrometric analysis of tobacco-specific nitrosamine-DNA adducts in smokers and nonsmokers. *Chem. Res. Toxicol.*, **4**, 364–368.
192. Alexandrov,K., Rojas,M., Geneste,O., Castegnaro,M., Camus,A.M., Petruzzelli,S., Giuntini,C. and Bartsch,H. (1992) An improved fluorometric assay for dosimetry of benzo[*a*]pyrene diol-epoxide-DNA adducts in smokers' lung: comparisons with total bulky adducts and aryl hydrocarbon hydroxylase activity. *Cancer Res.*, **52**, 6248–6253.
193. Mustonen,R., Schoket,B. and Hemminki,K. (1993) Smoking-related DNA adducts: ^{32}P-postlabeling analysis of 7-methylguanine in human bronchial and lymphocyte DNA. *Carcinogenesis*, **14**, 151–154.
194. Schoket,B., Kostic,S. and Vincze,I. (1993) *Determination of Smoking-Related DNA Adducts in Lung-Cancer and Non-Cancer Patients*. IARC Scientific Publications, IARC, Lyon, pp. 315–319.
195. Sherman,M.P., Aeberhard,E.E., Wong,V.Z., Simmons,M.S., Roth,M.D. and Tashkin,D.P. (1995) Effects of smoking marijuana, tobacco or cocaine alone or in combination on DNA damage in human alveolar macrophages. *Life Sci.*, **56**, 2201–2207.
196. Andreassen,A., Kure,E.H., Nielsen,P.S., Autrup,H. and Haugen,A. (1996) Comparative synchronous fluorescence spectrophotometry and ^{32}P-postlabeling analysis of PAH-DNA adducts in human lung and the relationship to TP53 mutations. *Mutat. Res.*, **368**, 275–282.
197. Ryberg,D., Skaug,V., Hewer,A., Phillips,D.H., Harries,L.W., Wolf,C.R., Ogreid,D., Ulvik,A., Vu,P. and Haugen,A. (1997) Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk. *Carcinogenesis*, **18**, 1285–1289.
198. Rojas,M., Alexandrov,K., Cascorbi,I. *et al*. (1998) High benzo[a]pyrene diol-epoxide DNA adduct levels in lung and blood cells from individuals with combined CYP1A1 MspI/Msp-GSTM1*0/*0 genotypes. *Pharmacogenetics*, **8**, 109–118.
199. Butkiewicz,D., Cole,K.J., Phillips,D.H., Harris,C.C. and Chorazy,M. (1999) GSTM1, GSTP1, CYP1A1 and CYP2D6 polymorphisms in lung cancer patients from an environmentally polluted region of Poland: correlation with lung DNA adduct levels. *Eur. J. Cancer Prev.*, **8**, 315–323.
200. Piipari,R., Savela,K., Nurminen,T., Hukkanen,J., Raunio,H., Hakkola,J., Mantyla,T., Beaune,P., Edwards,R.J., Boobis,A.R. and Anttila,S. (2000) Expression of CYP1A1, CYP1B1 and CYP3A and polycyclic aromatic hydrocarbon-DNA adduct formation in bronchoalveolar macrophages of smokers and non-smokers. *Int. J. Cancer*, **86**, 610–616.
201. Cheng,Y.W., Chen,C.Y., Lin,P., Huang,K.H., Lin,T.S., Wu,M.H. and Lee,H. (2000) DNA adduct level in lung tissue may act as a risk biomarker of lung cancer. *Eur. J. Cancer*, **36**, 1381–1388.
202. Schoket,B., Papp,G., Levay,K., Mrackova,G., Kadlubar,F.F. and Vincze,I. (2001) Impact of metabolic genotypes on levels of biomarkers of genotoxic exposure. *Mutat. Res.*, **482**, 57–69.
203. Jahnke,G.D., Thompson,C.L., Walker,M.P., Gallagher,J.E., Lucier,G.W. and DiAugustine,R.P. (1990) Multiple DNA adducts in lymphocytes of smokers and nonsmokers determined by ^{32}P-postlabeling analysis. *Carcinogenesis*, **11**, 205–211.
204. Savela,K. and Hemminki,K. (1991) DNA adducts in lymphocytes and granulocytes of smokers and nonsmokers detected by the ^{32}P-postlabelling assay. *Carcinogenesis*, **12**, 503–508.
205. Mustonen,R. and Hemminki,K. (1992) 7-Methylguanine levels in DNA of smokers' and non-smokers' total white blood cells, granulocytes and lymphocytes. *Carcinogenesis*, **13**, 1951–1955.
206. Santella,R.M., Grinberg-Funes,R.A., Young,T.L., Dickey,C., Singh,V.N., Wang,L.W. and Perera,F.P. (1992) Cigarette smoking related polycyclic aromatic hydrocarbon-DNA adducts in peripheral mononuclear cells. *Carcinogenesis*, **13**, 2041–2045.
207. Popp,W., Schell,C., Kraus,R., Vahrenholz,C., Wolf,R., Radtke,J., Bierwirth,K. and Norpoth,K. (1993) DNA strand breakage and DNA adducts in lymphocytes of oral cancer patients. *Carcinogenesis*, **14**, 2251–2256.
208. Rojas,M., Alexandrov,K., van Schooten,F.J., Hillebrand,M., Kriek,E. and Bartsch,H. (1994) Validation of a new fluorometric assay for benzo[a]pyrene diolepoxide-DNA adducts in human white blood cells: comparisons with ^{32}P-postlabeling and ELISA. *Carcinogenesis*, **15**, 557–560.
209. Ichiba,M., Hagmar,L., Rannug,A., Hogstedt,B., Alexandrie,A.K., Carstensen,U. and Hemminki,K. (1994) Aromatic DNA adducts, micronuclei and genetic polymorphism for CYP1A1 and GST1 in chimney sweeps. *Carcinogenesis*, **15**, 1347–1352.
210. Rojas,M., Alexandrov,K., Auburtin,G., Wastiaux-Denamur,A., Mayer,L., Mahieu,B., Sebastien,P. and Bartsch,H. (1995) Anti-benzo[a]pyrene diolepoxide–DNA adduct levels in peripheral mononuclear cells from coke oven workers and the enhancing effect of smoking. *Carcinogenesis*, **16**, 1373–1376.
211. Binkova,B., Lewtas,J., Miskova,I., Lenicek,J. and Sram,R. (1995) DNA adducts and personal air monitoring of carcinogenic polycyclic aromatic hydrocarbons in an environmentally exposed population. *Carcinogenesis*, **16**, 1037–1046.
212. Schell,C., Popp,W., Kraus,R., Vahrenholz,C. and Norpoth,K. (1995) ^{32}P-postlabeling analysis of DNA adducts in different populations. *Toxicol. Lett.*, **77**, 299–307.
213. Arnould,J.P., Hermant,A., Levi-Valensi,P. and Belegaud,J. (1998) Detection of benzo[a]pyrene-DNA adducts from leukocytes from heavy smokers. *Pathol. Biol. (Paris)*, **46**, 787–790.
214. Pavanello,S., Favretto,D., Brugnone,F., Mastrangelo,G., Dal Pra,G. and Clonfero,E. (1999) HPLC/fluorescence determination of anti-BPDE-DNA adducts in mononuclear white blood cells from PAH-exposed humans. *Carcinogenesis*, **20**, 431–435.
215. Poli,P., Buschini,A., Spaggiari,A., Rizzoli,V., Carlo-Stella,C. and Rossi,C. (1999) DNA damage by tobacco smoke and some antiblastic drugs evaluated using the Comet assay. *Toxicol. Lett.*, **108**, 267–276.
216. Georgiadis,P., Samoli,E., Kaila,S., Katsouyanni,K. and Kyrtopoulos,S.A. (2000) Ubiquitous presence of O^6-methylguanine in human peripheral and cord blood DNA. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 299–305.
217. Rojas,M., Cascorbi,I., Alexandrov,K., Kriek,E., Auburtin,G., Mayer,L., Kopp-Schneider,A., Roots,I. and Bartsch,H. (2000) Modulation of benzo[*a*]pyrene diolepoxide-DNA adduct levels in human white blood cells by CYP1A1, GSTM1 and GSTT1 polymorphism. *Carcinogenesis*, **21**, 35–41.
218. Duell,E.J., Wiencke,J.K., Cheng,T.J., Varkonyi,A., Zuo,Z.F., Ashok,T.D., Mark,E.J., Wain,J.C., Christiani,D.C. and Kelsey,K.T. (2000) Polymorphisms in the DNA repair genes XRCC1 and ERCC2 and biomarkers of DNA damage in human blood mononuclear cells. *Carcinogenesis*, **21**, 965–971.
219. Matullo,G., Palli,D., Peluso,M. *et al*. (2001) XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis*, **22**, 1437–1445.
220. van Delft,J.H., Steenwinkel,M.S., van Asten,J.G. *et al*. (2001) Biological monitoring of the exposure to polycyclic aromatic hydrocarbons of coke oven workers in relation to smoking and genetic polymorphisms for GSTM1 and GSTT1. *Ann. Occup. Hyg.*, **45**, 395–408.

221. Whyatt,R.M., Jedrychowski,W., Hemminki,K., Santella,R.M., Tsai,W.Y., Yang,K. and Perera,F.P. (2001) Biomarkers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 581–588.
222. Hou,S.M., Falt,S., Yang,K., Nyberg,F., Pershagen,G., Hemminki,K. and Lambert,B. (2001) Differential interactions between GSTM1 and NAT2 genotypes on aromatic DNA adduct level and HPRT mutant frequency in lung cancer patients and population controls. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 133–140.
223. Hou,S.M., Falt,S., Angelini,S., Yang,K., Nyberg,F., Lambert,B. and Hemminki,K. (2002) The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. *Carcinogenesis*, **23**, 599–603.
224. Teixeira,J.P., Gaspar,J., Martinho,G., Silva,S., Rodrigues,S., Mayan,O., Martin,E., Farmer,P.B. and Rueff,J. (2002) Aromatic DNA adduct levels in coke oven workers: correlation with polymorphisms in genes GSTP1, GSTM1, GSTT1 and CYP1A1. *Mutat. Res.*, **517**, 147–155.

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