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REVIEW
Biomonitoring of tobacco smoke carcinogenicity by dosimetry of DNA adducts and genotyping and phenotyping of biotransformational enzymes: a review on polycyclic aromatic hydrocarbons

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In this review article, we summarize the data on tobacco smoke carcinogenicity in relation to DNA adduct dosimetry and genotyping and phenotyping of biotransformational enzymes. A major class of carcinogens, polycyclic aromatic hydrocarbons, present in substantial quantities in tobacco smoke, is discussed. The historical background and an overview of the metabolic pathways are given. The epidemiological and biological data in particular on dosimetry of the representative DNA adducts and genotyping and phenotyping of the respective activating and detoxifying enzymes are presented. The salient findings are highlighted, the uncertainties are underlined and, finally, recommendations for future research are made.

Keywords: biotransformation, cancer, DNA adducts, polycyclic aromatic hydrocarbons (PAHs), tobacco smoke.

Historical background

Historically, tobacco was offered to Columbus on his landing on San Salvador in the Bahamas on October 12, 1492 by the House of Arawaks. Subsequently, the seafaring European merchants brought seeds of tobacco plants to Spain and Portugal and promoted the American Indians' custom of smoking. In 1585, Jean Nicot, the French ambassador to Portugal, introduced tobacco at the Royal Court of Paris, and the tobacco plant was named *Nicotiana* in his honour. Tobacco was used first in England in 1565, followed by Italy, Germany, Scandinavia and Russia through to the year 1600 (Hoffmann and Hoffmann 1997).

Initially, tobacco was chewed or smoked in pipes, but soon cigarettes and cigars became popular. At first, cigarettes were hand-made by stuffing tobacco into a hollow reed or cane or by rolling crushed tobacco leaves in a cornhusk or in leaves of other vegetables as a wrapper. The first machine-made cigarettes were manufactured in Cuba in 1853, in London in 1856 and in North America in 1860 (Tso 1990).

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Tobacco consumption was modest in the US, UK, continental Europe and Japan until World War I. In 1920, the annual consumption of cigarettes per capita (over the age of 15 years) in Western countries ranged from 400 to 800. It then increased drastically up to 4400 until the mid-1960s (Lee 1975). Following the report of the Royal College of Physicians of London in 1962, and that of the Surgeon General of the US Public Health Service in 1964 on the health hazards of smoking, cigarette consumption declined significantly in the developed world (US Department of Health Education and Welfare 1964). In the UK, the annual consumption of cigarettes per adult male dropped from 4030 in 1960 to 1982 in 1993 (50.8% reduction) (European Bureau of Tobacco and Smoking Prevention 1994). Likewise, in the US the yearly cigarette consumption per adult male decreased from 4345 in 1963 to 2493 in 1994 (42.6% reduction). In 1965, 51.9% and 33.9% of all adult American men and women, respectively, were cigarette smokers. In 1993, the respective numbers were 27.7% and 22.5% (Giovino *et al.* 1994, WHO 1997a). Conversely, in developing countries there has been a substantial increase in annual per capita cigarette consumption since 1970 (rising from 800 to 1400 cigarettes) (World Bank 1999). Worldwide, there has also been a shift in the age group of current smokers towards younger ages (WHO 1997b). Recent surveys show an appreciable increase in smoking prevalence in youth, for example from 27.5% in 1991 to 36.4% in 1997 in the US (MMWR 1998).

Tobacco smoking and cancer

Several decades of epidemiological research have identified tobacco smoking as the main cause of preventable mortality in developed countries (WHO 1997c, Ref. Landis *et al.* 1999). In the US, approximately one fifth of all deaths and one third of all cancers are attributable to smoking (World Cancer Research Fund/Academic Institute for Cancer Research 1997). Data from the mid-1980s confirm that among smokers aged 35–69 years, the death rate is three times higher than that in non-smokers, and that there is at least a two-fold excess mortality from all causes in old age (WHO 1997c). It is estimated that at least 50% of regular smokers who begin smoking during adolescence will eventually be killed by tobacco (WHO 1997c). Global cancer statistics show a prevailing link between tobacco smoking and a variety of human cancers, including cancers of the aerodigestive tract, lower respiratory tract, digestive and gastrointestinal system, renal and urinary tract, and colorectal system (Ferlay *et al.* 1998, Pisani *et al.* 1999, Parkin *et al.* 2001). There are one billion smokers throughout the world, one third of whom live in China, where a major smoke-associated cancer epidemic is predicted (Wald and Hackshaw 1996, Peto *et al.* 1999, Peto and Lopez 2001, Proctor 2001, Simonato *et al.* 2001): the world's most populous nation, which manufacturers and smokes 1.7 trillion cigarettes per year, will witness 100 million tobacco-related deaths in the first half of this century if current smoking trends are not changed (Peto *et al.* 1999, Proctor 2001). Taken together, although the argument for further tobacco control and improved smoking cessation strategies is powerful, the facts and figures imply that the utopian goal of a smoke-free society is still a long way off (Proctor 2001, Raw 2001). Instead, an understanding of smoke-induced carcinogenesis can lead to new strategies for decreasing risk, for identifying highly susceptible individuals and for developing innovative techniques for early detection.

Principal chemical constituents of tobacco smoke

Tobacco combustion results in the formation of mainstream (MS) smoke and sidestream (SS) smoke (Ingebrethsen 1986). MS smoke is generated during puff drawing in the burning cone and hot zone of a tobacco product and travels through the tobacco column out toward the mouthpiece. SS smoke is formed between puffing and is emitted from the smouldering coal of the tobacco product into the ambient air (Hoffmann and Wynder 1986). Both MS smoke and SS smoke are composed of (i) a vapour phase containing volatile agents such as benzene, vinyl chloride, acrolein, etc. and (ii) a particulate phase (tar) containing semi-volatile and non-volatile agents such as alkaloids like nicotine and its derivatives, aromatic amines, polycyclic aromatic hydrocarbons (PAHs), etc. (table 1) (US Environmental Protection Agency 1992, Jaakkola and Jaakkola 1997).

Since SS smoke is generated at a lower burning temperature, its chemical composition in both the vapour and particulate phases differs from that of MS smoke (National Research Council 1986a). The ratios of selected tobacco smoke constituents in MS smoke to those in SS smoke from non-filtered cigarettes are listed in table 1.

Environmental tobacco smoke

Environmental tobacco smoke (ETS) originates from the smouldering end of the tobacco product in between puffs and from the smoker's exhaled smoke. Other contributors to ETS include minor amounts of smoke that escape during the puff drawing from the burning cone of the tobacco product and some vapour phase agents that diffuse through the wrapping materials, such as the cigarette paper, into the environment (National Research Council 1986b). This mixture is released into the environment and is subsequently diluted by the surrounding air. It may then aggregate with pollutants already present in the environment and change character. Physicochemically, the composition of this complex mixture may differ considerably from that of MS smoke (National Research Council 1986a). Nonetheless, most toxic or carcinogenic components of MS smoke are also present in ETS, but in different concentrations (Hoffmann *et al.* 1984, National Research Council 1986a).

Tobacco smoke carcinogens

Of the 44 chemical agents classified as 'group I carcinogens' by the International Agency for Research on Cancer (IARC) (IARC 1994), nine – benzene, cadmium, arsenic, nickel, chromium, 2-naphthylamine, vinyl chloride, 4-aminobiphenyl (4-ABP) and beryllium – are present in tobacco smoke (Smith *et al.* 1997). Based on their mode of action, these compounds are divided into two distinct types: DNA-reactive and epigenetic (Hecht 1996). Carcinogens of the DNA-reactive type possess specific structures that yield electrophilic reactants either directly or after bioactivation. Thus they can form covalently bound DNA adducts, which in turn may give rise to mutagenic events leading to, for example, DNA strand breaks, chromosomal aberrations, oncogene activation and tumour suppressor gene inactivation (Hemminki 1993, Nesnow *et al.* 1995, Ross *et al.* 1995, Denissenko *et al.* 1996, Yoon *et al.* 2001). In addition, these compounds can have other cellular and tissue epigenetic effects, such as cell proliferation and

Table 1. Emissions of some major constituents of tobacco smoke in fresh, undiluted mainstream smoke (MS) and diluted sidestream smoke (SS) from non-filtered cigarettes. Adapted from Hoffmann and Wynder 1986.

	Emissions in MS	SS/MS ratio
Vapour phase		
Carbon monoxide	10–23 mg	2.5–4.7
Carbon dioxide	20–60 mg	8–11
Carbon sulphide	18–42 μg	0.03–0.13
Benzene	12–48 μg	10
Toluene	160 μg	6–8
Formaldehyde	70–100 μg	0.1– ~50
Acrolein	60–100 μg	8–15
Acetone	100–250 μg	2–5
Pyridine	16–40 μg	7–20
3-Vinylpyridine	15–30 μg	20–40
Hydrogen cyanide	400–500 μg	0.1–0.25
Hydrazine	32 ng	3.0
Ammonia	50–150 μg	40–170
Methylamine	17.5–28.7 μg	4.2–6.4
Dimethylamine	7.8–10 μg	3.7–5.1
Nitrogen oxides	100–600 μg	4–10
N-Nitrosodimethylamine	10–40 μg	20–100
N-Nitrosopyrrolidine	6–30 ng	6–30
Formic acid	210–478 μg	1.4–1.6
Acetic acid	330–810 μg	1.9–3.9
Particulate phase		
Particulate matter	15–40 mg	1.3–1.9
Nicotine	1.7–3.3 mg	1.8–3.3
Anatabine	2.4–20.1 μg	0.1–0.5
Phenol	60–140 μg	1.6–3.0
Catechol	100–360 μg	0.6–0.9
Hydroquinone	110–300 μg	0.7–0.9
Aniline	360 ng	30
o-Toluidine	160 ng	19
2-Naphthylamine	1.7 ng	30
4-Aminobiphenyl	4.6 ng	31
Benzo[*a*]anthracene	20–70 ng	2.2–4.0
Benzo[*a*]pyrene	20–40 ng	2.5–3.5
Cholesterol	14.2 μg	0.9
γ-Butyrolactone	10–22 μg	3.6–5.0
Quinoline	0.5–2 μg	8–11
Herman	1.7–3.1 μg	0.7–1.9
N′-Nitrosonornicotine	200–3000 ng	0.5–3.0
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	100–1000 ng	1–4
N-Nitrosodiethanolamine	20–70 ng	1.2
Cadmium	100 ng	3.6–7.2
Nickel	20–80 ng	0.2–30
Zinc	60 ng	0.2–6.7
Polonium-210	0.03–0.5 pCi[a]	1.06–3.7
Benzoic acid	14–28 μg	0.67–0.95
Lactic acid	63–174 μg	0.5–0.7
Glycolic acid	37–126 μg	0.6–0.95
Succinic acid	112–163 μg	0.43–0.62

[a] Picocurie (1 curie = 3.7×10^{10} becquerel).

growth promotion (Nesnow *et al.* 1995, Hecht 1996). Carcinogens of the epigenetic type, in contrast, do not generate reactive electrophiles and therefore are not likely to react with DNA (Williams *et al.* 1996). Instead, they display cellular effects such as neoplasm growth promotion, cytotoxicity, inhibition of tissue growth regulation, peroxisome proliferation, endocrine modification, immunosup-

pression and/or sustained tissue ischaemia that can be the basis for increases in neoplasia (Williams *et al*. 1996). Examples of carcinogens of the DNA-reactive and epigenetic types are 4-ABP and nickel, respectively.

To date, tobacco smoke carcinogenicity has been extensively studied using dosimetry of DNA adducts. Conceptually, DNA adducts are defined as biologically effective dose markers of exposure to carcinogens; they not only reflect prior exposure to carcinogens but also imply a tangible risk for cancer (Poirier *et al*. 2000, Vineis and Perera 2000, Hemminki *et al*. 2001). The latter aspect should be interpreted quite cautiously since DNA adduct formation is only one step in the multi-stage process of carcinogenesis, and many other influential factors such as cellular proliferation are also involved in this process (Poirier *et al*. 2000, Vineis and Perera 2000, Hemminki *et al*. 2001). Therefore, it is premature to rely solely on dosimetry of DNA adducts for cancer risk estimation. At present, although recent studies have indicated the predictive value of DNA adducts for cancer (Tang *et al*. 1995, 2001, Wei *et al*. 2000), it is more realistic to use DNA adduct dosimetry as a means to evaluate carcinogenesis and its mechanisms rather than to assess cancer risk *per se* (Goldring and Lucier 1990, Hemminki 1993, Hou *et al*. 1999, Besarati Nia *et al*. 2000a).

DNA adduct-inducing agents in tobacco smoke

Several classes of chemicals present in tobacco smoke can induce DNA adducts, including tobacco-specific *N*-nitrosamines, aromatic amines and PAHs. Of these, PAHs are the subject of the present review.

PAHs

PAHs are formed as a product of tobacco combustion, and are found in considerable quantities in its particulate phase (IARC 1986). PAHs are also emitted into the environment as a result of incomplete pyrolysis of organic materials such as fossil fuels. Established environmental sources of PAHs are power plants, domestic heating systems, petrol and diesel engines, refuse burning and various industrial activities (Guerin 1978).

Thus far, more than 500 individual PAHs have been identified (Grimmer 1983). However, only a limited number of them exhibit carcinogenicity in test systems. Tobacco smoke contains 11 potent carcinogenic PAHs, out of which benzo[*a*] pyrene (B[*a*]P) is widely known as the representative compound (IARC 1986). Chemically, PAHs are relatively inert as they are formed at very high temperatures. Being lipid soluble, they tend to accumulate in organisms that come into contact with them. They may subsequently undergo oxidative metabolism, yielding water-soluble derivatives or highly reactive electrophiles. The latter, if not eliminated before DNA replication, can interact with cellular structures and molecules and give rise to carcinogenesis. Paradoxically, it is now known that if PAHs were not metabolized, they would not be carcinogenic (Hall and Grover 1990).

Biotransformation of PAHs

On entering the body, PAHs are enzymatically transformed to a series of either excretable end products (detoxification) or highly reactive metabolites that interact

with subcellular targets (activation) (Hall and Grover 1990). The initial step is epoxidation, which is the addition of one atom of oxygen across a double bond. This reaction is usually catalysed by cytochrome P450 (CYP450) enzymes, encoded by the *CYP* family of genes (Nebert and Gonzalez 1987). Other enzymes such as lipoxygenase, cyclooxygenases, myeloperoxidases and monoamine oxidases are also involved, but to a lesser extent (Burchell *et al.* 1997, Duffel 1997). The epoxides can undergo (i) hydration to yield diols, catalysed by epoxide hydrolases (EHs); (ii) isomerization to phenol; or (iii) conjugation with glutathione (GSH), catalysed by glutathione *S*-transferases (GSTs) (Hall and Grover 1990). The diol derivatives can conjugate to glucuronic and sulphuric acids, catalysed by uridine-5′-diphosphate-glucuronosyl-transferases (UGTs) and sulphatases, respectively (Burchell *et al.* 1997, Duffel 1997), or be further oxidated/hydoxylated to yield diol epoxides (Guenther and Oech 1981). The diol epoxides may then undergo conjugation with GSH (Armsrong 1997) or further metabolism to triols and tetraols or interaction with macromolecules to form, for example, DNA adducts (Hecht 1999). Likewise, the phenol derivatives can conjugate to glucuronic and sulphuric acids or be further metabolized to adduct-forming intermediates (Fu *et al.* 1978). The metabolic pathway of the model PAH compound, B[*a*]P, is simplistically outlined in figure 1.

Stereoisometrically, most PAH derivatives exist as pairs of enantiomers (*anti* or *syn*), two compounds related to one another as non-superimposable mirror images. Physically, each member of a given pair differs from its antipode in the direction by which it rotates the plane of polarized light (+ or−), and also in the absolute configuration of the groups around a chiral centre (*R* or *S*) (Thakker 1985). Biologically, two members of a pair of enantiomers may exhibit large differences, both in terms of their activity and in the details of their metabolism (Trager and Jones 1987). For example, the 7,8-diol 9,10-oxide-B[*a*]P (BPDE), generally regarded as the ultimate carcinogenic form of B[*a*]P, is produced as two diastereomers, each comprising two enantiomers. Of these four, the (+)-*anti*-7*R*,8*S*-dihydroxy-9*S*,10*R*-epoxy-7,8,9,10-tetrahydro-B[*a*]P [(+)-*anti*-BPDE] has been found to possess greater biological activity than the other three in most test systems (Dipple *et al.* 1984). (+)-*Anti*-BPDE can bind covalently to the 2-amino group of guanine at its C-10 position, thereby forming a major DNA adduct, (+)-*anti*-BPDE-dG (Weinstein *et al.* 1976).

Epidemiological and biological data on PAHs

In 1775, Percival Pott noted a high incidence of skin cancer in chimney sweeps, probably induced by B[*a*]P, which occurs at a concentration of 0.2% in soot. Around a century later, von Volkman demonstrated the link between skin cancer and exposure to PAH in German coal tar workers (Alloway and Ayres 1994). In 1947, Kennaway showed the high risk of lung cancer in workers in the coal tar and gas industries (Alloway and Ayres 1994). This work was extended in 1972 by Sir Richard Doll (Alloway & Ayres 1994), who made a longitudinal study of mortality in workers in the same industries. Subsequent studies in individuals exposed occupationally, medically or environmentally to PAHs implicated these compounds in the aetiology of several human cancers, including cancers of the lung, oral cavity, larynx, skin, stomach, rectum, cervix and breast (IARC 1994). The history of the evaluation of PAHs has been extensively reviewed by Sir Ernest Kennaway (Kennaway 1955).

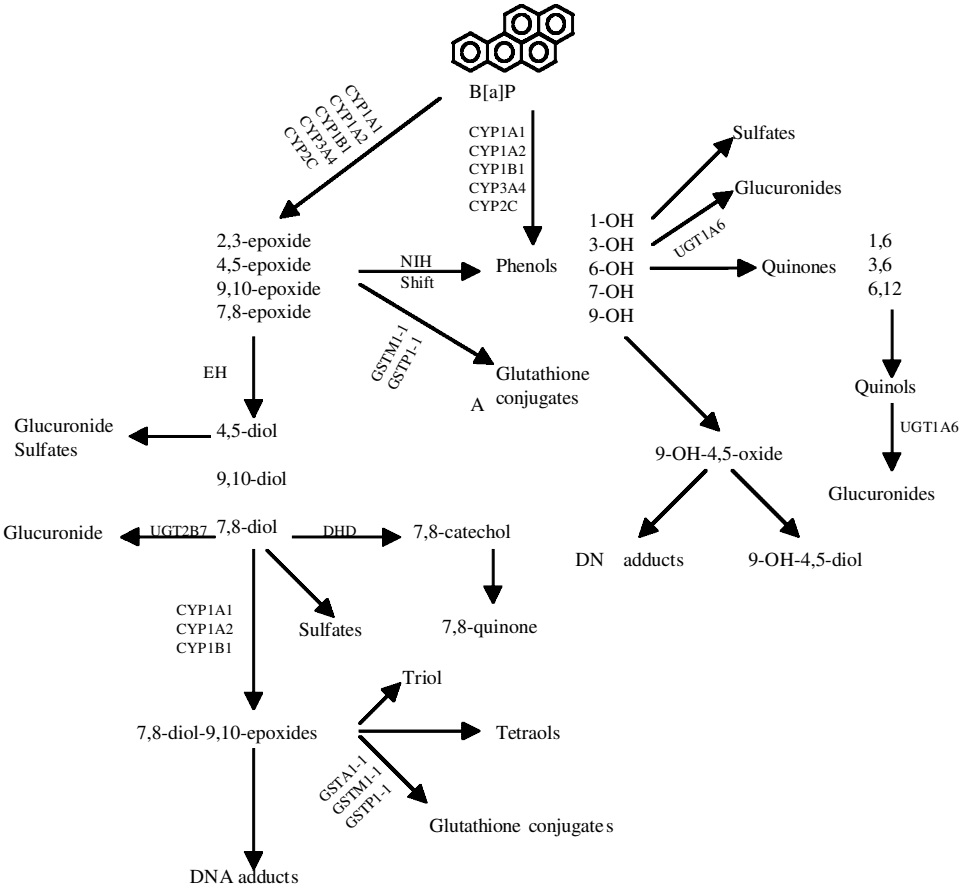

Figure 1. Metabolic pathways of B[*a*]P (adapted from Hecht 1999). The major enzymes involved in these reactions are indicated as CYP (cytochrome P450), DHD (dihydrodiol dehydrogenase), EH (epoxide hydrolase), GST (glutathione *S*-transferases), UGT (uridine-5′-diphosphate-glucuronosyl-transferases) and 1-OH, etc. (1-hydroxy B[*a*]P, etc.). Other abbreviations are defined in the text.

Thus far, the involvement of PAHs in smoking-associated cancers in humans has been investigated in the target organs (tumour-arising organs) or in the surrogate matrices such as peripheral blood cells (Phillips 1996, Poirier and Weston 1996). The latter have been used mainly in studies where the population under investigation consists of healthy individuals. Conceivably, most target organs for smoking-related cancers are not readily obtainable and require surgical or somewhat invasive procedures, precluding, for practical or ethical reasons, widespread population sampling or repeated sampling of the same individual (Phillips 1996).

Lung. In the lung, dosimetry of PAH–DNA adducts has consistently shown a higher level of adducts in smokers compared with non-smokers (Phillips *et al*. 1988, Van Schooten *et al*. 1990, Geneste *et al*. 1991, Lewtas *et al*. 1993, Lodovici *et al*. 1998, Hussain *et al*. 2001). In ^{32}P-postlabelling analyses, smokers show a typical diagonal radioactive zone (DRZ) generated by the resolution of labelled

DNA digests on two-dimensional thin-layer chromatograms. This is widely regarded as an indication of the formation of a complex mixture of adducts in the respiratory tract as a result of exposure to tobacco smoke (Phillips 1996). More recently, ammonium hydroxide-based thin layer chromatography solvents have been introduced, which resolve adduct DRZ into discrete spots and allow determination of adducts derived from various classes of compounds, including PAHs (Spencer-Beach *et al.* 1996). The PAH-derived nature of DNA adducts in the lungs of smokers is shown by the specific detection of BPDE-dG adducts using fluorescence spectrometry and enzyme-linked immunosorbent assay (ELISA) (Van Schooten *et al.* 1990, Weston and Bowman 1991). Also, using a robust and sensitive gas chromatography/mass spectrometry (GC/MS) method, Goldman *et al.* (Goldman *et al.* 2001) have recently shown dose-dependent formation of a spectrum of PAH–DNA adducts in the lungs of smokers. In contrast, non-urea-based ^{32}P-postlabelling analysis failed to demonstrate any typical PAH–DNA adducts in the lung tissue of smokers (Gupta *et al.* 1999).

Mechanistically, distribution of (±)-*anti*-BPDE-dG adducts has been mapped within the *p53* tumour suppressor gene, which is mutated in 60% of human lung cancers (Hollstein *et al.* 1991), in both HeLa cells and normal human bronchial epithelial cells (Denissenko *et al.* 1996). Accordingly, it has been shown that this major adduct of PAHs is preferentially formed at lung cancer mutational hotspots (codon 154, 157, 158, 245, 248, 249 and 273) (Hollstein *et al.* 1991, Denissenko *et al.* 1996). Subsequent mapping of other active diol epoxides of smoke-related PAHs (chrysene, 5-methylchrysene, 6-methylchrysene, benzo[*c*]phenanthrene and benzo[*g*]chrysene) in normal human bronchial epithelial cells has also shown that the DNA damage induced by these compounds is mostly located at the same codons previously reported for (±)-*anti*-BPDE (Smith *et al.* 2000). This indicates that other PAHs also contribute to the total load of adducts in the *p53* gene and probably impact on its mutational spectrum (Smith *et al.* 2000). Furthermore, it has been shown that the *p53* mutational spectra differ significantly between smokers and non-smokers in that smokers have a higher frequency of G→T transeversions (reviewed by Hainaut and Pfeifer 2001). Recently, the G→T transversion hotspots in *LacI*, *cII* and *SupF* transgenes have been shown to correlate with the preferential binding sites of BPDE at methylated CpG dinucletotides in these reporter genes (Yoon *et al.* 2001). Also, *in vitro* treatment of human bronchial epithelial cells with BPDE induced the frequency of G→T transversions at several mutational hotspots of the *p53* (Hussain *et al.* 2001). Altogether, these data reiterate a plausible link between the formation of smoke-derived PAH–DNA adducts and lung cancer.

Larynx. In the larynx, aromatic DNA adducts have been detected in smokers but not in non-smokers using both enrichment methods of the ^{32}P-postlabelling assay, the nuclease P1 (NP1) digestion and butanol extraction techniques (Degawa *et al.* 1994). Applying the NP1 version of this assay, Szyfter *et al.* (1994) demonstrated the formation of aromatic/hydrophobic DNA adducts in both tumour and non-tumour laryngeal tissues, with adduct levels being higher in the former. Analysis of laryngeal tumours by single-strand conformation polymorphism (SSCP) and direct DNA sequencing of exons 5–8 revealed that 46% of the mutations detected in these tissues were the typical *p53* mutations observed in lung cancer (G→T transversions, and G→A and C→T transitions) (Hainaut *et al.*

1998). Additionally, 54% of the detected mutations were within the hotspot region of the *p53* gene, covering codons 238–248 (Zhang *et al*. 1994).

Oral and nasal cavity. Initial reports on dosimetry of DNA adducts in the oral mucosa cells were inconclusive both in smokers versus non-smokers and in different groups of tobacco users, such as betel nut chewers, Khaini tobacco chewers and inverted smokers (Dunn and Stich 1986, Chacko and Gupta 1988). Subsequently, NP1- and butanol-enhanced ^{32}P-postlabelling analyses showed a higher level of adducts in the oral mucosa cells of smokers and suggested the aromatic amines and/or nitroaromatic nature of these adducts (Jones *et al*. 1993, Stone *et al*. 1995). Also, immunohistochemistry of PAH–DNA adducts showed a higher level of adducts in the oral cells of smokers compared with non-smokers (Hsu *et al*. 1997, Romano *et al*. 1999, Besarati Nia *et al*. 2000a). Likewise, in nasal epithelial cells obtained by biopsy or nasal lavage, smokers had a higher level of adducts compared with non-smokers in NP1-enriched ^{32}P-postlabelling analyses (Peluso *et al*. 1997, Zhao *et al*. 1997).

Cervix, placenta and sperm. In the cervical epithelium, smokers showed higher level of adducts compared with non-smokers in both versions of the ^{32}P-post-labelling assay; the differences were more pronounced when the butanol extraction method was utilized (Simons *et al*. 1993, 1995). A recent immunohistochemical study has also shown a higher level of PAH–DNA adducts in the cervical smears of smokers compared with non-smokers (Mancini *et al*. 1999). Also, elevated levels of DNA adducts were observed in the placentas of smoking pregnant women compared with their non-smoking counterparts by both ^{32}P-postlabelling assay and ELISA (Everson *et al*. 1988, Hansen *et al*. 1992, Arnould *et al*. 1997). In addition, the level of adducts in the umbilical cord vein was significantly lower than in the placenta, and marginally lower than in the umbilical cord artery of the same donor (Hansen *et al*. 1992, Arnould *et al*. 1997, Hoffmann and Hoffmann 1997). This implies a transplacental exposure of the fetus to smoke-derived carcinogens as well as the capability of the fetus to metabolize these compounds.

^{32}P-postlabelling analysis of human sperm cells failed to differentiate between smokers and non-smokers (Gallagher *et al*. 1993, Little and Vainio, 1994). However, pre-implemented embryos from smoking parents showed higher immunoreactivity for BPDE–DNA adducts compared with those from non-smoking parents (Zenzes *et al*. 1999a). The similar levels of immunostaining in embryos from both types of smoking couples (either one parent smoker or both parents smokers) in the same study (Zenzes *et al*. 1999a), together with the relatively higher immunoreactivity for BPDE–DNA adducts in the sperm cells of smokers compared with non-smokers in another study (Zenzes *et al*. 1999b), suggest that transmission of modified DNA is mainly through the spermatozoa.

Breast. In a pilot study in breast cancer cases and mammoplasty controls, only breast tissues (tumour and/or tumour-adjacent) from cases who were current smokers displayed a DRZ in NP1-enhanced ^{32}P-postlabelling analyses (Perera *et al*. 1995). Applying the same methodology, a large scale case control study showed a higher level of DNA adducts in the adjacent non-tumour tissues of the cases (Li *et al*. 1996). However, both studies were subsequently shown to be biased because of their inappropriate controls and unadjusted confounders. Recently, a well-designed hospital-based case control study has shown an association between

immunohistochemically detected PAH–DNA adducts and breast cancer risk (odds ratio = 4.43, 95% confidence interval = 1.09–18.01) after controlling for known risk factors for breast cancer and adjusting for confounding factors for PAH exposure. Smoking behaviour, however, did not correlate with the level of adducts or with the case-control status (Rundle *et al.* 2000a).

Miscellaneous. Scant ^{32}P-postlabelling data have shown the elevated level of DNA adducts in tumour tissues from gastric cancer patients compared with respective controls (Dyke *et al.* 1992). Also, in a pilot study in human colon mucosa, (+)-*anti*-BPDE-dG adducts were detected using high performance liquid chromatography with fluorescence detection (HPLC-FD) (Phillips 1996). In the skin, adduct measurement was performed in relation to occupational/medical exposure to PAHs rather than tobacco smoking. For example, in psoriasis patients treated with coal tar, specific nuclear staining for (±)-*anti*-BPDE-DNA adducts detected by the indirect immunofluorescence method was observed in skin biopsies (Zhang *et al.* 1990). The same samples revealed a pattern of multiple DNA adducts in NP1 ^{32}P-postlabelling analyses (Zhang *et al.* 1990). Similar results were also reported by our group in coal tar-treated eczema patients (Godschalk *et al.* 1998).

Peripheral blood, bronchoalveolar lavage and sputum. In peripheral blood cells, dosimetry of PAH–DNA adducts initially produced controversial results when this matrix was analysed in its entirety (Reddy *et al.* 1990, Rothman *et al.* 1990, Van Schooten *et al.* 1992). Later, it became evident that, since white blood cells (WBCs) are composed of different cell types with varying life spans and metabolizing capacities (Boggs and Winkelstein 1983), both the recency of exposure and the formation/persistence of adducts may vary within this matrix (Savela and Hemminki 1991). For instance, granulocytes, which comprise approximately 60% of the total WBCs and have a short half-life of 7–24 h (Boggs and Winkelstein 1983), had a considerably lower level of PAH–DNA adducts than mononuclear cells (monocytes [15%] plus lymphocytes [75%]) (Boggs and Winkelstein 1983, Savela and Hemminki 1991). Moreover, when treated separately with B[*a*]P *in vitro*, only short-lived monocytes (life span 8 h), but not unstimulated lymphocytes (life span 1–5 years), formed DNA adducts. Subsequent formation of DNA adducts in both cell types incubated simultaneously with B[*a*]P suggested that *in vivo* formation of adducts in lymphocytes occurs after other cell types activate the procarcinogens (Holz *et al.* 1991). Accordingly, use of total WBCs for the measurement of DNA adducts has increasingly been replaced by mononuclear cell DNA adduct dosimetry. Although more recent studies lend support to the relevance of DNA adduct analysis in subpopulations of WBCs for both exposure and risk assessment (Wiencke *et al.* 1999, Wei *et al.* 2000, Li *et al.* 2001, Tang *et al.* 2001), adduct dosimetry in fractionated WBCs is still subject to bias. Understandably, circulating blood cells are exposed to diverse routes of exposure, such as inhalation, ingestion and dermal absorption, thereby reflecting an integrated rather than a specific body burden for DNA adducts. This liability is of most concern when the carcinogen of interest enters the body via a specific route. Such non-specificity of DNA adducts in WBCs may explain the controversial data on PAH–DNA adducts in the WBCs of smokers versus non-smokers and of other groups of PAH-exposed versus non-exposed individuals (Tang *et al.* 1995, Phillips 1996, Poirier and Weston 1996, Palli *et al.* 2000, 2001). In addition, the lack of

correlation between the level of adducts in the WBCs and that in the target organs is to some extent expected (Phillips 1996, Poirier and Weston 1996). Interestingly, few studies (Izzotti *et al.* 1991, De Flora *et al.* 1993, Van Schooten *et al.* 1997, Piipari *et al.* 2000) have utilized bronchoalveolar lavage (BAL) cells, which are mainly (> 90%) composed of bronchoalveolar macrophages (BAMs), having an efficient metabolic machinery and a predominant exposure to inhalatory carcinogens (Harris *et al.* 1978, Petruzzelli *et al.* 1988). Although adduct analyses in BAL cells have been much more promising than those in WBCs, the invasiveness of the method of sampling hinders routine application of BAL cells in molecular dosimetry studies. Recently, we have examined the applicability of induced sputum, a non-invasively obtainable matrix from the lower respiratory tract, for dosimetry of inhalatory carcinogens (Besarati Nia *et al.* 2000b). Applying both versions of the ^{32}P-postlabelling assay, we found a higher level of DNA adducts in the induced sputum of smokers compared with non-smokers, with the differences being more explicit with the NP1 enrichment procedure (Besarati Nia *et al.* 2000b). Subsequent analysis of the samples by immunohistochemistry showed a non-significantly higher level of PAH–DNA adducts in the induced sputum of smokers compared with non-smokers ($p = 0.07$) (Besarati Nia *et al.* 2000c). These observations were confirmed in a follow-up study, in which we also demonstrated the superiority of adduct analysis in induced sputum to that in WBCs (Besarati Nia *et al.* 2000d).

Genotyping and phenotyping of biotransformational enzymes. A complementary area of interest is the polymorphisms of genes involved in the biotransformation of PAHs. The enzyme complex responsible for the initial activation of PAHs, arylhydrocarbon hydroxylase (AHH), consists of a battery of enzymes that includes CYP1A1 (Nelson *et al.* 1996). CYP1A1 mRNA reflects gene induction, while AHH and 7-ethoxyresirufun *O*-deethylase (EROD) are measures of enzyme activity (Cosma *et al.* 1993, Zhang *et al.* 1996). Among the four known polymorphisms identified in the *CYP1A1* gene, two closely linked mutations (mostly in Asians) have been extensively studied in relation to AHH inducibility, DNA damage and cancer risk: the *CYP1A1*2* allele, which results in a new restriction site (*Msp*I) in the 3′-untranslated region of the gene, and the *CYP1A1*3* allele, located in exon 7, which results in an amino acid exchange (Ile 462→Val) (Hayashi *et al.* 1991, Crofts *et al.* 1994). In most studies, but not all, these alleles have been associated with increases in the catalytic activity of the enzyme and with higher AHH inducibility (Cosma *et al.* 1993, Zhang *et al.* 1996). The alleles have also been associated with increased frequency of *p53* mutations in lung and oral tumour tissues (Kawajiri *et al.* 1996, Park *et al.* 1997). The extent of AHH activity in human lung microsomes has been positively correlated with the level of PAH–DNA adducts in the same lung samples (Geneste *et al.* 1991). In leukocytes, however, such a correlation has not been consistently shown (Ichiba *et al.* 1994, Rothman *et al.* 1995). Regarding *CYP1A1* polymorphism and susceptibility to DNA damage or cancer, contradictory results have been reported. This has mostly been the case when different populations with varying mutated allele frequencies were investigated or when the analyses were performed in surrogate tissues (Phillips 1996, Poirier and Weston 1996, Bartsch *et al.* 2000, Piipari *et al.* 2000). Overall, lack of agreement in these studies may have arisen from differences in the experimental approaches used to determine polymorph-

isms, to detect DNA adducts within specific organs, and to demonstrate subtle effects whilst having incomparable statistical power (IARC 1999a).

A major pathway of detoxification for PAHs is formed by the multi-gene family of cytosolic enzymes, the GSTs. These enzymes catalyse the conjugation of PAHs or their reactive metabolites to GSH, thereby making them readily excretable (Mannervik and Danielson 1988). Four different gene families of *GSTs* are known – a, μ, p and t (Hayes and Pulford 1995), although most of the available literature with regard to PAHs and cancer relates to *GSTμ* and *GSTp*. Each *GST* family comprises several genes; for example, the *GSTμ* family consists of at least five known genes, *GSTM1–5* (Hayes and Pulford 1995). The various GSTs have different but often overlapping substrate specificities (Hayes and Pulford 1995). Also, diverse patterns of expression in different cells and tissues have been shown (Hayes and Pulford 1995). The most studied human GST, GSTM1, is lacking in approximately 50% of Caucasian populations (Seidegård *et al.* 1988). Absence of this enzyme is caused by an 8 kb deletion at the entire coding region of the *GSTM1* gene, producing the null genotype (Seidegård *et al.* 1988). A meta-analysis of 12 case control studies has concluded that the *GSTM1* null genotype is a moderate risk factor for lung cancer (odds ratio = 1.41, 95% confidence interval = 1.23–1.61, $p < 0.0001$) (McWilliams *et al.* 1995). In some studies, but not all, this genotype has also been associated with an increased incidence of other types of cancer, including adenocarcinoma of stomach and caecum, multiple skin cancers, carcinoma of bladder, and oral nasopharyngeal and laryngeal cancer (Bell *et al.* 1993, Heagerty *et al.* 1994, Park *et al.* 1997, Nazar-Stewart *et al.* 1999). Additionally it has been shown that possession of the *GSTM1* null genotype may affect the susceptibility to DNA damage of various organs (Kato *et al.* 1995, Jourenkova *et al.* 1998, Bartsch *et al.* 1999). A number of studies have reported an association between the *GSTM1* null genotype and the level of PAH–DNA adducts in the non-tumorous tissues of lung cancer cases (Shields *et al.* 1993, Kato *et al.* 1995, Ryberg *et al.* 1997). Also, the *GSTM1* null genotype has been shown to be a major determinant of PAH–DNA adduct levels in both malignant and non-malignant breast tissues in cases but not in controls (Rundle *et al.* 2000b). However, the influence of this genotype on DNA adduct levels in leukocytes is not yet clear. Studies of subjects exposed occupationally, environmentally or medically to PAHs, e.g. chimney sweeps, coke oven and foundry workers, bus drivers, fire-fighters, soldiers and coal tar-treated patients, as well as studies of smokers have mainly shown no or an insignificant increase in the leukocyte DNA adduct levels dependent on the *GSTM1* null genotype (Butkiewicz *et al.* 1998, Bartsch *et al.* 1999, Rojas *et al.* 2000).

The *GSTP1* gene is also known to be polymorphic at different sites (Zimniak *et al.* 1994). A common polymorphism occurs in exon 5 of this gene, causing an exchange of amino acids (Ile 105→Val) with a mutant frequency of 30–35% (Butkiewicz *et al.* 2000). The two isoforms, *GSTP1-1/V-105* (GG: Val/Val, GA: Ile/Val) and *GSTP1-1/I-105* (AA: Ile/Ile), differ in activity, specificity and catalytic efficiency (Zimniak *et al.* 1994, Hu *et al.* 1997). Epidemiological studies indicate that individuals carrying the *GSTP1-1/V-105* rather than the *GSTP1-1/I-105* allele are more susceptible to tumour formation at sites such as the oral cavity, oesophagus, breast, bladder and testis (Harries *et al.* 1997, Helzlsouer *et al.* 1998, Katoh *et al.* 1999). In addition, the levels of hydrophobic DNA adducts in non-cancerous lung tissues from smoking lung cancer patients have been shown to

be associated with the *GSTP1-I/V-105* genotype (Ryberg *et al.* 1997). However, studies of *GSP1* polymorphisms in relation to PAH exposure are too few and limited to allow any definite conclusions (for more information on recent meta-analyses, see IARC 1999b).

Overall, it appears that a combination of different metabolic polymorphisms, rather than a single one, is a better determinant of DNA damage and cancer susceptibility (IARC 1999c, Bartsch *et al.* 2000, Rojas *et al.* 2000). For instance, in a study of non-small cell lung cancer (NSCLC), the prevalence of combined *GSTM1* null and *CYP1A1*2B* genotypes was significantly higher in the adeno-carcinoma group (Butkiewicz *et al.* 1999). In the same study, patients with the *GSTM1* null and *CYP1A1*3* combined genotypes had a significantly higher level of PAH–DNA adducts in non-tumorous tissues of the lung compared with those with other genotype combinations (Butkiewicz *et al.* 1999). Also, smoking lung cancer patients as well as coke oven workers who had a combination of *CYP1A1*2* or **3* and *GSTM1* null genotypes had a higher level of (+)-*anti*-BPDE–DNA adducts compared with those with *CYP1A1* and *GSTM1* wild-type genotypes at a similar or even lower smoking dose (Bartsch *et al.* 1999). Furthermore, smoking individuals with mutated *CYP1A1* and *GSTM1* null genotypes had a 100-fold higher level of bronchial BPDE–DNA adducts than those with other combined genotypes (Bartsch 1996). Among the moderate smokers, those who carried the combined *CYP1A1*2* and *GSTM1* null alleles showed the highest level of adducts (Ichiba *et al.* 1994). Likewise, lung cancer patients with *GSTM1* null and *GSTP1-I/V-105* combined genotypes had the highest level of hydrophobic DNA adducts in non-tumorous tissues of the lung ($p = 0.011$) (Ryberg *et al.* 1997). The distribution of combined *GSTM1* null and *GSTP1-1/V-105* genotypes was also significantly different in cases and controls (Ryberg *et al.* 1997). In a Japanese population, individuals who carried the mutated *GSTP1* and *GSTM1* null alleles had a higher risk of head and neck cancer compared with those who had other combined genotypes (odds ratio = 2.58, 95% confidence interval = 0.77–1.79) (Kihara *et al.* 1997). Also, the level of PAH–DNA adducts in the mononuclear WBCs of smokers with *GSTM1* null and *GSTP1-1/V-105* combined genotypes was non-significantly higher than that in smokers with other genotype combinations (Butkiewicz *et al.* 1998).

Taken together, the results of adduct dosimetry and genotyping/phenotyping studies partially support the contribution of PAHs to smoking-associated cancer, in particular cancer of the lower respiratory tract, in humans. The remaining uncertainties cannot be resolved unless the following issues are fully addressed: (i) the ubiquity of confounding exposure to PAHs; (ii) the complexity of exposure to a spectrum of PAHs with different carcinogenic potencies; and (iii) the statistical power criteria for genotyping/phenotyping of the relevant biotransformational and DNA repair enzymes. These, together with the need to validate the currently used surrogate matrices and/or to introduce innovative biological materials, should define the strategy for future research on PAHs, smoking and cancer.

Summary and conclusions

Although the carcinogenicity of tobacco smoke to humans is no longer a matter of dispute, the identity of the constituents involved in this process still is. Ample studies have attempted to address this issue by dosimetry of smoke-related DNA

adducts and genotyping/phenotyping of the relevant biotransformational enzymes. The majority of these investigations have implicated smoke-derived PAHs in the aetiology of several human cancers, in particular cancer of the lower respiratory tract. The results have been more straightforward when the target tissues have been analysed. Of course, since most target organs for PAH-associated cancers are only invasively accessible, routine biomonitoring of the general population has mainly been achieved by the analysis of the surrogate matrices. To some extent though, the incomparability of exposure patterns and the differences in cell compositions (with varying activation/detoxification and DNA repair capacities) have cast a shadow on the credibility of these matrices to portray the events occurring in the target sites. Such liabilities have introduced biases in the results of many studies. Nevertheless, cautious interpretations of the results have still shed light on the epidemiological observations and helped resolve partially the dilemma of causal/casual relationship between PAH exposure and cancer. This is best exemplified by a single recent nested-case control study in which dosimetry of PAH–DNA adducts in WBC could significantly predict the risk for lung cancer (Tang *et al*. 2001).

In the future, to better understand smoking-induced carcinogenesis in humans and the roles played by chemical carcinogens, large-scale research is needed. Such investigations should be conducted in a multi-disciplinary way to elucidate the impact of specific DNA adducts on target and relevant surrogate matrices, along with genotyping/phenotyping of biotransformational and DNA repair enzymes. Fortunately, great advances in adduct dosimetry techniques, enabling specific and sensitive detection of DNA adducts, together with major breakthroughs in DNA chip technology, which offer high-throughput gene analysis, have partly paved the way to embark upon such investigations.

References

Alloway, B. J. and Ayres, D. C. (editors) 1994, *Chemical Principles of Environmental Pollution* (Glasgow: Blackie Academic & Professional).

Armsrong, R. N. 1997, Glutathione-*S*-transferases. In *Comprehensive Toxicology: Biotransformation*, edited by F. P. Guengerich (Oxford: Elsevier Science), pp. 307–327.

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. *Human and Experimental Toxicology*, **16**, 716–721.

Bartsch, H. 1996, DNA adducts in human carcinogenesis: etiological relevance and structure–activity relationship. *Mutation Research*, **340**, 67–79.

Bartsch, H., Rojas, M., Nair, U., Nair, J. and Alexandrov, K. 1999, Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. *Cancer Detection and Prevention*, **23**, 445–453.

Bartsch, H., Nair, U., Risch, A., Rojas, M., Wikman, H. and Alexandrov, K. 2000, Genetic polymorphism of *CYP* genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiology, Biomarkers and Prevention*, **9**, 3–28.

Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L. and Lucier, G. W. 1993, Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. *Journal of the National Cancer Institute*, **85**, 1159–1164.

Besarati Nia, A., Van Straaten, H. W., Godschalk, R. W., Van Zandwijk, N., Balm, A. J., Kleinjans, J. C. and Van Schooten, F. J. 2000a, Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in mouth floor and buccal mucosa cells of smokers and nonsmokers. *Environmental and Molecular Mutagenesis*, **36**, 127–133.

Besarati Nia, A., Maas, L. M., Van Breda, S. G., Curfs, D. M., Kleinjans, J. C., Wouters, E. F. and Van Schooten, F. J. 2000b, Applicability of induced sputum for molecular dosimetry of exposure to inhalatory carcinogens: ^{32}P-postlabeling of lipophilic DNA adducts in smokers and nonsmokers. *Cancer Epidemiology, Biomarkers and Prevention*, **9**, 367–372.

Besarati Nia, A., Van Straaten, H. W., Kleinjans, J. C. and Van Schooten, F. J. 2000c, Immunoperoxidase detection of 4-aminobiphenyl- and polycyclic aromatic hydrocarbons-DNA adducts in induced sputum of smokers and non-smokers. *Mutation Research*, **468**, 125–135.

Besarati Nia, A., Maas, L. M., Brouwer, E. M., Kleinjans, J. C. and Van Schooten, F. J. 2000d, Comparison between smoking-related DNA adduct analysis in induced sputum and peripheral blood lymphocytes. *Carcinogenesis*, **21**, 1335–1340.

Boggs, D. R. and Winkelstein, A. (editors) 1983, *White Cells Manual* (Philadelphia: FA Davis).

Burchell, B., McGurk, K., Brierley, C. H. and Clarke, D. J. 1997, UDP-glucuronosyltransferases. In *Comprehensive Toxicology: Biotransformation*, edited by F. P. Guengerich (Oxford: Elsevier Science), pp. 401–436.

Butkiewicz, D., Grzybowska, E., Hemminki, K., Ovrebo, S., Haugen, A., Motykiewicz, G. and Chorazy, M. 1998, Modulation of DNA adduct levels in human mononuclear white blood cells and granulocytes by *CYP1A1, CYP2D6* and *GSTM1* genetic polymorphisms. *Mutation Research*, **415**, 97–108.

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. *European Journal of Cancer Prevention*, **8**, 315–323.

Butkiewicz, D., Grzybowska, E., Phillips, D. H., Hemminki, K. and Chorazy, M., 2000, Polymorphisms of the *GSTP1* and *GSTM1* genes and PAH-DNA adducts in human mononuclear white blood cells. *Environmental and Molecular Mutagenesis*, **35**, 99–105.

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.

Cosma, G., Crofts, F., Taioli, E., Toniolo, P. and Garte, S. 1993, Relationship between genotype and function of the human *CYP1A1* gene. *Journal of Toxicology and Environmental Health*, **40**, 309–316.

Crofts, F., Taioli, E., Trachman, J., Cosma, G. N., Currie, D., Toniolo, P. and Garte, S. J. 1994, Functional significance of different human *CYP1A1* genotypes. *Carcinogenesis*, **15**, 2961–2963.

De Flora, S., Izzotti, A., D'Agostini, F., Rossi, G. A. and Balansky, R. M. 1993, Pulmonary alveolar macrophages in molecular epidemiology and chemoprevention of cancer. *Environmental Health Perspectives*, **99**, 249–252.

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 Research*, **54**, 4915–4919.

Denissenko, M. F., Pao, A., Tang, M. and Pfeifer, G. P. 1996, Preferential formation of benzo[*a*]pyrene adducts at lung cancer mutational hotspots in *P53*. *Science*, **274**, 430–432.

Dipple, A., Moschel, R. C. and Bigger, C. A. H. 1984, Polynuclear aromatic carcinogens. In *Chemical Carcinogens,* edited by C. E. Searle (Washington DC: American Chemical Society), pp. 41–163.

Duel, M. W. 1997, Sulfotransferases. In *Comprehensive Toxicology: Biotransformation*, edited by F. P. Guengerich (Oxford: Elsevier Science), pp. 365–384.

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.

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.

European Bureau of Tobacco and Smoking Prevention. 1994, *Tobacco and Health in the European Union: An Overview* (Brussels: European Bureau of Tobacco and Smoking Prevention).

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. *Journal of National Cancer Institute*, **80**, 567–576.

Ferlay, J., Parkin, D.M. and Pisani, P. 1998, GLOBOCAN. In *Cancer Incidence and Mortality Worldwide* (CD ROM), edited by International Agency for Research on Cancer, Cancer Base (Lyon: International Agency for Research on Cancer).

Fu, P. P., Harvey, R. G. and Beland, F. A. 1978, Molecular orbital theoretical prediction of the isomeric products formed from reactions of arene oxide and related metabolites of polycyclic aromatic hydrocarbons. *Tetrahedron*, **34**, 857–866.

Gallagher, J. E., Vine, M. F., Schram, 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 Epidemiology, Biomarkers and Prevention*, **2**, 581–585.

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.

Giovino, G. A., Schooley, M. W., Zhu, B. P., Chrismon, J. H., Tomar, S. L., Peddicord, J. P., Merritt, R. K., Husten, C. G. and Eriksen, M. P. 1994, Surveillance for selected tobacco-use behaviours – United States, 1900–1994. *Morbidity and Mortality Weekly Report*, **43**, 1–43.

Godschalk, R. W., Ostertag, J. U., Moonen, E. J., Neumann, H. A., Kleinjans, J. C. and Van Schooten, F. J. 1998, Aromatic DNA adducts in human white blood cells and skin after dermal application of coal tar. *Cancer Epidemiology, Biomarkers and Prevention*, **7**, 767–773.

Goldman, R., Enewold, L., Pellizzari, E., Beach, J. B., Bowman, E. D., Krishnan, S. S. and Shields, P. G. 2001, Smoking increases carcinogenic polycyclic aromatic hydrocarbons in human lung tissue. *Cancer Research*, **61**, 6367–6371.

Goldring, J. and Lucier, G. 1990, Protein and DNA adducts. In *Biological Markers in Epidemiology*, edited by B. S. Hulka, T. Wilcosky and J. Griffith (New York: Oxford University Press), pp. 78–104.

Grimmer, G. (editor) 1983, *Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons* (Boca Raton: CRC Press).

Guenther, T. M. and Oech, F. 1981, Microsomal epoxide hydrolase and its role in polycyclic aromatic hydrocarbon transformation. In *Polycyclic Aromatic Hydrocarbons and Cancer*, edited by H. V. Gelboin and P. O. P. Ts'o (New York: Academic Press), pp. 183–212.

Guerin, M. R. 1978, Energy sources of polycyclic aromatic hydrocarbons. In *Polycyclic Hydrocarbons and Cancer*, edited by H. V. Gelboin and P. O. P. Ts'o, (New York: Academic Press), pp. 3–42.

Gupta, R. C., Arif, J. M. and Gairola, C. G. 1999, Enhancement of pre-existing DNA adducts in rodents exposed to cigarette smoke. *Mutation Research*, **424**, 195–205.

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.

Hainaut, P., Hernandez, T., Robinson, A., Rodriguez-Tome, P., Flores, T., Hollstein, M., Harris, C. C. and Montesano, R. 1998, IARC Database of *p53* gene mutations in human tumors and cell lines: updated compilation, revised formats and new visualisation tools. *Nucleic Acids Research*, **26**, 205–213.

Hall, M. and Grover, P. L. 1990, Polycyclic aromatic hydrocarbons: metabolism, activation and tumour initiation. In *Chemical Carcinogenesis and Mutagenesis I*, edited by C. S. Cooper and P. L. Grover (Berlin-Heidelberg: Springer-Verlag), pp. 327–372.

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. *Teratogenesis, Carcinogenesis and Mutagenesis*, **12**, 51–60.

Harries, L. W., Stubbins, M. J., Forman, D., Howard, G. C. and Wolf, C. R. 1997, Identification of genetic polymorphisms at the glutathione *S*-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis*, **18**, 641–644.

Harris, C., Hsu, I., Stoner, G., Trump, B. and Selkirk, J. 1978, Human pulmonary alveolar macrophages metabolise benzo[*a*]pyrene to proximate and ultimate mutagens. *Nature*, **272**, 633–634.

Hayashi, S., Watanabe, J., Nakachi, K. and Kawajiri, K. 1991, Genetic linkage of lung cancer-associated *MspI* polymorphisms with amino acid replacement in the heme binding region of the human cytochrome *P450IA1* gene. *Journal of Biochemistry*, **110**, 407–411.

Hayes, J. D. and Pulford, D. J. 1995, The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology*, **30**, 445–600.

Heagerty, A. H., Fitzgerald, D., Smith, A., Bowers, B., Jones, P., Fryer, A. A., Zhao, L., Alldersea, J. and Strange, R. C. 1994, Glutathione *S*-transferase GSTM1 phenotypes and protection against cutaneous tumours. *Lancet*, **343**, 266–268.

Hecht, S. S. 1996, Carcinogenesis due to tobacco: molecular mechanisms. In *Encyclopedia of Cancer*, edited by J. R. Bertino (San Diego: Academic Press), pp. 220–232.

Hecht, S. S. 1999, Tobacco smoke carcinogens and lung cancer. *Journal of the National Cancer Institute*, **91**, 1194–1210.

Helzlsouer, K. J., Selmin, O., Huang, H. Y., Strickland, P. T., Homan, S., Alberg, A. J., Watson, M., Comstock, G. W. and Bell, D. 1998, Association between glutathione *S*-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. *Journal of the National Cancer Institute*, **90**, 512–518.

Hemminki, K. 1993, DNA adducts, mutations and cancer. *Carcinogenesis*, **14**, 2007–2012.

Hemminki, K., Koskinen, M. and Zhao, C. 2001, DNA adducts as a marker for cancer risk? *International Journal of Cancer*, **92**, 923–926.

Hoffmann, D. and Homann, I. 1997, The changing cigarette, 1950–1995. *Journal of Toxicology and Environmental Health*, **50**, 307–364.

Hoffmann, D. and Wynder, E. L. 1986, Chemical constituents and bioactivity of tobacco smoke. In *Tobacco: A Major International Health Hazard*, edited by D. G. Zardize and R. Peto (Lyon: International Agency for Research on Cancer), pp. 145–165.

Hoffmann, D., Haley, N. J., Adams, J. D. and Brunnemann, K. D. 1984, Tobacco sidestream smoke: uptake by nonsmokers. *Preventive Medicine*, **13**, 608–617.

Hollstein, M., Sidransky, D., Vogelstein, B. and Harris, C. C. 1991, *p53* mutations in human cancers. *Science*, **253**, 49–53.

Holz, O., Krause, T. and Rudiger, H. W. 1991, Differences in DNA adduct formation between monocytes and lymphocytes after *in vivo* incubation with benzo[*a*]pyrene. *Carcinogenesis*, **12**, 2181–2183.

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.

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 Epidemiology, Biomarkers and Prevention*, **6**, 193–199.

Hu, X., O'Donnell, R., Srivastava, S. K., Xia, H., Zimniak, P., Nanduri, B., Bleicher, R. J., Awasthi, S., Awasthi, Y. C., Ji, X. and Singh, S. V. 1997, Active site architecture of polymorphic forms of human glutathione *S*-transferase P1-1 accounts for their enantioselectivity and disparate activity in the glutathione conjugation of 7beta,8alpha-dihydroxy-9alpha,10alpha-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Biochemical and Biophysical Research Communications*, **235**, 424–428.

Hussain, S. P., Amstad, P., Raja, K., Sawyer, M., Hofseth, L., Shields, P. G., Hewer, A., Phillips, D. H., Ryberg, D., Haugen, A. and Harris, C. C. 2001, Mutability of *p53* hotspot codons to benzo[*a*]pyrene diol epoxide (BPDE) and the frequency of *p53* mutations in nontumorous human lung. *Cancer Research*, **61**, 6350–6355.

IARC. 1986, *Tobacco Smoking*. Monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol. 38 (Lyon: International Agency for Research on Cancer), pp. 37–375.

IARC. 1994, *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1–60*. Monographs on the evaluation of the carcinogenic risk of chemicals to humans (Lyon: International Agency for Research on Cancer).

IARC. 1999a, *Metabolic Polymorphisms and Susceptibility to Cancer*, IARC Scientific Publications no. 148 (Lyon: International Agency for Research on Cancer), pp. 403–408.

IARC. 1999b, *Metabolic Polymorphisms and Susceptibility to Cancer*, IARC Scientific Publications no. 148 (Lyon: International Agency for Research on Cancer), pp. 323–393.

IARC. 1999c, *Metabolic Polymorphisms and Susceptibility to Cancer*, IARC Scientific Publications no. 148 (Lyon: International Agency for Research on Cancer), pp. 303–322.

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.

Ingebrethsen, B. J. 1986, Aerosol studies of cigarette smoke. *Recent Advances in Tobacco Science*, **12**, 54–142.

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.

Jaakkola, M. S. and Jaakkola, J. J. 1997, Assessment of exposure to environmental tobacco smoke. *European Respiratory Journal*, **10**, 2384–2397.

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 Research*, **53**, 1522–1528.

Jourenkova, N., Reinikainen, M., Bouchardy, C., Dayer, P., Benhamou, S. and Hirvonen, A. 1998, Larynx cancer risk in relation to glutathione *S*-transferase M1 and T1 genotypes and tobacco smoking. *Cancer Epidemiology, Biomarkers and Prevention*, **7**, 19–23.

Kato, S., Bowman, E. D., Harrington, A. M., Blomeke, B. and Shields, P. G. 1995, Human lung carcinogen-DNA adduct levels mediated by genetic polymorphisms *in vivo*. *Journal of the National Cancer Institute*, **87**, 902–907.

Katoh, T., Kaneko, S., Takasawa, S., Nagata, N., Inatomi, H., Ikemura, K., Itoh, H., Matsumoto, T., Kawamoto, T. and Bell, D. A. 1999, Human glutathione *S*-transferase P1 polymorphism and susceptibility to smoking related epithelial cancer, oral, lung, gastric, colorectal and urothelial cancer. *Pharmacogenetics*, **9**, 165–169.

Kawajiri, K., Eguchi, H., Nakachi, K., Sekiya, T. and Yamamoto, M. 1996, Association of *CYP1A1* germ line polymorphisms with mutations of the *p53* gene in lung cancer. *Cancer Research*, **56**, 72–76.

Kennaway, E. 1955, The identification of a carcinogenic compound in coal-tar. *British Medical Journal*, **ii**, 749–752.

KIHARA, M., KUBOTA, A., FURUKAWA, M. and KIMURA, H. 1997, *GSTM1* gene polymorphism as a possible marker for susceptibility to head and neck cancers among Japanese smokers. *Cancer Letters*, **112**, 257–262.

LANDIS, S. H., MURRAY, T., BOLDEN, S. and WINGO, P. A. 1999, Cancer statistics, 1999. *CA: A Cancer Journal for Clinicians*, **49**, 8–31.

LEE, P. N. (editor) 1975, *Tobacco Consumption in Various Countries* (London: Tobacco Research Council).

LEWTAS, J., MUMFORD, J., EVERSON, R. B., HULKA, B., WILCOSKY, T., KOZUMBO, W., THOMPSON, C., GEORGE, M., DOBIAS, L. and SRAM, R., *et al*. 1993, Comparison of DNA adducts from exposure to complex mixtures in various human tissues and experimental systems. *Environmental Health Perspectives*, **99**, 89–97.

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 Research*, **56**, 287–293.

LI, D., FIROZI, P. F., CHANG, P., WANG, L. E., XIONG, P., STURGIS, E. M., EICHER, S. A., SPITZ, M. R., HONG, W. K. and WEI. Q. 2001, *In vitro* BPDE-induced DNA adducts in peripheral lymphocytes as a risk factor for squamous cell carcinoma of the head and neck. *International Journal of Cancer*, **93**, 436–440.

LITTLE, J. and VAINIO, H. 1994, Mutagenic lifestyles? A review of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of 'recreational' drugs. *Mutation Research*, **313**, 131–151.

LODOVICI, M., AKPAN, V., GIOVANNINI, L., MIGLIANI, F. and DOLARA, P. 1998, Benzo[*a*]pyrene diol-epoxide DNA adducts and levels of polycyclic aromatic hydrocarbons in autoptic samples from human lungs. *Chemico-biological Interactions*, **116**, 199–212.

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. *Gynecologic Oncology*, **75**, 68–71.

MANNERVIK, B. and DANIELSON, U. H. 1988, Glutathione transferases – structure and catalytic activity. *CRC Critical Reviews in Biochemistry*, **23**, 283–337.

MCWILLIAMS, J. E., SANDERSON, B. J., HARRIS, E. L., RICHERT-BOE, K. E. and HENNER, W. D. 1995, Glutathione *S*-transferase M1 (*GSTM1*) deficiency and lung cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **4**, 589–594.

MMWR. 1998, Tobacco use among high school students – United States, 1997. *Morbidty and Mortality Weekly Reports*, **47**, 229–233.

NATIONAL RESEARCH COUNCIL. 1986a, The physicochemical nature of sidestream smoke and environmental tobacco smoke. In *Environmental Tobacco Smoke. Measuring Exposure and Assessing Health Effects*, edited by Committee on Passive Smoking Board on Environmental Studies and Toxicology (Washington DC: National Academy Press), pp. 25–53.

NATIONAL RESEARCH COUNCIL. 1986b, Executive summary. In *Environmental Tobacco Smoke. Measuring Exposure and Assessing Health Effects*, edited by Committee on Passive Smoking Board on Environmental Studies and Toxicology (Washington DC: National Academy Press), pp. 1–12.

NAZAR-STEWART, V., VAUGHAN, T. L., BURT, R. D., CHEN, C., BERWICK, M. and SWANSON, G. M. 1999, Glutathione *S*-transferase M1 and susceptibility to nasopharyngeal carcinoma. *Cancer Epidemiology, Biomarkers and Prevention*, **8**, 547–551.

NEBERT, D. W. and GONZALEZ, F. J. 1987, *P450* genes: structure, evolution, and regulation. *Annual Review of Biochemistry*, **56**, 945–993.

NELSON, D. R., KOYMANS, L., KAMATAKI, T., STEGEMAN, J.J., FEYEREISEN, R., WAXMAN, D. J., WATERMAN, M. R., GOTOH, O., COON, M. J., ESTABROOK, R. W., GUNSALUS, I. C. and NEBERT, D. W. 1996, *P450* superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, **6**, 1–42.

NESNOW, S., ROSS, J. A., STONER, G. D. and MASS, M. J. 1995, Mechanistic linkage between DNA adducts, mutations in oncogenes and tumorigenesis of carcinogenic environmental polycyclic aromatic hydrocarbons in strain A/J mice. *Toxicology*, **105**, 403–413.

PALLI, D., VINEIS, P., RUSSO, A., BERRINO, F., KROGH, V., MASALA, G., MUNNIA, A., PANICO, S., TAIOLI, E., TUMINO, R., GARTE, S. and PELUSO, M. 2000, Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. *International Journal of Cancer*, **87**, 444–451.

PALLI, D., RUSSO, A., MASALA, G., SAIEVA, C., GUARRERA, S., CARTURAN, S., MUNNIA, A., MATULLO, G. and PELUSO, M. 2001, DNA adduct levels and DNA repair polymorphisms in traffic-exposed workers and a general population sample. *International Journal of Cancer*, **94**, 121–127.

PARK, J. Y., MUSCAT, J. E., REN, Q., SCHANTZ, S. P., HARWICK, R. D., STERN, J. C., PIKE, V., RICHIE, J. P. JR and LAZARUS, P. 1997, *CYP1A1* and *GSTM1* polymorphisms and oral cancer risk. *Cancer Epidemiology, Biomarkers and Prevention*, **6**, 791–797.

PARKIN, D. M., BRAY, F., FERLAY, J. and PISANI, P. 2001, Estimating the world cancer burden: Globocan 2000. *International Journal of Cancer*, **94**, 153–156.

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.

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 Epidemiology, Biomarkers and Prevention*, **4**, 233–238.

Peto, R. and Lopez, A. 2001, Future worldwide health effects of current smoking patterns. In *Critical Issues in Global Health*, edited by C. E. Koop, C. E. Pearson and M. R. Schwartz (San Francisco: Jossey-Bass).

Peto, R., Chen, Z. M. and Boreham, J. 1999, Tobacco – the growing epidemic. *Nature Medicine*, **5**, 15–17.

Petruzzelli, S., Bernard, P., Paoletti, P., Rane, A., Giuntini, C. and Pacifici, G. 1988, Presence of epoxide hydrolase and glutathione *S*-transferase in human pulmonary alveolar macrophages. *European Journal of Clinical Pharmacology*, **34**, 419–421.

Phillips, D. H. 1996, DNA adducts in human tissues: biomarkers of exposure to carcinogens in tobacco smoke. *Environmental Health Perspectives*, **104**, supplement 3, 453–458.

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.

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. *International Journal of Cancer*, **86**, 610–616.

Pisani, P., Parkin, D. M., Bray, F. and Ferlay, J. 1999, Estimates of the worldwide mortality from 25 cancers in 1990. *International Journal of Cancer*, **83**, 18–29.

Poirier, M. C. and Weston, A. 1996, Human DNA adduct measurements: state of the art. *Environmental Health Perspectives*, **104**, supplement 5, 883–893.

Poirier, M. C., Santella, R. M. and Weston, A. 2000, Carcinogen macromolecular adducts and their measurement. *Carcinogenesis*, **21**, 353–359.

Proctor, R. N. 2001, Tobacco and the global lung cancer epidemic. *Nature Medicine*, **1**, 82–86.

Raw, M. 2001, Fighting tobacco dependence in Europe. *Nature Medicine* **7**, 13–14.

Reddy, M. V., Kenny, P. C. and Randerath, K. 1990, ^{32}P-assay of DNA adducts in white blood cells and placentas of pregnant women: lack of residential wood combustion-related adducts but presence of tissue-specific endogenous adducts. *Teratogenesis, Carcinogenesis and Mutagenesis*, **10**, 373–384.

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.

Romano, G., Sgambato, A., Boninsegna, A., Flamini, G., Curigliano, G., Yang, Q., La Gioia, V., Signorelli, C., Ferro, A., Capelli, G., Santella, R. M. and Cittadini, A. 1999, Evaluation of polycyclic aromatic hydrocarbon-DNA adducts in exfoliated oral cells by an immunohistochemical assay. *Cancer Epidemiology, Biomarkers and Prevention*, **8**, 91–96.

Ross, J. A., Nelson, G. B., Wilson, K. H., Rabinowitz, J. R., Galati, A., Stoner, G. D., Nesnow, S. and Mass, M. J. 1995, Adenomas induced by polycyclic aromatic hydrocarbons in strain A/J mouse lung correlate with time-integrated DNA adduct levels. *Cancer Research*, **55**, 1039–1044.

Rothman, N., Poirier, M. C., Baser, M. E., Hansen, J. A., Gentile, C., Bowman, E. D. and Strickland, P. T. 1990, Formation of polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells during consumption of charcoal-broiled beef. *Carcinogenesis*, **11**, 1241–1243.

Rothman, N., Shields, P. G., Poirier, M. C., Harrington, A. M., Ford, D. P. and Strickland, P. T. 1995, The impact of glutathione *s*-transferase M1 and cytochrome *P450 1A1* genotypes on white-blood-cell polycyclic aromatic hydrocarbon-DNA adduct levels in humans. *Molecular Carcinogenesis*, **14**, 63–68.

Royal College of Physicians of London. 1962, *Smoking and health* (London: Pitman Medical).

Rundle, A., Tang, D., Hibshoosh, H., Estabrook, A., Schnabel, F., Cao, W., Grumet, S. and Perera, F. P. 2000a, The relationship between genetic damage from polycyclic aromatic hydrocarbons in breast tissue and breast cancer. *Carcinogenesis*, **21**, 1281–1289.

Rundle, A., Tang, D., Zhou, J., Cho, S. and Perera, F. 2000b, The association between glutathione *S*-transferase M1 genotype and polycyclic aromatic hydrocarbon-DNA adducts in breast tissue. *Cancer Epidemiology, Biomarkers and Prevention*, **9**, 1079–1085.

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.

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.

Seidegard, J., Vorachek, W. R., Pero, R. W. and Pearson, W. R. 1988, Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proceedings of the National Academy of Sciences USA*, **85**, 7293–7297.

Shields, P. G., Bowman, E. D., Harrington, A. M., Doan, V. T. and Weston, A. 1993, Polycyclic aromatic hydrocarbon-DNA adducts in human lung and cancer susceptibility genes. *Cancer Research*, **53**, 3486–3492.

Simonato, L., Agudo, A., Ahrens, W., Benhamou, E., Benhamou, S., Boffetta, P., Brennan, P., Darby, S. C., Forastiere, F., Fortes, C., Gaborieau, V., Gerken, M., Gonzales, C. A., Jockel, K. H., Kreuzer, M., Merletti, F., Nyberg, F., Pershagen, G., Pohlabeln, H., Rosch, F., Whitley, E., Wichmann, H. E. and Zambon, P. 2001, Lung cancer and cigarette smoking in Europe: an update of risk estimates and an assessment of inter-country heterogeneity. *International Journal of Cancer*, **91**, 876–887.

Simons, A. M., Phillips, D. H. and Coleman, D. V. 1993, Damage to DNA in cervical epithelium related to smoking tobacco. *British Medical Journal*, **306**, 1444–1448.

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. *British Journal of Cancer*, **71**, 246–249.

Smith, C. J., Livingston, S. D. and Doolittle, D. J. 1997, An international literature survey of 'IARC Group I carcinogens' reported in mainstream cigarette smoke. *Food and Chemical Toxicology*, **35**, 1107–1130.

Smith, L. E., Denissenko, M. F., Bennett, W. P., Li, H., Amin, S., Tang, M. and Pfeifer, G. P. 2000, Targeting of lung cancer mutational hotspots by polycyclic aromatic hydrocarbons. *Journal of the National Cancer Institute*, **92**, 803–811.

Spencer-Beach, G. G., Beach, A. C. and Gupta, R. C. 1996, High-resolution anion-exchange and partition thin-layer chromatography for complex mixtures of ^{32}P-postlabeled DNA adducts. *Journal of Chromatography B Biomedical Applications*, **677**, 265-273.

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 Research*, **55**, 1267–1270.

Szyfter, K., Hemminki, K., Szyfter, W., Szmeja, Z., Banaszewski, J. and Yang, K. 1994, Aromatic DNA adducts in larynx biopsies and leukocytes. *Carcinogenesis*, **15**, 2195–199.

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 Epidemiology, Biomarkers and Prevention*, **4**, 341–346.

Tang, D., Phillips, D. H., Stampfer, M., Mooney, L. A., Hsu, Y., Cho, S., Tsai, W. Y., Ma, J., Cole, K. J., She, M. N. and Perera, F. P. 2001, Association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the physicians health study. *Cancer Research*, **61**, 6708–6712.

Thakker, D. R. 1985, Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In *Bioactivation of Foreign Compounds*, edited by M. W. Anders (New York: Academic Press), pp. 177–242.

Trager, W. F. and Jones, J. P. 1987, Stereochemical considerations in drug metabolism. *Progress in Drug Metabolism*, **10**, 55–83.

Tso, T. C. (editor) 1990, *Production, Physiology and Biochemistry of Tobacco Plant* (Beltsville, Maryland: Ideals).

US Department of Health Education and Welfare. Public Health Services, Services. 1964, Smoking and Health Report of the Advisory Committee to the Surgeon General of the Public Health Services, Public Health Service Publication, Washington DC.

US Environmental Protection Agency. 1992, Respiratory health effects of passive smoking: lung cancer and other disorders, In US Environmental Protection Agency, Office of Health and Environmental Assessment, Office of Research and Development. EPA/600/6-90/006F, Washington DC.

Van Schooten, F. J., Hillebrand, M. J., Van Leeuwen, F. E., Lutgerink, J. T., Van Zandwijk, N., Jansen, H. M. and Kriek, E. 1990, Polycyclic aromatic hydrocarbon-DNA adducts in lung tissue from lung cancer patients. *Carcinogenesis*, **11**, 1677–1681.

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.

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. *Mutation Research*, **378**, 65–75.

Vineis, P. and Perera, F. 2000, DNA adducts as markers of exposure to carcinogens and risk of cancer. *International Journal of Cancer*, **88**, 325–328.

Wald, N. J. and Hackshaw, A. K. 1996, Cigarette smoking: an epidemiological overview. *British Medical Bulletin*, **52**, 3–11.

Wei, Q., Cheng, L., Amos, C. I., Wang, L. E., Guo, Z., Hong, W. K. and Spitz, M. R. 2000, Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *Journal of the National Cancer Institute*, **92**, 1764–1772.

Weinstein, I. B., Jerey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H. and Nakanishi, K. 1976, Benzo[*a*]pyrene diol epoxides as intermediates in nucleic acid binding *in vitro* and *in vivo*. *Science*, **193**, 592–595.

Weston, A. and Bowman, E. D. 1991, Fluorescence detection of benzo[*a*]pyrene-DNA adducts in human lung. *Carcinogenesis*, **12**, 1445–1449.

WHO, 1997a, Smoking prevalence. In *Tobacco or Health: A Global Status Report*, edited by World Health Organization (Geneva: World Health Organization), pp. 10–18.

WHO, 1997b, Tobacco consumption. In *Tobacco or Health: A Global Status Report*, edited by World Health Organization (Geneva: World Health Organization), pp. 19–30.

WHO, 1997c, Health effects In *Tobacco or Health: A Global Status Report*, edited by World Health Organization (Geneva: World Health Organization), pp. 43–48.

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. *Journal of the National Cancer Institute*, **91**, 614–619.

Williams, G. M., Iatropoulos, M. J. and Weisburger, J. H. 1996, Chemical carcinogen mechanisms of action and implications for testing methodology. *Experimental and Toxicologic Pathology*, **48**, 101–111.

World Bank. 1999, *Curbing the Epidemic: Governments and the Economics of Tobacco Control* (Washington DC: The World Bank).

World Cancer Research Fund/Academic Institute for Cancer Research (editors) 1997, *Food, Nutrition and the Prevention of Cancer: A Global Perspective* (Washington DC: American Institute for Cancer Research).

Yoon, J. H., Smith, L. E., Feng, Z., Tang, M., Lee, C. S. and Pfeifer, G. P. 2001, Methylated CpG dinucleotides are the preferential targets for G-to-T transversion mutations induced by benzo[*a*]pyrene diol epoxide in mammalian cells: similarities with the *p53* mutation spectrum in smoking-associated lung cancers. *Cancer Research*, **61**, 7110–7117.

Zenzes, M. T., Bielecki, R. and Reed, T. E. 1999a, Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. *Molecular Human Reproduction*, **5**, 125–131.

Zenzes, M. T., Bielecki, R. and Reed, T. E. 1999b, Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in sperm of men exposed to cigarette smoke. *Fertility and Sterility*, **72**, 330–335.

Zhang, Y. J., Li, Y., DeLeo, V. A. and Santella, R. M. 1990, Detection of DNA adducts in skin biopsies of coal tar-treated psoriasis patients: immunofluorescence and ^{32}P postlabeling. *Skin Pharmacology*, **3**, 171–179.

Zhang, L. F., Hemminki, K., Szyfter, K., Szyfter, W. and Soderkvist, P. 1994, *p53* mutations in larynx cancer. *Carcinogenesis*, **15**, 2949–2951.

Zhang, Z. Y., Fasco, M. J., Huang, L., Guengerich, F. P. and Kaminsky, L. S. 1996, Characterization of purified human recombinant cytochrome *P4501A1-Ile462* and *-Val462*: assessment of a role for the rare allele in carcinogenesis. *Cancer Research*, **56**, 3926–3933.

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.

Zimniak, P., Nanduri, B., Pikula, S., Bandorowicz-Pikula, J., Singhal, S. S., Srivastava, S. K., Awasthi, S. and Awasthi, Y. C. 1994, Naturally occurring human glutathione *S*-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. *European Journal of Biochemistry*, **224**, 893–899.