5 DNA Adducts and Their Consequences

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5.1 INTRODUCTION

A central tenet of chemical carcinogenesis is that the covalent binding of carcinogens to DNA is causally related to tumorigenesis. This belief is supported by a number of observations, including the facts that:

1. the majority of carcinogens are also mutagens;
2. the mutagenic and carcinogenic properties of many carcinogens depend upon their conversion to electrophilic derivatives that react with nucleophilic sites within DNA;
3. the extent of DNA adduct formation can often be correlated with the magnitude of mutagenic and carcinogenic responses; and
4. the activation of certain proto-oncogenes can be accomplished through the interaction of carcinogens with DNA.

DNA adducts are typically formed in very low concentrations *in vivo* (<100 fmol/μg DNA; <3 adducts/10^5 nucleotides), which has made their detection difficult; however, with the advent of immunoassays (Poirier, 1984) and ^{32}P-postlabelling (Gupta *et al.*, 1982), their occurrence in exposed human populations has become possible to investigate. In this review, the DNA adducts obtained from four classes of carcinogens for which there is substantial evidence of human exposure are considered: *N*-nitrosamines, aflatoxins, aromatic amines, and polycyclic aromatic hydrocarbons. For each class, the metabolic activation pathways that lead to DNA adduct formation will be discussed briefly. The adducts that have been identified *in vitro* will be compared with those found *in vivo* in experimental animals and humans. Whenever possible, the discussion will include dose–response relationships, the significance of particular adducts in tumorigenesis, the

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heterogeneity of adduct distribution and processing, and the role of the adducts in oncogene activation.

5.2 *N*-NITROSAMINES

Humans are exposed to *N*-nitrosamines from a wide variety of sources, including foods, beverages, tobacco, cosmetics, cutting oils, hydraulic fluids, and rubber products (Preussmann and Eisenbrand, 1984). Over 300 *N*-nitrosamines have been demonstrated to be carcinogenic in experimental animals (Preussmann and Stewart, 1984). As with the majority of chemical carcinogens, these chemically inert compounds are metabolized to reactive electrophiles before binding to cellular macromolecules (Lawley, 1984). This process typically involves the oxidation of the carbon adjacent to the amine nitrogen (α-hydroxylation), as is illustrated by the ubiquitously distributed *N*-nitrosodimethyl amine, and the tobacco-specific 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Hecht *et al.*, 1986). The resultant α-hydroxy-*N*-nitrosoalkylamines are unstable, and rapidly decompose to produce aldehydes and alkyl diazohydroxides, the latter of which have the ability to alkylate DNA. *N*-Nitrosodimethylamine is a symmetrical *N*-nitrosamine; thus, α-hydroxylation of either carbon will yield the same methylating agent. Since 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is asymmetrical, DNA is methylated or pyridyloxobutylated depending upon the carbon that undergoes α-hydroxylation. To date, the *N*-nitrosamine DNA adducts that have been identified and studied most rigorously result from either methylation or ethylation of DNA.

As noted earlier, *N*-nitrosamines do not react directly with DNA; therefore, to characterize the methylated and ethylated DNA adducts resulting from exposure to these compounds, experiments have been conducted with direct-acting compounds that yield the same electrophilic intermediates. Table 5.1 illustrates the distribution of adducts observed in DNA from *in vitro* reactions with a series of dialkylsulphates, alkyl methanesulphonates, and *N*-alkylnitrosoureas (Singer and Grunberger, 1983). A general rule regarding these alkylating agents is that the potential exists to form adducts with all exocyclic oxygens and ring nitrogens, with the exception of the N^1 site of guanine. Except for *N*-ethylnitrosourea, the primary site of substitution is N^7 of guanine. Ethylating agents bind to a greater extent than methylating agents with phosphodiesters, and exocyclic oxygens are preferentially modified by *N*-alkylnitrosoureas as compared with dialklysulphates and alkyl methanesulphonates.

The distribution of DNA adducts found *in vivo* from direct-acting alkylating agents is similar to that observed *in vitro*, with two notable exceptions: N^3-methyladenine and O^6-methylguanine are present in decreased quantities *in vivo*, suggesting that these adducts are subject to enzymatic repair processes. Since *N*-alkylnitrosoureas are more carcinogenic than dialkylsulphates and alkyl methanesulphonates, a comparison of the distribution of adducts from these agents indicates that substitution of exocyclic oxygens (e.g., O^6 of guanine) is more

important for the induction of tumours than reaction with the ring nitrogens (e.g., N^7 of guanine; Frei and Lawley, 1976; Frei *et al.*, 1978). This interpretation is supported by experiments demonstrating that O^6-alkylguanines and O^4-alkylthymines give rise to base substitution mutations, of which the majority are transitions, whereas alkylation of ring nitrogens does not cause miscoding (Abbott and Saffhill, 1977, 1979; Saffhill and Abbott, 1978).

Table 5.1. Alkylation of DNA *in vitro* and *in vivo*[†]

Alkylating agent (DNA Source)	Percentage of total alkylation											
	Adenine			Guanine			Thymine			Cytosine		Phospho-diester
	N^1	N^3	N^7	N^3	O^6	N^7	O^2	N^3	O^4	O^2	N^3	
Dimethylsulphate												
(*In vitro*)	1.9	18	1.9	1.1	0.2	74	—	—	—	nd	<2.0	1
Diethylsulphate												
(*In vitro*)	2.0	10	1.5	0.9	0.2	67	—	nd	—	—	0.7	16
Methyl methane-sulphonate												
(*in vitro*)	3.8	10	1.8	0.6	0.3	85	nd	0.1	nd	nd	<1.0	1
Ethyl methane-sulphonate												
(*in vitro*)	1.7	4.9	1.1	0.9	2.0	65	nd	nd	nd	nd	0.6	13
(*in vivo*)	—	3.3	—	—	1.5	70	—	—	—	—	—	—
n-Methyl-nitrosourea												
(*in vitro*)	1.3	9.0	1.7	0.8	6.3	67	0.1	0.3	0.4	0.1	0.6	16
(*in vivo*)	—	3.6	—	—	3.6	70	—	—	—	—	—	—
n-Ethyl-nitrosourea												
(*in vitro*)	0.2	4.0	0.3	0.6	7.8	12	7.4	0.8	2.5	3.5	0.2	57
(*in vivo*)	—	4.1	0.6	1.4	7.2	14	7.4	—	2.3	1.3	—	60
n-Nitroso-dimethylamine												
(*in vivo*)	0.8	2.4	1.5	0.6	6.6	69	—	0.4	—	—	0.6	9
n-Nitroso-diethylamine												
(*in vivo*)	—	3.7	—	—	5.6	15	6.0	—	0.7	—	—	—

[†]Data are from Singer and Grunberger (1983). *In vivo* refers to the alkylation pattern in rat liver DNA. A dash indicates that the adduct was not analyzed; "nd" indicates that the adduct was not detected.

The adduct distribution obtained from *N*-nitrosodimethylamine and *N*-nitrosodiethylamine after metabolism *in vivo* is shown in Table 5.1. The pattern is very similar to that observed with the respective *N*-alkylnitrosourea, which supports the proposition that they share a common reactive electrophile. These data are from rat liver; however, the ratio of adducts in other tissues and species appears similar (Lawley, 1976). In general, the administration of direct-acting alkylating agents, such as alkyl methanesulphonates or *N*-alkylnitrosoureas, results in a similar adduct concentration in all tissues. In contrast with *N*-nitrosodialkylamines which require metabolism, the concentration of adducts depends upon the capability of the specific tissue to catalyze α-hydroxylation (liver tissue > kidney tissue > lung tissue; Lawley, 1976). The ability of *N*-nitrosodialkylamines to undergo α-hydroxylation can also vary within cell types of a particular tissue. For example, Belinsky *et al.* (1987) found preferential alkylation of Clara cells as compared with alveolar small cells or type II cells in the lungs of rats administered 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. This trend was more pronounced at low doses of the compound, and was not found with *N*-nitrosodimethylamine, which is weakly carcinogenic for lung tissue.

A non-random distribution of alkylation within DNA exists. With *N*-methylnitrosourea, for instance, the *in vitro* formation of O^6- and N^7-methylguanine in small oligonucleotides is favoured in positions with an adenine or thymine 5′ to the adducted guanine (Dolan *et al.*, 1988). Other alkylating agents, including dimethylsulphate, have been shown to react preferentially *in vitro* with guanine-cytosine-rich sequences in the 5′-flanking region of the c-*Ha-ras* oncogene (Mattes *et al.*, 1988). A similar degree of resolution has not been obtained *in vivo*; nevertheless, using immune electron microscopy, Nehls *et al.* (1984) demonstrated a non-random distribution of O^6-ethylguanine in brain DNA from fetal rats treated with *N*-ethylnitrosourea. Likewise, Ryan *et al.* (1986) found enhanced O^6-methylguanine formation in transcriptionally active chromatin and nuclear matrix-associated DNA as compared with bulk chromatin in livers from rats administered *N*-nitrosodimethylamine. Recently, Milligan and Archer (1988) observed an excess of strand breaks, presumably due to 7-methylguanine and 3-methyladenine, in the transcriptionally active albumin gene as compared with the non-transcribed *IgE* gene in the livers of rats treated with the same carcinogen. The non-random distribution of adducts is also reflected in the observed mutations. In *Escherichia coli* treated with *N*-methylnitrosourea, 95% of the mutations are guanine-to-adenine transitions, and these are 10 times more likely to occur in a 5′-purine-guanine-3′ sequence than in a 5′-pyrimidine-guanine-3′ sequence (Burns *et al.*, 1988). *Ras* oncogene activation in rats (Zarbl *et al.*, 1985) and mice (Belinsky *et al.*, 1989) administered alkylating agents is associated primarily with a guanine-to-adenine transition in a 5′-purine-guanine-3′ sequence.

The kinetics of DNA adduct formation *in vivo* with alkylating agents are dependent upon the treatment regimen. Following the administration of single doses of *N*-ethylnitrosourea or *N*-methylnitrosourea to mice (Frei *et al.*, 1978) or a linear relationship was observed between dose and adduct concentration with the

adduct profile being similar to that shown in Table 5.1. In rats given a single dose of *N*-nitrosodimethylamine, the hepatic concentrations of N^7-methylguanine also increased linearly with dose; however, the amount of O^6-methylguanine increased in a sublinear manner, which was attributed to the saturation of repair of O^6-methylguanine at higher doses of *N*-nitrosodimethylamine (Pegg and Hoi, 1978). A similar sublinear dose–response relationship was found for the formation of O^6-ethylguanine in the livers of rats treated with a single dose of *N*-nitrosodiethylamine (Scherer *et al.*, 1977). In contrast, in the lungs of rats treated multiple times with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, the correlation between administered dose and the concentration of O^6-methylguanine was supralinear (Belinsky *et al.*, 1987). This effect was suggested to result from a decreased rate of metabolism at higher doses of the carcinogen. During continuous administration of *N*-nitrosodiethylamine, an essentially linear relationship between dose and DNA adduct concentration appears to exist (Boucheron *et al.*, 1987); however, the adduct profile can differ markedly from that observed after a single treatment. For example, in hepatic DNA from rats treated once with *N*-nitrosodiethylamine, three to four times more O^6-ethylguanine is formed compared with O^4-ethylthymine, whereas the amount of O^4-ethylthymine produced during continuous administration is 50 times greater than that of O^6-ethylguanine (Richardson *et al.*, 1985). Likewise, following a single dose of the rat hepatocarcinogen, 1,2-dimethylhydrazine, the ratio of O^6-methylguanine to O^4-methylthymine is 100:1, whereas this ratio falls to <2:1 during chronic exposure (Richardson *et al.*, 1985). The change in adduct profiles that occurs during continuous dosing is the result of the preferential repair of O^6-alkylguanines by alkyltransferases. Differences in alkyltransferase activity between liver cell types also may account for the preferential accumulation of O^6-methylguanine in non-parenchymal cells as compared with hepatocytes in rats administered 1,2-dimethylhydrazine (Lewis and Swenberg, 1980).

With the exception of the induction of oral cancer in snuff dippers (Winn, 1986; Montesano *et al.*, 1988), no conclusive epidemiological evidence exists for the carcinogenicity of *N*-nitrosamines in humans. Nevertheless, the types of DNA adducts formed in exposed individuals are similar to those observed in experimental animals. For example, Herron and Shank (1980) found N^7- and O^6-methylguanine in the liver of a victim poisoned with *N*-nitrosodimethylamine. More recently, Umbenhauer *et al.* (1985) detected O^6-methylguanine in stomach and oesophageal tissue and oesophageal tumor DNA from Chinese cancer patients. This adduct was also found at lower levels in the same tissues in Europeans, who are at lower risk for developing oesophageal tumors. Similar results were obtained by Saffhill *et al.* (1988) when comparing individuals in Southeast Asia with those in England. Likewise, Hsieh *et al.* (1988) found higher levels of O^4-ethylthymine in DNA from Japanese cancer patients compared with non-tumor-bearing control patients.

5.3 AFLATOXINS

Humans are exposed to aflatoxins through the consumption of mouldy cereals, grains, and nuts (Busby and Wogan, 1984). Four major naturally occurring aflatoxins, aflatoxin B_1 (AFB_1), aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2, have been characterized. AFB_1 is the most abundant as well as the most carcinogenic. The metabolic activation of AFB_1 involves oxidation of the 8,9-olefinic bond to give AFB_1-8,9-oxide (Baertschi *et al.*, 1988), which reacts with DNA to yield *trans*-8,9-dihydro-8-(deoxyguanosin-7-yl)-9-hydroxy AFB_1 (AFB_1-N7-dG; Essigmann *et al.*, 1977), a structure consistent with *trans* opening of the epoxide ring and simultaneous attack on N^7 of guanine. AFB_1-N7-dG carries a positive charge and is, therefore, unstable. It can undergo depurination to give *trans*-8,9-dihydro-8-(guan-7-yl)-9-hydroxy AFB_1 (AFB_1-N7-Gua) (Essigmann *et al.*, 1977) or base-catalyzed opening of the imidazole ring to yield two pyrimidine adducts: 8,9-dihydro-8-(2,6-diamino-4-oxo-3,4-dihydropyrimid-5-yl formamido)-9-hydroxy AFB_1 (AFB_1-N7-P4r major) and 8,9-dihydro-8-(2-amino-6-formamido-4-oxo-3,4-dihydropyrimid-5-yl)-9-hydroxy AFB_1 (AFB_1-N7-P4r minor) (Hertzog *et al.*, 1982).

Several investigators have demonstrated that AFB_1-8,9-oxide does not react randomly with DNA *in vitro* (D'Andrea and Haseltine, 1978; Misra *et al.*, 1983; Muench *et al.*, 1983; Marien *et al.*, 1987). This finding has been studied in greatest detail by Benasutti *et al.* (1988) who showed that the reactivity of a particular guanine was markedly affected by the 5′- and 3′- flanking bases. The most reactive sequence was 5′-GGG-3′ followed by 5′-CGG-3′ and 5′-GGT-3′; these were approximately 20-fold more reactive than the least reactive sequence, 5′-TGA-3′. Whether a similar sequence specificity occurs *in vivo* is unknown; however, AFB_1 preferentially binds (a) linker, as compared with core, DNA sequences of nucleosomes in trout liver (Bailey *et al.*, 1980), (b) mitochondrial, as compared with nuclear, DNA in rat liver (Niranjan *et al.*, 1982), and (c) transcriptionally active, as compared with bulk, DNA in rat liver (Irvin and Wogan, 1984).

The hepatocarcinogenicity of AFB_1 varies among species with the relative order of sensitivity being: trout > rat >> hamster ≈ mouse ≈ salmon (Busby and Wogan, 1984; Bailey *et al.*, 1988). A similar relationship appears to hold for the extent of binding to liver DNA; thus, the magnitude of adduct formation correlates with the relative degree of hepatocarcinogenicity (Garner and Wright, 1975; Lutz *et al.*, 1980; Ueno *et al.*, 1980; Croy and Wogan, 1981b; Bailey *et al.*, 1988). Adduct formation is also dose-related; for instance, following a single administration of AFB_1 to rats, the extent of hepatic DNA binding is linear over a 10^5-fold dose range (Appleton *et al.*, 1982; Lutz, 1986; Wild *et al.*, 1986). The analysis of AFB_1 DNA binding is complicated by the instability of AFB_1-N7-dG and the persistence of AFB_1-N7-P4r major and AFB_1-N7-P4r minor. In rat liver, AFB_1-N7-dG has a half-life of 7.5 hours (Croy and Wogan, 1981a), compared with 3 to 4 weeks in trout liver (Goeger *et al.*, 1986). Therefore, DNA binding measured during the chronic administration of AFB_1 reflects a mixture of adducts, which will presumably be species-dependent.

The instability of AFB_1-N7-dG also complicates the analysis of mutations induced by AFB_1. Base-substitution and frameshift mutations have been detected in

Salmonella, and these have been attributed to AFB_1-N7-dG (Stark *et al.*, 1979); whereas the guanine-to-thymine transversions in *E. coli* (Foster *et al.*, 1983) and the mutations induced by AFB_1 in human diploid lymphoblasts (Kaden *et al.*, 1987) have been ascribed to the existence of apurinic sites that result from the loss of AFB_1-N7-Gua. Likewise, the induction of liver tumors in rats by AFB_1 has been associated with a guanine-to-adenine transition at codon 12 of the *ras* oncogene (McMahon *et al.*, 1987); yet whether this effect is due to AFB_1-N7-dG, its imidazole-ring-opened derivatives, or the apurinic site remains unknown. It has been noted that tumor initiation best correlates with initial levels of DNA damage (Kensler *et al.*, 1986; Bailey *et al.*, 1988), suggesting that AFB_1-N7-dG may be the critical lesion.

During the continuous administration of AFB_1, steady-state hepatic DNA-adduct levels are observed after approximately 2 to 6 weeks in rats (Wild *et al.*, 1986; Buss and Lutz, 1988) and after 3 weeks in trout (Bailey *et al.*, 1988). In both species, steady-state adduct concentrations appear to be linearly related to the concentration of AFB_1 administered chronically (Dashwood *et al.*, 1988, 1989; Buss and Lutz, 1988). Furthermore, if steady-state adduct levels are compared with hepatic tumor incidence, a nearly identical linear relationship is observed for both species (Bechtel, 1989).

In humans, a positive correlation exists between the amount of AFB_1 ingested and the incidence of liver cancer (Busby and Wogan, 1984). Furthermore, AFB_1 DNA adducts have been detected in tissues and urine from exposed humans (Autrup *et al.*, 1983; Groopman *et al.*, 1985; Hsieh *et al.*, 1988). Recently, a highly significant correlation was observed between daily consumption of AFB_1 and concentrations of serum adducts of AFB_1 and urinary AFB_1-N7-Gua (Gan *et al.*, 1988; Groopman *et al.*, 1989), representing perhaps steady-state levels.

5.4 AROMATIC AMINES

Human exposure to aromatic amines and amides occurs from a number of sources, including various industrial processes, cigarette smoke, and certain foods (Beland and Kadlubar, 1990). Widespread exposure also exists to nitropolycyclic aromatic hydrocarbons, which are products of incomplete combustion and are converted to aromatic amines by nitroreduction (Tokiwa and Ohnishi, 1986). The initial activation of aromatic amines and amides generally consists of an *N*-oxidation to yield *N*-hydroxy arylamines and *N*-hydroxy arylamides (arylhydroxamic acids), respectively. Similarly, the first step in the activation of nitropolycyclic aromatic hydrocarbons is a nitroreduction to an *N*-hydroxy arylamine. *N*-Hydroxy arylamines can react directly with DNA or be further activated through the formation of acetate and sulphate esters. Arylhydroxamic acids are not directly electrophilic and must be further metabolized to reactive esters (Figure 5.1). Typically, major adducts from these electrophilic intermediates are formed through covalent linkage of the amine or amide nitrogen to the C^8 of guanine, whereas

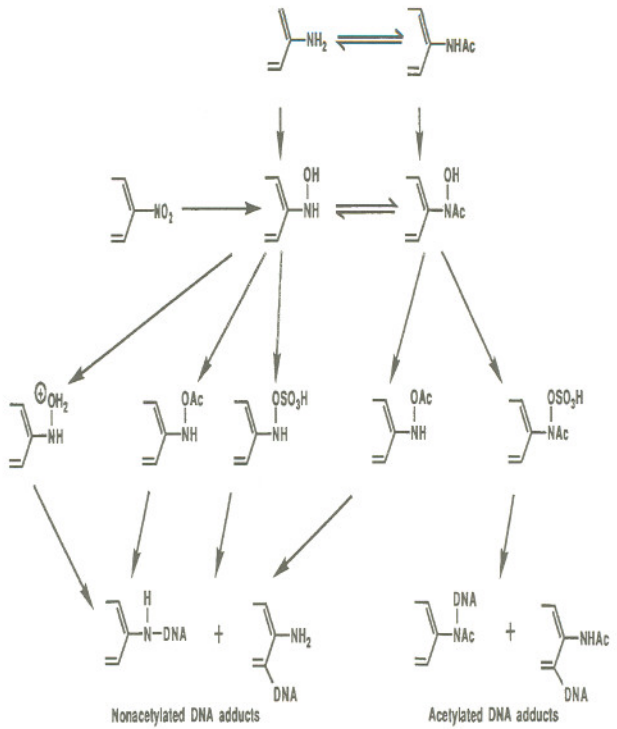

Figure 5.1. Metabolic activation pathways of aromatic amines, aromatic amides, and nitropolycyclic aromatic hydrocarbons

minor adducts arise from reactions between carbons in the *ortho* position in relation to the amine or amide nitrogen and the exocyclic nitrogens and oxygens of guanine and adenine. Non-acetylated C^8-substituted guanine adducts are the predominant products at doses normally used in carcinogenesis experiments, even when aromatic amides or arylhydroxamic acids are administered.

The distribution and mutagenic processing of aromatic amine DNA adducts appear to be non-random and influenced by the nature of the surrounding nucleotides at the binding site of the carcinogen. While this phenomenon has been studied most extensively with the adducts derived from 2-acetylaminofluorene, it applies to other compounds as well. For example, in plasmid DNA reaction with model electrophile *N*-acetoxy-2-acetylaminofluorene, the extent of modification of specific guanines varied 40-fold (Fuchs, 1983). Similar differences in reactivity, although smaller in magnitude, have been observed with *N*-hydroxy-2-aminofluorene (Bichara and Fuchs, 1985), *N*-acetoxy-2-trifluoroacetoxy-2-aminofluorene (Mah *et al.*, 1989), and *N*-hydroxy-1-aminopyrene (Yang *et al.*, 1988). In each case, no obvious reasons for the differences are apparent. A non-random induction of mutation is also found in the modified plasmids; however, the nucleotides most frequently mutated do not always correspond to those containing

the most adducts. Plasmids modified with *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene and transformed into *E. coli* cause frameshift mutations almost exclusively, and these occur in guanine repeatitions or in cytosine-guanine sequences (Koffel-Schwartz *et al.*, 1984). In the same system, the non-acetylated adduct *N*-(deoxyguanosin-8-yl)-2-aminofluorene induces mainly guanine-to-thymine transversions, but with no apparent sequence specificity (Bichara and Fuchs, 1985). Plasmids modified with *N*-(deoxyguanosin-8-yl)-2-aminofluorene or *N*-(deoxyguanosin-8-yl)-1-aminopyrene and transformed into human cells cause primarily guanine-to-thymine mutations in 5′-purine-guanine-purine-3′ sequences (Yang *et al.*, 1988; Mah *et al.*, 1989). Guanine-to-thymine transversions are also observed in Chinese hamster ovary cells exposed to *N*-acetoxy-2-acetylaminofluorene to produce *N*-(deoxyguanosin-8-yl)-2-aminofluorene-modified DNA (Carothers *et al.*, 1989). Similar mutations occur in the c-*Ha-ras* proto-oncogene in the livers of $B_6C_3F_1$ mice administered *N*-hydroxy-2-acetylaminofluorene (Wiseman *et al.*, 1986), presumably through the formation of *N*-(deoxyguanosin-8-yl)-2-aminofluorene (Lai *et al.*, 1985). The reaction of aromatic amine metabolites with DNA is strongly influenced by chromatin structure. In cells treated with *N*-acetoxy-2-acetylaminofluorene, for example, more extensive binding occurs in internucleosomal linker regions as compared with the nucleosome core (Kaneko and Cerutti, 1980; Lang *et al.*, 1982). Likewise, in the livers of rats administered 2-acetylaminofluorene or its arylhydroxamic acid, more extensive binding has been observed in staphylococcal nuclease-sensitive (Metzger *et al.*, 1976) and DNAse I-resistant (Ramanathan *et al.*, 1976; Metzger *et al.*, 1977; Baranyi-Furlong and Goodman, 1984) regions of chromatin, transcriptionally active DNA (Moyer *et al.*, 1977; Schwartz and Goodman, 1979; Walker *et al.*, 1979), and repetitive DNA sequences (Gupta, 1984).

2-Acetylaminofluorene is more hepatocarcinogenic in rats than in mice; in both species, a linear relationship exists between the concentration of a single oral dose of carcinogen and the extent of hepatic DNA binding, with higher binding levels being observed in rats (Pereira *et al.*, 1981). A linear relationship has also been observed between the amount of benzidine administered intraperitoneally to mice and hepatic DNA adduct levels (Talaska *et al.*, 1987). In rats, the highest quantities of aromatic amine DNA adducts are typically found in liver (Neumann, 1983; Gupta *et al.*, 1988, 1989), but while this organ is a target tissue for 2-acetylaminofluorene tumorigenesis, it is refractory to tumor induction by a number of other aromatic amine carcinogens (Garner *et al.*, 1984; Beland and Kadlubar, 1990). Within rat liver, more extensive adduct formation from 2-acetylaminofluorene occurs in hepatocytes, the presumed target cells for this aromatic amide, as compared with non-parenchymal cells (Westra *et al.*, 1983; Swenberg *et al.*, 1983; Poirier *et al.*, 1989).

During the continuous administration of aromatic amine carcinogens to rats or mice, steady-state concentrations of the amine in blood (Jackson *et al.*, 1980; Green *et al.*, 1984) and tissue (Jackson *et al.*, 1980), hepatic DNA adducts (Poirier *et al.*, 1984; Buss and Lutz, 1988; Beland *et al.*, 1990a), and bladder DNA adducts

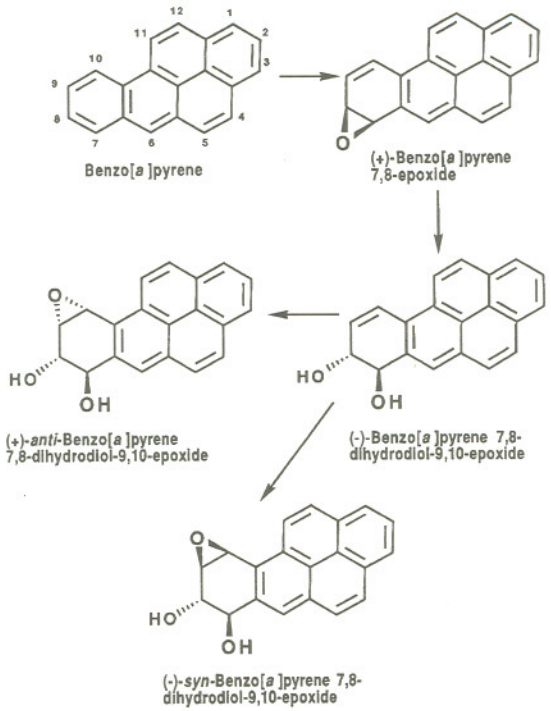

Figure 5.2. Metabolism of benzo[*a*]pyrene to *syn*- and *anti*-benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxides

(Beland *et al.*, 1990a; Talaska *et al.*, 1990) appear after approximately 1 month of dosing. These steady-state levels are dose-related (Jackson *et al.*, 1980; Buss and Lutz, 1988; Beland *et al.*, 1990a); and a linear correlation exists between the DNA-adduct concentration and the hepatic tumor incidence in rats (Buss and Lutz, 1988), in mice administered 2-acetylaminofluorene (Beland *et al.*, 1990a), and in mice treated with 4-aminobiphenyl (Beland *et al.*, 1990b). A linear relationship has also been observed between bladder tumor incidence and bladder DNA adduct concentration in male mice given 4-aminobiphenyl (Beland *et al.*, 1990b); however, in female mice fed 2-acetylaminofluorene, the correlation between the occurrence of bladder tumors and DNA-adduct concentration is non-linear (Beland *et al.*, 1990a). Hepatic tumor induction by 2-acetylaminofluorene in mice and rats is sex-related. In mice, tumorigenesis is correlated with the extent of DNA adduct formation; in rats, nearly equal concentrations of adducts are found in both sexes, but males have a much higher tumor yield (Pereira *et al.*, 1981; Beland *et al.*, 1982).

Aromatic amines are clearly implicated in the induction of bladder cancer in humans (Parkes and Evans, 1984). Haemoglobin adducts of 4-aminobiphenyl have been detected in blood samples from humans, and these concentrations are higher

in smokers than in non-smokers (Bryant *et al.*, 1987, 1988), which is consistent with tobacco-related increases in the incidence of bladder cancer (Mommsen and Aagaard, 1983). In other studies, ^{32}P-postlabelling and immunoassays have indicated the presence of 4-aminobiphenyl DNA adducts in human lung and bladder samples (Kadlubar *et al.*, 1988, 1989; Wilson *et al.*, 1989; Talaska *et al.*, 1990), with the concentration in the bladder increasing in the following order: smokers > ex-smokers > non-smokers (Kadlubar *et al.*, 1989).

5.5 POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAHs) are by-products of combustion processes, resulting in ubiquitous human exposure to this class of carcinogens (Dipple, 1985). The initial step in the activation of PAHs (*e.g.*, benzo[*a*]pyrene) typically involves an epoxidation in a terminal benzo ring (*e.g.*, carbons 7, 8, 9, and 10 of benzo[*a*]pyrene; Figure 5.2) (Dipple *et al.*, 1984). This step is followed by hydrolysis of the epoxide to a dihydrodiol and then an additional epoxidation in the same benzo ring to give a vicinal dihydrodiol epoxide. Since PAHs are generally asymmetrical, more than one geometric dihydrodiol epoxide can be formed; for example, the metabolism of benzo[*a*]pyrene can give rise to both a 7,8-dihydrodiol-9,10-epoxide and a 9,10-dihydrodiol-7,8-epoxide. However, tumor data as well as theoretical calculations (Jerina and Daly, 1977) support the concept that the most active derivatives are those in which the epoxide function is pointed toward the angular "bay" region of the PAH (*e.g.*, carbons 9, 10, 11, and 12 in benzo[*a*]pyrene). The metabolism of PAHs is further complicated by the fact that, in addition to geometric isomers, both diastereomeric (*e.g.*, *syn*- and *anti*-benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide) and enantiomeric (*e.g.*, (+)- and (-)-*anti*-benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide) stereoisomers can be formed that have markedly different biological properties. For example, (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide is more mutagenic in mammalian cells (Brookes and Osborne, 1982) and tumorigenic in experimental animals (Buening *et al.*, 1978; Slaga *et al.*, 1979) than the (-)-*anti* or (±)-*syn* isomers. Likewise, (-)-*anti*-benzo[*c*]phenanthrene-3,4-dihydrodiol-1,2-epoxide is the most mutagenic (Wood *et al.*, 1983) and tumorigenic (Levin *et al.*, 1986) dihydrodiol epoxide stereoisomer obtained from benzo[*c*]phenanthrene.

DNA adducts of PAH dihydrodiol epoxides have been studied exhaustively for benzo[*a*]pyrene (Baird and Pruess-Schwartz, 1988) and to a lesser extent for benz[*a*]anthracene (Hemminki *et al.*, 1980), chrysene (Hodgson *et al.*, 1983), dibenzo[*a*,*e*]fluoranthene (Pèrin-Roussel *et al.*, 1984), benzo[*c*]phenanthrene (Agarwal *et al.*, 1987), 7,12-dimethylbenz[*a*]anthracene (Cheng *et al.*, 1988), dibenz[*a*,*j*]anthracene (Chadha *et al.*, 1989), and fluoranthene (Gorelick and Wogan, 1989). Adduct formation normally involves *cis* or *trans* opening of the epoxide ring with covalent attachment at the benzylic carbon (*e.g.*, carbon 10 of benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide) of the dihydrodiol epoxide. Generally, guanine is the preferred base for reaction; however, depending upon the

PAH, considerable binding can also occur with adenine and cytosine (Table 5.2). Furthermore, the extent of reaction with a particular nucleic acid base will depend upon the particular stereoisomer being considered. For instance, with (+)-*syn*-benzo[*c*]phenanthrene-3,4-dihydrodiol-1,2-epoxide, ≈10% of the binding is to guanine; this binding increases to ≈40% with the (-)-*anti* isomer (Dipple *et al.*, 1987).

As with other carcinogens, PAH dihydrodiol epoxides do not appear to bind randomly to DNA. When studied by a photochemical cutting technique, (±)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide was found to bind preferentially to guanines in 5′-XGG-3′ and 5′-GGX-3′ sequences (Boles and Hogan, 1986), whereas alkali-labile lesions occur mainly in 5′-pyrimidine-guanine-3′ sequences (Lobanenkov *et al.*, 1986). The differences between these results may be due the nature of the adduct being examined, with the former reflecting N^2-deoxyguanosine lesions and the latter indicating the location of N^7-deoxyguanosine adducts.

The mutagenic processing of PAH dihydrodiol epoxide DNA adducts is also nonrandom. Guanine to thymine transversions are the primary mutations found in Chinese hamster ovary cells treated with (±)-*anti*-benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide and these tend to occur in 5′-AG_nA-3′ sequences (Mazur and Glickman, 1988). Similar mutations are observed in human cells treated with an (±)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-modified shuttle vector, and these are found mainly in runs of guanines (Yang *et al.*, 1987). In the same human cell system, (-)-*anti*-benzo[*c*]phenanthrene-3,4-dihydrodiol-1,2-epoxide causes adenine-to-thymine and guanine-to-cytosine (or cytosine-to-guanine) transversions primarily in 5′-AGA-3′, 5′-AAC-3′, and 5′-GAG-3′ sequences (Bigger *et al.*, 1989). Guanine-to-thymine transversions are associated with the activation of the c-*Ha-ras* proto-oncogene treated with (±)-*anti*-benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide and transvected into NIH 3T3 cells (Vousden *et al.*, 1986). In contrast, the activation of the *ras* proto-oncogene by 7,12-dimethylbenz[α]anthracene appears to involve an adenine-to-thymine transversion (Zarbl *et al.*, 1985; Bizub *et al.*, 1986; Dandekar *et al.*, 1986; Quintanilla *et al.*, 1986).

Higher level chromatin structure also affects the binding of PAH metabolites. In *in vitro* incubations with rat liver or lung tissue, cells, or nuclei, benzo[*a*]pyrene has been found to bind to a greater extent to nuclear matrix associated DNA than bulk chromatin (Blazsek *et al.*, 1979; Hemminki and Vainio, 1979; Ueyama *et al.*, 1981; Mironov *et al.*, 1983; Obi *et al.*, 1986). Similar observations have been made with 7,12-dimethylbenz[α]anthracene and (±)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide in rat liver nuclei (Mironov *et al.*, 1983) and with dibenzo[*a*,*e*]fluoranthene in mouse fibroblasts (Pèrin-Roussel *et al.*, 1988). In other *in vitro* experiments, benzo[*a*]pyrene and its (±)-*anti*- and (±)-*syn*-7,8-dihydrodiol-9,10 epoxides demonstrated greater binding to linker sequences than to the core sequences of nucleosomes (Koostra and Slaga; 1980; Jack and Brookes, 1981) and to transcribed as compared to nontranscribed regions of DNA (Arrand and Murray, 1982; Obi *et al.*, 1986). Fewer studies have been conducted *in vivo*; however, a single dose of

benzo[*a*]pyrene was found to bind preferentially to the nuclear matrix DNA isolated from lungs and livers of rats (Hemminki and Vaino, 1979).

Table 5.2. Sites of modification in DNA *in vivo* by aromatic amine and nitropolycyclic aromatic hydrocarbon carcinogens

Carcinogen	Nucleic acid base				
	Guanine			Adenine	
	C^8	N^2	O^6	C8	N^6
1-Naphthylamine	++				
2-Naphthylamine	++	+			
4-Aminobiphenyl	++	+		+	
4-Acetylaminobiphenyl	++[b]	+			
4-Nitrobiphenyl	++	+		+	
4′-Fluoro-4-acetylaminobiphenyl	++[b]	+			
2-Aminofluorene	++				
2-Acetylaminofluorene	++[b]	+			
Benzidine	++				
NN′-Diacetylbenzidine	++[b]				
4-Aminoazobenzene	++				
N-Methyl-4-aminoazobenzene	++[c]	+		+	
NN-Dimethyl-4-aminoazobenzene	++[c]	+			
2-Acetylaminophenanthrene[d]	++	+			
4-Acetylaminostilbene	++				
1-Nitropyrene	++				
1,6- and 1,8-Dinitropyrene	++				

[a]Data are from Beland and Kadlubar (1985, 1990). Results are from target tissues, typically after the administration of a single dose. For aromatic amines or amides, their *N*-hydroxy derivatives may have been given.
++ = >75% binding; + = <25% binding.
[b]Both *N*-acetylated and non-acetylated adducts are found in a ratio of ≈1:3.
[c]Both *N*-methylated and non-methylated adducts are found.
[d]Data are from Gupta *et al.* (1989).

PAHs induce primarily skin, stomach, lung, and mammary gland tumors in experimental animals (Dipple *et al.*, 1984). Following a single dose, PAH–DNA adducts are formed in both target and nontarget tissues, with the concentration being relatively uniform between tissues (Stowers and Anderson, 1985). A number of dose–response studies have also been conducted with PAHs; in nearly all of these, DNA adducts are linearly related to dose. In mice administered benzo[*a*]pyrene orally over a 10^5-fold range in dose, the extent of DNA binding in liver and stomach increased in a linear fashion (Dunn, 1983). In another study, a decrease in the extent of adduct formation was observed at higher doses of

benzo[*a*]pyrene, which may be due to a saturation of the metabolic activation pathways (Adriaenssens *et al.*, 1983). Supralinear dose–response relationships between cancer incidence and the binding to epidermal DNA have also been observed in mice treated topically with benzo[*a*]pyrene (Pereira *et al.*, 1979; Perera *et al.*, 1982; Nakayama *et al.*, 1984) and 7,12-dimethylbenz[*a*]anthracene (Phillips *et al.*, 1978). The correlation between administered dose and DNA-adduct concentration does not appear to have been examined in the mammary gland; however, an adduct formed by the reaction of (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide with N^2 of deoxyguanosine is the major product detected in mammary gland DNA when benzo[*a*]pyrene is administered orally to rats (Seidman *et al.*, 1988). DNA-adduct dose–response experiments have not been reported for the continuous administration of PAHs.

PAHs are probably carcinogenic in humans (IARC, 1983); nevertheless, attempts to detect DNA adducts from these compounds in exposed populations have met with mixed success. Since cigarette smoke contains substantial quantities of PAHs (Surgeon General, 1982), a number of studies have compared DNA adduct concentrations in various tissues from smokers and non-smokers. Immunoassays with antibodies elicited against (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide-modified DNA have revealed that smokers generally have slightly higher PAH–DNA adduct levels (termed benzo[*a*]pyrene antigenicity) than non-smokers (Everson *et al.*, 1986, 1988; Perera *et al.*, 1987), although this is not always the case (Perera *et al.*, 1982, 1988; Harris *et al.*, 1985; Shamsuddin *et al.*, 1985; Haugen *et al.*, 1986). More dramatic differences have been found when assays are based upon ^{32}P-postlabelling. Depending upon assay conditions, smokers have had higher levels of discrete adducts (Everson *et al.*, 1986, 1988; Randerath *et al.*, 1986), or a diffuse area of adducts (Phillips *et al.*, 1988; Randerath *et al.*, 1989), than non-smokers. However, none of these adducts has been characterized chemically, and most cannot be attributed to specific PAH DNA adducts. The presence of benzo[*a*]pyrene dihydrodiol epoxide DNA adducts in human placenta has been established by subjecting placental DNA to immunoaffinity chromatography followed by high pressure liquid chromatography and synchronous fluorescence spectroscopy, as well as gas chromatography–mass spectrometry; however, the levels did not appear to be related to smoking (Manchester *et al.*, 1988). Exposure-related increases in benzo[*a*]pyrene antigenicity have been reported in peripheral blood lymphocytes from iron-foundry workers (Perera *et al.*, 1988) and individuals ingesting charcoal-broiled beef (Rothman *et al.*, 1990). Positive results for PAH–DNA adducts (or benzo[*a*]pyrene antigenicity) have also been found in blood samples from coke oven workers (Harris *et al.*, 1985), foundry workers, roofers (Shamsuddin *et al.*, 1985), and fire fighters (Liou *et al.*, 1989).

5.6 SUMMARY

Some interesting similarities and differences observed between the classes of

carcinogen–DNA adducts considered in this review include the following:

1. With alkylating agents, mutations and, presumably, tumors result from minor adducts (*e.g.*, O^6-alkylguanine and O^4-alkylthymine); whereas with the other carcinogens, the biological responses are normally associated with the major forms of DNA damage.
2. The site of substitution for biologically important adducts appear to be chemical-class specific. Mutations and tumor induction from alkylating agents are correlated with O^6-guanine and O^4-thymine substitution. For aflatoxins, these responses are best correlated with reaction at the N^7 site of guanine; with aromatic amines, C^8-guanine substitution generally appears to be the critical lesion; and for PAHs, N^2 of guanine and/or N^6 of adenine appear to be the important sites for substitution.
3. Reactions with DNA are clearly non-random. Sequence specificity for adduct formation has been demonstrated *in vitro* with chemicals of each of class; however, presently not enough information is available to determine if significant differences exist between classes or what factors are critical in determining the sequence specificity for both the formation and processing of the adducts. Furthermore, essentially nothing is known about the sequence specificity of adduct formation and processing *in vivo*.
4. Higher order chromatin structure affects the binding of carcinogens to DNA. With each of the classes, for example, more extensive binding has been found with transcriptionally active DNA, presumably due to its open conformation. Nevertheless, most observations concerning the distribution of DNA adducts in chromatin have been made after single doses of carcinogen, rather than after dosing regimens that give rise to tumors. The distribution of DNA adducts during continuous dosing reflects not only a dynamic process of adduct formation and removal, but also changes in chromatin structure as a function of time. How these factors affect the final distribution of adducts is unknown.
5. For several carcinogens, the relationship between administered dose and DNA-adduct concentrations is linear after both single doses and continuous administration. In certain instances, however, correlations are non-linear, which may be due to saturation of activation or detoxification pathways at high doses of carcinogen. Thus, caution must be exercised in extrapolating from high to low doses.
6. Steady-state DNA-adduct concentrations are obtained during continuous carcinogen administration, and this condition typically occurs after approximately 1 month of chronic dosing. Because steady-state DNA-adduct levels occur, an estimation of the risk for developing a tumor cannot be obtained from the DNA-adduct concentration by itself, but must include both the DNA adduct concentration and the length of carcinogen exposure.
7. DNA adducts have been measured in target tissues following dosing regimens that induce tumors. DNA adduct concentrations necessary to induce a 50%

tumor incidence have been measured. The results are both interesting and troubling. For example, a 10-fold higher concentration of O^6-methylguanine as compared with O^6-ethylguanine is required to induce a 50% incidence of thymic lymphoma. This difference may reflect the fact that the former is repaired more readily than the latter. Likewise, the inability of O^4-ethylguanine to be repaired probably accounts for the even lower concentrations of this adduct necessary to induce the equivalent hepatic tumor incidence. The observation that in two species the identical levels of AFB_1–DNA adducts induce the same incidence of hepatic tumors indicates that making cross-species comparisons may be possible. In addition, the data from mice treated with 2-acetylaminofluorene and 4-aminobiphenyl suggest that structurally similar DNA adducts in different organs are processed in a similar manner. Nonetheless, the apparent 100-fold range in the ability of DNA adducts to induce the same tumor incidence raises concerns about the prospect of being able to predict the tumor induction potential of unknown DNA adducts.

8. DNA adducts can be detected in humans using immunoassays and ^{32}P-postlabelling. In some instances exposure-related increases in DNA-adduct concentrations have been demonstrated. At times, however, the correlation between different types of assays has been unsatisfactory; for example, positive results have been obtained for benzo[*a*]pyrene antigenicity, and yet identifiable PAH–DNA adducts have not been detected in the same samples by ^{32}P-postlabelling. When used alone, neither technique has chemical specificity, but when combined with other adduct-detection procedures (e.g., immunoaffinity chromatography followed by high-pressure liquid chromatography or gas chromatography/mass spectrometry), these methods have allowed the identification of specific DNA adducts in human samples. Such an approach holds promise for much improved sensitivity and specificity.

5.7 REFERENCES

Abbott, P.J. and Saffhill R. (1977) DNA synthesis with methylated poly(dA-dT) templates: possible role of O^4-methylthymidine as a pro-mutagenic base. *Nucleic Acids Res.* **4**, 761–769.

Abbott, P.J., and Saffhill R. (1979) DNA synthesis with methylated poly(dC-dG) templates. Evidence for a competitive nature to miscoding by O^6-methylguanine. *Biochim. Biophys. Acta*, **562**, 51–61.

Adriaenssens, P.I., White, C.M., and Anderson M.W. (1983) Dose–response relationships for the binding of benzo[*a*]pyrene metabolites to DNA and protein in lung, liver, and forestomach of control and butylated hydroxyanisole-treated mice. *Cancer Res.* **43**, 3712–3719.

Agarwal, S.K., Sayer, J.M., Yeh, H.J.C. Pannell, L.K. Hilton, B.D. Pigott, M.A. Dipple, A. Yagi, H., and Jerina D.M. (1987) Chemical characterization of DNA adducts derived from

the configurationally isomeric benzo[*c*]phenanthrene-3,4-diol 1,2-epoxides. *J. Am. Chem. Soc.* **109**, 2497–2504.

Appleton, B.S., Goetchius, M.P., and Campbell T.C. (1982) Linear dose-response curve for the hepatic macromolecular binding of aflatoxin B_1 in rats at very low exposures. *Cancer Res.* **42**, 3659–3662.

Arrand, J.E. and Murray A.M. (1982) Benzpyrene groups bind preferentially to the DNA of active chromatin in human lung cells. *Nucleic Acids Res.* **10**, 1547–1555.

Autrup, H., Bradley, K.A., Shamsuddin, A.K.M., Wakhisi, J., and Wasunna A. (1983) Detection of putative adduct with fluorescence characteristics identical to 2,3-dihydro-2-(7′-guanyl)-3-hydroxyaflatoxin B_1 in human urine collected in Murang'a district, Kenya. *Carcinogenesis* **4**, 1193–1195.

Baertschi, S.W., Raney, K.D., Stone, M.P., and Harris T.M. (1988) Preparation of the 8,9-epoxide of the mycotoxin aflatoxin B_1: the ultimate carcinogenic species. *J. Am. Chem. Soc.* **110**, 7929–7931.

Bailey, G.S., Williams, D.E., Wilcox, J.S., Loveland, P.M., Coulombe, R.A., and Hendricks J.D. (1988) Aflatoxin B_1 carcinogenesis and its relation to DNA adduct formation and adduct persistence in sensitive and resistant salmonid fish. *Carcinogenesis* **9**, 1919–1926.

Bailey, G.S., Nixon, J.E., Hendricks, J.D., Sinnhuber, R.O., and Van Holde, K.E. (1980) Carcinogen aflatoxin B_1 is located preferentially in internucleosomal deoxyribonucleic acid following exposure *in vivo* in rainbow trout. *Biochemistry* **19**, 5836–5842.

Baird, W.M. and Pruess-Schwartz, D. (1988) Polycyclic aromatic hydrocarbon–DNA adducts and their analysis: a powerful technique for characterization of pathways of metabolic activation of hydrocarbons to ultimate carcinogenic metabolites. In: Yang, S.K. and Silverman, B.D. (Eds) *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure–Activity Relationships*, Vol. II, pp. 141–179. CRC Press, Boca Raton, Florida.

Baranyi-Furlong, B.L. and Goodman, J.I. (1984) Non-random interaction of *N*-2-acetylaminofluorene and its *N*-hydroxy metabolite with DNA of rat hepatic parenchymal and non-parenchymal cell nuclei following *in vivo* administration of carcinogen. *Chem.-Biol. Interactions* **48**, 15–28.

Bechtel, D.H. (1989) Molecular dosimetry of hepatic aflatoxin B_1-DNA adducts: Linear correlations with hepatic cancer risk. *Regulatory Toxicol. Pharmacol.* **10**, 74–81.

Beland, F.A. and Kadlubar, F.F. (1985) Formation and persistence of arylamine DNA adducts *in vivo*. *Environ. Health Perspectives*, **62**, 19–30.

Beland, F.A. and Kadlubar, F.F. (1990) Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons. In: Cooper, C.S. and Grover, P.L. (Eds) *Handbook of Experimental Pharmacology*, Vol. 94/I: *Chemical Carcinogenesis and Mutagenesis I*, pp. 267–325. Springer-Verlag, Heidelberg.

Beland, F.A., Dooley, K.L., and Jackson, C.D. (1982) Jackson Persistence of DNA adducts in rat liver and kidney after multiple doses of the carcinogen *N*-hydroxy-2-acetylaminofluorene. *Cancer Res.* **42**, 1348–1354.

Beland, F.A., Fullerton, N.F., Kinouchi, T., Smith, B.A., and Poirier, M.C. (1990a) DNA adduct formation in relation to tumorigenesis in mice chronically fed 2-acetylaminofluorene. In: Stevenson, D.E., Popp, J.A., Ward, J.M., McClain, R.M., Slaga, T.J., and Pitot, H.C. (Eds) *Mouse Liver Carcinogenesis: Mechanisms and Species Comparisons*, pp. 121–129. Alan R. Liss, Cleveland, Ohio.

Beland, F.A., Fullerton, N.F., Marques, M.M., Melchior, Jr., W.B., Smith, B.A., and Poirier, M.C. (1990b) DNA adduct formation in relation to tumorigenesis in mice continuously administered 4-aminobiphenyl. In: Mendelsohn, M.L. (Ed) *Proceedings from the Fifth*

International Conference on Environmental Mutagens. Alan R. Liss, Cleveland, Ohio.

Belinsky, S.A., White, C.M., Devereux, T.R., Swenberg, J.A., and Anderson, M.W. (1987) Cell selective alkylation of DNA in rat lung following low dose exposure to the tobacco specific carcinogen 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Res.* **47**, 1143–1148.

Belinsky, S.A., Devereux, T.R., Maronpot, R.R., Stoner, G.D., and Anderson, M.W. (1989) Relationship between the formation of promutagenic adducts and the activation of the *K-ras* protooncogene in lung tumors from A/J mice treated with nitrosamines. *Cancer Res.* **49**, 5305–5311.

Benasutti, M., Ejadi, S., Whitlow, M.D., and Loechler, E.L. (1988) Mapping the binding site of aflatoxin B_1 in DNA: systematic analysis of the reactivity of aflatoxin B_1 with guanines in different DNA sequences. *Biochemistry* **27**, 472–481.

Bichari, M. and Fuchs, R.P.P. (1985) DNA binding and mutation spectra of the carcinogen *N*-2-aminofluorene in *Escherichia coli*: A correlation between the conformation of the premutagenic lesion and the mutation specificity. *J. Mol. Biol.* **183**, 341–351.

Bigger, C.A.H., Strandberg, J., Yagi, H., Jerina, D.M., and Dipple, A. (1989) Mutagenic specificity of a potent carcinogen, benzo[*c*]phenanthrene (4R,3S)-dihydrodiol (2S,1R)-epoxide, which reacts with adenine and guanine in DNA. *Proc. Natl. Acad. Sci. USA* **86**, 2291–2295.

Bizub, D., Wood, A.W., and Skalka, A.M. (1986) Mutagenesis of the *Ha-ras* oncogene in mouse skin tumors induced by polycyclic aromatic hydrocarbons. *Proc. Natl. Acad. Sci. USA* **83**, 6048–6052.

Blazsek, I., Vauhkonen, M., and Hemminki, K. (1979) Binding of benzo[*a*]pyrene into lung and thymocyte nuclear fractions. *Res. Commun. Chem. Pathol. Pharmacol.* **23**, 611–626.

Boles, T.C. and Hogan, M.E. (1986) High-resolution mapping of carcinogen binding sites on DNA. *Biochemistry* **25**, 3039–3043.

Boucheron, J.A., Richardson, F.C., Morgan, P.H., and Swenberg, J.A. (1987) Molecular dosimetry of O^4-ethyldeoxythymidine in rats continuously exposed to diethylnitrosamine. *Cancer Res.* **47**, 1577–1581.

Brookes, P. and Osborne, M.R. (1982) Mutation in mammalian cells by stereoisomers of anti-benzo[*a*]pyrene-diolepoxide in relation to the extent and nature of the DNA reaction products. *Carcinogenesis* **3**, 1223–1226.

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.

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.

Buening, M.K., Wislocki, P.G., Levin, W., Yagi, H., Thakker, D.R., Akagi, H., Koreeda, M., Jerina, D.M., and Conney A.H. (1978) Tumorigenicity of the optical enantiomers of the diastereomeric benzo[*a*]pyrene 7,8-diol-9,10-epoxides in newborn mice: Exceptional activity of (+)-7ß,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene. *Proc. Natl Acad. Sci. USA* **75**, 5358–5361.

Burns, P.A., Gordon, A.J.E., and Glickman B.W. (1988) Mutational specificity of *N*-methyl-*N*-nitrosourea in the *lacI* gene of *Escherichia coli*. *Carcinogenesis* **9**, 1607–1610.

Busby, Jr., W.F., and Wogan, G.N. (1984) Aflatoxins. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 182, pp. 945–1136. American Chemical Society, Washington, DC.

Buss, P., and Lutz, W.K. (1988) Steady-state DNA adduct level in rat liver after chronic

exposure to low doses of aflatoxin B_1 and 2-acetylaminofluorene. *Proc. Am. Assoc. Cancer Res.* **29**, 96.

Carothers, A.M., Steigerwalt, R.W., Urlaub, G., Chasin, L.A., and Grunberger D. (1989) DNA base changes and RNA levels in *N*-acetoxy-2-acetylaminofluorene-induced dihydrofolate reductase mutants of Chinese hamster ovary cells. *J. Mol. Biol.* **208**, 417–428.

Chadha, A., Sayer, J.M., Yeh, H.J.C., Yagi, H., Cheh, A.M., Pannell L.K., and Jerina, D.M. (1989) Structures of covalent nucleoside adducts formed from adenine, guanine, and cytosine bases of DNA and the optically active bay-region 3,4-diol 1,2-epoxides of dibenz[*a,j*]anthracene. *J. Am. Chem. Soc.* **111**, 5456–5463.

Cheng, S.C., Prakash, A.S., Pigott, M.A., Hilton, B.D., Roman, J.M., Lee, H., Harvey, R.G., and Dipple A. (1988) Characterization of 7,12-dimethylbenz[α]anthracene-adenine nucleoside adducts. *Chem. Res. Toxicol.* **1**, 216–221.

Croy, R.G. and Wogan, G.N. (1981a) Temporal patterns of covalent DNA adducts in rat liver after single and multiple doses of aflatoxin B_1. *Cancer Res.* **41**, 197–203.

Croy, R.G. and Wogan, G.N. (1981b) Quantitative comparison of covalent aflatoxin-DNA adducts formed in rat and mouse livers and kidneys. *J. Natl. Cancer Inst.* **66**, 761–768.

D'Andrea, A.D. and Haseltine, W.A. (1978) Modification of DNA by aflatoxin B_1 creates alkali-labile lesions in DNA at positions of guanine and adenine. *Proc. Natl. Acad. Sic. USA* **75**, 4120–4124.

Dandekar, S., Sukumar, S., Zarbl, H., Young, L.J.T., and Cardiff R.D. (1986) Specific activation of the cellular *Harvey-ras* oncogene in dimethylbenzanthracene-induced mouse mammary tumors. *Mol. Cell. Biol.* **6**, 4104–4108.

Dashwood, R.H., Arbogast, D.N., Fong, A.T., Hendricks, J.D., and Bailey, G.S. (1988) Mechanisms of anti-carcinogenesis by indole-3-carbinol: detailed *in vivo* DNA binding dose–response studies after dietary administration with aflatoxin B_1. *Carcinogenesis* **9**, 427–432.

Dashwood, R.H., Arbogast, D.N., Fong, A.T., Pereira, C., Hendricks, J.D., and Bailey, G.S. (1989) Quantitative inter-relationships between aflatoxin B_1 carcinogen dose, indole-3-carbinol anti-carcinogen dose, target organ DNA adduction and final tumor response. *Carcinogenesis* **10**, 175–181.

Dipple, A. (1985) Polycyclic aromatic hydrocarbon carcinogenesis: An introduction. In: Harvey, R.G. (Ed) *Polycyclic Hydrocarbons and Carcinogenesis*, ACS Symposium Series 283, pp. 1–17. American Chemical Society, Washington, DC.

Dipple, A., Moschel, R.C., and Bigger, C.A.H. (1984) Polynuclear aromatic carcinogens. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 182, pp. 41–163. American Chemical Society, Washington, DC.

Dipple, A., Pigott, M.A., Agarwal, S.K., Yagi, H., Sayer, J.M., and Jerina, D.M. (1987) Optically active benzo[*c*]phenanthrene diol epoxides bind extensively to adenine in DNA. *Nature* **327**, 535–536.

Dolan, M.E., Oplinger, M., and Pegg A.E. (1988) Sequence specificity of guanine alkylation and repair. *Carcinogenesis* **9**, 2139–2143.

Dunn, B.P. (1983) Wide-range linear dose–response curve for DNA binding of orally administered benzo[*a*]pyrene in mice. *Cancer Res.* **43**, 2654–2658.

Essigmann, J.M., Croy, R.G., Nadzan, A.M., Busby, Jr., W.F., Reinhold, V.N., Büchi, G., and Wogan, G.N. (1977) Structural identification of the major DNA adduct formed by aflatoxin B_1 *in vitro*. *Proc. Natl. Acad. Sci. USA* **74**, 1870–1874.

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.

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.

Foster, P.L., Eisenstadt, E., and Miller, J.H. (1983) Base substitution mutations induced by metabolically activated aflatoxin B_1. *Proc. Natl Acad. Sci. USA* **80**, 2695–2698.

Frei, J.V. and Lawley, P.D. (1976) Tissue distribution and mode of DNA methylation in mice by methyl methanesulphonate and *N*-methyl-*N'*-nitro-*N*-nitroso guanidine: lack of thymic lymphoma induction and low extent of methylation of target tissue DNA at *O*-6 of guanine. *Chem.-Biol. Interactions* **13**, 215–222.

Frei, J.V., Swenson, D.H., Warren, W., and Lawley, P.D. (1978) Alkylation of deoxyribonucleic acid *in vivo* in various organs of C57BL mice by the carcinogens *N*-methyl-*N*-nitrosourea, *N*-ethyl-*N*-nitrosourea and ethyl methanesulphonate in relation to induction of thymic lymphoma. Some applications of high-pressure liquid chromatography. *Biochem. J.* **174**, 1031–1044.

Fuchs, R.P.P. (1983) DNA binding spectrum of the carcinogen *N*-acetoxy-*N*-2-acetyl aminofluorene significantly differs from the mutation spectrum. *J. Mol. Biol.* **177**, 173–180.

Gan, L.S., Skipper, P.L., Peng, X., Groopman, J.D., Chen, J.S., Wogan, G.N., and Tannenbaum, S.R. (1988) Serum albumin adducts in the molecular epidemiology of aflatoxin carcinogenesis: correlation with aflatoxin B_1 intake and urinary excretion of aflatoxin M_1. *Carcinogenesis* **9**, 1323–1325.

Garner, R.C., and Wright, C.M. (1975) Binding of [^{14}C]aflatoxin B_1 to cellular macromolecules in the rat and hamster. *Chem.-Biol. Interactions* **11**, 123–131.

Garner, R.C., Martin, C.N., and Clayson, D.B. (1984) Carcinogenic aromatic amines and related compounds. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 182, pp. 175–276. American Chemical Society, Washington, DC.

Goeger, D.E., Shelton, D.W., Hendricks, J.D., and Bailey, G.S. (1986) Mechanisms of anti-carcinogenesis by indole-3-carbinol: effect on the distribution and metabolism of aflatoxin B_1 in rainbow trout. *Carcinogenesis* **7**, 2025–2031.

Gorelick, N.J. and Wogan, G.N. (1989) Fluoranthene-DNA adducts: identification and quantification by an HPLC-^{32}P-postlabelling method. *Carcinogenesis* **10**, 1567–1577.

Green, L.C., Skipper, P.L., Turesky, R.J., Bryant, M.S., and Tannenbaum, S.R. (1984) *In vivo* dosimetry of 4-aminobiphenyl in rats via a cysteine adduct in hemoglobin. *Cancer Res.* **44**, 4254–4259.

Groopman, J.D., Donahue, P.R., Zhu, J., Chen, J., and Wogan, G.N. (1985) Aflatoxin metabolism in humans: Detection of metabolites and nucleic acid adducts in urine by affinity chromatography. *Proc. Natl Acad. Sci. USA* **82**, 6492–6496.

Groopman, J.D., Gan, L., Skipper, P., Chen, J., Tannenbaum, S.R., and Wogan, G.W. (1989) Dosimetry of aflatoxin DNA and serum albumin adducts in people living in Guangxi Province, PRC. *Proc. Am. Assoc. Cancer Res.* **30**, 317.

Gupta, R.C. (1984) Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylamino fluorene to repetitive sequences of rat liver DNA *in vivo*. *Proc. Natl Acad. Sci. USA* **81**, 6943–6947.

Gupta, R.C., Reddy, M.V., and Randerath, K. (1982) ^{32}P-postlabelling analysis of non-radioactive aromatic carcinogen-DNA adducts. *Carcinogenesis* **3**, 1081–1092.

Gupta, R.C., Earley, K., and F.F. Becker (1988) Analysis of DNA adducts in putative

premalignant hepatic nodules and nontarget tissues of rats during 2-acetylaminofluorene carcinogenesis. *Cancer Res.* **48**, 5270–5274.

Gupta, R.C., Earley, K., Fullerton, N.F., and Beland, F.A. (1989) Formation and removal of DNA adducts in target and nontarget tissues of rats administered multiple doses of 2-acetyl-aminophenanthrene. *Carcinogenesis* **10**, 2025–2033.

Harris, C.C., Vahakangas, K., Newman, M.J., Trivers, G.E., Shamsuddin, A., Sinopoli, N., Mann, D.L., and Wright, W.E. (1985) Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in peripheral blood lymphocytes and antibodies to the adducts in serum from coke oven workers. *Proc. Natl Acad. Sci. USA* **82**, 6672–6676.

Haugen, A., Becher, G., Benestad, C., Vahakangas, K., Trivers, G.E., Newman, M.J., and Harris, C.C. (1986) Determination of polycyclic aromatic hydrocarbons in the urine, benzo[*a*]pyrene diol epoxide-DNA adducts in lymphocyte DNA, and antibodies to the adducts in sera from coke oven workers exposed to measured amounts of polycyclic aromatic hydrocarbons in the work atmosphere. *Cancer Res.* **46**, 4178–4183.

Hecht, S.S., Foiles, P.G., Carmella, S.G., Trushin, N., Rivenson, A., and Hoffmann, D. (1986) Recent studies on the metabolic activation of tobacco-specific nitrosamines: prospects for dosimetry in humans. In: Hoffmann, D. and Harris, C.C. (Eds) *Mechanisms in Tobacco Carcinogenesis*, Banbury Report 23, pp. 245–257. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Hemminki, K. and Vainio, H. (1979) Preferential binding of benzo[*a*]pyrene into nuclear matrix fraction. *Cancer Lett.* **6**, 167–173.

Hemminki, K., Cooper, C.S., Ribeiro, O., Grover, P.L., and Sims, P. (1980) Reactions of "bay-region" and non-"bay-region" diol-epoxides of benz[a])anthracene with DNA: evidence indicating that the major products are hydrocarbon-N^2-guanine adducts. *Carcinogenesis* **1**, 277–286.

Herron, D.C. and Shank, R.C. (1980) Methylated purines in human liver DNA after probable dimethylnitrosamine poisoning. *Cancer Res.* **40**, 3116–3117.

Hertzog, P.J., Smith, J.R.L., and Garner, R.C. (1982) Characterisation of the imidazole ring-opened forms of trans-8,9-dihydro-8-(7-guanyl)9-hydroxy aflatoxin B_1. *Carcinogenesis* **3**, 723–725.

Hodgson, R.M., Cary, P.D., Grover, P.L., and Sims, P. (1983) Metabolic activation of chrysene by hamster embryo cells: evidence for the formation of a "bay-region" diol-epoxide-N^2-guanine adduct in RNA. *Carcinogenesis* **4**, 1153–1158.

Hsieh, L-L., Hsu, S-W., Chen, D-S., and Santella, R.M. (1988) Immunological detection of aflatoxin B_1-DNA adducts formed *in vivo*. *Cancer Res.* **48**, 6328–6331.

IARC (1983) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental and Experimental Data*, Vol. 32, pp. 1–477. International Agency for Research on Cancer, Lyon.

Irvin, T.R. and Wogan, G.N. (1984) Quantitation of aflatoxin B_1 adduction within the ribosomal RNA gene sequences of rat liver DNA. *Proc. Natl Acad. Sci. USA* **81**, 664–668.

Jack, P.L. and Brookes, P. (1981) The distribution of benzo[*a*]pyrene DNA adducts in mammalian chromatin. *Nucleic Acids. Res.* **9**, 5533–5552.

Jackson, C.D., Weis, C., and Shellenberger, T.E. (1980) Tissue binding of 2-acetylaminofluorene in BALB/c and $C57B_1$/6 mice during chronic oral administration. *Chem.-Biol. Interactions* **32**, 63–81.

Jerina, D.M. and Daly, J.W. (1976) Oxidation at carbon. In: Parke, D.V. and Smith, R.L. (Eds) *Drug Metabolism*, pp. 13–32. Taylor and Francis: London.

Kaden, D.A., Call, K.M., Leong, P-M., Komives, E.A., and Thilly, W.G. (1987) Killing and mutation of human lymphoblast cells by aflatoxin B_1: evidence for an inducible repair response. *Cancer Res.* **47**, 1993–2001.

Kadlubar, F.F., Talaska, G., Lang, N.P., Benson, R.W., and Roberts, D.R. (1988) Assessment of exposure and susceptibility to aromatic amine carcinogens. In: Bartsch, H., Hemminki, K., and O'Neill, I.K. (Eds) *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, pp. 166–174. International Agency for Research on Cancer, Lyon.

Kadlubar, F.F., Talaska, G., Culp, S.J., Roberts, D.R., Al-Juburi, A.Z.S.S., and Lang, N.P. (1989) Molecular epidemiological studies on the presence of carcinogen-DNA adducts in human urinary bladder and peripheral lung tissues. Proceedings of the Fourth Aspen Cancer Conference on Carcinogenesis: From Molecular Machanisms to Molecular Epidemiology. Aspen, Colorado, USA, July 23-26, 1989. *Toxicol. Pathol.* **17**(4. Part 2), 836–837.

Kaneko, M. and Cerutti, P.A. (1980) Excision of *N*-acetoxy-2-acetylaminofluorene-induced DNA adducts from chromatin fractions of human fibroblasts. *Cancer Res.* **40**, 4313–4319.

Kensler, T.W., Egner, P.A., Davidson, N.E., Roebuck, B.D., Pikul, A., and Groopman, J.D. (1986) Modulation of aflatoxin metabolism, aflatoxin-*N*7-guanine formation, and hepatic tumorigenesis in rats fed ethoxyquin: role of induction of glutathione S-transferases. *Cancer Res.* **46**, 3924–3931.

Koffel-Schwartz, N., Verdier, J.M., Bichara, M., Freund, A-M., Daune, M.P., and Fuchs, R.P.P. (1984) Carcinogen-induced mutation spectrum in wild-type, *uvrA* and *umuC* strains of *Escherichia coli*. Strain specificity and mutation-prone sequences. *J. Mol. Biol.* **177**, 33–51.

Koostra, A. and Slaga, T.J. (1980) Binding of isomers of benzo[*a*]pyrene diol-epoxide to chromatin. *Biochem. Biophys. Res. Commun.* **93**, 954–959.

Lai, C.C., Miller, J.A., Miller, E.C., and Liem, A. (1985) *N*-Sulphoxy-2-amino fluorene is the major ultimate electrophilic and carcinogenic metabolite of *N*-hydroxy-2-acetylaminofluorene in the livers of infant male C57BL/6J x C3H/HeJ F1 ($B_6C_3F_1$) mice. *Carcinogenesis* **6**, 1037–1045.

Lang, M.C., de Murcia, G., Mazen, A., Fuchs, R.P.P., Leng, M., and Daune, M. (1982) Non-random binding of *N*-acetoxy-*N*-2-acetylaminofluorene to chromatin subunits as visualized by immunoelectron microscopy. *Chem.-Biol. Interactions* **41**, 83–93.

Lawley, P.D. (1976) Carcinogenesis by alkylating agents. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 173, pp. 83–244. American Chemical Society, Washington, DC.

Lawley, P.D. (1984) Carcinogenesis by alkylating agents. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 182, pp. 325–484. American Chemical Society, Washington, DC.

Levin, W., Chang, R.L., Wood, A.W., Thakker, D.R., Yagi, H., Jerina, D.M., and Conney, A.H. (1986) Tumorigenicity of optical isomers of the diastereomeric bay-region 3,4-diol-1,2-epoxides of benzo(*c*)phenanthrene in murine tumor models. *Cancer Res.* **46**, 2257–2261.

Lewis, J.G. and Swenberg, J.A. (1980) Differential repair of O^6-methylguanine in DNA of rat hepatocytes and nonparenchymal cells. *Nature* **288**, 185–187.

Liou, S., Jacobson-Kram, D., Poirier, M.C., Nguyen, D., Strickland, P.T., and Tockman, M.S. (1989) Biological monitoring of fire fighters: sister chromatid exchange and polycyclic aromatic hydrocarbon-DNA adducts in peripheral blood cell. *Cancer Res.* **49**,

4929–4935.

Lobanenkov, V.V., Plumb, M., Goodwin, G.H., and Grover, P.L. (1986) The effect of neighboring bases on G-specific DNA cleavage mediated by treatment with the anti-diol epoxide of benzo[*a*]pyrene *in vitro*. *Carcinogenesis* **7**, 1689–1695.

Lutz, W. K. (1986) Quantitative evaluation of DNA binding data for risk estimation and for classification of direct and indirect carcinogens. *J. Cancer Res. Clin. Oncol.* **112**, 85–91.

Lutz, W.K., Jaggi, W., L'thy, J., Sagelsdorff, P., and Schlatter, C. (1980) *In vivo* covalent binding of aflatoxin B_1 and aflatoxin M_1 to liver DNA of rat, mouse and pig. *Chem.-Biol. Interactions* **32**, 249–256.

Mah, M.C-M., Maher, V.M., Thomas, H., Reid, T.M., King, C.M., and McCormick, J.J. (1989) Mutations induced by aminofluorene-DNA adducts during replication in human cells. *Carcinogenesis* **10**, 2321–2328.

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.

Marien, K., Moyer, R., Loveland, P., Van Holde, K., and Bailey, G. (1987) Comparative binding and sequence interaction specificities of aflatoxin B_1, aflatoxicol, aflatoxin M_1, and aflatoxicol M_1 with purified DNA. *J. Biol. Chem.* **262**, 7455–7462.

Mattes, W.B., Hartley, J.A., Kohn, K.W., and Matheson, D.W. (1988) GC-rich regions in genomes as targets for DNA alkylation. *Carcinogenesis* **9**, 2065–2072.

Mazur, M. and Glickman, B.W. (1988) Sequence specificity of mutations induced by benzo[*a*]pyrene-7,8-diol-9,10-epoxide at endogenous *aprt* gene in CHO cells. *Somatic Cell Mol. Genet.* **14**, 393–400.

McMahon, G., Davis, E., and Wogan, G.N. (1987) Characterization of *c-Ki-ras* oncogene alleles by direct sequencing of enzymatically amplified DNA from carcinogen-induced tumors. *Proc. Natl Acad. Sci. USA* **84**, 4974–4978.

Metzger, G., Wilhelm, F.X., and Wilhelm, M.L. (1976) Distribution along DNA of the bound carcinogen *N*-acetoxy-*N*-2-acetylaminofluorene in chromatin modified *in vitro*. *Chem-Biol. Interactions* **15**, 257–265.

Metzger, G., Wilhelm, F.X., and Wilhelm, M.L. (1977) Non-random binding of a chemical carcinogen to the DNA in chromatin. *Biochem. Biophys. Res. Commun.* **75**, 703–710.

Milligan, J.R., and Archer, M.C. (1988) Alkylation of individual genes in rat liver by the carcinogen *N*-nitrosodimethylamine. *Biochem. Biophys. Res. Commun.* **155**, 14–17.

Mironov, N.M., Grover, P.L., and Sims, P. (1983) Preferential binding of polycyclic hydrocarbons to matrix-bound DNA in rat-liver nuclei. *Carcinogenesis* **4**, 189–193.

Misra, R.P., Muench, K.F., and Humayun, M.Z. (1983) Covalent and noncovalent interactions of aflatoxin with defined deoxyribonucleic acid sequences. *Biochemistry* **22**, 3351–3359.

Mommsen, S. and Aagaard, J. (1983) Tobacco as a risk factor in bladder cancer. *Carcinogenesis* **4**, 335–383.

Montesano, R., Brosil, H., Degan, P., Martel-Planche, G., Serres, M., and Wild, C.P. (1988) Detection in human cells of alkylated macromolecules attributable to exposure to nitrosamines. In: Bartsch, H., Hemminki, K., and O'Neill, I.K. (Eds) *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*. IARC Scientific Publications No. 89, pp. 75–82. International Agency for Research on Cancer, Lyon.

Moyer, G.H., Gumbiner, B., and Austin, G.E. (1977) Binding of *N*-hydroxy acetylaminofluorene to eu- and heterochromatin fractions of rat liver *in vivo*. *Cancer Lett.*

2, 259–266.

Muench, K.F., Misra, R.P., and Humayun, M.Z. (1983) Sequence specificity in aflatoxin-B_1-DNA interactions. *Proc. Natl Acad. Sci. USA* **80**, 6–10.

Nakayama, J., Yuspa, S.H., and Poirier, M.C. (1984) Benzo[*a*]pyrene-DNA adduct formation and removal in mouse epidermis *in vivo* and *in vitro*: Relationship of DNA binding to initiation of skin carcinogenesis. *Cancer Res.* **44**, 4087–4095.

Nehls, P., Rajewsky, M.F., Spiess, E., and Werner, D. (1984) Highly sensitive sites for guanine-O^6 ethylation in rat brain DNA exposed to *N*-ethyl-*N*-nitrosourea *in vivo*. *EMBO Journal* **3**, 327–332.

Neumann, H.G. (1983) Role of tissue exposure and DNA lesions for organ-specific effects of carcinogenic *trans*-4-acetylaminostilbene in rats. *Environ. Health Perspect.* **49**, 51–58.

Niranjan, B.G., Bhat, N.K., and Avadhani, N.G. (1982) Preferential attack of mitochondrial DNA by aflatoxin B_1 during hepatocarcinogenesis. *Science* **215**, 73–75.

Obi, F.O., Ryan, A.J., and Billett, M.A. (1986) Preferential binding of the carcinogen benzo[*a*]pyrene to DNA in active chromatin and the nuclear matrix. *Carcinogenesis* **7**, 907–913.

Parkes, H.G., and Evans, A.E.J. (1984) Epidemiology of aromatic amine cancers. In: Searle, C.E. (Ed) *Chemical Carcinogens*, ACS Monograph 182, pp. 277–301. American Chemical Society, Washington, DC.

Pegg, A.E. and Hoi, G. (1978) Formation and subsequent removal of O^6-methylguanine from dooxynucleic acid in rat liver and kidney after small doses of dimethylnitrosamine. *Biochem. J.* **173**, 739–748.

Pereira, M.A., Burns, F.J., and Albert, R.E. (1979) Dose response for benzo[*a*]pyrene adducts in mouse epidermal DNA. *Cancer Res.* **39**, 2556–2559.

Pereira, M.A., Lin, L-H.C., and Chang, L.W. (1981) Dose-dependency of 2-acetylamino-fluorene binding to liver DNA and hemoglobin in mice and rats. *Toxicol. Appl. Pharmacol.* **60**, 472–478.

Perera, F.P., Poirier, M.C., Yuspa, S.H., Nakayama, J., Jaretzki, A., Curnen, M.M., Knowles, D.M., and Weinstein, I.B. (1982) A pilot project in molecular cancer epidemiology: Determination of benzo[*a*]pyrene-DNA adducts in animal and human tissues by immunoassays. *Carcinogenesis* **3**, 1405–1410.

Perera, F.P., Santella, R.M., Brenner, D., Poirier, M.C., Munshi, A.A., Fischman, H.K., and Ryzin, J.V. (1987) DNA adducts, protein adducts, and sister chromatid exchange in cigarette smokers and nonsmokers. *J. Natl. Cancer Inst.* **79**, 449–456.

Perera, F.P., Hemminki, K., Young, T.L., Brenner, D., Kelly, G., and Santella, R.M. (1988) Detection of polycyclic aromatic hydrocarbon-DNA adducts in white blood cells of foundry workers. *Cancer Res.* **48**, 2288–2291.

Pèrin-Roussel, O., Barat, N., and Zajdela, F. (1988) Non-random distribution of dibenzo[*a*,*e*]fluoranthene-induced DNA adducts in DNA loops in mouse fibroblast nuclei. *Carcinogenesis* **9**, 1383–1388.

Phillips, D.H., Grover, P.L., and Sims, P. (1978) The covalent binding of polycyclic hydrocarbons to DNA in the skin of mice of different strains. *Int. J. Cancer* **22**, 487–494.

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.

Poirier, M.C. (1984) The use of carcinogen–DNA adduct antisera for quantitation and localization of genomic damage in animal models and the human population. *Environ. Mutagen.* **6**, 879–887.

Poirier, M.C., Hunt, J.M., True, B., Laishes, B.A., Young, J. F., and Beland, F.A. (1984)

DNA adduct formation, removal and persistence in rat liver during one month of feeding 2-acetylaminoflourene. *Carcinogenesis* **5**, 1591–1596.

Poirier, M.C., Beland, F.A., Deal, F.H., and Swenberg, J.A. (1989) DNA adduct formation and removal in specific liver cell populations during chronic dietary administration of 2-acetylaminofluorene. *Carcinogenesis* **10**, 1143–1145.

Preussmann, R. and Eisenbrand, G. (1984) *N*-Nitroso carcinogens in the environment. In: Searle, C.E. (Ed) *Chemical Carcinogens*, Vol. 2, ACS Monograph 182, pp. 829–868. American Chemical Society, Washington, DC.

Preussmann, R., and Stewart, B.W. (1984) *N*-Nitroso carcinogens. In: Searle, C.E. (Ed) *Chemical Carcinogens*, Vol. 2, ACS Monograph 182, pp. 643–828. American Chemical Society, Washington, DC.

Quintanilla, M., Brown, K., Ramsden, M., and Balmain, A. (1986) Carcinogen-specific mutation and amplification of *Ha-ras* during mouse skin carcinogenesis. *Nature* **322**, 78–80.

Ramanathan, R., Rajalakshmi, S., and Sarma, D.S.R. (1976) Non-random nature of *in vivo* interaction of ^{3}H-*N*-hydroxy-2-acetylaminofluorene and its subsequent removal from rat liver chromatin-DNA. *Chem.-Biol. Interactions* **14**, 375–377.

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.

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-postlabelling assay. *J. Natl Cancer Inst.* **81**, 341–347.

Richardson, F.C., Dyroff, M.C., Boucheron, J.A., and Swenberg, J.A. (1985) Differential repair of O^4-alkythymidine following exposure to methylating and ethylating hepatocarcinogens. *Carcinogenesis* **6**, 625–629.

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.

Ryan, A.J., Billett, M.A., and O'Connor, P.J. (1986) Selective repair of methylated purines in regions of chromatin DNA. *Carcinogenesis* **7**, 1497–1503.

Saffhill, R. and Abbott, P.J. (1978) Formation of O^2-methylthymine in poly(dA-dT) on methylation with *N*-methyl-*N*-nitrosourea and dimethyl sulphate. Evidence that O^2-methylthymine does not miscode during DNA synthesis. *Nucleic Acids Res.* **5**, 1971–1978.

Saffhill, R., Badawi, A.F., and Hall, C.N. (1988) Detection of O^6-methylguanine in human DNA. In: Bartsch, H., Hemminki, K., and O'Neill, I.K. (Eds) *Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention*, IARC Scientific Publications No. 89, pp. 301–305. International Agency for Research on Cancer, Lyon.

Scherer, E., Steward, A.P., and Emmelot, P. (1977) Kinetics of formation of O^6-ethylguanine in, and its removal from liver DNA of rats receiving diethylnitrosamine. *Chem.-Biol. Interactions* **19**, 1–11.

Schwartz, E.L. and Goodman, J.I. (1979) Quantitative and qualitative aspects of the binding of *N*-hydroxy-2-acetylaminofluorene to hepatic chromatin fractions. *Chem.-Biol. Interactions* **26**, 287–303.

Seidman, L.A., Moore, C.J., and Gould, M.N. (1988) ^{32}P-Postlabelling analysis of DNA adducts in human and rat mammary epithelial cells. *Carcinogenesis* **9**, 1071–1077.

Shamsuddin, A.K.M., Sinopoli, N.T., Hemminki, K., Boesch, R.R., and Harris, C.C. (1985) Detection of benzo[*a*]pyrene: DNA adducts in human white blood cells. *Cancer Res.* **45**, 66–68.

Singer, B. and Grunberger, D. (1983) *Molecular Biology of Mutagens and Carcinogens*, pp. 45–96. Plenum Press, New York.

Slaga, T.J., Bracken, W.J., Gleason, G., Levin, W., Yagi, H., Jerina, D.M., and Conney, A.H. (1979) Marked differences in the skin tumor-initiating activities of the optical enantiomers of the diastereomeric benzo[*a*]pyrene 7,8-diol-9,10-epoxides. *Cancer Res.* **39**, 67–71.

Stark, A.A., Essigmann, J.M., Demain, A.L., Skopek, T.R., and Wogan, G.N. (1979) Aflatoxin B_1 mutagenesis, DNA binding, and adduct formation in *Salmonella typhimurium*. *Proc. Natl Acad. Sci. USA* **76**, 1343–1347.

Stowers, S.J., and Anderson, M.W. (1985) Formation and persistence of benzo[*a*]pyrene metabolite-DNA adducts. *Environ. Health Perspect.* **62**, 31–39.

Surgeon General (1982) *The Health Consequences of Smoking. Cancer. A Report of the Surgeon General*, DHHS (PHS) Publ. No. 82-50179, pp. 193–233. US Department of Health and Human Services, Public Health Service, Office of Smoking and Health, Rockville, Maryland.

Swenberg, J.A., Rickert, D.E., Baranyi, B.L., and Goodman, J.I. (1983) Cell specificity in DNA binding and repair of chemical carcinogens. *Environ. Health Perspectives* **49**, 155–163.

Talaska, G., Au, W.W., Ward, Jr., J.B., Randerath, K., and Legator, M.S. (1987) The correlation between DNA adducts and chromosomal aberrations in the target organ of benzidine exposed, partially-hepatectomized mice. *Carcinogenesis* **8**, 1899–1905.

Talaska, G., Dooley, K.L., and Kadlubar, F.F. (1990) Detection and characterization of carcinogen-DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by ^{32}P-postlabelling and subsequent thin-layer and high pressure liquid chromatography. *Carcinogenesis*, **11**, 639–646.

Tokiwa, H. and Ohnishi, Y. (1986) Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *CRC Crit. Rev. Toxicol.* **17**, 23–60.

Ueno, I., Friedman, L., and Stone, C.L. (1980) Species difference in the binding of aflatoxin B_1 to hepatic macromolecules. *Toxicol. Appl. Pharmacol.* **52**, 177–180.

Ueyama, H., Matsuura, T., Nomi, S., Nakayasu, H., and Ueda, K. (1981) Binding of benzo[*a*]pyrene to rat liver nuclear matrix. *Life Sci.* **29**, 655–661.

Umbenhauer, D., Wild, C.P., Montesano, R., Saffhill, R., Boyle, J.M., Huh, N., Kirstein, U., Thomale, J., Rajewsky, M.F., and Lu, S.H. (1985) O^6-Methyl deoxyguanosine in oesophageal DNA among individuals at high risk of oesophageal cancer. *Int. J. Cancer* **36**, 661–665.

Vousden, K.H., Bos, J.L., Marshall, C.J., and Phillips, D.H. (1986) Mutations activating human c-*Ha-ras1* protooncogene (*HRAS1*) induced by chemical carcinogens and depurination. *Proc. Natl Acad. Sci. USA* **83**, 1222–1226.

Walker, M.S., Becker, F.F., and Rodriguez, L.V. (1979) *In vivo* binding of *N*-2-acetylaminofluorene and its *N*-hydroxy derivative to the DNA of fractionated rat liver chromatin. *Chem.-Biol. Interactions* **27**, 177–190.

Westra, J.G., Visser, A., and Tulp, A. (1983) Binding and repair of 2-acetylaminofluorene adducts in distinct liver cell populations. *Environ. Health Perspect.* **49**, 87–92.

Wild, C.P., Garner, R.C., Montesano, R., and Tursi, F. (1986) Aflatoxin B_1 binding to plasma albumin and liver DNA upon chronic administration to rats. *Carcinogenesis* **7**,

853–858.

Wilson, V.L., Weston, A., Manchester, D.K., Trivers, G.E., Roberts, D.W., Kadlubar, F.F., Wild, C.P., Montesano, R., Willey, J.C., Mann, D.L., and Harris, C.C. (1989) Alkyl and aryl carcinogen adducts detected in human peripheral lung. *Carcinogenesis*, **10**, 2149–2153.

Winn, D.M. (1986) Smokeless tobacco and oral/pharynx cancer: the role of cofactors. In: Hoffmann, D. and Harris, C.C. (Eds) *Mechanisms in Tobacco Carcinogenesis*, Banbury Report 23, pp. 361–375. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Wiseman, R.W., Stowers, S.J., Miller, E.C., Anderson, M.W., and Miller J.A. (1986) Activating mutations of the *c-Ha-ras* protooncogene in chemically induced hepatomas of the male B6C3 F1 mouse. *Proc. Natl Acad. Sci. USA* **83**, 5825–5829.

Wood, A.W., Chang, R.L., Levin, W., Yagi, H., Thakker, D.R., van Bladeren, P.J., Jerina, D.M., and Conney, A.H. (1983) Mutagenicity of the enantiomers of the diastereomeric bay-region benz(*a*)anthracene 3,4-diol-1,2-epoxides in bacterial and mammalian cells. *Cancer Res.* **43**, 5821–5825.

Yang, J-L., Maher, V.M., and McCormick, J.J. (1987) Kinds of mutations formed when a shuttle vector containing adducts of (+)-7ß,8α-dihydroxy-9α,10α-7 epoxy, 8,9,10-tetrahydrobenzo[*a*]pyrene replicates in human cells. *Proc. Natl. Acad. Sci. USA* **84**, 3787–3791.

Yang, J-L., Maher, V.M., and McCormick, J.J. (1988) Kinds and spectrum of mutations induced by 1-nitrosopyrene adducts during plasmid replication in human cells. *Mol. Cell. Biol.* **8**, 3364–3372.

Zarbl, H., Sukumar, S., Arthur, A.V., Martin-Zanca, D., and Barbacid, M. (1985) Direct mutagenesis of *Ha-ras-1* oncogenes by *N*-nitroso-*N*-methylurea during initiation of mammary carcinogenesis in rats. *Nature* **315**, 382–386.