4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Toxicokinetics

4.1.1 *Absorption, distribution, metabolism and excretion*

(a) Overview

This section provides an overview of the toxicokinetics of polycyclic aromatic hydrocarbons (PAHs). Other more comprehensive reviews of the toxicokinetics of PAHs include those by the Environmental Protection Agency (1991), the Agency for Toxic Substances and Disease Registry (ATSDR, 1995) and the International Programme on Chemical Safety (IPCS, 1998), as well as reviews on the metabolism and bioactivation of PAHs by Conney (1982), Cooper *et al*. (1983), Shaw and Connell (1994), Penning *et al*. (1999), Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2005) and Xue and Warshawsky (2005). Little is known about the toxicokinetics of mixtures of or individual PAHs in humans. Multiple studies have been conducted to monitor urinary metabolites of PAHs and PAH–DNA adducts in the lymphocytes of workers exposed to mixtures of PAHs. However, most of the available data on toxicokinetic parameters for PAHs derive from studies of benzo[*a*]pyrene in animals.

Because of their lipophilicity, PAHs dissolve into and are transported by diffusion across lipid/lipoprotein membranes of mammalian cells, thus facilitating their absorption by the respiratory tract, gastrointestinal tract and skin. PAHs with two or three rings can be absorbed more rapidly and extensively than those with five or six rings. Once absorbed, PAHs are widely distributed throughout the body, with some preferential distribution to or retention in fatty tissues. They are rapidly metabolized to more soluble metabolites (epoxides, phenols, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, quinones and tetrols), and conjugates of these metabolites are formed with sulfate, glutathione (GSH) or glucuronic acid. The covalent binding of reactive PAH metabolites to form DNA adducts may represent a key molecular event in the formation of mutations and the initiation of cancer. The structures of the DNA adducts that are formed provide an inference of the precursor metabolites. PAHs are eliminated from the body principally as conjugated metabolites in the faeces, via biliary excretion, and in the urine.

Most PAHs with potential biological activity range in size from two to six fused aromatic rings. Because of this vast range in molecular weight, several of the physicochemical properties that are critical to their biological activity vary greatly. Five properties in particular have a decisive influence on the biological activity of PAHs: their vapour pressure, their adsorption onto surfaces of solid carrier particles, their absorption into liquid carriers, their lipid/aqueous partition coefficient in tissues and their limits of solubility in the lipid and aqueous phases of tissues.

These properties are intrinsically linked with the metabolic activation of the most toxic PAHs, and an understanding of the nature of this interaction may facilitate the interpretation of studies on their deposition and disposition that are occasionally conflicting.

Transporters may play a role in the biological activity of PAHs. Adenosine triphosphate (ATP)-binding cassette (ABC) transporters (49 genes characterized in humans) transport specific molecules across lipid membranes including hydrophobic compounds and metabolites (Schinkel & Jonker, 2003). P-Glycoprotein transports mainly non-metabolized compounds and multidrug resistance-associated protein-1 (MRP1) and -2 conjugates of foreign compounds (Leslie *et al.*, 2001; Haimeur *et al.*, 2004). Several ABC transporters are polymorphic (Sakaeda *et al.*, 2004). Benzo[*a*]pyrene conjugates may be substrates for ABC transporters, such as the GSH conjugate of benzo[*a*]pyrene-7,8-diol-9,10-oxide which is a substrate for MRP2 (Srivastava *et al.*, 2002) and benzo-[*a*]pyrene-3-glucuronide which is a substrate for breast cancer resistance protein (BCRP) (Ebert *et al.*, 2005). PAHs are ubiquitous in the environment due to volcanic eruptions and forest fires, and their presence in various media (i.e. air and soil) constitutes a background level of exposure. Additional exposure occurs through the ingestion of grilled or cured meats (see Section 1). These exposures should be taken into consideration when assessing health risks due to exposure to PAHs.

(b) Absorption through the respiratory tract

Vapour pressure is a major determinant for the distribution of a PAH between the particulate and gaseous phase of the aerosol by which the substance is emitted into the atmosphere. The vapour pressure of PAHs decreases drastically with increasing molecular weight (Lohmann & Lammel, 2004), so that two-ringed naphthalenes are mostly found in the gas phase whereas five-ringed PAHs such as benzo[*a*]pyrene are mostly adsorbed on airborne particles at room temperature (Lane & Gundel, 1996). Strong sorption of a PAH onto particles can further increase the particle-bound fraction of that substance (Lohmann & Lammel, 2004). Because the most carcinogenic PAHs of greater size are, to a large degree, particle-associated, there is considerable potential for covariance with an inflammatory response that is induced by the carrier particles alone. Typical carbonaceous carrier particles of PAHs that have no adsorbed genotoxic material have been shown to be carcinogenic, particularly in rats (see IARC, 2010). This mechanism is, however, outside the scope of this monograph. Gas/particle partitioning is also of great importance during inhalation exposure in order to determine the probable sites of deposition within the respiratory tract. The smaller gaseous PAHs are deposited mostly as soluble vapours, whereas five- to six- ringed aromatic compounds are mostly particle-associated at ambient temperatures and can be expected to be deposited with the carrier particles. The rate and extent of absorption by the respiratory tract of PAHs from PAH-containing particles are dependent on particle size (i.e. aerodynamic diameter, which influences regional deposition in the respiratory tract) and the rate of release of PAHs from the particle. Because the release of PAHs from particles is extraneous in

exposure to vapours, the rate and extent of absorption of inhaled vapour-phase PAHs are different from those of particle-bound PAHs.

After deposition in the respiratory tract, the sorptive properties of PAHs are a major determinant for the bioavailability of the substance in the organism. The timing of the release from carrier particles in particular affects the toxicity of inhaled PAHs at the site of entry. For solid particles, the major determinant for the release is the rate of desorption of the hydrocarbons from the surface, whereas for liquid aerosols, either the dissolution of the entire particle or desorption from insoluble carrier particles is a decisive factor. A rapid release from carrier particles gives a close correlation between the deposition pattern of inhaled aerosols and the site-of-entry exposures to particle-associated PAHs. Slower release alters the exposures, and shows a clearance pattern of inhaled particles. Substantial fractions of inhaled PAHs deposited in the tracheobronchial region and upper airways can be redistributed by the mucociliary escalator to the gastrointestinal tract, which thereby changes the exposure route from inhalation to ingestion (Sun *et al*., 1982).

Following deposition and desorption from their carrier particles, PAHs are absorbed through the epithelial barriers onto which they are deposited. The slow diffusion of highly lipophilic substances into the tissues is fundamental to the behaviour of PAHs in biological systems. This is a strictly physicochemical mechanism that needs to be considered in all measurements of the kinetics of PAHs in tissues. A highly lipophilic substance dissolves readily in the first lipid membrane it encounters, but is then transported slowly into the next layer (Gerde *et al*., 1993a). This is due to the low concentration of lipophilic solute in the aqueous gaps between cell membranes, which results in a high concentration of solute in the epithelium at the site of entry, comparatively slow absorption into the circulation and a low concentration in all tissues distal to the site of entry. The absorption process is strongly dependent on the lipophilicity of the PAHs and their metabolites: with higher lipophilicities, the mobility of the substances by diffusion into tissues is lower and, with thicker entrance epithelium, the half-life of absorption into the capillary bed of the submucosa is longer. Highly lipophilic PAHs that are released from particles deposited in the conducting and bronchial airways are retained for several hours and absorbed slowly by a diffusion-limited process, whereas PAHs that are released from particles in alveolar airways are absorbed within minutes (Gerde *et al*., 1991a,b; 1993a,b,c; Gerde & Scott, 2001). The relative thickness of the epithelium of the conducting airways compared with the thin epithelium of the alveolar region has been proposed as a contributing factor to this regional difference in duration of absorption following deposition. Slow absorption through the epithelium of the conducting airways probably leads rapidly to saturation in the mucous lining layer and airway epithelium with increasing levels of exposure (Gerde *et al.*, 1991a,b). A probable consequence is an increase in the fraction of undissolved/undesorbed PAHs that is transported to the gastrointestinal tract by the mucociliary escalator.

A major effect of the metabolic conversion of PAHs of lower molecular weight is to decrease their lipophilicity and thus accelerate their mobility in tissues (Gerde *et al*., 1997). Phase I metabolites are slightly more mobile and phase II metabolites are

considerably more mobile than the parent compound. As a result, the overall effect of metabolism in the epithelium at the site of entry is to accelerate transport of a lipophilic substrate into the circulation and thereby directly decrease high, acute exposures to this particular epithelial cell population. This local metabolism in airway epithelia probably explains the high levels of benzo[*a*]pyrene-related DNA adducts that have been measured in pure preparations of bronchial epithelial cells from patients with lung cancer (Rojas *et al.*, 2004).

The low mobility of the highly lipophilic PAHs in tissues is an important factor that complicates the toxicokinetics/pharmacokinetics of such substances. The fundamental condition of perfusion-limited compartments in physiologically based pharmacokinetic models does not hold for highly lipophilic PAHs. According to this condition, a solute is in equilibrium between the tissues of an organ and the blood stream that leaves that organ (Bischoff & Dedrick, 1970). However, because of low mobility, the lipophilic solute does not have time to reach equilibrium before the blood exits the tissue. This effect is more pronounced for slowly perfused tissues. A typical example of such a tissue is adiposal fat which, despite a very high tissue/blood partition coefficient, contains low concentrations of PAHs shortly after exposure but has the highest relative concentrations longer after exposures (Withey *et al*., 1993a). For physiologically based pharmacokinetic models to predict better the systemic distribution of PAHs and their metabolites, the delayed equilibration between blood and tissue needs to be taken into account.

Another aspect of modelling that needs further study is an accurate description of site-of-entry dosimetry. Because of diffusion-limited absorption, the local dose to the epithelium of the site of entry is probably considerably higher than that predicted by the well-mixed compartment assumption in classical physiologically based pharmacokinetic models. Even if the absorption rates in classical models are adjusted with an empirical mass-transfer coefficient from the environment to the blood, the models cannot predict the limited distribution of the highly exposed cell populations at the site of entry. Conversely, models that describe site-of-entry dosimetry with greater resolution can predict both absorption rates and local tissue doses with reasonable accuracy (Gerde & Scott, 2001), but such models are rather complex and cannot describe the systemic distribution of the solutes in any detail. For these reasons, no validated physiologically based pharmacokinetic models for the deposition, absorption and systemic distribution of PAHs are available.

Results from studies of rats exposed by inhalation to radiolabelled benzo[*a*]pyrene indicate that inhaled PAHs can be rapidly absorbed by the respiratory tract (for reviews, see Environmental Protection Agency, 1991; ATSDR, 1995; IPCS, 1998). For example, in a study in which pregnant rats were exposed to 200 and 800 mg/m^3 [^{14}C]-labelled benzo[*a*]pyrene aerosol for 95 min on gestation day 17, radiolabel was detected, as the parent compound and metabolites, in maternal blood samples collected immediately after exposure (Withey *et al*., 1993a). The detection of high levels of radioactivity in the lung and liver of these rats is consistent with the occurrence of mucociliary clearance from the

respiratory tract and ingestion into the oesophagus and gastrointestinal tract, as well as rapid absorption, metabolism and biliary excretion of metabolites (Withey *et al.*, 1994).

The relatively longer retention of PAHs released in the conducting airways (compared with the air-exchange region) may allow for substantial metabolism within this region of deposition. In a study in which anaesthetized dogs were instilled intratracheally with single doses of [^{3}H]-labelled benzo[*a*]pyrene dissolved in a saline/phospholipid suspension, absorption into the trachea showed a half-time of about 73 min (Gerde *et al*., 1997). Three hours after instillation, a total of about 90% of the radioactivity retained in the tracheal epithelium was associated with metabolites of benzo[*a*]pyrene. Results from similar experiments with pyrene indicated that, although pyrene is more rapidly absorbed in the tracheal mucosa than benzo[*a*]pyrene, significant metabolism occurred within the tracheal epithelium (Gerde *et al*., 1998). Similar results that indicate extensive metabolism in the lung have been reported for rats exposed by inhalation for 4 h to aerosols of carbon black with adsorbed benzo[*a*]pyrene (Ramesh *et al*., 2001). In these experiments, only metabolites of benzo[*a*]pyrene, and not the parent material, were detected in lung tissue sampled at 30, 60, 120 or 240 min after cessation of exposure.

(c) Absorption through the gastrointestinal tract

PAHs are absorbed by the gastrointestinal tract via both diffusion across cellular membranes, based on their lipophilicity, and normal absorption of dietary lipids (O'Neill *et al*., 1991). Absorption of specific PAHs, such as benzo[*a*]pyrene, has been demonstrated following oral administration of radiolabelled compounds to laboratory animals (for review, see Environmental Protection Agency, 1991; ATSDR, 1995; IPCS, 1998). Results from animal studies indicate that absorption is rapid (Rees *et al*., 1971; Modica *et al*., 1983), that fractional absorption of lower-molecular-weight PAHs, such as two-ringed naphthalene, may be more complete than that of higher-molecular-weight PAHs, such as five-ringed benzo[*a*]pyrene (Chang, 1943; Modica *et al*., 1983), and that the presence of other materials, such as bile salts or components of the diet, can influence the rate or extent of absorption of PAHs from the intestine (Rahman *et al*., 1986).

(d) Absorption through the skin

Evidence of the dermal absorption of PAHs includes the detection of elevated levels of PAH metabolites, such as 1-hydroxypyrene, in the urine of humans exposed dermally to complex mixtures of PAHs, such as coke-oven emissions in the workplace (van Rooij *et al*., 1993a) or coal-tar ointments (van Rooij *et al*., 1993b; Godschalk *et al*., 1998). Results from animal studies indicate that dermal absorption of PAHs can be rapid and extensive. For example, the reported half-times of disappearance of radiolabel from the skin of rats treated with 2, 6 or 15 mg/kg [^{14}C]-labelled pyrene in acetone applied to a 4-cm^2 area ranged from about 0.5 to 0.8 days, and about 50% of the applied radiolabel was recovered in urine and faeces collected within 6 days of application (Withey *et al*., 1993b).

(e) Distribution

Results from studies in rats indicate that: (i) absorbed PAHs are widely distributed to most organs and tissues; (ii) fatty tissues can serve as storage sites to which PAHs may be gradually absorbed and from which they are then released; and (iii) the gastrointestinal tract can contain high levels of PAHs and their metabolites following exposure (by any route) due to mucociliary clearance from the respiratory tract and hepatobiliary excretion of metabolites (Mitchell & Tu, 1979; Mitchell, 1982, 1983; Sun *et al*., 1984; Withey *et al*., 1991,1993a). For example, immediately after a 95-min inhalation exposure of pregnant rats to [^{3}H]-labelled benzo[*a*]pyrene aerosols, levels of radiolabel associated with parent compound in the tissues decreased in the following order: lung > blood > liver > kidney > fat > fetus; 6 h after cessation of exposure, the order was fat > lung > kidney > liver > blood > fetus (Withey *et al*., 1993a). Similarly, about 7 h after a 95-min inhalation exposure of male rats to [^{14}C]-labelled pyrene aerosol at a concentration of 800 mg/m^{3}, levels of radiolabel associated with parent compound decreased in the following order: fat > kidney > lung > liver > testes > spleen > brain (Withey *et al*., 1994).

In other studies that involved inhalation exposure of rats for 1 h to [^{3}H]-benzo[*a*]-pyrene aerosols (500 μg/L; mass median aerodynamic diameter [MMAD], 1–2 μm), clearance of radiolabel from the respiratory tract was biphasic, with 50% of the radiolabel cleared within 2–3 h (Mitchell, 1982). Elimination half-times for radiolabel that remained in the lung after this time ranged from about 25 to 50 h, depending on the location in the lung. Half an hour after termination of exposure, concentrations of radiolabel were higher in the stomach and small intestine than in any other tissue, although significant amounts were detected in the liver and kidneys. During the first day after exposure, the amount of radiolabel in the faeces was about 10-fold greater than that in the urine. In experiments with rats exposed to pyrene aerosols (500 μg/L; MMAD, 0.3–0.8 μm), similar rapid clearance from the respiratory tract and distribution to the gastrointestinal tract, liver and kidneys was observed (Mitchell & Tu, 1979).

In experiments with rats exposed by inhalation to radiolabelled benzo[*a*]pyrene adsorbed onto ultrafine particles (e.g. gallium oxide or diesel exhaust particles; MMAD, ~0.1 μm), similar rapid clearance from the respiratory tract was observed. The relative amount of radiolabel in the stomach was higher following exposure to benzo[*a*]pyrene adsorbed onto gallium oxide or diesel exhaust particles than after exposure to pure aerosols of benzo[*a*]pyrene (Sun *et al*., 1982, 1984). In rats exposed to [^{3}H]-benzo[*a*]-pyrene-coated diesel engine exhaust particles (MMAD, ~0.14 μm; Sun *et al*., 1984), the amounts of radiolabel retained in the lung during the second, slow phase of lung clearance were higher than those in the study of gallium oxide-associated ^{3}H-benzo[*a*]pyrene study (Sun *et al*., 1982). [The results from these studies are consistent with the concept that PAHs are, in general, cleared rapidly from the initial sites of deposition in the respiratory tract and distributed to a significant extent in the gastrointestinal tract, liver and kidney; the kinetics and patterns of distribution, however, can be influenced by size and compo-

sitional characteristics of the particulate matter, as well as by the chemical properties of the PAHs themselves.]

(f) Metabolism

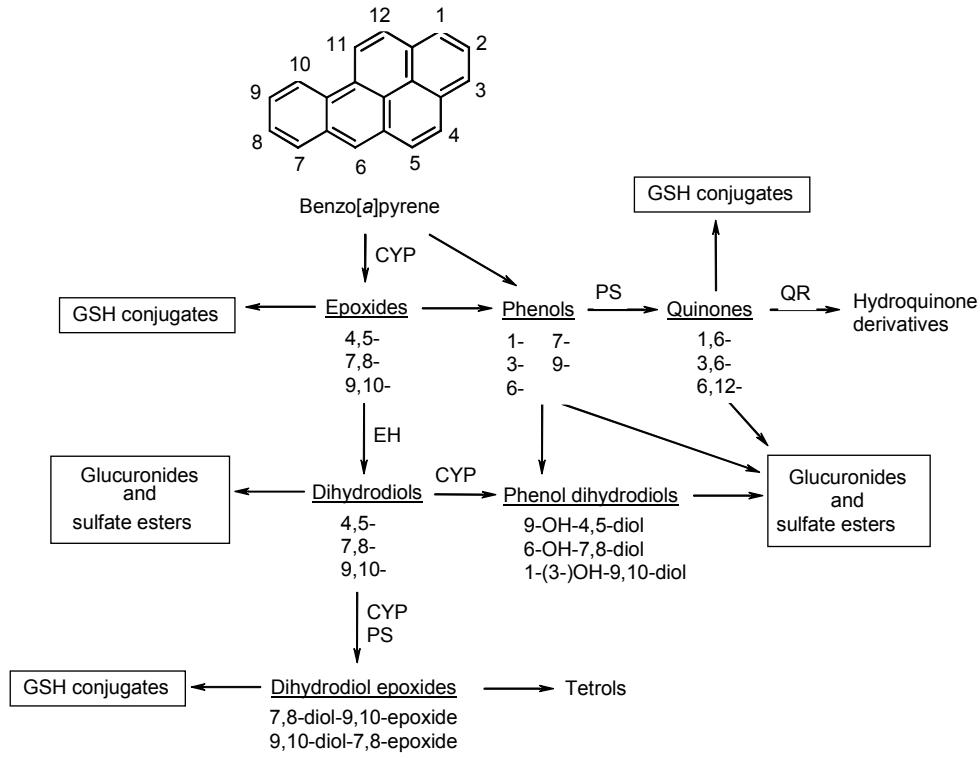
The metabolism of benzo[*a*]pyrene has been studied extensively in human and animal tissues, and generally serves as a model for the metabolism of other PAHs (for review, see Environmental Protection Agency, 1991; ATSDR 1995; IPCS 1998). A metabolic schema for benzo[*a*]pyrene is presented in Figure 4.1 which shows pathways to the formation of epoxides, phenols, quinones, hydroquinones, dihydrodiols, phenol dihydrodiols, dihydrodiol epoxides, tetrols and other potentially reactive intermediates. Table 4.1 contains examples of PAHs and PAH metabolites that are substrates for human enzymes. The tissue localization of human enzymes indicates that metabolic activation can occur in key target organs. It should be noted that differences between in-vivo and in-vitro studies of metabolism have been observed in some cases for benzo[*a*]pyrene and other PAHs.

The mechanism of PAH oxidation by cytochrome P450 (CYP) mono-oxygenase is complex and can involve an odd electron abstraction rebound mechanism as well as a one-electron radical cation mechanism (Cavalieri *et al.*, 1988; Guengerich, 2001; Cavalieri & Rogan, 2002; Mulder *et al.*, 2003).

Benzo[*a*]pyrene is initially metabolized by CYP mono-oxygenases to several epoxides. CYP1A1 can metabolize a wide range of PAHs, but other CYPs, including CYP1A2 and members of the CYP1B, CYP2B, CYP2C and CYP3A families of enzyme, have been demonstrated to catalyse the initial oxidation of benzo[*a*]pyrene and other PAHs to varying extents (for review, see IPCS, 1998; Xue & Warshawsky, 2005). PAHs are recognized inducers of CYP enzymes, and exposure to PAHs can therefore influence the balance of phase I and phase II enzymes, which can determine whether or not a toxic cellular response occurs. The mammalian CYP genes that encode CYP1A1, 1A2 and 1B1 are regulated in part by the aryl hydrocarbon receptor (AhR). Differences in AhR affinities in inbred mice correlate with variations in the inducibility of CYP and may be associated with differences in the risk for cancer from PAHs (Nebert *et al.*, 2004). A correlation between the variability in AhR affinity in humans and differences in cancer risk remains unproven. Therefore, the role of CYP in activation versus detoxification probably depends on multiple factors such as the subcellular content and location, the degree of phase II metabolism and the pharmacokinetics of the chemical.

Epoxides may rearrange spontaneously to phenols, be hydrated via epoxide hydrolase catalysis to dihydrodiols or be conjugated with GSH, either spontaneously or via GSH-*S*-transferase (GST) catalysis. It has been proposed that the formation of 1-, 3- and 6-hydroxybenzo[*a*]pyrene from benzo[*a*]pyrene and their subsequent conversion to quinones involve CYP isoforms (Cavalieri *et al.*, 1988) and 6-hydroxybenzo[*a*]pyrene can also be formed by prostaglandin H synthase (Cooper *et al.*, 1983; for a review, see IPCS, 1998). Quinones can be converted to hydroquinone derivatives by quinone reductase or be conjugated with GSH, sulfate or glucuronic acid.

Figure 4.1. Metabolic schema for benzo[*a*]pyrene



Adapted from Cooper *et al.* (1983); ATSDR (1995); IPCS (1998).
CYP, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; PS, prostaglandin H synthase; QR, quinone reductase

Dihydrodiol derivatives can be further oxidized by CYPs to form phenol dihydrodiols or dihydrodiol epoxides. Phenols, phenol dihydrodiols and dihydrodiols can be conjugated with glucuronic acid or sulfate. Dihydrodiol epoxides may also be formed from dihydrodiols by reaction with peroxyl radicals generated from the oxidative biosynthesis of prostaglandins from fatty acids via prostaglandin H synthase (Marnett, 1981, 1987; Reed *et al.*, 1988; Eling *et al*., 1990). The metabolic fate of dihydrodiol epoxides includes conjugation with GSH or covalent modification of cellular macromolecules that possibly lead to mutagenic and carcinogenic responses.

Dihydrodiols may also be metabolized to *ortho*-quinones by aldo-keto reductases (AKR1C1–AKR1C4, AKR1A1). *ortho*-Quinone derivatives have been demonstrated *in vitro* to produce, via redox cycling with nicotinamide adenine dinucleotide (phosphate) (with or without phosphate) (NAD(P)H) and copper, reactive oxygen species that cause

Table 4.1. PAHs and PAH metabolites as substrates of human enzymes and localization in human tissues outside the liver (extrahepatic metabolism)

Enzyme	PAH substrates	Human tissues
CYP1A1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene and its 11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene and its 7,8-diols Cyclopenta[*cd*]pyrene Dibenzo[*a*,*l*]pyrene Dibenzo[*a*,*l*]pyrene-11,12-diol 5-Methylchrysene 5-Methylchrysene-1,2-diol	Lung of smokers (mRNA, prot., act.). Oesophagus Stomach Small intestine Colon Skin Placenta of smokers Fetal liver
CYP1A2	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene-7,8-diol Cyclopenta[*cd*]pyrene Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene and its 11,12-diol 5-Methylchrysene-1,2-diol	Lung (mRNA +/–, prot., +/–, no act.) Oesophagus Stomach Colon
CYP1B1	Benzo[*g*]chrysene-11,12-diol Benzo[*b*]fluoranthene-9,10-diol Benzo[*a*]pyrene and its 7,8-diol Dibenzo[*a*,*l*]pyrene Dibenzo[*a*,*l*]pyrene-11,12-diol 5-Methylchrysene-1,2-diol	Lung (mRNA, prot., act.) Small intestine Colon Skin Fetal liver
CYP2A		Oesophagus
CYP2A6		Lung (mRNA, prot. +/–, no act.) Nasal mucosa Trachea
CYP2A13[a, b, c]		Nasal mucosa (highest expression) Trachea Lung
CYP2B6	Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene	Lung (mRNA, prot., act.) Nasal mucosa Trachea
CYP2C	Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene	Lung (mRNA +/–, prot. +/–) Nasal mucosa Stomach Small intestine Early placenta Fetal liver (2C8)

Table 4.1 (contd)

Enzyme	PAH substrates	Human tissues
CYP2D6		Lung (mRNA +/–, prot. +/–, no act.) Small intestine Early placenta Fetal liver
CYP2E1	Dibenz[*a*,*h*]anthracene	Lung (mRNA, prot., act.) Oesophagus Small intestine Fetal liver
CYP2F1[a]		Lung (mRNA)
CYP2J2		Lung (mRNA, prot., act.) Nasal mucosa Oesophagus Stomach Small intestine Colon
CYP2S1[a]		Lung (mRNA, prot.) Trachea Stomach Small intestine
CYP3A	Dibenz[*a*,*h*]anthracene (3A3)	Nasal mucosa
CYP3A4	Cyclopenta[*c*,*d*]pyrene Dibenz[*a*,*h*]anthracene Dibenzo[*a*,*l*]pyrene 5-Methylchrysene	Lung (mRNA +/–, prot.) Stomach Small intestine Colon Fetal liver
CYP3A5[a,d]	Dibenz[*a*,*h*]anthracene	Lung (mRNA, prot., act.) Oesophagus Small intestine Colon Kidney Fetal liver
CYP3A7		Lung (mRNA +/–) Early placenta Fetal liver
CYP4B1[a]		Lung (mRNA, prot.) Placenta
PS	Benzo[*a*]pyrene Cyclopenta[*cd*]pyrene	Most tissues

Table 4.1 (contd)

Enzyme	PAH substrates	Human tissues
Aldo-keto-reductase (AKR)	Benz[*a*]anthracene Benzo[*g*]chrysene Benzo[*a*]pyrene 5-Methylchrysene	Most tissues
AKR1C1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon[1], bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], ovary[1]
AKR1C2	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon, bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], prostate[1]
AKR1C3	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol-	Liver Hep G2 cells[1,2,3] Lung adenocarcinoma A549 cells[1,2,3] Trachea[1], colon[1], bladder[1], small intestine[1], heart[1], aorta[1], stomach[1], testis[1], mammary gland, prostate[1]
AKR1C4	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Liver-specific[1,2,3]
AKR1A1	Benz[*a*]anthracene-3,4-diol Benzo[*g*]chrysene-11,12-diol Benzo[*c*]phenanthrene-3,4-diol Benzo[*a*]pyrene-7,8-diol 5-Methylchrysene-7,8-diol	Kidney, liver[1], salivary gland[1], trachea[1], stomach[1], duodenum[1], pancreas, fetal lung[1], prostate[1], placenta[1], mammary gland[1], lung[1], lung adenocarcinoma[1], bladder[1], oesophagus[1], ovary[1], testis[1]

From Hakkola *et al.* (1998a,b); Palackal *et al.* (2001a); Hukkanen *et al.* (2002); Palackal *et al.* (2002a); Ding & Kaminsky (2003); Steckelbroeck *et al.* (2004); Shimada & Fujii-Kuriyama (2004)
+/–, conflicting evidence; act., catalytic activities; CYP, cytochrome P450; mRNA, messenger ribonucleicacid; PAH, polycyclic aromatic hydrocarbon; prot., protein; PS, prostaglandin H synthetase

[a] Preferentially expressed in lung
[b] Preferentially expressed in nasal mucosa
[c] Preferentially expressed in trachea
[d] Main isoform in kidney
[1] RNA analysis, array data with AKR1C proto or isoform specific reverse transcriptase polymerase chain reaction
[2] Protein
[3] Enzyme activity

DNA fragmentation and mutation of p53 (Flowers *et al*., 1996, 1997; Penning *et al*., 1999; Yu *et al.*, 2002a). PAH *ortho*-quinones produced by this pathway are also ligands for AhR (Burczynski & Penning, 2000). This effect of *ortho*-quinones may play a role in the mutagenicity and carcinogenicity of benzo[*a*]pyrene and other PAHs.

The stereochemistry of the dihydrodiol epoxide derivatives of benzo[*a*]pyrene is important in the toxicity of benzo[*a*]pyrene and other PAHs (Conney, 1982; Shaw & Connell, 1994; for a review, see IPCS, 1998). Of the four possible stereoisomers of the 7,8-dihydrodiol-9,10-epoxide benzo[*a*]pyrene derivative, the predominant one formed in mammaliam systems, (+)-*anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, has been shown to have the highest tumour-initiation activity and to be the predominant metabolite that forms DNA adducts in mammalian tissues exposed to benzo[*a*]pyrene. The formation of DNA adducts may be a first step in the initiation of carcinogenesis by PAHs.

(g) Elimination

Results from studies of animals exposed to PAHs indicate that their metabolites are largely excreted as conjugates of GSH, glucuronic acid or sulfate in the faeces via biliary excretion and in the urine (for review, see ATSDR, 1995; IPCS, 1998).

4.1.2 *Enzymes involved in the metabolism of PAHs and their genetic variability*

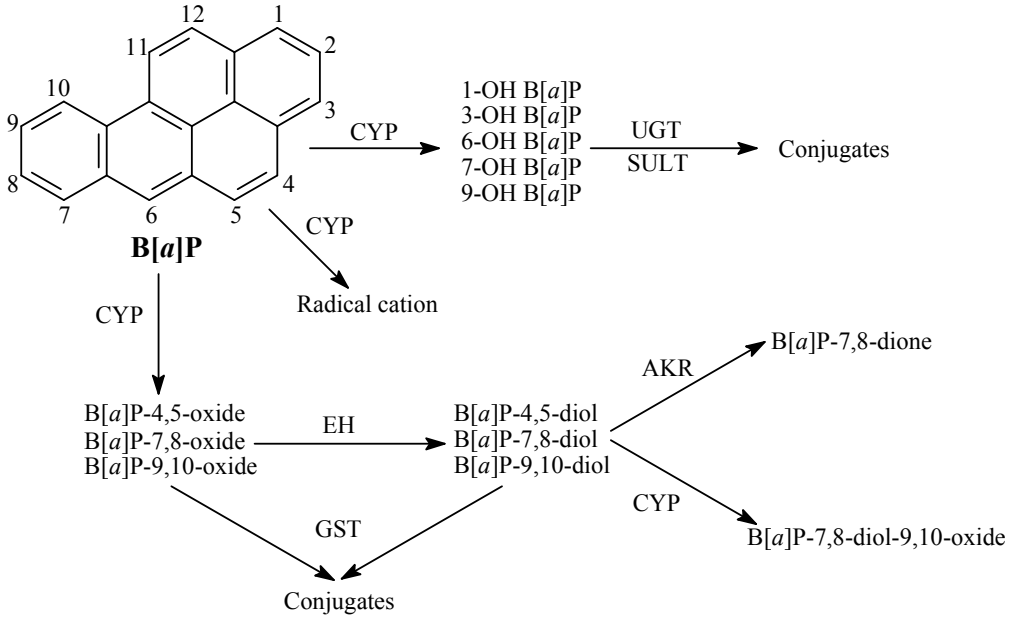
(a) Individual enzymes

(i) *Drug-metabolizing enzymes involved in the metabolism of PAHs*

PAHs are metabolized to water-soluble conjugates by the combined action of phase I enzymes that are involved in their functionalization (e.g. CYP, epoxide hydrolase) and phase II enzymes that form conjugates with the functionalized metabolites (e.g. GST, uridine 5′-diphosphate (UDP)-glucuronosyltransferase (UGT) and sulfotransferase (SULT)). In addition, AKRs and NAD(P)H quinone oxidoreductase 1 (NQO1) play important roles in the further metabolism of diols and quinones, respectively (Conney, 1982; Gonzalez *et al.*, 1991; Guengerich & Shimada, 1991; Hecht, 1998; Penning *et al.*, 1999; Hecht, 2002a).

CYPs are the major phase I enzymes that initially catalyse mono-oxygenation of PAHs (such as benzo[*a*]pyrene) to phenols and epoxides (Figure 4.2; Conney, 1982; Shimada *et al.*, 1989a,b; Guengerich & Shimada, 1991). The oxygenated (intermediate) products are further metabolized to more polar products by several conjugating enzymes such as epoxide hydrolase, GST, UGT and SULT (Nebert *et al.*, 1999). AKRs catalyse the oxidation of *trans*-dihydrodiols to catechols which are non-enzymatically oxidized to *ortho*-quinones, while NQO1 catalyses the two-electron reduction of *para*-quinones to hydroquinones. The catechols or hydroquinones in each instance can undergo conjugation. The conjugated metabolites thus formed are usually more soluble in water than the parent compounds and can be excreted from the body.

Figure 4.2. Metabolism of benzo[*a*]pyrene by CYPs and other drug-metabolizing enzymes

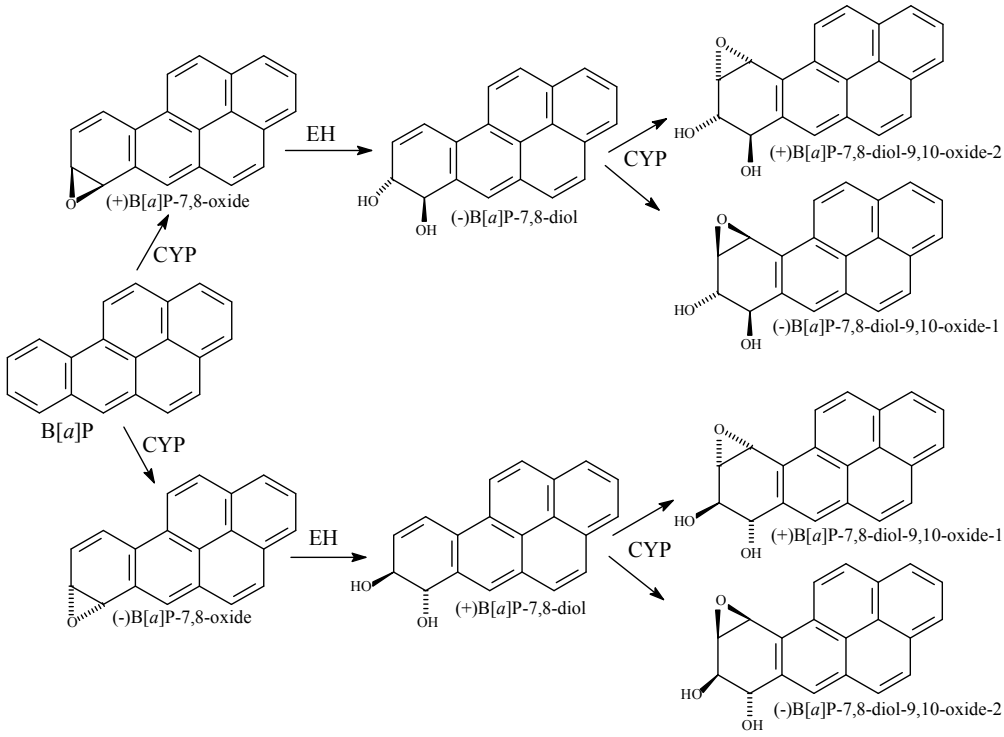


AKR, aldo-keto reductase; B[*a*]P, benzo[*a*]pyrene; CYP, cytochrome P450; EH, microsomal epoxide hydrolase; GST, glutathione *S*-transferase; SULT, sulfotransferase; UGT, Uridine 5′-diphosphate-glucuronosyltransferase

(ii) *Metabolic activation of PAHs by CYPs*

Bay-region diol epoxides represent major electrophilic PAH metabolites at the core of one of the proposed mechanisms of PAH activation (see Section 4.2.1). The pathways that lead to the formation of bay-region epoxides of benzo[*a*]pyrene were investigated extensively in rat liver microsomes and reconstituted systems containing purified rat CYPs and epoxide hydrolase (Figure 4.3) and serve as a model for other bay-region PAHs (Thakker *et al.*, 1977a,b; Lu & West, 1979; Levin *et al.*, 1980; Conney, 1982; Levin *et al.*, 1986). Benzo[*a*]pyrene is first oxidized by liver microsomes of 3-methylcholanthrene-treated rats to (+)- and (–)-benzo[*a*]pyrene-7,8-oxides; the rate of conversion of the (+) enantiomer is much higher than that of the (–) form. Subsequently, microsomal epoxide hydrolase hydrolyses these oxides to (–)- and (+)-benzo[*a*]pyrene-7,8-diols (Thakker *et al.*, 1977a,b; Levin *et al.*, 1980), which are finally activated by CYP isoforms to the highly reactive bay-region epoxides, (–)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-1, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-2, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-1 and (–)-benzo[*a*]pyrene-7, 8-diol-9,10-epoxide-2 (Thakker *et al.*, 1977b; Kapitulnik *et al.*, 1978a; Levin *et al.*, 1980). Although these four diol epoxides were

Figure 4.3. Metabolic activation of benzo[*a*]pyrene by CYPs and epoxide hydrolase



B[*a*]P, benzo[*a*]pyrene; CYP, cytochrome P450; EH, epoxide hydrolase

highly mutagenic in Ames *Salmonella* tester strains and Chinese hamster V79 cells, (+)-benzo[*a*]pyrene-7,8-diol-9,10-epoxide-2 was identified as the most reactive in producing tumours in newborn mice (Wood *et al.*, 1976; Buening *et al.*, 1978). Since (+)-benzo[*a*]-pyrene-7,8-diol-9,10-epoxide-2 had almost the same level of carcinogenicity as benzo[*a*]-pyrene itself or (–)-benzo[*a*]pyrene-7,8-diol, this diol epoxide is considered to be an ultimate carcinogenic metabolite (Kapitulnik *et al.*, 1978a; Conney, 1982; Levin *et al.*, 1986).

The bay-region theory has also been applied to other PAHs (Conney, 1982; Pelkonen & Nebert, 1982; Adams *et al.*, 1995). On the basis of this common mechanism, it is important to know that the major CYP isoforms involved in the formation of the respective arene oxides and diol epoxides are CYP1A1, CYP1A2 and CYP1B1.

(iii) *CYP1A1 and activation of PAHs*

CYP1A1 was thought to be uniquely responsible for the metabolic activation of most of the carcinogenic PAHs (Lu & West, 1979; Conney, 1982; Pelkonen & Nebert, 1982; Guengerich, 1988; Shimada *et al.*, 1989b; Ryan & Levin, 1990). CYP1A1 was first termed aryl hydrocarbon hydroxylase because it had high activity for benzo[*a*]pyrene 3-

hydroxylation (Nebert *et al.*, 1975; Nebert & Jensen, 1979), and was considered to be the major enzyme responsible for the metabolic activation of benzo[*a*]pyrene and other carcinogenic PAHs until CYP1B1 enzymes were found in mice, rats and humans (Pottenger & Jefcoate, 1990; Otto *et al.*, 1991; Pottenger *et al.*, 1991; Sutter *et al.*, 1991; Savas *et al.*, 1994; Sutter *et al.*, 1994). CYP1A1 has relatively similar substrate specificities to CYP1B1 (see below) in the metabolism of PAHs and other carcinogens, although some striking differences are observed, depending on the substrates (Guengerich *et al.*, 1986; Shimada *et al.*, 1992; Guengerich & Shimada, 1998; Shimada *et al.*, 1998a, 2001a). CYP1A1 also catalyses the oxidation of several xenobiotic compounds, including 7-ethoxyresorufin, theophylline, caffeine, 7-ethoxycoumarin and chlorzoxazone (Shimada *et al.*, 1997a), and the endogenous steroids, 17β-estradiol and estrone (Shimada *et al.*, 1999a, 2001b).

Recently, Uno *et al*. (2004) showed that CYP1A1 is important in the detoxification and protection against the oral toxicity of benzo[*a*]pyrene rather than in its metabolic activation in studies using CYP1A1 knockout mice. CYP1A1-null mice died within 30 days after oral administration of 125 mg/kg bw benzo[*a*]pyrene, while wild-type mice did not show any signs of toxicity for 1 year. In addition, benzo[*a*]pyrene–DNA adduct levels were found to be higher in CYP1A1-null mice than in the wild-type mice (Uno *et al.*, 2004; Nebert, 2005a).

CYP1A1 is expressed in human lung and other tissues including the prostate, peripheral blood cells (lymphocytes, monocytes), mammary gland, pancreas, thymus, small intestine, colon and uterus, but not in the liver, in adults (Shimada *et al.*, 1996; Ding & Kaminsky, 2003); it is also expressed in fetal liver (Kitada *et al.*, 1991). CYP1A1 is induced by PAHs, polyhalogenated biphenyls and polyhalogenated dibenzofurans and dioxins such as 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), through the AhR (Pelkonen & Nebert, 1982; Gonzalez *et al.*, 1991; Hankinson, 1995). In this paradigm, PAHs induce their own metabolism.

(iv) *CYP1A2 and activation of PAHs*

CYP1A2 is mainly expressed in the liver (Shimada *et al.*, 1994). It is regulated in part by AhR and is induced by AhR ligands, such as PAHs and TCDD (Hankinson, 1995; Sogawa *et al.*, 2004). Tobacco-smoke condensates and broiled foods, such as grilled meat and fish, induce CYP1A2 (Jaiswal *et al.*, 1987; Gonzalez, 1988; Guengerich & Shimada, 1991). The levels of CYP1A2 protein are ~10–15% that of total CYP in human adult livers on average, and the expression levels vary about 40-fold among individuals (Shimada *et al.*, 1994).

CYP1A2 catalyses the metabolic activation of a variety of aryl and heterocyclic amines to reactive metabolites (Shimada & Okuda, 1988; Shimada *et al.*, 1989a,b; Shimada & Guengerich, 1991). The procarcinogens that are activated by CYP1A2 include 2-aminoanthracene, 2-acetylaminofluorene, 2-aminofluorene, 6-aminochrysene and other aromatic amines. CYP1A2 catalyses the activation of PAH diols to reactive metabolites, although at slower rates than CYP1A1 and CYP1B1 (Shimada *et al.*, 2001a).

CYP1A2 also catalyses the oxidation of several xenobiotic compounds, including acetaminophen, antipyrine, caffeine, 7-ethoxyresorufin, lidocaine, phenacetin, theophylline and *R*-warfarin (Guengerich & Shimada, 1991; Shimada *et al.*, 1994, 1997a).

(v) *CYP1B1 and activation of PAHs*

In 1990, mouse CYP1B1 was purified from C3H/10T1/2 CL8 cells derived from an embryonic fibroblast and the purified enzyme was found to metabolize 7,12-dimethylbenz[*a*]anthracene (DMBA) (Pottenger & Jefcoate, 1990; Pottenger *et al.*, 1991). Spectral interaction studies suggested that purified mouse CYP1B1 can interact with several PAHs, such as benzo[*a*]pyrene, benz[*a*]anthracene, DMBA, 3-methylcholanthrene and 1-ethynylpyrene (Savas *et al.*, 1994, 1997). Human *CYP1B1* cDNA was isolated and was introduced into a yeast expression vector (Sutter *et al.*, 1991, 1994; Shimada *et al.*, 1996). Yeast microsomes that contained the expressed CYP1B1 protein activated diverse procarcinogens including PAHs, aryl and heterocyclic amines and nitroarenes to mutagenic products as determined by an *umu* gene expression system in the *Salmonella typhimurium* NM2009 tester strain (Shimada *et al.*, 1996; Guengerich & Shimada, 1998; Shimada *et al.*, 1998a, 2001a). A close resemblance was found in the substrate specificities of CYP1A1 and CYP1B1 towards various procarcinogens and promutagens, particularly when dihydrodiol derivatives of PAHs were used as substrates (Shimada *et al.*, 1998a, 2001a). Other human CYPs such as 2C9, 2C19 and 3A4 were all relatively weak at activating these PAH compounds.

Buters *et al.* (1999) reported that cultured embryonic fibroblasts isolated from CYP1B1-null mice were unable to metabolize DMBA and were resistant to its cytotoxic and carcinogenic effects. Similarly, recombinant human CYP1A1 and 1B1 were reported to differ in their regio- and stereochemical selectivity for the activation of dibenzo[*a,l*]pyrene; CYP1B1 was reported to play a more important role than CYP1A1 in the formation of fjord region dibenzo[*a,l*]pyrene-11,12-diol-13,14-epoxides (Luch *et al.*, 1997, 1999a). In support of these findings, Buters *et al*. (2002) reported that dibenzo[*a,l*]-pyrene does not produce any type of malignant or benign tumour in the ovaries or lymphoid tissues of *CYP1B1*-null mice. CYP1B1 is therefore thought to play a key role in the metabolic activation of the two prototype carcinogens, dibenzo[*a,l*]pyrene and DMBA, to toxic and carcinogenic metabolites.

Shimada *et al*. (1999b) reported that CYP1B1, together with epoxide hydrolase, catalyses the conversion of benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol at much higher rates that CYP1A1. The catalytic activity of CYP1B1 for the formation of benzo[*a*]-pyrene-7,8-diol was about 10-fold higher than that of CYP1A1. Other human CYPs (including CYP1A2, 2E1 and 3A4) had very low or undetectable activities for the formation of benzo[*a*]pyrene-7,8-diol.

Native human CYP1B1 protein has not yet been isolated from human tissue samples; the enzyme is predominantly expressed in extrahepatic organs, probably at low levels (Sutter *et al.*, 1994; Shimada *et al.*, 1996). A human CYP1B1 cDNA clone was isolated from a human cDNA library and characterized (Sutter *et al.*, 1991, 1994; Tang *et al*.,

1996). The isolated clone that maps to chromosome 2 at 2p21-22 contained a 5.1-kb sequence and a single open-reading frame of 1629 base pairs that predicts a protein of 543 amino acids (Sutter *et al.*, 1994; Shimada *et al.*, 1996; Tang *et al*., 1996). The similarity of amino acid sequence of human CYP1B1 to that of rat and mouse CYP1B1 enzymes was determined to be 80% and 81%, respectively, and that of CYP1A1 and CYP1A2 was reported to be ~40% in three animal species examined (Otto *et al.*, 1991; Savas *et al.*, 1994; Sutter *et al.*, 1994; Walker *et al.*, 1995).

In adults, CYP1B1 mRNA is expressed at significant levels in the mammary gland, prostate, uterus, kidney, adrenal and pituitary glands, ovary, colon, thymus, spleen, lung, small intestine and heart (Shimada *et al.*, 1996; Spink *et al.*, 1998). In human fetal tissues, CYP1B1 mRNA is also detected in the heart, brain, lung and kidney (Shimada *et al.*, 1996). CYP1B1 is expressed constitutively in several types of human cell, including mammary epithelial cells, mammary stromal fibroblasts, broncho-alveolar cells, lymphocytes, bone-marrow stromal cells and monocytes and macrophages (Shimada *et al.*, 1996; Baron *et al.*, 1998; Spink *et al.*, 1998; Spencer *et al.*, 1999; Shimada, 2000; Shimada & Fujii-Kuriyama, 2004). Human CYP1B1 is also reported to be expressed significantly in several tumours including breast cancer cells, uterine myoma and renal adenocarcinoma cells (Liehr *et al.*, 1995; Huang *et al.*, 1996; Spink *et al.*, 1997; Baron *et al.*, 1998; Spink *et al.*, 1998; Spencer *et al.*, 1999).

Human CYP1B1 plays an important role in the activation of diverse procarcinogens (Shimada *et al.*, 1996; Shimada, 2000; Toide *et al.*, 2003; Shimada & Fujii-Kuriyama, 2004). It catalyses the monoxygenation of such procarcinogens as PAHs and their dihydrodiol derivatives, heterocyclic and aryl amines and aminoazo dyes, and nitroaromatic hydrocarbons. These include benzo[*a*]pyrene, benzo[*a*]pyrene-4,5-diol, (+)-benzo[*a*]pyrene-7,8-diol, (–)-benzo[*a*]pyrene-7,8-diol, dibenzo[*a,l*]pyrene, dibenzo[*a,l*]-pyrene-11,12-diol, benz[*a*]anthracene, benz[*a*]anthracene-3,4-diol, benzo[*g*]chrysene-11,12-diol, benzo[*b*]fluoranthene-9,10-diol, benzo[*c*]phenanthrene-3,4-diol, chrysene-1,2-diol, dibenzo[*a,l*]pyrene-11,12-diol, 7,12-dimethylbenz[*a*]anthracene-3,4-diol, 5,6-dimethyl-chrysene-1,2-diol, 5-methylchrysene, 5-methylchrysene-1,2-diol, 6-aminochrysene-1,2-diol, 2-amino-3-methylimidazo[4,5-*f*]quinoline, 2-amino-3,4-dimethylimidazo[4,5-*f*],-quinoline, 2-amino-3,8-dimethylimidazo[4,5*f*]quinoxaline, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]-indole, 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine, 2-aminoanthracene, 2-amino-fluorene, 4-aminobiphenyl, 6-aminochrysene, 2-aminofluorene, 3-methoxy-4-amino-azobenzene, 2-nitropyrene and 6-nitrochrysene (Shimada *et al.*, 1996; Shimada, 2000; Shimada & Fujii-Kuriyama, 2004).

(vi) *CYP2C9 and activation of PAHs*

Other CYPs (CYP2C9 and CYP3A4) play minor roles in PAH metabolism. CYP2C9 is mainly located in the liver and the levels of its expression are estimated to account for an average of 20% of total CYP in the liver (Shimada *et al.*, 1994). It catalyses the metabolism of clinically used drugs such as flurbiprofen, phenytoin, tolbutamide and warfarin (Shimada *et al.*, 1994; Yamazaki & Shimada, 1997; Yamazaki *et al.*, 1997,

1998a). CYP2C9 also mediates 3-hydroxylation of benzo[*a*]pyrene and the metabolic activation of several PAH diols to active metabolites that induce *umu* gene expression in *Salmonella* tester strains (Yun *et al.*, 1992; Shimada *et al.*, 2001a). These activities, however, are lower than those induced by CYP1 enzymes (Shimada *et al.*, 2001a).

(vii) *CYP3A4 and activation of PAHs*

CYP3A4 is the most abundant CYP in human liver, and the levels of its expression in adults average about 30% of total CYP (Guengerich *et al.*, 1986; Shimada *et al.*, 1994). The enzyme is induced by various barbiturates, antibiotics and macrolide antibiotics such as rifampicin and troleandomycin (Gonzalez, 1988; Guengerich & Shimada, 1991; Wilkinson, 1996; Guengerich, 1999). CYP3A4 metabolizes about 50% of clinically used drugs (Guengerich *et al.*, 1986; Wilkinson, 1996; Guengerich, 1999). Procarcinogens activated by CYP3A4 include aflatoxin B_1, aflatoxin G_1, sterigmatocystin, benzo-[*a*]pyrene-7,8-diol and other PAH diols, and 6-aminochrysene (Shimada & Guengerich, 1989; Shimada *et al.*, 1989a; Guengerich & Shimada, 1991).

(viii) *Induction of CYP1A1, 1A2 and 1B1 by PAHs*

The AhR regulates the induction of CYP1A1, CYP1A2 and CYP1B1 and other drug-metabolizing enzymes by carcinogenic PAHs and other chemicals and plays an important role in the toxicity and carcinogenesis of these chemicals (Nebert, 1978, 1980; Gonzalez, 1990; Okey *et al.*, 1994; Hankinson, 1995; Mimura & Fujii-Kuriyama, 2003). Planar PAHs bind to the AhR in cytosol, which dissociates from chaperone proteins and heterodimerizes with AhR nuclear translocator (ARNT) in the nucleus. The ligand-occupied heterodimer then binds to the xenbiotic response elements of the *CYP1A1*, *CYP1A2* and *CYP1B1* genes to cause an increase in gene transcription. Genetically engineered $AhR^{-/-}$ mice are resistant to TCDD-induced teratogenesis (Mimura *et al.*, 1999; Mimura & Fujii-Kuriyama, 2003) and benzo[*a*]pyrene-induced carcinogenesis (Shimizu *et al.*, 2000). Mechanisms that underly the resistance of these animals might be due to their inability to express significant levels of CYP and other enzymes that play major roles in the activation of xenobiotic and endobiotic chemicals (Shimizu *et al.*, 2000; Mimura & Fujii-Kuriyama, 2003). The levels of expression of CYP1A1 and 1B1 are of particular interest, since these CYPs principally catalyse the activation of carcinogenic PAHs to reactive metabolites that initiate cancer (Shimada, 2000; Williams & Phillips, 2000; Murray *et al.*, 2001; Shimada & Fujii-Kuriyama, 2004).

Recently, potent carcinogenic PAHs, such as benzo[*a*]pyrene, DMBA, dibenzo[*a,l*]-pyrene, 3-methylcholanthrene, 1,2,5,6-dibenz[*a,h*]anthracene benzo[*b*]fluoranthene and benz[*a*]anthracene, have been shown to induce liver and lung CYP1A1 and 1B1 mRNAs significantly in $AhR^{+/+}$ mice but not in $AhR^{-/-}$ mice (Shimada *et al.*, 2002). Using male and female $AhR^{+/+}$ and $AhR^{-/-}$ C57BL/6J mice, Shimada *et al*. (2003a) found tissue-specific induction of CYP1A1 and 1B1 mRNAs by PAHs, 3,4,3′,4′-tetrachlorobiphenyl and the polychlorinated biphenyl mixtures, Kanechlor-300, -400 and -500. In both male and female mice, CYP1A1 mRNA was found to be constitutively expressed at very low

levels in various organs of $AhR^{+/+}$ and $AhR^{-/-}$ mice and was induced in various organs by PAHs and 3,4,3′,4′-tetrachlorobiphenyl only in $AhR^{+/+}$ mice. In contrast, CYP1B1 mRNA was expressed constitutively at significant levels in various organs, except for liver and lung, in both male and female $AhR^{+/+}$ and $AhR^{-/-}$ mice. Levels of induction of CYP1B1 by PAHs and tetrachlorobiphenyl in $AhR^{+/+}$ mice were not so significant in organs in which the constitutive levels of CYP1B1 were high.

The extents of induction of hepatic mRNAs of CYP1A1, 1A2 and 1B1 were also determined in $AhR^{+/+}$ mice that had been treated intraperitoneally with each of 23 PAHs or 3,4,3′,4′-tetrachlorobiphenyl at a dose level of 100 mg/kg bw (Shimada *et al.*, 2003b). Both CYP1A1 and 1B1 were highly induced by the PAHs that are potent carcinogens in experimental animals (Conney, 1982; Pelkonen & Nebert, 1982; Shimada *et al.*, 2002, 2003a). These PAHs include benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, 3-methylcholanthrene, dibenz[*a,h*]anthracene, benz[*a*]anthracene and dibenzo[*a,l*]pyrene. Other PAHs, such as dibenz[*a,j*]acridine, dibenz[*a,c*]anthracene, DMBA, 5-methylchrysene, 6-aminochrysene, chrysene and dibenz[*a,e*]pyrene, also induced mRNAs of liver CYP1A1 and 1B1. CYP1A2 was constitutively expressed in the livers of the mice but was induced to a much lesser extent than CYP1A1 and CYP1B1. Liver microsomal 7-ethoxyresorufin *O*-deethylation activities were well correlated with levels of liver CYP1A1 and 1B1 mRNAs in $AhR^{+/+}$ mice.

(ix) *Inhibitors of CYP1A1, 1A2 and 1B1*

Since most of the carcinogenic PAHs require metabolic activation by CYP and other enzymes to evoke their carcinogenic potentials, chemical inhibitors of CYP and other enzymes may potentially act as chemopreventive agents (Cai *et al.*, 1997; Shimada *et al.*, 1997b, 1998b; Chun *et al.*, 1999; Shimada, 2000; Chun *et al.*, 2001; Chun & Kim, 2003; Guengerich, 2003).

Some of these inhibitors of CYP1A1 and 1B1 suppress the tumorigenesis that is induced by a variety of chemical carcinogens (Gelboin *et al.*, 1970; Kinoshita & Gelboin, 1972; Slaga *et al.*, 1977a; El-Bayoumy *et al.*, 1992; Jang *et al.*, 1997; Kleiner *et al.*, 2002, 2003; El-Bayoumy & Sinha, 2004). In a rat mammary tumour model using DMBA, *para*-1,4-phenylene-bis(methylene)selenocyanate [also called xyleneselenocyanate] has been shown to have chemopreventive activity (El-Bayoumy *et al.*, 1992; Ip *et al.*, 1994; Prokopczyk *et al.*, 2000) and to inhibit the formation of DMBA–DNA adducts in the rat mammary gland (El-Bayoumy *et al.* 1992). The synthetic organoselenium compounds were found to be potent inhibitors of human CYP1A1 and CYP1B1, and were selective for CYP1B1 (Shimada *et al.*, 1997b). Similarly, 1-ethynylpyrene inhibits covalent binding of DMBA and benzo[*a*]pyrene to epidermal DNA (Viaje *et al.*, 1990) and prevents tumour formation caused by DMBA and benzo[*a*]pyrene in mouse skin (Alworth *et al.*, 1991); 1-ethynylpyrene inhibited CYP1B1 to a greater extent than CYP1A1 (Shimada *et al.*, 1998b). Resveratrol, which is found in red grapes and has been shown to have cancer preventive activity, is a potent inhibitor of human CYP1A1 and 1B1 (Jang *et al.*, 1997; Chun *et al.*, 1999; Potter *et al.*, 2002).

(x) *Epoxide hydrolase*

Microsomal epoxide hydrolase catalyses the hydrolysis of various epoxides and reactive epoxide intermediates of numerous endobiotic and xenobiotic chemicals into less reactive and more polar dihydrodiols (Omiecinski *et al.*, 2000; Hosagrahara *et al.*, 2004). Although it is mainly known to be a protective enzyme by decomposing numerous epoxide intermediates, it also mediates activation of PAHs to highly reactive diol epoxides, in conjunction with CYPs (Conney, 1982; Gonzalez *et al.*, 1991; Hecht, 2002a). Thus, microsomal epoxide hydrolase plays a role in both the inactivation and activation of xenobiotic chemicals (Lu & West, 1979; Pelkonen & Nebert, 1982).

(xi) *Glutathione* S-*transferases*

GSTs are constitutively expressed in a wide variety of mammalian tissues. Characteristic patterns of *GST* genes have a superfamily of at least 16 genes that consist of six subfamilies, named *alpha* (*GSTA*), *mu* (*GSTM*), *omega* (*GSTO*), *pi* (*GSTP*), *theta* (*GSTT*) and *zeta* (*GSTZ*) (Williams & Phillips, 2000; Strange *et al.*, 2001; Daly, 2003). GSTs encoded by polymorphic members of the *mu* (*GSTM1*), *pi* (*GSTP1*) and *theta* (*GSTT1*) gene families play important roles in the detoxication of a variety of reactive toxic and carcinogenic compounds, including PAH epoxides and diol epoxides (Hecht, 2002a). Isoforms involved in the formation of glutathionyl conjugates with PAH diol epoxides have been assigned (Jernström *et al.*, 1996; Sundberg *et al.*, 1998, 2002) and their rank order of importance based on catalytic efficiency (catalytic constant (k_{cat})/Michaelis constant (K_m)) varies by PAH diol epoxide and individual stereochemistry. For example, for the conjugation of the (–)-*anti*-diol epoxide dibenzo[*a*,*l*]pyrene (*R*-configuration at the benzylic oxirane carbon in the fjord-region), the preference is GSTA1-1 > GSTM1-1 > GSTP1-1. In contrast, the rank order of catalytic efficiency for the conjugation of (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide is GSTP1-1 > GSTM1-1 > GSTA1-1.

(xii) *Uridine 5′-diphosphate-glucuronosyltransferase*

UGTs catalyse the glucuronidation of a variety of endogenous and exogenous compounds to more polar metabolites that are excreted from the body (Hecht, 2002b; Daly, 2003). Glucuronidation is a major pathway of detoxification of numerous carcinogens such as PAHs and aryl and heterocyclic amines (Hecht, 2002a). The UGT1A family is the most relevant for PAH diol conjugation. Using the (+)-7*S*,8*S*-*trans*-dihydrodiol and the (–)7*R*,8*R*-*trans*-dihydrodiol of benzo[*a*]pyrene, the liver UGT1A1 and UGT2B7 isoforms conjugated the 7*S*,8*S*-enantiomer to form the 7*S*-glucuronide as the major diastereomer, while UGT1A8 and UGT1A10, the extrahepatic enzymes present in the aerodigestive tract, preferentially conjugated the 7*R*,8*R*-enantiomer to form the 7*R*-glucuronide and 8*R*-glucuronide (Fang *et al.*, 2002). The K_m values of individual UGT enzymes, which were taken to reflect affinity, gave the following rank order for the conjugation of (–)-7*R*,8*R*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene: UGT1A10 > UGT1A9 > UGT1A1 > UGT1A7; in contrast, the K_m values of individual UGT enzymes gave the

following rank order for the conjugation of (+)-7*S*,8*S*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene: UGT1A10 > UGT1A9 > UGT2B7 ≈ UGT1A1 > UGT1A7 (Fang *et al.*, 2002).

(xiii) *Sulfotransferases*

Cytosolic SULTs catalyse the sulfonation of a variety of carcinogens including PAHs (Hecht, 2002a; Moreno *et al.*, 2005). Sulfonation is generally thought to be a detoxication process; however, the enzyme also activates certain promutagens, e.g. 5-methylchrysene (Blanchard *et al.*, 2004), DMBA, cyclopenta[*c,d,*]pyrene and benzo[*a*]pyrene (Watabe *et al.*, 1982; Surh *et al.*, 1987, 1993; Surh & Tannenbaum, 1995) by a mechanism that involves the conjugation of benzylic alcohols. Six cytosolic SULTs are known to be expressed in humans, including phenol SULTs, hydroxy SULTs and estrogen SULTs (Raftogianis *et al.*, 1997; Daly, 2003; Moreno *et al.*, 2005). Phenol SULTs include SULT1A1, SULT1A2 and SULT1A3. SULT1A1 is highly expressed in numerous organs and catalyses the sulfonation of xenobiotic chemicals including PAHs and endogenous compounds (Raftogianis *et al*, 1997; Moreno *et al.*, 2005). SULT2A is the hydroxy SULT most heavily implicated in the activation of benzylic alcohols (Blanchard *et al*., 2004).

(xiv) *Aldo-keto reductases*

AKRs, the soluble NAD(P)H-dependent oxidoreductases, comprise a gene superfamily consisting of 14 families with 114 members among prokaryotes and eukaryotes (Penning, 2004; http://www.med.upenn.edu/akr). The AKR1 family contains several human enzymes, including the AKR1As (aldehyde reductases), AKR1Bs (aldose reductases), AKR1Cs (hydroxysteroid/dihydrodiol dehydrogenases) and AKR1Ds (steroid 5β-reductases) (Hyndman *et al.*, 2003). Human AKRs have been shown to be involved in the metabolism of PAHs (Palackal *et al*., 2001b, 2002a; Jiang *et al.*, 2005a). The enzymes have dihydrodiol dehydrogenation activities and can convert PAH dihydrodiols to reactive and redox-active *ortho*-quinones, which are key intermediates in one proposed mechanism of PAH activation. Such reactions are principally catalysed by human AKR1A1, 1C1, 1C2, 1C3 and 1C4 (Burczynski *et al*., 1998; Palackal *et al*., 2001b, 2002a).

Differences exist in the specificity of AKR1 enzymes for PAH *trans*-dihydrodiols. For example, AKR1A1 preferentially oxidizes the stereospecific oxidation of (–)-benzo-[*a*]pyrene-7*R*,8*R*-dihydrodiol whereas the AKR1C isoforms oxidize both stereoisomers of PAH *trans*-dihydrodiol mixtures and have a preference for methylated PAH *trans*-dihydrodiols (Palackal *et al*., 2001b).

AKR1C1 is induced by bi- and monofunctional inducers and by reactive oxygen species consistent with regulation by an anti-oxidant response element (Burczynski *et al.*, 1999a). In addition, PAH *ortho*-quinone products are ligands for the AhR and can induce CYP1A1 in human hepatoma HepG2 cells (Burczysnki & Penning, 2000).

AKR1C1 enzymes are also potently inhibited by non-steroidal anti-inflammatory drugs and may exert some of their chemopreventive properties by inhibition at this level (Penning & Talalay, 1983).

(xv) *NAD(P)H quinone oxidoreductase 1*

NQO1, formerly referred to as DT-diaphorase, catalyses two-electron reduction of quinones (Ross & Siegel, 2004). This conversion is important in detoxification since the resulting hydroquinone is eliminated as a sulfate or glucuronide conjugate; in addition, one electron-reduction of the quinone to the highly reactive semiquinone anion radical mediated by P450-NADPH oxidoreductases is avoided. Paradoxically, if the hydroquinone is not conjugated, it can be oxidized back to the quinone-generating reactive oxygen species, which would be a toxification event. For example, NQO1 converts benzene-derived quinones to less active hydroquinones and is considered to affect benzene-induced haematotoxicity (Ross, 2000; Ross & Siegel, 2004). However, certain quinones that are used to treat tumours, such as mitomycin C and streptonigrin, are activated by NQO1 to more toxic metabolites (Siegel *et al*., 1990; Beall *et al*., 1996; Ross & Siegel, 2004). Using benzo[*a*]pyrene as a representative PAH, extended dicarbonyls are produced, e.g. the 1,6-, 3,6- and 6,12-diones, from CYP isoforms. In addition, PAH *ortho*-quinones are formed from the oxidation of non-K region *trans*-dihydrodiols, e.g. benzo[*a*]pyrene-7,8-dione, by AKRs. Of these, only the extended diones are substrates for NQO1 (Flowers-Geary *et al*., 1992). NQO1 has been shown to prevent the formation of PAH quinone–DNA adducts (Joseph & Jaiswal, 1994) and NQO1-deficient mice show an increased incidence of skin cancer when exposed to benzo[*a*]pyrene and DMBA (Long *et al*., 2000, 2001). The human enzyme is induced by bi-functional (PAHs and chlorinated dibenzodioxins) and monofunctional inducers (Dinkova-Kostova *et al*., 2004; Ross & Siegel, 2004).

(b) Genetic variability in PAH-metabolizing enzymes

All of the genes involved in PAH activation/detoxification are polymorphic and may define individual susceptibility to exposure to PAHs. Polymorphisms can exist in the promoter regions, introns and exons of a given gene and therefore can effect transcription, mRNA processing, functional activity or enzyme stability, respectively. Many of the polymorphisms (gene deletion, multiple point mutation, single nucleotide variation, with an incidence > 1% in the population) have been studied in isolation, yet end-points of exposure to PAHs such as metabolic profile, DNA adducts (type and amount) and tumour incidence may result from multigene interactions. Relating a single nucleotide polymorphism within a single gene to these end-points can be problematic unless it is considered that complex *gene x gene x gene x PAH exposure* paradigms exist. The importance of single nucleotide polymorphisms exceeds the enzymes listed in this section and could include those involved in DNA-adduct repair as well as those involved in DNA-lesion by-pass. In addition, the frequency of allelic variation and its distribution within the human population should be considered. This section is limited to a discussion of the polymorphisms that have been identified in individual PAH-metabolizing enzymes. When these polymorphisms have been used to determine correlations between single

nucleotide polymorphism and incidence of disease in a human population, these studies are described in a later section (Section 4.3).

(i) *Genetic variability of* CYP1A1

The *CYP1A1* gene has seven exons and the cDNA is about 70% identical to that of *CYP1A2* (Jaiswal *et al.*, 1985, 1987).

At least 11 alleles of *CYP1A1*, including wild-type *CYP1A1*1A*, have been identified (see http://www.imm.ki.se/CYPalleles; see GeneAtlas website). *CYP1A1*2B*, **2C*, **3*, **4*, **5*, **6*, **7*, **8*, **9*, **10* and **11* showed amino acid changes (Nagata & Yamazoe, 2002). The extent to which these amino acid changes cause alterations in catalytic activities for the oxidation of xenobiotics, including PAHs, is still unknown. For example, a variant (Ile462Val) of CYP1A1 did not show any major differences in catalytic specificities towards various substrates when compared with the wild-type enzyme CYP1A1*1 (Zhang *et al.*, 1996; Persson *et al.*, 1997).

Two mutually linked polymorphisms of the human *CYP1A1* gene, the *MspI* polymorphism (**2A*) located in the 3'-flanking region of the gene and the *Ile-Val* polymorphism (**2B*) at amino acid residue 462 in the haeme-binding region, were reported to be associated with susceptibility to tobacco smoking-associated squamous-cell carcinoma of the lung in Japanese populations (Hayashi *et al.*, 1991; Kawajiri & Fujii-Kuriyama, 1991; Nakachi *et al.*, 1993; Kawajiri *et al.*, 1996; Masson *et al.*, 2005). However, this association has not been found in other ethnic groups (Tefre *et al.*, 1991; Hirvonen *et al.*, 1992). Other studies on the role of genetic polymorphisms of *CYP1A1* in cancer susceptibility in humans are discussed in Section 4.3.1. In many cases, race-related differences have been reported in the occurrence of genetic polymorphisms in *CYP1A1* (Kiyohara *et al.*, 2002a).

(ii) *Genetic variability of* CYP1A2

At least 24 genetic polymorphisms of *CYP1A2* have been identified (see http://www.imm.ki.se/CYPalleles). The *CYP1A2*7* allele contains a splicing defect at the site of intron 6 (G3534A) (Allorge *et al.*, 2003). This mutation was found in a 71-year-old, nonsmoking, Caucasian woman who showed very high concentrations of clozapine, a substrate of CYP1A2, when she was treated with a standard dose (Allorge *et al.*, 2003). *CYP1A2*1C* has been shown to decrease the clearance of caffeine in Japanese smokers *in vivo* (Nakajima *et al.*, 1999). Allele frequency in 116 Japanese showed 0.77 and 0.23 for the wild-type and variant *CYP1A2* alleles, respectively. Another study showed that subjects with *CYP1A2*1K* (-T739G, -C729T and -C163A) had significantly decreased CYP1A2 activity *in vivo* as determined by the caffeine clearance test (Aklillu *et al.*, 2003).

Alleles *CYP1A2*2*, **3*, **4*, **5*, **6*, **8*, **9*, **10*, **11*, **12*, **13*, **14*, **15* and **16* show amino acid changes (Nakajima *et al.*, 1999; Nagata & Yamazoe, 2002). In-vitro studies revealed that a Phe186Leu (*CYP1A2*11*) variant caused a decrease in enzymatic activity

toward 7-ethoxyresorufin *O*-deethylation (Murayama *et al.*, 2004). Little is known about the effects of other mutations of *CYP1A2* on its catalytic properties (Chida *et al*., 1999).

(iii) *Genetic variability of* CYP1B1

Several genetic polymorphisms occur in the human *CYP1B1* gene (see http:www.imm.ki.se/CYPalleles). At least 23 variant forms of *CYP1B1* exist in humans, 17 of which show amino acid changes (Nagata & Yamazoe, 2002). Null alleles of *CYP1B1* are reported to relate to hereditary glaucoma (Stoilov *et al.*, 1997, 1998). To determine the catalytic alterations due to mutations, *CYP1B1* variants with amino acid changes at 48 (Arg48Gly), 119 (Ala119Ser), 432 (Leu432Val) and 453 (Asn453Ser) have been studied extensively in different laboratories (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Shimada *et al.*, 1999a; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Shimada *et al.*, 2001b). 17β-Estradiol 4-hydroxylation activities have been measured in different protein expression systems. Although there are some differences in catalytic activities in the variant forms of *CYP1B1*, it is not clear whether these mutations cause major alterations in the catalytic activities of variant *CYP1B1s* (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Aklillu *et al.*, 2002). For example, the maximum velocity (V_{max})/K_m ratio of 17β-estradiol 4-hydroxylation activities in eight *CYP1B1* variants with combinations of amino acid changes at 48, 119, 432 and 453 changed only from 0.12 to 0.25 $\mu M^{-1}min^{-1}$ (Shimada *et al.*, 2001b).

Shimada *et al*. (2001a) examined the catalytic specificities of four enzymes coded by *CYP1B1* variants, *CYP1B1*1* (wild-type, $Arg^{48}Ala^{119}Leu^{432}Asn^{453}$), *CYP1B1*2* ($Gly^{48}Ser^{119}Leu^{432}Asn^{453}$), *CYP1B1*3* ($Arg^{48}Ala^{119}Val^{432}Asn^{453}$) and *CYP1B1*6* ($Gly^{48}Ser^{119}Val^{432}Asn^{453}$) in the activation of PAHs, PAH diols and 9-hydroxybenzo[*a*]pyrene. Essentially similar catalytic specificities were determined in four *CYP1B1* variants when (+)-, (–)- and (±)-benzo[*a*]pyrene-7,8-diol, 5-methylchrysene-1,2-diol, 7,12-DMBA-3,4-diol, dibenzo[*a,l*]pyrene-11,12-diol, benzo[*b*]fluoranthene-9,10-diol, benzo[*c*]chrysene, 5,6-dimethylchrysene-1,2-diol, benzo[*c*]phenanthrene-3,4-diol, 7,12-DMBA, benzo[*a*]-pyrene, 5-methylchrysene and benz[*a*]anthracene were used as substrates.

(iv) *Genetic variability of* CYP2C9

Genetic polymorphisms of *CYP2C9* have been reported and more than 24 alleles have been identified (see http://www.imm.ki.se/CYPalleles) (Yamazaki *et al.*, 1998a,b; Nagata & Yamazoe, 2002). Alleles *CYP2C9*2*, **3*, **4* and **5* lead to amino acid replacements, and these mutations have been shown to decrease catalytic activities *in vitro* (Rettie *et al.*, 1994; Haining *et al.*, 1996; Sullivan-Klose *et al.*, 1996; Dickmann *et al.*, 2001; Kidd *et al.*, 2001; Takahashi *et al.*, 2004). *CYP2C9*3* is reported to lead to lower activities *in vivo* (Sullivan-Klose *et al.*, 1996; Shintani *et al.*, 2001; Takahashi *et al.*, 2004). It is not known whether these genetic polymorphisms are related to cancer susceptibility in humans (Garcia-Martin *et al.*, 2002a).

(v) *Genetic variability of* CYP3A4

More than 18 genetic polymorphisms of *CYP3A4* have been identified (see http://www.imm.ki.se/CYPalleles) (Nagata & Yamazoe, 2002). However, it is not known to what extent these mutations affect catalytic activities, except that CYP3A4*18 has higher activities and CYP3A4*17 has lower activities *in vitro* (Dai *et al.*, 2001). The significance of these polymorphisms for cancer susceptibility remains unclear.

(vi) *Genetic variability of epoxide hydrolase*

Two genetic polymorphisms of the *mEH* gene that encodes microsomal epoxide hydrolase have been reported to affect its catalytic activities (Omiecinski *et al.*, 2000; Kiyohara *et al.*, 2002a; Gsur *et al.*, 2003; Hosagrahara *et al.*, 2004). The *Tyr113His* variant (*EH3*) was found in exon 3 and caused a decrease in catalytic activities. The other *His139Arg (EH4)* variant in exon 4 is reported to have high catalytic activity compared with that of the wild-type enzyme (Hassett *et al.*, 1994; Kiyohara *et al.*, 2002a; Zhao *et al.*, 2002; Huang *et al.*, 2005; Park, J.Y. *et al.*, 2005). However one study showed that the specific activity of microsomal epoxide hydrolase was similar for each variant (Hassett *et al.*, 1994). The structural differences encoded by the *Tyr113His* and *His139Arg* variants have been reported to exert only a modest impact on the activities of microsomal epoxide hydrolase, using *cis*-stilbene oxide and benzo[*a*]pyrene-4,5-oxide as substrates (Hassett *et al.*, 1994; Omiecinski *et al.*, 2000; Hosagrahara *et al.*, 2004).

(vii) *Genetic variability of glutathione S-transferase*

The levels of individual *GST*s may be influenced by induction and by genetic polymorphism. The roles of *GSTM*, *GSTT1* and *GSTP1* polymorphisms have been reported to be important in determining cancer susceptibilities in humans (Williams & Phillips, 2000; Kiyohara *et al.*, 2002a).

GSTM1 amd *GSTT1* are polymorphic due to large deletions in the structural gene. Meta-analysis of 12 case–control studies showed a significant association between the homozygous deletion and an increased risk for lung cancer (Hengstler *et al.*, 1998).

The *GST-Pi* gene has allelic variants that give rise to two different encoded proteins with isoleucine (GSTP1-1/Ile-105) or valine (GSTP1-1/Val-105) at position 105. The latter was threefold more active in catalysing conjugation reactions with *anti*-diol epoxides of the *R*-absolute configuration at the benzylic oxirane carbon than the variant with isoleucine. Individuals with the Val-105 allele show a higher susceptibility to maligancy, which indicates that this allele cannot account for the increase (Sundberg *et al.*, 1998).

(viii) *Genetic variability of uridine 5′-diphosphate glucuronosyltransferase*

The *UGT* superfamily consists of two families, *UGT1* and *UGT2* (Guillemette *et al.*, 2000a,b; Mackenzie *et al.*, 2005). *UGT1* contains five exons and has a unique gene structure in which there are 13 individual promoters/first exons, from *UGT1A1* to

UGT1A13P, and exons 2–5 are used in common in all mRNAs (Guillemette *et al.*, 2000b; Gong *et al.*, 2001; Jinno *et al.*, 2003). Since all members of this family (*UGT1A1–UGT1A13*) share exons 2–5, the resulting isoforms are identical in their last 245 amino acids. The full-length proteins are 530 amino acids in length and transcripts are formed by the splicing of alternative exons (1.1–1.13). The *UGT2* subfamily consists of six members. UGT1 catalyses the conjugation of many drugs and carcinogens as well as bilirubin and steroids, and genetic polymorphisms in exon 1 of this gene family have been reported (Guillemette *et al.*, 2000b; Vogel *et al.*, 2002; Wells *et al.*, 2004).

The UGTs implicated in forming benzo[*a*]pyrene-glucuronide conjugates belong to the UGT1A family. The *UGT1A* variant allele which resides in the TATA box renders the entire *UGT1A* locus inactive. When this allele is present, the production of benzo-[*a*]pyrene glucuronides is dramatically reduced, which leads to elevated levels of DNA adducts. These data support the role of the UGTs in PAH detoxification (Tukey & Starassburg, 2000).

(ix) *Genetic variability of sulfotransferase*

The two sulfotransferases most implicated in PAH metabolism are SULT1A1 (phenol sulfotransferase) which eliminates hydroxybenzo[*a*]pyrenes and SULT2A which conjugates benzylic alcohols of PAHs (e.g. 5-methylchrysene) and leads to their bio-activation (Glatt *et al.*, 2000; Blanchard *et al.*, 2004). Four allelic variants of *SULT1A1* (*SULT1A1*1*, *SULT1A1*2*, *SULT1A1*3* and *SULT1A1*4*) have been reported. The most common single nucleotide polymorphism is *SULT1A1*2* which has a nucleotide change of G to A that causes a change from arginine to histidine at codon 213. This allozyme has lower enzymic activity and thermostability than the wild-type enzyme (Raftogianis *et al.*, 1997; Engelke *et al.*, 2000). An association between genetic polymorphisms of *SULT1A1* and cancer susceptibility has been reported (Dalhoff *et al.*, 2005).

(x) *Genetic variability of aldo-keto reductase*

AKRs implicated in the metabolic activation of PAHs in humans include AKR1A1, and AKR1C1–AKR1C4 (Burczynski *et al.*, 1998; Palackal *et al.*, 2001a, 2002a). Each gene encoding for the enzyme is polymorphic and contains non-synonymous single nucleotide polymorphisms as follows: *AKR1C1* has *Thr38Ala*, *Thr38Ile* and *Ary47His* allelic variants; *AKR1C2* has *Val38Ala*, *Val38Ile*, *Phe46Tyr*, *His47Ary*, *Val111Ala*, A*ry170Pro*, *Ary170Leu*, *Gly176Gln*, *Lys179Glu* and *Glu185Gln* allelic variants; *AKR1C3* has *Gln5His*, *Met175Ile*, *Arg295Ser*, and *Met293Ile* allelic variants; and *AKR1C4* has *Gly135Glu*, *Ser145Cys*, *Arg250Gln* and *Leu311Val* allelic variants (Hyndman *et al.*, 2003; see also http://www.med.upenn.edu./akr). Of these, the variant examined in a population-based study for increased risk for lung cancer was *AKR1C3*Gln5His* (Lan *et al.*, 2004).

(xi) *Genetic variability of NAD(P)H quinone oxidoreductase 1*

A genetic polymorphism of *NQO1*, a C to T transition at nucleotide 609 in the cDNA, which yields a proline to serine substitution in codon 187, is associated with reduced

NQO1 activity (Saldivar *et al.*, 2005). Some reports have examined the relationship between *NOQ1* polymorphism and cancer susceptibilities (see below) (Chen *et al.*, 1999; Xu *et al.*, 2001).

The single nucleotide polymorphism in *NQO1* of most prominence is the *NQO1*2* allele where a C to T transition at position 609 of cDNA results in a Pro187Ser codon change (Saldivar *et al.*, 2005). The *NQO1*2/*2* allele has only 2–4% of the activity of the wild-type protein because of a diminished ability to bind flavin adenine dinucleotide. This allele also leads to poor protein expression due to accelerated degradation. The frequency of the *NQO1*2/*2* allele across ethnic groups ranges from 4% in Caucasians, 5% in African–Americans, 16% in Mexican Hispanics to 22% in Chinese populations (Ross *et al.*, 2000).

A second polymorphism in *NQO1* (*NQO1*3*) has also been characterized. This is a *C456T* change which changes arginine to tryptophan. The affect of this polymorphism on phenotype is substrate-dependent and the frequency of this polymorphism is low (Ross *et al.*, 2000).

4.2 Mechanisms of carcinogenesis

4.2.1 *Bay- and fjord-region PAH diol epoxide and cyclopenta-ring oxidation*

(a) Mechanisms

(i) *Bay- and fjord-region PAH diol epoxides*

The generalized diol epoxide mechanism was developed from the bay-region theory proposed by Jerina *et al.* (1976), based on the earlier observations of the nature of PAH metabolites (see Boyland & Sims, 1964), and results from a quantum mechanical model. This theory recognized that angular benzo ring fusions on PAHs created a topological indentation on the polycyclic ring structure, called the bay region. In benzo[*a*]pyrene, the bay region encompasses four carbons (carbons 10, 10a, 10b and 11) and three carbon–carbon bonds. In the example of benzo[*a*]pyrene, metabolism by the CYP isozymes at the C7–C8 aromatic double bond creates an arene oxide, benzo[*a*]pyrene-7,8-oxide, that disrupts the aromatic nucleus by saturating that carbon–carbon bond. Benzo[*a*]pyrene-7,8-oxide is hydrated by epoxide hydrolase to a form a dihydrodiol (diol), benzo[*a*]-pyrene-7,8-diol. Benzo[*a*]pyrene-7,8-diol is further metabolized (epoxidized) by the CYP isozymes at the C9–C10 double bond to give the bay-region diol epoxide, benzo[*a*]-pyrene-7,8-diol-9,10-oxide. This diol epoxide possesses an inherent activity to undergo carbon–oxygen bond scission or ring opening to form a carbonium ion on carbon 10 (i.e. a positively charged carbon atom). Carbonium ions are highly reactive species that react with nucleophiles, such as DNA and proteins, to form covalent adducts. One of the postulated quantitative measures of the reactivity of diol epoxides is the carbonium ion delocalization energies ($\Delta E_{deloc}/\beta$) which is based on perturbational molecular orbital calculations that predict the ease of carbonium ion formation. The greater the $\Delta E_{deloc}/\beta$ value, the more reactive the carbonium ion; greater values were associated with PAHs

that exhibited higher tumorigenic activities (Jerina *et al.*, 1976). This theory was expanded to include PAH structures with deeper peripheral indentations in their structure — those that contain a fjord region (e.g. dibenzo[*a*,*l*]pyrene). The fjord region encompasses five carbons and four carbon–carbon bonds; in some cases, the steric interactions between atoms within the fjord region of the PAH forces the PAH ring system out of planarity (Katz *et al.*, 1998). Some PAH fjord-region diol epoxides are non-planar (Lewis-Bevan *et al.*, 1995), and these non-planar PAH diol epoxides possess even higher reactivities than those predicted by $\Delta E_{deloc}/\beta$ alone. The enzymes primarily responsible for phase 1 metabolism of PAHs are CYP1A1, CYP1A2 and CYP1B1 and NADPH CYP reductase, which convert PAHs to different arene oxides, and epoxide hydrolase that catalyses the addition of water to the arene oxides to form *trans* diols. PAH phenols are also formed either by rearrangement of arene oxides or by direct oxygen insertion into a carbon–hydrogen bond. Quinones are formed by further oxidation of phenols or by the enzymatic action of AKRs on PAH diols. The phase 2 enzymes, UGT, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) SULTs and GST, conjugate PAH diols, phenols and epoxides to glucuronic acid, sulfate and GSH, respectively (see Section 4.1.2).

The stereochemistry of the metabolic transformation of PAHs to diols and diol epoxides is an important component of this mechanism of action and affects the biological activities of these metabolites. CYPs can be regio- and stereospecific in their action. The stereospecific metabolizing activity of each CYP, in combination with the capacity of many PAH carbons to form chiral centres through metabolism, can create multiple forms of many PAH metabolites. For example, benz[*a*]anthracene is metabolized in a stereospecific manner at the C3–C4 bond to give two benzo[*a*]anthracene-3,4-oxides (benzo[*a*]anthracene-3*S*,4*R*-oxide and benzo[*a*]anthracene-3*R*,4*S*-oxide) in different amounts (Yang, 1988) which are then hydrated in a stereospecific manner by epoxide hydrolase to give two benzo[*a*]anthracene-3,4-diols (benzo[*a*]anthracene-3*R*,4*R*-diol and benzo[*a*]anthracene-3*S*,4*S*-diol) in different amounts (Yang, 1988). Each diol can form two diol epoxides that vary depending on the relative position of epoxide function in relation to one of the diol hydroxyls — a *syn*-benzo[*a*]anthracene diol epoxide and an *anti*-benzo[*a*]anthracene diol epoxide — for a total of four benzo[*a*]anthracene diol epoxides. While diol epoxides are not subject to enzymatic hydrolysis by epoxide hydrolase (Thakker *et al.*, 1976; Wood *et al.*, 1976), they are non-enzymatically hydrolysed to tetrols (Jankowiak *et al.*, 1997) and are enzymatically detoxified by GSTs (Dreij *et al.*, 2002). Therefore, the formation and degradation of stereochemically specific diol epoxides are dependent on species, strain, sex, organ, tissue, type of CYP and phase 2 enzymes.

One of the original tenets of the mechanism for bay-region or fjord-region diol epoxides is that, as the PAH is metabolically activated in sequence through the diol to the diol epoxide, the process creates intermediates that generally possess greater biological activities than their precursors. This effect has been observed for a number of PAHs. An example is benz[*a*]anthracene. One of the *anti*-diol epoxides of benzo[*a*]anthracene (*anti*-benzo[*a*]anthracene-3,4-diol-1,2-oxide) possesses greater tumour-initiating or carcino-

genic activity in mouse skin or mouse lung than its precursor diol (benzo[*a*]anthracene-3,4-diol), which in turn posseses greater activity than the parent PAH, benzo[*a*]anthracene (Levin *et al.*, 1978; Wislocki *et al.*, 1979). While this effect is observed for some PAHs, it is not universal for all PAHs that are metabolized to diols and diol epoxides due to a number of confounding factors (e.g. reactivity with water and biological constituents or cytotoxicity). Also, the formation of a bay-region PAH diol epoxide by itself does not confer carcinogenicity to that PAH, as is the case for phenanthrene; both phenanthrene and its bay-region diol epoxide (*anti*-phenanthrene-1,2-diol-3,4 oxide) are inactive as carcinogens in newborn mice (Buening *et al.*, 1979a).

Bay-region and fjord-region diol epoxides possess many biological activities; one of the most important of these is their ability to form stable covalent adducts with DNA. The nature and sequence specificity of these DNA adducts are based, in part, on the absolute configuration, molecular conformation and stereochemistry of the diol epoxide, the specific purine (or pyrimidine base) that is adducted, the site of adduction and the nature and sequence of the DNA that is adducted (Jerina *et al.*, 1986). As described previously, each PAH diol can form four diastereomeric *syn*- and *anti*-diol epoxides. When diol epoxides react with DNA (mainly at the purines, deoxyguanosine and deoxyadenosine), each can form both *cis* and *trans* adducts thus giving a total of 16 possible DNA adducts. However, in most cases, far fewer DNA adducts are actually observed. While PAH–DNA adducts represent a type of DNA damage, they can be converted into heritable mutations by misrepair or faulty DNA synthesis (Watanabe *et al.*, 1985; Rodriguez & Loechler, 1995). Bay- or fjord-region diol epoxide–DNA adducts are repaired by nucleotide excision repair (Geacintov *et al.*, 2002). Numerous examples have shown that bay- and fjord-region diol epoxides of PAHs are mutagenic in bacteria, cause damage to DNA or induce chromosomal damage in human and mammalian cells in culture and induce skin, lung or liver tumours in mice, similarly to the parent PAH. Furthermore, PAHs or their bay- or fjord-region diol epoxides induced mutations in critical genes associated with chemical carcinogenesis such as proto-oncogenes (Prahalad *et al.*, 1997; Chakravarti *et al.*, 1998) and tumour-suppressor genes (Ruggeri *et al.*, 1993; Rämet *et al.*, 1995). A strong relationship exists between the nature of the DNA adducts of the diol epoxide and the type of *ras* proto-oncogene mutations observed in DNA from tumours induced by PAHs. In general, PAHs that form DNA adducts at deoxyguanosine primarily induce mutations in the *ras* gene at codons 12 or 13, while those that form DNA adducts at deoxyadenosine induce mutations in the *ras* gene at codon 61. PAHs that induce adducts at both purine bases induced both types of mutations (Ross & Nesnow, 1999). In addition to their genotoxic effects, some bay- or fjord-region diol epoxides are reported to induce apoptosis and cell-cycle arrest in mammalian cells (Chramostova *et al.*, 2004).

The diol epoxide–DNA adducts of PAHs have also been identified in populations exposed to complex mixtures that contain PAHs, i.e. foundry workers (Hemminki *et al.*, 1988; Perera *et al.*, 1988), coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999a), cigarette smokers (Rojas *et al.*, 1995; Lodovici *et al.*, 1998), chimney sweeps (Pavanello *et al.*, 1999a) and people exposed to mixtures in smoke emissions from coal combustion

(Mumford *et al.*, 1993). Some bay- or fjord-region diol epoxides form DNA adducts in the human *TP53* tumour-suppressor gene at sites that are hotspots for lung cancer (Smith *et al.*, 2000).

Interpretation of data on DNA adducts requires a full understanding of the strengths and weaknesses of the methods used to identify and quantitate each adduct. An international workshop on genotoxicity test procedures summarized the strengths and weaknesses of test systems that are commonly used to determine DNA adducts (Phillips *et al*., 2000) and discussed the strengths, limitations and sensitivity of three separate methods as follows.

^{32}P-Postlabelling assay

Strengths. ^{32}P-Postlabelling assay is highly sensitive, requires small quantities of DNA (1–10 μg) and can measure DNA adducts of chemicals with diverse structure in a wide variety of tissues.

Limitations. It does not provide structural information directly, is less useful for low-molecular-weight DNA adducts and requires rigorous internal standards to adjust for labelling efficiency and recovery.

Sensitivity. This varies from one to 100 adducts in 10^9 nucleotides.

Immunoassays

Strengths. They are inexpensive and relatively easy to perform, and can be highly sensitive and specific for classes of adducts.

Limitations. This approach requires the production of antibodies, prior knowledge of the adduct being measured and relatively large amounts of DNA for sensitivity, does not give precise structural information and can be non-specific depending on antibody use.

Sensitivity. One adduct is yielded in approximately 10^8 nucleotides.

Mass spectrometry

Strengths. Mass spectrometry has greatest potential for chemical sensitivity and unequivocal characterization of DNA adducts, analytical backgrounds are very low and it is applicable to many classes of DNA adducts.

Limitations. Stable isotope-labelled internal standards are generally required for quantitation and immuno-affinity pre-purification may be required.

Sensitivity. It yields one adduct in 10^9 nucleotides.

There are several variants on the diol epoxide mechanism. Bis-diol epoxide–DNA adducts were formed from dibenz[*a*,*h*]anthracene (Platt & Schollmeier, 1994) and dibenz[*a*,*j*]anthracene (Vulimiri *et al.*, 1999). A bis-diol epoxide was proposed as a mechanism for the carcinogenesis for dibenz[*a*,*h*]anthracene, while its biological significance for dibenz[*a*,*j*]anthracene is unknown. A phenolic diol epoxide–DNA adduct was formed from benz[*b*]fluoranthene and was proposed to contribute to its biological activity (Weyand *et al.*, 1993a,b). Although not a true diol epoxide, a phenolic oxide–DNA adduct of benzo[*a*]pyrene has been described but its biological significance is unknown (Fang *et al.*, 2001).

(ii) *Cyclopenta-ring oxidation*

The cyclopenta-ring oxidation mechanism involves the formation of the arene oxide at a highly electron-rich isolated double bond that is located at a five-membered ring within a PAH. The cyclopenta ring is an external five-membered carbocyclic ring that is situated on a carbocyclic hexameric fused-ring system. For example, a cyclopenta-ring derivative of benz[*a*]anthracene is benz[*j*]aceanthrylene and that of pyrene is cyclopenta[*cd*]pyrene. In general, cyclopenta-ring derivatives of PAHs are more mutagenic than their unsubstituted counterparts (e.g. anthracene is non-mutagenic while its cyclopenta-ring counterpart, aceanthrylene, is highly mutagenic) (Kohan *et al.*, 1985). Similarly, cyclopenta-ring derivatives of PAHs are generally more carcinogenic than their unsubstituted counterparts (e.g. pyrene is not carcinogenic while cyclopenta[*cd*]pyrene is highly carcinogenic) (Nesnow *et al.*, 1998a). Since the cyclopenta ring is usually the region of highest electron density, it is a major site of oxidation by the CYP isozymes (Nesnow *et al.*, 1984, 1988). Rat and mouse liver preparations, human and rodent cells in culture, human CYP1A1, CYP1A2 and CYP3A4, human liver microsomes and rats in vivo metabolize cyclopenta-fused PAHs at the cyclopenta-ring double bond to give cyclopenta-ring oxides and diols (Gold & Eisenstadt, 1980; Mohapatra *et al.*, 1987; Kwon *et al.*, 1992; Nyholm *et al.*, 1996; Johnsen *et al.*, 1998a,b; Hegstad *et al.*, 1999). Cyclopenta-ring oxides are reactive intermediates and bind to DNA to form DNA adducts in vitro and in vivo mainly at deoxyguanosine (Beach & Gupta, 1994; Hsu *et al.*, 1997, 1999). Cyclopenta-ring oxides, like their parent cyclopenta-PAHs, are mutagenic in bacteria and mammalian cells and can morphologically transform immortalized cells in culture (Bartczak *et al.*, 1987; Nesnow *et al.*, 1991). Cyclopenta-ring oxides are hydrated by epoxide hydrolase to diols. Some cyclopenta-ring diols are conjugated to sulfate esters by PAPS SULT. The cyclopenta-ring oxides are mutagenic and can bind to DNA to form adducts (Surh *et al.*, 1993). As an example, a cyclopenta-PAH, cyclopenta[*cd*]pyrene, induced mutations at the Ki-*Ras* proto-oncogene in lung tumours of treated mice (Nesnow *et al.*, 1994a).

(b) Individual compounds

Benz[*j*]aceanthrylene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*j*]aceanthrylene has been documented in a number studies in rat liver microsomes, rodent and human cells in culture and in rats *in vivo*. Three potential centres exist for biochemical oxidation in benz[*j*]aceanthrylene: the cyclopenta-ring (C1–C2), the bay region (C7–C10) and the K-region (C11–C12) are all found to be involved. Aroclor 1254-induced and phenobarbital-induced rat liver microsomes metabolized benz[*j*]aceanthrylene to the cyclopenta-ring diol, *trans*-1,2-dihydroxy-1,2-dihydrobenz[*j*]-aceanthrylene (benz[*j*]aceanthrylene-1,2-diol), the K-region diol, *trans*-11,12-dihydroxy-11,12-dihydrobenz[*j*]aceanthrylene (benz[*j*]aceanthrylene-11,12-diol), the precursor to the bay-region diol epoxide, *trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene (benz-

[*j*]aceanthrylene-9,10-diol) and 10-hydroxy-benz[*j*]aceanthrylene (Nesnow *et al.*, 1984, 1988). The major metabolite was benz[*j*]aceanthrylene-1,2-diol which represented approx 60% of the total metabolites in either type of induced microsomes (Nesnow *et al.*, 1988). Liver microsomes from control and Aroclor 1254-induced rats also produced additional metabolites: benz[*j*]aceanthrylene-1,2,9,10-tetrahydrotetrol, 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxy-benz[*j*]aceanthrylene-1,2-diol. Induction with Aroclor 1254 increased the overall conversion of benz[*j*]aceanthrylene to metabolites, particularly those formed by oxidation at the cyclopenta-ring (Johnsen *et al.*, 1998b). Lung microsomes from control and Aroclor 1254-induced rats produced benz[*j*]aceanthrylene-1,2-diol as the main metabolite with an increase due to induction (Johnsen *et al.*, 1997). Human liver microsomes from five donors produced only benz[*j*]aceanthrylene-1,2-diol and benz[*j*]-aceanthrylene-2-one (Johnsen *et al.*, 1998a,b). Few data are available that characterize the specific CYP isoforms involved in the biotransformation of benz[*j*]aceanthrylene. A series of primary and conjugated metabolites of benz[*j*]aceanthrylene were identified using human and rodent mammalian cells in culture. Hepatocytes from untreated and Aroclor 1254-induced rats produced a large number of metabolites including benz[*j*]-aceanthrylene-1,2-diol, benz[*j*]aceanthrylene-11,12-diol, benz[*j*]aceanthrylene-9,10-diol, 10-hydroxybenz[*j*]aceanthrylene, benz[*j*]aceanthrylene-1,2,9,10-tetrahydrotetrol, 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxybenz[*j*]aceanthrylene-1,2-diol and sulfate and glucuronide conjugates of many of the primary metabolites (Nyholm *et al.*, 1996). C3H10T1½Cl8 mouse embryo fibroblasts that are known to contain CYP1B1 metabolized benz[*j*]aceanthrylene to benz[*j*]aceanthrylene-1,2-diol, benz[*j*]aceanthrylene-11,12-diol, benz[*j*]aceanthrylene-9,10-diol, 10-hydroxybenz[*j*]aceanthrylene and to sulfate and glucuronide conjugates; however, the major metabolite formed was benz[*j*]-aceanthrylene-9,10-diol (55%) (Mohapatra *et al.*, 1987). Benz[*j*]aceanthrylene was metabolized solely to benz[*j*]aceanthrylene-1,2-diol by isolated human blood lymphocytes (Johnsen *et al.*, 1998a). Rats treated with benz[*j*]aceanthrylene produced biotransformation products in faeces, urine and bile. The major metabolites in faeces were 8-hydroxybenz[*j*]aceanthrylene-1,2-diol and 10-hydroxybenz[*j*]aceanthrylene-1,2-diol, which were also found as conjugated metabolites in the bile. The glucuronide conjugate of 10-hydroxybenz[*j*]aceanthrylene-1,2-diol was also a major metabolite in urine. Two sulfate conjugates of oxidized benz[*j*]aceanthrylene, a sulfate conjugate of a benz-[*j*]aceanthrylene-diol-phenol and benz[*j*]aceanthrylene-1,2-diol-10-sulfate were also detected in bile. Benz[*j*]aceanthrylene-1,2-diol was detected in urine, faeces and bile (Hegstad *et al.*, 1999).

(ii) *Formation of DNA adducts*

DNA adducts have been identified in calf thymus DNA, mammalian cells in culture and in rodents after exposure to benz[*j*]aceanthrylene. The major benz[*j*]aceanthrylene–calf thymus DNA adduct formed in the presence of liver microsomes from rats (control or treated with Aroclor 1254) or from humans was a benz[*j*]aceanthrylene-1,2-oxide adduct (Johnsen *et al.*, 1998b). CYP1A2 was found to be a major CYP involved in the covalent

binding of benz[*j*]aceanthrylene to DNA in the presence of Aroclor 1254-induced rat liver microsomes (Johnsen *et al.*, 1998b). Two major groups of adducts were identified in C3H10T½Cl8 mouse embryo cells exposed to benz[*j*]aceanthrylene: one group was the result of the interaction of benz[*j*]aceanthrylene-1,2-oxide with deoxyguanosine and deoxyadenosine; the second group was a result of the interaction of *anti-trans*-9,10-dihydroxy-9,10-dihydrobenz[*j*]aceanthrylene-7,8-oxide (*anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide) with deoxyguanosine and deoxyadenosine. Qualitative and quantitative analysis of the postlabelling data suggest that benz[*j*]aceanthrylene is metabolically activated by two distinct routes: the bay-region diol epoxide and the cyclopenta-ring oxide; the former was the most significant (Nesnow *et al.*, 1991). In isolated rat hepatocytes and rabbit lung cells, benz[*j*]aceanthrylene-1,2-oxide adducts of both deoxyadenosine and deoxyguanosine were observed (Holme *et al.*, 1993; Johnsen *et al.*, 1998b). Rat Clara cells, type 2 cells and macrophages exposed to benz[*j*]aceanthrylene produced only benz[*j*]aceanthrylene-1,2-oxide–DNA adducts (Johnsen *et al.*, 1997). Human peripheral lymphocytes and HL-60 cells exposed to benz[*j*]aceanthrylene also produced only benz[*j*]aceanthrylene-1,2-oxide adducts (Johnsen *et al.*, 1998a). Addition of Aroclor 1254-induced rat liver microsomes to human lymphocytes increased the levels of DNA adducts (Johnsen *et al.*, 1998a). *In vivo*, only benz[*j*]aceanthrylene-1,2-oxide–DNA adducts were detected in the lymphocytes and lungs of Wistar rats (Johnsen *et al.*, 1998a). Strain A mice exposed intraperitoneally to benz[*j*]aceanthrylene formed a number of adducts in the lung; benz[*j*]aceanthrylene-1,2-oxide adducts of deoxyadenosine and deoxyguanosine and benz[*j*]aceanthrylene-9,10-diol-7,8-oxide adducts of deoxyadenosine and deoxyguanosine were identified (Mass *et al.*, 1993).

(iii) *Genotoxicity of benz[j]aceanthrylene*

Benz[*j*]aceanthrylene was mutagenic in *S. typhimurium* TA98 in the presence of metabolic activation by Aroclor 1254-induced rat liver (Nesnow *et al.*, 1984), control rat liver or human liver microsomes (Johnsen *et al.*, 1998b). It was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) in the presence of Aroclor 1254-induced rat liver metabolic activation (Nesnow *et al.*, 1984) and induced morphological cell transformation in C3H10T½Cl8 cells in culture (Mohapatra *et al.*, 1987; Nesnow *et al.*, 1991). Only a small increase in single-strand breaks was observed in benz[*j*]-aceanthrylene-treated isolated human lymphocytes in the presence of cytosine-^{1}-β-D-arabinofuranoside and hydroxy urea (added 1 h prior to analysis to prevent strand breaks rejoining), whereas large increases were observed in lymphocytes co-incubated with Aroclor 1254-induced rat liver microsomes (Johnsen *et al.*, 1998b).

(iv) *Benz[j]aceanthrylene-induced mutations in proto-oncogenes*

Benz[*j*]aceanthrylene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from benz[*j*]aceanthrylene-treated strain A mice (GGT → CGT (65%) and GGT → GTT (30.4%)), indicating that guanine was a primary target for this PAH (Mass *et al.*, 1993).

(v) *Other effects of benz[j]aceanthrylene*

Benz[*j*]aceanthrylene induced apoptosis and formation of the active form of caspase-3, cleavage of poly(ADP-ribose)polymerase (PARP), DNA fragmentation and an accumulation of the tumour-suppressor protein p53 in mouse hepatoma Hepa1c1c7 cells. However, it did not trigger apoptosis in primary cultures of rat lung cells (Clara cells, type 2 cells or lung alveolar macrophages) (Solhaug *et al.*, 2004a).

(vi) *Evidence for a mechanism of cyclopenta-ring oxide metabolic activation*

Genotoxicity of benz[*j*]aceanthrylene-1,2-oxide

Benz[*j*]aceanthrylene-1,2-oxide was mutagenic in *S. typhimurium* TA98 in the absence and presence of Aroclor 1254-induced rat liver metabolic activation (Bartczak *et al.*, 1987). It induced morphological cell transformation and DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Conclusion

Benz[*j*]aceanthrylene is metabolically activated by cyclopenta-ring oxidation in rat and human liver microsomes, in mouse, rat and human cells in culture and in rats *in vivo*. In addition to the unconjugated metabolites, sulfate, glucuronide and possibly GSH conjugates have been reported. The key evidence for a mechanism of cyclopenta-ring oxide metabolic activation is based on the measurement of the stable cyclopenta-ring dihydrodiol metabolite, benz[*j*]aceanthrylene-1,2-diol, in rodent liver and lung tissues, hepatocytes and fibroblasts and human liver microsomes *in vitro* and in rodents *in vivo*. Benz[*j*]aceanthrylene-1,2-oxide is genotoxic in bacteria and mammalian cells and its adducted form with deoxyadenosine and deoxyguanosine were produced in mouse lung. Benz[*j*]aceanthrylene-induced tumours expressed Ki-*ras* codon 12 guanine mutations.

(vii) *Evidence for a mechanism of diol epoxide metabolic activation*

Genotoxicity of benz[*j*]aceanthrylene-9,10-oxide

Benz[*j*]aceanthrylene-9,10-oxide was mutagenic in *S. typhimurium* TA98 in the presence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Genotoxicity of benz[*j*]aceanthrylene-9,10-diol

Benz[*j*]aceanthrylene-9,10-diol was mutagenic in *S. typhimurium* TA98 in the presence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed *anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Genotoxicity of benz[*j*]aceanthrylene-9,10-diol-7,8-oxide

Benz[*j*]aceanthrylene-9,10-diol-7,8-oxide was mutagenic in *S. typhimurium* TA98 in the presence and absence of Aroclor 1254-induced rat liver metabolic activation (Newcomb *et al.*, 1993). It induced morphological cell transformation and formed *anti*-benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts in C3H10T½Cl8 mouse embryo cells in culture (Nesnow *et al.*, 1991).

Conclusion

Benz[*j*]aceanthrylene is metabolically activated by the diol epoxide pathway in mammalian cells based on the measurement of the diol metabolite benz[*j*]aceanthrylene-9,10-diol in rat liver microsomes and hepatocytes and mouse embryo fibroblasts and the genotoxicity in bacteria and mammalian cells of benz[*j*]aceanthrylene-9,10-diol and benz[*j*]aceanthrylene-9,10-diol-7,8-oxide. *anti*-Benz[*j*]aceanthrylene-9,10-diol-7,8-oxide–DNA adducts were observed in the lungs of strain A mice that are susceptible to benz[*j*]aceanthrylene-induced lung tumorigenesis.

Benz[*l*]aceanthrylene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*l*]aceanthrylene has been documented in a number studies in rat liver microsomes and rodent cells in culture. Aroclor 1254-induced and phenobarbital-induced rat liver microsomes metabolized benz[*l*]aceanthrylene to the cyclopenta-ring diol, *trans*-1,2-dihydroxy-1,2-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-1,2-diol), the K-region diol, *trans*-7,8-dihydroxy-7,8-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-7,8-diol) and a naphtho-ring diol, *trans*-4,5-dihydroxy-4,5-dihydrobenz[*l*]aceanthrylene (benz[*l*]aceanthrylene-4,5-diol) (Gold *et al.*, 1980; Nesnow *et al.*, 1988). The major metabolite was benz[*l*]aceanthrylene-7,8-diol which represented approximately 28–40% of the total metabolites depending on the type of induced microsomes. The cyclopenta-ring diol benz[*l*]aceanthrylene-1,2-diol was the second most abundant metabolite representing 16–20% of the total metabolites formed depending on the inducer type (Nesnow *et al.*, 1988). C3H10T½CL8 mouse embryo fibroblasts that are known to contain CYP1B1 metabolized benz[*l*]aceanthrylene to benz[*l*]aceanthrylene-7,8-diol (45%), benz[*l*]aceanthrylene-4,5-diol (6%), benz[*l*]aceanthrylene-1,2-diol (5%) and unidentified metabolites (44%) as well as sulfate and glucuronide conjugates (Mohapatra *et al.*, 1987).

(ii) *Formation of DNA adducts*

DNA adducts have been identified in calf thymus DNA and in mammalian cells in culture after exposure to benz[*l*]aceanthrylene. Benz[*l*]aceanthrylene formed four unidentified DNA adducts in human peripheral blood lymphocytes and in the human promyelocytic leukaemia HL 60 cell line (Johnsen *et al.*, 1998a). Addition of Aroclor 1254-induced rat liver microsomes to human lymphocytes increased the levels of DNA adducts (Johnsen *et al.*, 1998a). These adducts were also observed in isolated rat

hepatocytes from untreated and Aroclor 1254-treated rats, and in calf thymus DNA incubated with control, Aroclor 1254-induced rat liver microsomes or human microsomes (Johnsen *et al.*, 1998b). Unidentified DNA adducts were observed in rat lung cells and uninduced Clara cells, type 2 cells and alveolar macrophages exposed to benz[*l*]-aceanthrylene. Aroclor 1254-induced rat Clara cells and type 2 cells produced slightly higher levels of DNA adducts (Johnsen *et al.*, 1997). Unidentified adducts were observed in rabbit Clara cells exposed to benz[*l*]aceanthrylene (Holme *et al.*, 1993).

(iii) *Genotoxicity of benz[l]aceanthrylene*

Benz[*l*]aceanthrylene was mutagenic in *S. typhimurium* TA98, in the presence of Aroclor 1254-induced rat liver microsomes, control rat liver microsomes or human liver microsomes (Nesnow *et al.*, 1984; Johnsen *et al.*, 1997, 1998b) and in *S. typhimurium* TA98 co-incubated with rat hepatocytes (Holme *et al.*, 1993). It induced unscheduled DNA synthesis and DNA damage (by alkaline elution) in primary rat hepatocytes and in human lymphocytes co-incubated with Aroclor 1254-induced rat liver microsomes (Holme *et al.*, 1993). Benz[*l*]aceanthrylene was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) in the presence of Aroclor 1254-induced rat liver metabolic activation, and in V79 cells co-cultured with irradiated primary Syrian hamster embryo cells (Nesnow *et al.*, 1984). It induced morphological cell transformation in C3H10T½C8 cells in culture (Mohapatra *et al.*, 1987) and induced anchorage-independent growth in normal human diploid fibroblasts (Nesnow *et al.*, 1990). Benz[*l*]aceanthrylene was both a gene mutagen and a chromosomal mutagen in L5178Y/TK$^{+/-}$ mouse lymphoma cells in the presence of an Aroclor 1254-induced rat liver metabolic activation. It induced sister chromatid exchange in the peripheral blood lymphocytes of mice (Kligerman *et al.*, 1986).

(iv) *Evidence for a cyclopenta-ring metabolic activation as mechanism of carcinogenesis*

Genotoxicity of benz[*l*]aceanthrylene-1,2-oxide

Benz[*l*]aceanthrylene-1,2-oxide was mutagenic in *S. typhimurium* TA98 in the absence and presence of Aroclor 1254-induced rat liver metabolic activation (Nesnow *et al.*, 1984; Bartczak *et al.*, 1987) and induced 6-thioguanine resistant mutations in Chinese hamster V79 cells (Nesnow *et al.*, 1984).

Conclusion

Benz[*l*]aceanthrylene is metabolically activated by cyclopenta-ring oxidation; epoxidation at the cyclopenta ring is one biotransformation pathway for benz[*l*]aceanthrylene in rat liver microsomes and mouse cells in culture. The cyclopenta-ring oxide, benz[*l*]aceanthrylene-1,2-oxide, is genotoxic in bacteria and mammalian cells. The stable cyclopenta-ring dihydrodiol metabolite, benz[*l*]aceanthrylene-1,2-diol, is formed by microsomal and cell-based systems. However, no benz[*l*]aceanthrylene–DNA adducts

have been identified in bioassay systems that confirm the intermediacy of the cyclopenta-ring oxide.

Benz[*a*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of benz[*a*]anthracene has been documented in an number of studies in rat liver microsomes and mouse skin explants. There are three centres of metabolism: the 1–2 and 3–4 bonds that lead to bay-region diol epoxides, the K-region (5–6 bond) and the terminal benzo region (8–9 and 10–11 bonds). Benz[*a*]anthracene is metabolized by control and 3-methylcholanthrene-induced rat liver microsomal fractions and a reconstituted purified cytochrome P-448 system to the major metabolites: the K-region diol, 5,6-dihydroxy-5,6-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-5,6-diol) and the terminal benzo ring diol, 8,9-dihydroxy-8,9-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-8,9-diol) with minor amounts of 3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene (benz[*a*]-anthracene-3,4-diol), 10,11-dihydroxy-10,11-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-10,11-diol) and 1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene (benz[*a*]anthracene-1,2-diol) (Thakker *et al.*, 1979a). Benz[*a*]anthracene is metabolized by mouse skin explants to a series of diols: benz[*a*]anthracene-3,4-diol, benz[*a*]anthracene-8,9-diol, benz[*a*]anthracene-5,6-diol and benz[*a*]anthracene-10,11-diol (MacNicoll *et al.*, 1980). It was metabolized by 3-phenobarbital-induced rat liver microsomes to (–)-benz[*a*]-anthracene-3*R*,4*R*-diol as the predominant stereochemical form (Thakker *et al.*, 1979a,b).

(ii) *Formation of DNA adducts*

Two major DNA adducts were formed after topical administration of benz[*a*]-anthracene to mouse skin: an *anti*-3,4-dihydroxy-1,2,3,4-tetrahydrobenz[*a*]anthracene-1,2-oxide (*anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide)–DNA adduct and an *anti*-8,9-dihydroxy-8,9,10,11-tetrahydrobenz[*a*]anthracene-10,11-oxide (*anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide)–DNA adduct (Cooper *et al.*, 1980a). These adducts were further characterized in hamster embryo cells as *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine and *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide–deoxyguanosine. The non-'bay-region' diol epoxide *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide reacts also with guanosine and adenosine in RNA (Cooper *et al.*, 1980b).

(iii) *Genotoxicity of benz[*a*]anthracene*

In a previous monograph, benz[*a*]anthracene was reported to induce DNA damage and mutation in bacteria, mutation in *Drosphila melanogaster*, DNA damage, mutation, chromosomal effects and morphological cell transformation in mammalian cells, and chromosomal effects *in vivo* in mammals (IARC, 1983). Benz[*a*]anthracene was mutagenic in human B lymphoblastoid cells h1A1v2 (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benz[*a*]anthracene-3,4-diol

Groups of 28–29 female CD-1 mice, 51–65 days of age, received a single dermal application of 2 μmol [525 μg] racemic benz[*a*]anthracene-3,4-diol in acetone followed 7 days later by 16.2 nmol [10 μg] 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in acetone twice a week for 26 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 27 weeks after treatment was 85% (4.7) compared with 6% (0.1) in mice treated with TPA alone (Slaga *et al.*, 1978a).

Groups of 29–30 female CD-1 mice, 60 days of age, received a single dermal application of 0.4, 1 or 2 μmol [105, 262.3 or 525 μg] racemic benz[*a*]anthracene-3,4-diol in acetone dimethyl sulfoxide (DMSO) (95:5) followed 18 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 22.5 weeks after treatment was 77% (2.4), 79% (3.6) or 80% (4.8) compared with 3% (0.03) in mice treated with TPA alone (Wood *et al.*, 1977a).

Groups of 30 female CD-1 mice, 52–66 days of age, received a single dermal application of 400 nmol [105 μg] racemic benz[*a*]anthracene-3,4-diol, (+)-benz[*a*]-anthracene-3,4-diol or (–)-benz[*a*]anthracene-3,4-diol in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 45% (1.3), 20% (0.4) or 50% (1.8) compared with 3% (0.03) in mice treated with TPA alone (Levin *et al.*, 1978).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benz[*a*]anthracene-3,4-diol (total dose, 2.8 μmol) [734.5 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 22 weeks. In 28 male and female mice, pulmonary adenomas developed in 96% of the surviving mice (54.3 tumours/mouse) and 24% of the surviving mice had malignant lymphomas. In 20 male and female mice treated with DMSO alone, pulmonary adenomas developed in 5% of the surviving mice (0.05 tumours/mouse) but no malignant lymphoma. Under the same treatment conditions, benz[*a*]anthracene-1,2-diol, benz[*a*]-anthracene-5,6-diol, benz[*a*]anthracene-8,9-diol or benz[*a*]anthracene-10,11-diol did not induce statistically significant numbers of pulmonary tumours (Wislocki *et al.*, 1978a).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic benz[*a*]anthracene-3,4-diol, (–)-benz[*a*]anthracene-3*R*,4*R*-diol or (+)-benz[*a*]anthracene-3*S*,4*S*-diol (total dose, 280 nmol) [735 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26 weeks. In 68, 58 or 67 male and female mice, pulmonary tumours were observed in 35, 72 or 9% of the surviving mice (0.37, 1.88 or 0.09 tumours/mouse). In 67 male and female mice treated with DMSO alone, pulmonary tumours developed in 13% of the surviving mice (0.15 tumours/mouse) (Wislocki *et al.*, 1979).

Metabolism of benz[*a*]anthracene-3,4-diol

(–)-Benz[*a*]anthracene-3*R*,4*R*-diol was metabolized by phenobarbital- or 3-methylcholanthrene-induced rat liver microsomes to (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, benz[*a*]anthracene-3,4-quinone and bis-diols. (+)-Benz[*a*]anthracene-3*S*,4*S*-diol was not metabolized to a diol epoxide but was metabolized to benz[*a*]anthracene-3,4-quinone and bis-diols (Thakker *et al.*, 1982).

Other benz[*a*]anthracene diol metabolites (e.g. benz[*a*]anthracene-8,9-diol and benz-[*a*]anthracene-10,11-diol) have been reported to form non-bay-region diol epoxides. The non-bay-region diol epoxide, *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide, was formed (measured as a DNA adduct) in hamster embryo cells and in mouse skin treated with benz[*a*]anthracene (Cooper *et al.*, 1980c). *syn*- and *anti*-Benz[*a*]anthracene-10,11-diol-8,9-oxides were formed from benz[*a*]anthracene-10,11-diol by 3-methylcholanthrene-induced rat liver microsomes, but these diol epoxides did not contribute to the covalent binding of benz[*a*]anthracene to DNA (Cooper *et al.*, 1980d). Benz[*a*]anthracene-8,9-diol and benz[*a*]anthracene-10,11-diol and their diol epoxides are not described further in this monograph because both the diols and diol epoxides were inactive as tumour initiators in mouse skin, possessed either no or low mutagenic activity in mammalian cells and did not induce tumours in newborn mice (Slaga *et al.*, 1978a; Wislocki *et al.*, 1978a).

Genotoxicity of benz[*a*]anthracene-3,4-diol

Benz[*a*]anthracene-3,4-diol was mutagenic to Chinese hamster V79 cells (ouabain resistance) co-cultured with irradiated golden hamsters, and was the most mutagenic of all four metabolic benz[*a*]anthracene diols evaluated (Slaga *et al.*, 1978a).

Carcinogenicity studies of benz[*a*]anthracene-3,4-diol-1,2-oxide

Groups of 27–29 female CD-1 mice, 51–65 days of age, received a single dermal application of 2 μmol [556.6 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide in acetone followed 7 days later by 16.2 nmol [10 μg] TPA in acetone twice a week for 26 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 27 weeks after treatment was 100% (5) compared with 6% (0.1) in mice treated with TPA alone (Slaga *et al.*, 1978a).

Groups of 30 female CD-1 mice, 52–66 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide or *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 70% (1.9) or 43% (0.6), respectively, compared with 3% (0.03) in mice treated with TPA alone (Levin *et al.*, 1978).

Groups of 27–30 female CD-1 mice, 58–65 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide or (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide in acetone followed 14 days later by 16 nmol [10 μg] TPA in acetone

twice a week for 25 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 50% (1.63), 90% (3.35) or 14% (0.14), respectively, compared with 7% (0.07) in mice treated with TPA alone (Levin *et al.*, 1984).

Groups of 27–30 female CD-1 mice, 58–65 days of age, received a single dermal application of 400 nmol [111.5 μg] racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide or (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide in acetone followed 14 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after treatment was 19% (0.36), 47% (0.73) or 17% (0.17), respectively, compared with 7% (0.07) in mice treated with TPA alone (Levin *et al.*, 1984).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide or racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide (total dose, 280 nmol) [78 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26 weeks. In 38 or 57 male and female mice, pulmonary tumours developed in 100 or 42% of the surviving mice (13.34 or 0.56 tumours/mouse). In 67 male and female mice treated with DMSO alone, 13% of the surviving mice (0.15 tumours/mouse) had pulmonary tumours (Wislocki *et al.*, 1979).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide, (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide or (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide (total dose, 140 nmol) [39 μg] in DMSO. Mice were weaned at age 25 days, separated by sex and killed at age 26–32 weeks. Only (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide and (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide induced statistically significant responses compared with control mice. In 38 or 48 male and female mice, pulmonary tumours developed in 100 or 31% of the surviving mice (23.11 or 0.38 tumours/mouse). In 65 male and female mice treated with DMSO alone, 8% of the surviving mice (0.08 tumours/mouse) had pulmonary tumours (Levin *et al.*, 1984).

DNA adducts of benz[*a*]anthracene-3,4-diol-1,2-oxide

anti-Benz[*a*]anthracene-3,4-diol-1,2-oxide produced a single unidentified DNA adduct in calf thymus DNA and isolated DNA (Cooper *et al.*, 1980a; King *et al.*, 1994), which was probably *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (Cooper *et al.*, 1980b).

Genotoxicity of benz[*a*]anthracene-3,4-diol-1,2-oxide

Racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide was mutagenic in *S. typhimurium* TA100 and in Chinese hamster V79 cells (8-azaguanine resistance) in the absence of a metabolic activation system. This diol epoxide was 15–35 times more mutagenic in bacteria and 65–125 times more mutagenic in mammalian cells than the isomeric racemic *anti*-benz[*a*]anthracene-8,9-diol-10,11-oxide or *anti*-benz[*a*]anthracene-10,11-diol-8,9-

oxide (Wood *et al.*, 1977b). Racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide, racemic *syn*-benz[*a*]anthracene-3,4-diol-1,2-oxide, (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide, (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, (–)-*syn*-benz[*a*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide and (+)-*syn*-benz[*a*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide were all mutagenic in *S. typhimurium* TA98 and TA100 and in Chinese hamster V79 cells (8-azaguanine resistance) in the absence of exogenous metabolic activation. In strain TA98, the (–)-*anti*-benz[*a*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide isomer was the most active while in strain TA100 and in V79 cells the (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide was the most active (Wood *et al.*, 1983).

Conclusion

Benz[*a*]anthracene is activated metabolically by the diol epoxide mechanism at the bay region (i.e. C1–C4) in mouse skin and mouse lung. Benz[*a*]anthracene was metabolized by control and induced rat liver microsomes, reconstituted CYP systems and mouse skin explants to benz[*a*]anthracene-3,4-diol. In rat liver microsomes, the major enantiomeric form was (–)-benz[*a*]anthracene-3*R*,4*R*-diol. Racemic benz[*a*]anthracene-3,4-diol was genotoxic in mammalian cells and the (–)-benz[*a*]anthracene-3*R*,4*R*-diol enantiomer had the highest activity as a tumour initiator in mouse skin and as a pulmonary carcinogen in newborn mice. (–)-Benz[*a*]anthracene-3*R*,4*R*-diol was metabolized by rat liver microsomes to (+)-*anti*-benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide which had the highest mutagenic activity of all of the isomers in mammalian cells. In mouse skin, benz[*a*]anthracene was metabolized to the diol epoxide, *anti*-benz[*a*]-anthracene-3,4-diol-1,2-oxide, which formed a single DNA adduct, probably *anti*-benz-[*a*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine. (+)-*anti*-Benz[*a*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide was the most active isomer as a pulmonary carcinogen in mice and racemic *anti*-benz[*a*]anthracene-3,4-diol-1,2-oxide was the most active isomer as a tumour initiator in mouse skin.

Benzo[*g*]chrysene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*g*]chrysene has been documented in rat liver microsomes. There is one major centre for biochemical oxidation in benzo[*g*]chrysene, the fjord region (C11–C14). Benzo[*g*]chrysene forms two diols after application to mouse skin *in vivo*: (–)-*trans*-benzo[*g*]chrysene-11*R*,12*S*-diol and (+)-*trans*-benzo[*g*]chrysene-11*S*,12*R*-diol. This conclusion was based on the structure of DNA adducts formed, not by direct measurement of the diols (Giles *et al.*, 1996).

(ii) *Formation of DNA adducts*

Benzo[*g*]chrysene is metabolically activated in human mammary carcinoma MCF-7 cells to form DNA adducts through both *syn*-11,12-dihydroxy-11,12-dihydrobenzo[*g*]-chrysene-13,14-oxide (*syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide) and *anti*-11,12-di-hydroxy-11,12-dihydrobenzo[*g*]chrysene-13,14-epoxide (*anti*-benzo[*g*]chrysene-11,12-

diol-13,14-oxide). Several adducts were identified: an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct, an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct, a *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct and a *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct (Agarwal *et al.*, 1997). Benzo[*g*]chrysene forms DNA adducts after application to mouse skin *in vivo*. These adducts were derived through the formation of (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide. Five adducts were (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide-derived, two with deoxyadenosine and three with deoxyguanosine, and two adducts were (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide-derived, one with deoxyadenosine and one with deoxyguanosine. The adenine adducts accounted for 64% of the total major adducts formed in benzo[*g*]chrysene-treated mouse skin (Giles *et al.*, 1996). Benzo[*g*]chrysene binds to different extents to the DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in Ahr$^{-/-}$ mice, suggesting that CYP1A1 is involved in the bioactivation of benzo[*g*]chrysene (Kleiner *et al.*, 2004).

(iii) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Metabolism of benzo[*g*]chrysene-11,12-diol

Benzo[*g*]chrysene-11,12-diol is metabolized by Aroclor 1254-induced rat liver microsomes to a series of diastereomeric tetraols formed through the intermediary *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides, predominantly in the *anti* form (86:14) (Luch *et al.*, 1998a).

DNA adducts of benzo[*g*]chrysene-11,12-diol

Benzo[*g*]chrysene-11,12-diol produces DNA adducts in human mammary carcinoma MCF-7 cells consistent with the metabolic intermediate, *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide. One adduct was identified as an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyguanosine adduct and the other as an *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide–deoxyadenosine adduct; the latter adduct was the major one (Agarwal *et al.*, 1997). Both (–)-*trans*-benzo[*g*]chrysene-11*R*,12*S*-diol and (+)-*trans*-benzo[*g*]chrysene-11*S*,12*R*-diol formed DNA adducts on mouse skin consistent with the adducts formed by application of benzo[*g*]chrysene (Giles *et al.*, 1996).

Genotoxicity of benzo[*g*]chrysene-11,12-diol

CYP1A1, CYP1A2 and CYP1B1 enzymes activate benzo[*g*]chrysene-11,12-diol metabolically to DNA-damaging forms in cDNA-based recombinant (*Escherichia coli* or *Trichoplusia ni*) systems that express these isoforms of human CYP (Shimada *et al.*, 2001a).

Carcinogenicity studies of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Groups of newborn Hsd:ICR mice were injected intraperitoneally on days 1, 7 and 15 of life with racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide (total dose, 25 nmol) in DMSO. Mice were weaned at age 21 days, separated by sex and killed at age 35 weeks. In treated mice, pulmonary tumours developed in 96.4% of tumour-bearing females (24 animals; 31.6 tumours/mouse) and 100% of the tumour-bearing males (25 animals; 24.4 tumours/mouse); liver tumours developed in 21.4% of the tumour-bearing females (0.54 tumours/mouse) and in 66.7% of tumour-bearing males (eight tumours/mouse). In mice treated with DMSO alone, pulmonary tumours occurred in 14.8% of the tumour-bearing females (26 animals; 0.19 tumours/mouse) and 7.4% of the tumour-bearing males (27 animals; 0.074 tumours/mouse); liver tumours developed in 11.1% of females (0.11 tumours/mouse) and 3.7% of males (0.037 tumours/mouse). The difference between the treated and control groups in males and females for pulmonary tumours, and that in males for hepatic tumours were statistically significant (Amin *et al.*, 1995a).

Groups of 30-day-old female Charles River CD rats were injected twice with a total dose of 1.2 μmol *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide dissolved in DMSO under the three nipples on the left side. The DMSO control was injected under the nipples on the right side. At 41 weeks, 95% of 20 rats had mammary tumours (adenomas, adenocarcinomas, sarcomas), with a mean latent period of 26.5 weeks. In the DMSO controls, 11% of 19 rats had mammary tumours (adenomas, adenocarcinomas), with a mean latent period of 39 weeks. The difference between the treated and control group was statistically significant (Amin *et al.*, 1995b).

DNA adducts of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide forms several DNA adducts in human mammary MCF-7 cells, many of which were the same as those obtained in mice treated with benzo[*g*]chrysene, benzo[*g*]chrysene-11,12-diol and *anti*-benzo[*g*]-chrysene-11,12-diol-13,14-oxide (Agarwal *et al.*, 1997). The major adduct formed between calf thymus DNA and racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was a *trans-anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine adduct (Szeliga *et al.*, 1995). Several major adducts were formed with calf thymus DNA and racemic *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide, a *trans-syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide–deoxyadenosine adduct and a possible mixture of a *trans-syn*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*R*,14*S*-oxide–deoxyguanosine and *trans-syn*-benzo[*g*]-chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide–deoxyguanosine (Szeliga *et al.*, 1994). Both *R*,*S*,*S*,*R* and *S*,*R*,*R*,*S* enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides formed deoxyguanosine and deoxyadenosine DNA adducts in human breast carcinoma MCF-7 cells (Khan *et al.*, 1998).

Genotoxicity of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Both *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104, induced SOS response in *E. coli* (SOS

chromotest in strain PQ37) and were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) (Glatt *et al.*, 1991). Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was mutagenic in the shuttle vector pSP189 with 39% of the mutations at AT pairs which suggested a relationship between adduct formation at deoxyadenosine and mutation (Szeliga *et al.*, 1995). Racemic *syn*-benzo[*g*]chrysene-11,12-diol-13,14-oxide was mutagenic in the shuttle vector pSP189 system giving A→T and G→T mutations (Szeliga *et al.*, 1994) and in the dihydrofolate reductase gene in Chinese hamster ovary hemizygous UA21 cells giving A→T transversion base substitution in 59% of the total induced changes (Yuan *et al.*, 1995). Both *syn*- and *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) and formed four unidentified DNA adducts in these cells. The potent mutagenicity of these fjord-region diol epoxides appears to be due to the high frequency with which they form DNA adducts in V79 cells, rather than to formation of adducts with greater mutagenic potential (Phillips *et al.*, 1991). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide gave base-specific mutations of GC→TA (41%), GC→CG (13%), GC→AT (7%), AT→TA (23%), AT→CG (5%) and AT→GC (11%) in supF DNA using a SV40-based shuttle vector system. *syn*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide gave base-specific mutations of GC→TA (28%), GC→CG (12%), GC→AT (12%), AT→TA (40%), AT→CG (5%) and AT→GC (3%) (Bigger *et al.*, 2000). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide formed deoxyguanosine adducts particularly within codon 158 of exon 5, codons 237 and 248 of exon 7 and codons 273 and 290 of exon 8 in the human *TP53* tumour-suppressor gene. These codons were also hotspots for mutations in the *TP53* gene of lung cancer patients (Smith *et al.*, 2000).

Other effects of benzo[*g*]chrysene-11,12-diol-13,14-oxides

Human O41 TR cells exposed to 1.2 μM *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide formed four major deoxyadenosine and deoxyguanosine adducts. Similar adduct levels were detected in both p53-proficient and p53-deficient cells, and removal of adducts was not observed in either case. At lower concentrations, p53-proficient cells had fewer adducts than p53-deficient cells. p53 Appeared to minimize the appearance of benzo[*g*]chrysene adducts in human cells by up-regulating global nucleotide excision repair and reducing the maximum adduct levels (Lloyd & Hanawalt, 2002). Racemic *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide and each of the *R*,*S*,*S*,*R* and *S*,*R*,*R*,*S* enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxides delayed human breast carcinoma MCF-7 cells in the S phase by delaying DNA synthesis; both enantiomers induced p53 (Khan *et al.*, 1997, 1998). Benzo[*g*]chrysene-11,12-diol-13,14-oxide was found to bind preferentially to methylated CpG sequences at mutational hotspots in the *p53* gene (Chen *et al.*, 1998). *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide is a relatively poor substrate for murine GST 9.5, mGSTP1-1, mGSTM1-1 and mGSTA3-3 (Hu & Singh, 1997).

Conclusion

Benzo[*g*]chrysene can be activated metabolically by a fjord-region diol epoxide mechanism. On mouse skin, benzo[*g*]chrysene was metabolized to DNA adducts through both the *R,R* and *S,S* enantiomers of benzo[*g*]chrysene-11,12-diol. Racemic benzo[*g*]-chrysene-11,12-diols were genotoxic in bacteria and were bioactivated by human CYP1A1, CYP1A2 and CYP1B1. On mouse skin, both diols were further metabolized to the diol epoxides (–)-*anti*-benzo[*g*]chrysene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-benzo[*g*]chrysene-11*S*,12*R*-diol-13*S*,14*R*-oxide, both of which formed deoxyadenosine and deoxyguanosine adducts — the same adducts as those found after treatment with benzo[*g*]chrysene. CYP1A1 is involved in this biotransformation process. *anti*-Benzo[*g*]-chrysene-11,12-diol-13,14-oxide was genotoxic in bacteria and mammalian cells and induced mammary cancer in rats. It was tumorigenic when injected into newborn mice, inducing pulmonary tumours. Both enantiomers of *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide formed deoxyguanosine and deoxyadenosine adducts in human mammary carcinoma cells, and the same pattern of DNA adducts was observed in mouse skin. In human cells, *anti*-benzo[*g*]chrysene-11,12-diol-13,14-oxide adducts were repaired by nucleotide excision repair. *anti*-Benzo[*g*]chrysene-11,12-diol-13,14-oxide induced p53 in human mammary carcinoma cells and mutated the *p53* tumour-suppressor gene.

Benzo[*b*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*b*]fluoranthene has been documented in a number of studies in rat liver microsomes and in mouse epidermis *in vivo*. Benzo[*b*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to 4-hydroxy-, 5-hydroxy-, 6-hydroxy- or 7-hydroxybenzo[*b*]fluoranthene. The major diol metabolite was *trans*-11,12-dihydro-11,12-dihydroxybenzo[*b*]fluoranthene (benzo[*b*]fluoranthene-11,12-diol), and the minor diol metabolite was 1,2-dihydro-1,2-dihydroxybenzo[*b*]fluoranthene (benzo[*b*]-fluoranthene-1,2-diol). No evidence was obtained for the formation of 7b,8-dihydro-7b,8-dihydroxybenzo[*b*]fluoranthene or *trans*-9,10-dihydro-9,10-dihydroxybenzo[*b*]fluor-anthene (benzo[*b*]fluoranthene-9,10-diol) (Amin *et al.*, 1982). Benzo[*b*]fluoranthene is metabolized in mouse skin epidermis *in vivo* to 4-, 5- and 6-hydroxybenzo[*b*]fluoranthene as well as to sulfate and glucuronide conjugates. Minor metabolites included 12-hydroxy-benzo[*b*]fluoranthene, benzo[*b*]fluoranthene-1,2-diol and benzo[*b*]fluoranthene-11,12-diol. Phenolic gluronides and sulfate conjugates of benzo[*b*]fluoranthene were also observed. Benzo[*b*]fluoranthene-9,10-diol was not detected (Geddie *et al.*, 1987).

(ii) *Formation of DNA adducts*

Benzo[*b*]fluoranthene forms two unidentified DNA adducts in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987). Benzo[*b*]fluoranthene administered to male Sprague-Dawley rats produced DNA adducts in the lung, liver and peripheral blood lymphocytes. Only one minor adduct was identified that derived from *anti-trans*-9,10-dihydro-9,10-dihydroxy-benzo[*b*]fluoranthene-11,12-oxide (*anti*-benzo[*b*]fluoranthene-9,10-diol 11,12-

oxide) (Ross *et al.*, 1992). Treatment with benzo[*b*]fluoranthene of mouse epidermis *in vivo*, of human skin maintained in short-term organ culture and single-stranded DNA incubated with Aroclor 1254-induced rat liver microsomes gave two adducts — a major adduct suggested to be a bay-region triol epoxide (containing a phenolic OH-group on carbon 5 or 6 of the peninsula ring) and a minor adduct from *anti*-benzo[*b*]fluoranthene-9,10-diol-11,12-oxide (Pfau *et al.*, 1992). Benzo[*b*]fluoranthene forms a single major adduct with four additional minor DNA adducts in mouse epidermis *in vivo*. The DNA adducts formed with 5-hydroxybenzo[*b*]fluoranthene-9,10-diol were identical to the major and one of the minor adducts observed for benzo[*b*]fluoranthene. *anti*-5-Hydroxybenzo[*b*]fluoranthene-9,10-diol–DNA is the major adduct formed and is a deoxyguanosine adduct (Weyand *et al.*, 1993b). The major benzo[*b*]fluoranthene–DNA adduct in the lungs of benzo[*b*]fluoranthene-treated strain A mice was identified as an *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine adduct (Mass *et al.*, 1996).

(iii) *Genotoxic effects of benzo[*b*]fluoranthene*

In a previous monograph, benzo[*b*]fluoranthene was reported to induce mutations in *S. typhimurium* TA98 and TA100 and to induce chromosomal effects in Chinese hamster bone-marrow cells *in vivo* (IARC, 1983). Benzo[*b*]fluoranthene was mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Amin *et al.*, 1985a) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996). Benzo[*b*]fluoranthene administered to male Sprague-Dawley rats produced sister chromatid exchange in peripheral blood lymphocytes (Ross *et al.*, 1992).

(iv) *Benzo[*b*]fluoranthene-induced mutations in proto-oncogenes*

Benzo[*b*]fluoranthene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from treated strain A mice — GGT→TGT (56%), GGT→GTT (36%), GGT→GAT (4%) and GGT→CGT (4%) — indicating that deoxyguanosine was a primary target for this PAH (Mass *et al.*, 1996).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benzo[*b*]fluoranthene-9,10-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 10, 30 or 100 μg [35, 105 or 350 nmol] racemic benzo[*b*]fluoranthene-9,10-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the treated groups 21 weeks after treatment was 95% (8.4), 63% (3.8) and 26% (1.0), respectively. No tumours were observed in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 100 nmol [29 μg] racemic benzo[*b*]fluoranthene-9,10-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 85% (4.0). The response in control mice treated with TPA alone was not stated (Geddie *et al.*, 1987).

Genotoxicity of benzo[*b*]fluoranthene-9,10-diol

CYP1A1 and CYP1B1 enzymes metabolized benzo[*b*]fluoranthene-9,10-diol to DNA-damaging forms using cDNA-based recombinant (*E. coli* or *T. ni*) systems that express these forms of human CYP (Shimada *et al.*, 2001a). The further metabolism of synthetic benzo[*b*]fluoranthene-9,10-diol by Aroclor 1254-induced rat liver microsomes gave the major metabolites 5- and 6-hydroxybenzo[*b*]fluoranthene-9,10-diol. Little if any benzo[*b*]fluoranthene-9,10,11,12-tetraol was detected (Geddie *et al.*, 1987).

Genotoxicity of benzo[*b*]fluoranthene-9,10-diol-11,12-oxide

anti-Benzo[*b*]fluoranthene-9,10-diol-11,12-oxide induced DNA lesions in human cell-free extracts. This damage was repaired by both nucleotide excision repair and base excision repair of induced apurinic/apyrimidinic sites (Braithwaite *et al.*, 1998).

Conclusion

There is some evidence for two possible routes for the diol epoxide metabolic activation mechanism for benzo[*b*]fluoranthene — a phenolic diol epoxide, *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide, and a diol epoxide, *anti*-benzo[*b*]-fluoranthene-9,10-diol-11,12-oxide. In mouse skin, benzo[*b*]fluoranthene was metabolized to the 5-hydroxybenzo[*b*]fluoranthene metabolite and formed *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine as the major DNA adduct *in vivo*. Benzo[*b*]fluoranthene-9,10-diol was mutagenic in bacteria and initiated mouse skin tumours. *anti*-Benzo[*b*]fluoranthene-9,10-diol-11,12-oxide damaged DNA; in mouse lung *in vivo*, the major DNA adduct was *anti*-5-hydroxybenzo[*b*]fluoranthene-9,10-diol-11,12-oxide–deoxyguanosine. Lung tumours from benzo[*b*]fluoranthene-treated mice exhibited Ki-*ras* mutations at deoxyguanosine residues.

Benzo[*j*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*j*]fluoranthene has been documented in a number studies in rat liver microsomes and in mouse epidermis *in vivo*. Benzo[*j*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to two diol metabolites, one of which was identified as *trans*-9,10-dihydro-9,10-dihydroxybenzo[*j*]fluoranthene (benzo[*j*]fluoranthene-9,10-diol) (LaVoie *et al.*, 1980). Administration of benzo[*j*]fluoranthene to mouse epidermis produced benzo[*j*]fluoranthene-9,10-diol and 4,5-dihydro-4,5-dihydroxybenzo[*j*]fluoranthene (benzo[*j*]fluoranthene-4,5-diol) as major metabolites. 4-Hydroxy-

and 10-hydroxybenzo[*j*]fluoranthene and benzo[*j*]fluoranthene-4,5-dione were also tentatively identified as metabolites (Rice *et al.*, 1987).

(ii) *Formation of DNA adducts*

The major benzo[*j*]fluoranthene–DNA adduct produced in *S. typhimurium* TA97a and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation was an *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine adduct (Marshall *et al.*, 1993), which was also the major DNA adduct formed in mouse epidermis treated with benzo[*j*]fluoranthene (Weyand *et al.*, 1987, 1993a).

(iii) *Genotoxic effects of benzo[j]fluoranthene*

In a previous monograph, benzo[*j*]fluoranthene was reported to induce mutations in *S. typhimurium* TA100 in the presence of an exogenous metabolic activation system (IARC, 1983). Benzo[*j*]fluoranthene was also mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of benzo[*j*]fluoranthene-4,5-diol

Groups of 18–19 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 1 or 3 μmol [287 or 859 μg] benzo[*j*]fluoranthene-4,5-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 100% (5.0) and 78% (4.3), respectively. The response in control mice treated with TPA alone was 10% (0.1) (Rice *et al.*, 1987).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with benzo[*j*]fluoranthene-4,5-diol at total doses of 275 or 1100 nmol [79 or 31.5 μg] in DMSO. Mice were weaned at age 28 days, separated by sex and killed at week 52 of the bioassay. Alveolar/bronchiolar carcinomas were observed in 41.2% of 34 and 89.5% of 19 surviving low- and high-dose female mice (0.44 and 3.63 tumours/mouse), respectively, and in 38.5% of 26 and 91.9% of 37 surviving low- and high-dose male mice (0.50 and 4.22 tumours/mouse), respectively, at 52 weeks. Hepatic tumours were observed in 30.8 and 56.8% of the surviving low- and high-dose males (0.5 and 1.43 tumours/mouse), respectively, but not in female mice, and 5.4% of the males had hepatic carcinomas. In 33 females and 33 males treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 and 18.2% of the surviving mice (0.24 and 0.18 tumours/mouse) and hepatic tumours developed in 0 and 9.1% (0 and 0.18 tumours/mouse). No hepatic carcinomas were observed in the DMSO controls (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-4,5-diol

Benzo[*j*]fluoranthene-4,5-diol formed a number of DNA adducts in mouse epidermis, and was determined to be the major proximate diol produced in mouse epidermis after treatment with benzo[*j*]fluoranthene by DNA adduct analyses. The major DNA adduct formed was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-4,5-diol

Benzo[*j*]fluoranthene-4,5-diol induced mutations in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

Groups of newborn Charles River CD-1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide at total doses of 110 or 275 nmol [79 or 31.5 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. Alveolar/bronchiolar carcinomas occurred in 88.9% of 36 and 95.8% of 24 surviving low- and high-dose females (2.14 and 8.63 tumours/mouse), respectively, and in 97% of 33 and 100% of 30 surviving low- and high-dose males (2.94 and 5.03 tumours/mouse), respectively. Hepatic tumours developed in 42.4 and 83.3% of the surviving males at 52 weeks (0.79 and 4.2 tumours/mouse), respectively, but not in female mice; hepatic carcinomas were observed in 12.1 and 33.3% of the surviving males, respectively. In mice treated with DMSO alone, alveolar/bronchiolar carcinomas in were observed in 21.2% of 33 female and 18.2% of 33 male surviving mice (0.24 and 0.18 tumours/mouse) and hepatic tumours developed in 0 and 9.1% of the surviving female and male mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed in DMSO controls (LaVoie *et al.*, 1994).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 29, 37 or 26 female mice, alveolar/bronchiolar carcinomas developed in 41.4, 21.6 or 11.5% of the surviving mice (0.52, 0.50 or 0.12 tumours/mouse), respectively. In 31, 28 or 34 male mice, alveolar/bronchiolar carcinomas developed in 64.5, 39.3 or 17.6% of the surviving mice (0.9, 0.5 or 0.2 tumours/mouse), hepatic tumours in 74.4, 28.6 or 23.5% of the surviving mice (3.27, 0.5 or 0.35 tumours/mouse) and hepatic carcinomas in 16.1, 14.3 and 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were maintained on a high-fat diet and administered *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide (total dose, 1.2 μmol) [343 μg] in DMSO by six direct applications to the tissue underlying each of the thoracic nipples beneath the mammary glands. The experiment was terminated after 44 weeks. In 20 rats, fibroadenomas/adenocarcinomas/dysplastic fibroadenomas incidence was 55% with a tumour latency of 36.2 weeks. In the DMSO-treated rats, mammary tumour incidence was 15% with a tumour latency of 39.7 weeks (Hecht *et al.*, 1996).

DNA adducts of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

anti-Benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide formed a number of DNA adducts with calf thymus DNA (King *et al.*, 1994). The major DNA adduct produced by *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide in *S. typhimurium* TA97a and TA100 in the absence of an exogenous metabolic activation system was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Marshall *et al.*, 1993). Both *anti*- and *syn*-benzo[*j*]-fluoranthene-4,5-diol-6,6a-oxides formed DNA adducts in mouse epidermis and the *anti* isomer had a higher adduct level. The major DNA adduct formed from the *anti* isomer was *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-4,5-diol-6,6a-oxides

Both *anti*- and *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide were mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the absence of an exogenous metabolic activation system (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-9,10-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 30, 100 or 1000 μg [105, 349 or 3496 nmol] benzo[*j*]fluoranthene-9,10-diol in acetone followed 10 days later by 2.5 μg [4 nmol] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 5% (0.1), 20% (0.3) and 84% (4.5), respectively. No skin tumours were observed in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses every other day for a total initiating application of 3 μmol [859 μg] benzo[*j*]fluoranthene-9,10-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 60% (1.7). The responses in control mice treated with TPA alone was 10% (0.1) (Rice *et al.*, 1987).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with benzo[*j*]fluoranthene-9,10-diol at total doses of 275 or 1100 nmol [31.5 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 39 or 22 female mice, alveolar/bronchiolar carcinomas developed in 43.6 or 22.7% of the surviving mice (0.82 or 0.27 tumours/mouse)

and hepatic tumours in 7.9 or 4.5% of the surviving mice (0.21 or 0.05 tumours/mouse), respectively. In 22 or 40 male mice, alveolar/bronchiolar carcinomas developed in 63.6 or 27.5% of the surviving mice (1.0 or 0.53 tumours/mouse) and hepatic tumours in 81.8 or 25% of the surviving mice (2.82 or 0.32 tumours/mouse), respectively. The incidence of hepatic carcinomas was 27.3 or 5%. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-9,10-diol

Benzo[*j*]fluoranthene-9,10-diol formed three major and seven minor DNA adducts in mouse epidermis. The major DNA adducts seem to be related to both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide based on application of these diol epoxides to mouse epidermis (Weyand *et al.*, 1993b).

Genotoxicity of benzo[*j*]fluoranthene-9,10-diol

Benzo[*j*]fluoranthene-9,10-diol induced mutations in *S. typhimurium* TA97a, TA98 and TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993).

Carcinogenicity studies of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 21, 34 or 33 female mice, alveolar/bronchiolar carcinomas developed in 100, 55.9 or 30.3% of the surviving mice (15.1, 1.0 or 0.3 tumours/mouse) and hepatic tumours in 0, 8.8 or 0% of the surviving mice (0, 0.08 or 0 tumours/mouse), respectively. In 22, 22 or 33 male mice, alveolar/bronchiolar carcinomas developed in 100, 95.4 or 27.3% of the surviving mice (11.1, 2.82 or 0.27 tumours/mouse), hepatic tumours in 95.5, 77.3 or 18.2% of the surviving mice (5.51, 2.82 or 0.21 tumours/mouse) and hepatic carcinomas in 18.2, 13.6 or 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were maintained on a high-fat diet and administered *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide (total dose, 1.2 μmol) in DMSO by six direct applications to the tissue underlying each of the thoracic nipples beneath the mammary glands. The experiment was terminated after 44 weeks. In 20 rats, multiple tumour histologies were observed, the major type being fibroadenomas in 70% of the surviving rats, with a tumour latency of 21 weeks. In the DMSO-treated

rats, mammary tumour incidence was 15%, with a tumour latency of 39.7 weeks (Hecht *et al.*, 1996).

Groups of newborn Charles River CD1 mice were injected intraperitoneally on days 1, 8 and 15 of life with *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide at total doses of 110, 275 or 1100 nmol [31.5, 79 or 315 μg] in DMSO. Mice were weaned at 28 days of age, separated by sex and killed at week 52 of the bioassay. In 25, 25 or 28 female mice, alveolar/bronchiolar carcinomas developed in 68, 24 or 14.3% of the surviving mice (0.88, 0.36 or 0.14 tumours/mouse), respectively, but no hepatic tumours. In 38, 23, or 35 male mice, alveolar/bronchiolar carcinomas developed in 55.2, 26 or 37.1% of the surviving mice (0.78, 0.26 or 0.43 tumours/mouse), hepatic tumours in 68.4, 34.8 or 25.7% of the surviving mice and hepatic carcinomas in 26.3, 4.34 or 0% of the surviving mice, respectively. In 33 female or 33 male mice treated with DMSO alone, alveolar/bronchiolar carcinomas developed in 21.2 or 18.2% of the surviving mice (0.24 or 0.18 tumours/mouse) and hepatic tumours in 0 or 9.1% of the surviving mice (0 or 0.18 tumours/mouse), respectively. No hepatic carcinomas were observed (LaVoie *et al.*, 1994).

DNA adducts of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

anti-Benzo[*j*]fluoranthene-9,10-diol-11,12-oxide formed several adducts with calf thymus DNA (King *et al.*, 1994). One major DNA adduct was produced by *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide in *S. typhimurium* TA97a and TA100 in the absence of an exogenous metabolic activation system but not after treatment with benzo[*j*]fluoranthene in the presence of Aroclor 1254-induced rat liver metabolic activation (Marshall *et al.*, 1993). Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxides formed DNA adducts after application to mouse epidermis. However, these adducts were not observed in the epidermis of mice treated with benzo[*j*]fluoranthene (Weyand *et al.*, 1993a).

Genotoxicity of benzo[*j*]fluoranthene-9,10-diol-11,12-oxides

Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide were mutagenic in *S. typhimurium* TA97a, TA98 and TA100 in the absence of an exogenous metabolic activation system (Marshall *et al.*, 1993).

Conclusion

Benzo[*j*]fluoranthene is metabolically activated by a diol epoxide mechanism.

In mouse skin, benzo[*j*]fluoranthene was metabolized to two diols, benzo[*j*]-fluoranthene-4,5-diol and benzo[*j*]fluoranthene-9,10-diol. Benzo[*j*]fluoranthene-4,5-diol was mutagenic to bacteria, initiated mouse skin tumours and induced both pulmonary and hepatic tumours in newborn mice. Both benzo[*j*]fluoranthene and benzo[*j*]fluoranthene-4,5-diol formed *anti*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide–deoxyguanosine DNA adducts in mouse skin. Both *anti*- and *syn*-benzo[*j*]fluoranthene-4,5-diol-6,6a-oxide were mutagenic to bacteria, and induced pulmonary and hepatic tumours in newborn mice and DNA adducts on mouse skin. Benzo[*j*]fluoranthene-9,10-diol was mutagenic to bacteria,

initiated tumours and induced pulmonary and hepatic tumours in newborn mice. Benzo[*j*]fluoranthene-9,10-diol formed DNA adducts in mouse skin which are related to both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide. Both *anti*- and *syn*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide induced pulmonary and hepatic tumours in newborn mice and DNA adducts in mouse skin; however, these DNA adducts were not observed in mouse skin after treatment with benzo[*j*]fluoranthene. In the rat mammary model, both *anti*-benzo[*j*]fluoranthene-9,10-diol-11,12-oxide and *anti*-benzo[*j*]-fluoranthene-4,5-diol-6,6a-oxide induced mammary tumours, although the former produced a higher tumour incidence. In summary, for mouse skin tumorigenesis, there is evidence for a diol epoxide mechanism for benzo[*j*]fluoranthene through benzo[*j*]fluoranthene-4,5-diol.

Benzo[*k*]fluoranthene

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*k*]fluoranthene has been documented in several studies in rat liver microsomes. Benzo[*k*]fluoranthene is metabolized by Aroclor 1254-induced rat liver microsomes to the major metabolite, *trans*-8,9-dihydro-8,9-dihydroxybenzo[*k*]fluoranthene (benzo[*k*]fluoranthene-8,9-diol), benzo[*k*]fluoranthene-2,3-quinone and 3-, 8- and 9- hydroxybenzo[*k*]fluoranthene (LaVoie *et al.*, 1980; Weyand *et al.*, 1988).

(ii) *Formation of DNA adducts*

Benzo[*k*]fluoranthene forms a single unidentified DNA adduct in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987).

(iii) *Genotoxic effects of benzo[k]fluoranthene*

In a previous monograph, benzo[*k*]fluoranthene was reported to induce mutations in *S. typhimurium* in the presence of an exogenous metabolic activation system (IARC, 1983). It is mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Weyand *et al.*, 1988) and in human B lymphoblastoid h1A1v1 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

DNA adducts of benzo[*k*]fluoranthene-8,9-oxide

Benzo[*k*]fluoranthene-8,9-oxide formed one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Carcinogenicity study of benzo[*k*]fluoranthene-8,9-diol

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal application of 10 subdoses every other day for total initiating applications of 105, 349, or 3496 nmol [30, 100 or 1000 μg] benzo[*k*]fluoranthene-8,9-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 21 weeks after treatment

was 15% (0.1), 10% (0.1) and 10% (0.4), respectively. No skin tumours were found in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Genotoxicity of benzo[*k*]fluoranthene-8,9-diol

Benzo[*k*]fluoranthene-8,9-diol is mutagenic in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (LaVoie *et al.*, 1980; Weyand *et al.*, 1988).

DNA adducts of benzo[*k*]fluoranthene-8,9-diol-10,11-epoxide

Benzo[*k*]fluoranthene-8,9-diol-10,11-epoxide formed one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Conclusion

There are insufficient data on benzo[*k*]fluoranthene to propose a route of metabolic activation. Benzo[*k*]fluoranthene is metabolized to benzo[*k*]fluoranthene-8,9-diol by rat liver preparations. Benzo[*k*]fluoranthene-8,9-diol is mutagenic to bacteria but is inactive as a tumour initiator in mouse skin.

Benzo[*c*]phenanthrene

The Working Group did not review all the mechanistic data on benzo[*c*]phenanthrene. However, there is evidence that benzo[*c*]phenanthrene can be activated metabolically by the diol epoxide mechanism. Benzo[*c*]phenanthrene was metabolized to benzo[*c*]phenanthrene-3,4-diol and benzo[*c*]phenanthrene-5,6-diol and unidentified monohydroxy derivatives in the presence of rat liver preparations (Ittah *et al*., 1983). The proximate fjord-region diol, benzo[*c*]phenanthrene-3,4-diol, was metabolized by rat liver microsomes to diastereomeric fjord-region diol epoxides that were mutagenic to bacterial and mammalian cells. One of both diastereomeric diol epoxides was considered to be an ultimate carcinogen (Wood *et al*., 1980). Benzo[*c*]phenanthrene-3,4-diol-1,2-oxide was a tumour initiator in mouse skin (Levin *et al*., 1980, 1986) and it induced mammary tumours in rats (Hecht *et al*., 1994; Amin *et al*., 1995a) and pulmonary tumours in newborn mice (Amin *et al*., 1995b).

Benzo[*a*]pyrene

It is universally accepted that most PAHs are biologically inert and require metabolism to reactive electrophilic metabolites that bind to DNA in the target tissue to initiate carcinogenesis. Using cell culture systems and isolated organelles from rodent and human tissues, PAHs have been shown to be metabolized by various pathways. Three major pathways are known by which PAHs are metabolized. These are: (i) metabolism by CYP enzymes and epoxide hydrolases to diol epoxides; (ii) metabolism through radical cation formation; and (iii) metabolism via quinones to yield reactive oxygen species. These pathways lead to active metabolites that are capable of binding to macromolecules. DNA lesions, if not repaired, may lead to mutations that may constitute the basis of the carcinogenic process. Other cellular pathways affected by benzo[*a*]pyrene may be

important in carcinogenesis, such as effects on cell proliferation, apoptosis and gap-junctional intercellular communication.

(i) *Metabolism and metabolic activation*

The metabolism of benzo[*a*]pyrene has been documented in an extremely large number of in-vitro and in-vivo studies that encompass many phyla. The majority of these studies have used mouse, rat and human tissues. While there are many sites of metabolism on the benzo[*a*]pyrene ring system, there is one primary centre of metabolism and metabolic activation where formation of the bay-region diol epoxide takes place on the benzo ring, at the C7–C8 and C9–C10 bonds. The enzymes primarily responsible for the metabolism of benzo[*a*]pyrene are CYP1A1 and CYP1B1, which convert it to several different arene oxides that are enzymatically converted to diols by epoxide hydrolase.

In general, the most commonly identified metabolites of benzo[*a*]pyrene are three diols: the proximate bay-region diol, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (benzo[*a*]pyrene-7,8-diol), 9,10-dihydroxy-9,10-dihydrobenzo[*a*]pyrene (benzo[*a*]pyrene-9,10-diol) and the K-region diol, 4,5-dihydroxy-4,5-dihydrobenzo[*a*]pyrene (benzo[*a*]-pyrene-4,5-diol); three quinones: benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone and benzo[*a*]pyrene-6,12-quinone; and several phenols: benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol. Under some conditions, benzo[*a*]pyrene-4,5-oxide may be observed, while benzo[*a*]pyrene-7,8-oxide and benzo[*a*]pyrene-9,10-oxide are too unstable to be isolated. Other minor metabolites that have been detected are 11,12-dihydroxy-11,12-dihydrobenzo[*a*]pyrene and 6-hydroxymethylbenzo[*a*]pyrene. Benzo-[*a*]pyrene was metabolized by liver microsomes or cultured hepatocytes from control, 5,6-benzoflavone-induced and phenobarbital-induced rats to the following metabolites: benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]-pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]-pyrene-3-phenol and benzo[*a*]pyrene-9-phenol (Nesnow *et al.*, 1980).

In studies with cultured rodent cells, benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol were identified after incubation of benzo[*a*]pyrene with primary rat hepatocytes (Shen *et al.*, 1980), mouse embryo cells (Nesnow *et al.*, 1981), primary mouse epidermal cells (excluding benzo[*a*]pyrene-4,5-diol) (DiGiovanni *et al.*, 1982a), hamster embryo cells (excluding benzo[*a*]pyrene-4,5-diol) (Nemoto *et al.*, 1979) and short-term organ cultures of rat and hamster trachea (Mass & Kaufman, 1978; Moore & Cohen, 1978).

In studies with cultured human cells, benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-9,10-diol, benzo[*a*]pyrene-4,5-diol, benzo[*a*]pyrene-1,6-quinone, benzo[*a*]pyrene-3,6-quinone, benzo[*a*]pyrene-6,12-quinone, benzo[*a*]pyrene-3-phenol and benzo[*a*]pyrene-9-phenol were identified after incubation of benzo[*a*]pyrene with cultured human bronchial explants (Hsu *et al.*, 1978), human bronchial epithelial cells (excluding benzo[*a*]pyrene-4,5-diol) (Siegfried *et al.*, 1986), human epidermal keratinocytes (excluding benzo[*a*]-pyrene-4,5-diol) (Kuroki *et al.*, 1980) and human skin fibroblasts (excluding

benzo[*a*]pyrene-4,5-diol and quinones) (Cunningham *et al.*, 1989). Benzo[*a*]pyrene-7,8-diol, benzo[*a*]pyrene-4,5-diol and quinones and 3- and 9-hydroxybenzo[*a*]pyrene were measured in lung and liver tissues from newborn mice after intraperitoneal administration of benzo[*a*]pyrene (Melikian *et al.*, 1989).

Human recombinant liver CYP1B1 and CYP1A1 in combination with epoxide hydrolase metabolized benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol; CYP1A1 was 10 times less active than CYP1B1 (Shimada *et al.*, 1999b). Benzo[*a*]pyrene binds at different levels to DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in the Ahr$^{-/-}$ mice, which suggests that CYP1A1 is involved in the bioactivation of benzo[*a*]pyrene (Kleiner *et al.*, 2004). The metabolism of benzo[*a*]pyrene to benzo[*a*]pyrene-7,8-diol was stereospecific. Uninduced, 3-methylcholanthrene-induced and phenobarbital-induced rat liver microsomes metabolized benzo[*a*]pyrene to benzo[*a*]pyrene-7*R*,8*R*-diol which was a result of the in-situ formation of benzo[*a*]pyrene-7*R*,8*S*-oxide (Yang, 1988).

(ii) *Formation of DNA adducts*

The most ubiquitous adduct detected in isolated mammalian DNA after metabolic activation of benzo[*a*]pyrene, in metabolically competent mammalian cells in culture or in mammals is the N^2-deoxyguanosine adduct, (+)-N^2-10*S*-(7*R*,8*S*,9*R*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene)-yl)–2′-deoxyguanosine, derived from 7*R*,8*S*-dihydroxy-9*R*,10*R*-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide). It was first fully identified from benzo[*a*]pyrene-treated human and bovine bronchial explants (Jeffrey *et al.*, 1977), and was actually visualized by electron microscopy on mouse embryo cell DNA as an antibody complex (Paules *et al.*, 1985).

This *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been detected in incubations of benzo[*a*]pyrene with calf thymus DNA or with Chinese hamster lung V79 cells or V79 cell nuclei in the presence of an Aroclor 1254-induced rat liver preparation (Sebti & Baird, 1984; Bodell *et al.*, 1989), in mouse, rat and hamster embryo cells exposed to benzo[*a*]pyrene in culture (Sebti *et al.*, 1985), in mouse skin DNA after topical treatment with benzo[*a*]pyrene (Ashurst *et al.*, 1983; Bodell *et al.*, 1989; Chen *et al.*, 1996), in epidermal and dermal skin of mice treated topically with benzo[*a*]pyrene (Huckle *et al.*, 1986), in mouse lung and liver after treatment with benzo[*a*]pyrene by gavage (Kulkarni & Anderson, 1984), in rat liver, lung and peripheral blood lymphocytes after intraperitoneal treatment with benzo[*a*]pyrene (Garner *et al.*, 1985; Ross *et al.*, 1990), in mouse skin explant cultures exposed to benzo[*a*]pyrene (Huckle *et al.*, 1986), in human bronchus and peripheral lung explants exposed to benzo[*a*]pyrene (Shinohara & Cerutti, 1977; Garner *et al.*, 1985), in human mammary epithelial cells exposed to benzo[*a*]pyrene (Moore *et al.*, 1987), in human MRC5CV1 fibroblast cells exposed to benzo[*a*]pyrene in the presence of an Aroclor 1254-induced rat liver fraction (Hanelt *et al.*, 1997) and in human epithelial lung BEAS-2B cells exposed to benzo[*a*]pyrene without an activation system (van Agen *et al.*, 1997).

In humans, the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been detected in autopsy samples from human lungs of smokers and nonsmokers (Lodovici *et al.*, 1998), in white blood cells of coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999a), cigarette smokers (Rojas *et al.*, 1995) and chimney sweeps (Pavanello *et al.*, 1999a).

(iii) *Genotoxicity of benzo[*a*]pyrene*

In a previous monograph, benzo[*a*]pyrene was reported to induce DNA repair, bacteriophages and mutations in bacteria, mutations in *Drosphila melanogaster*, DNA binding, DNA repair, sister chromatid exchange, chromosomal aberrations, point mutations and morphological cell transformation in mammalian cells; and point mutations, sister chromatid exchange, chromosomal aberrations, sperm abnormality and somatic mutations in bioassays in mammals *in vivo* (IARC, 1983). Benzo[*a*]pyrene induced DNA strand breaks and hypoxanthine(guanine)phosphoribosyltransferase (*HPRT*) gene mutation (6-thioguanine resistance) in human MRC5CV1 fibroblast cells in the presence of an Aroclor 1254-induced rat liver fraction (Hanelt *et al.*, 1997). It induced micronuclei and anchorage-independent growth (cell transformation) in human epithelial lung BEAS-2B cells (van Agen *et al.*, 1997).

(iv) *Benzo[*a*]pyrene-induced mutations in proto-oncogenes and tumour-suppressor genes*

Benzo[*a*]pyrene induced three types of Ki-*ras* codon 12 mutations in lung adenomas from treated strain A/J mice: GGT→TGT (56.3%), GGT→GTT (25%) and GGT→GAT (19%), indicating that deoxyguanosine was a primary target for this PAH in mouse lung (Mass *et al.*, 1993). Codon 13 c-Ha-*ras* mutations (GGC→GTC and GGC→CGC) (DiGiovanni *et al.*, 1993) and codon 61 mutations (CAA→CTA) were detected in Sencar mouse skin papillomas induced by benzo[*a*]pyrene (Chakravarti *et al.*, 1995).

Liu *et al.* (2005) have shown that, in murine embryonic fibroblasts from human *TP53* knock-in (Hupki) mice, benzo[*a*]pyrene induced mutations similar to those found in smoking-related lung cancer: a predominance of G→T mutations, unequivocal strand bias of the transversions and mutational hotspots at codons 157 and 158 (see below (viii)).

(v) *Evidence for the diol epoxide pathway as a mechanism of carcinogenesis*

Benzo[*a*]pyrene-7,8-oxide

*Carcinogenicity studies of benzo[*a*]pyrene-7,8-oxide*

Groups of 30–39 female C57BL/6J mice, 63 days of age, received dermal applications of 100 or 400 nmol [27 or 108 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1) every two weeks for 60 weeks. In three or 25 mice autopsied at 60 weeks, two or 28 had squamous-cell carcinomas of the skin. There were no skin tumours in mice treated with acetone:ammonium hydroxide (1000:1) alone (Levin *et al.*, 1976).

A group of 29 female CD-1 mice, 51–65 days of age, received a single dermal application of 200 nmol [53 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:-ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of tumours in the dosed group 30 weeks after promotion was 89% (2.52 tumours/mouse). No data were reported on skin tumours in mice treated with TPA alone (Slaga *et al.*, 1976).

Groups of 30 female CD-1 mice, 52–59 days of age, received a single dermal application of 100 or 400 nmol [27 or 108 μg] racemic benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours in the two dose groups 26 weeks after promotion was 50 or 60% (0.83 or 1.67 tumours/mouse), respectively. Mice treated with TPA alone had a skin tumour incidence of 7% (0.07 tumours/mouse) (Levin *et al.*, 1980).

Groups of 30 female CD-1 mice, 52–59 days of age, received a single dermal application of 100 or 400 nmol [27 or 108 μg] (+)-benzo[*a*]pyrene-7,8-oxide or (–)-benzo[*a*]pyrene-7,8-oxide in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of skin tumours in the two dose groups 26 weeks after promotion was 18 or 55% (0.54 or 1.03 tumours/mouse) or 11 or 36% (0.11 or 0.43 tumours/mouse), respectively. Mice treated with TPA alone had a tumour incidence of 7% (0.07 tumours/mouse) (Levin *et al.*, 1980).

Groups of newborn Swiss Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-oxide (total dose, 1.4 μmol) [375 μg] in DMSO:ammonium hydroxide (1000:1). Mice were weaned at 25 days of age, separated by sex and killed at 24 weeks of age. In 53 male and female mice, pulmonary tumours developed in 72% of the surviving mice (2.1 tumours/mouse). In 40 male and female mice treated with DMSO:ammonium hydroxide (1000:1) alone, pulmonary tumours developed in 8% of the surviving mice (0.08 tumours/mouse) (Wislocki *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-oxide, (+)-benzo[*a*]pyrene-7,8-oxide or (–)-benzo[*a*]pyrene-7,8-oxide (total dose, 700 nmol) [188 μg] in DMSO:ammonium hydroxide (1000:1). Mice were weaned at 23 days of age, separated by sex and killed at 31–35 weeks of age. In 63, 74 or 59 male and female mice, pulmonary tumours were observed in 89, 84 or 20% of the surviving mice (3.60, 2.28 or 0.25 tumours/mouse), respectively. In 74 male and female mice treated with DMSO:ammonium hydroxide (1000:1) alone, pulmonary tumours developed in 13% of the surviving mice (0.14 tumours/mouse) (Levin *et al.*, 1980).

Genotoxicity of benzo[a]pyrene-7,8-oxide

Benzo[*a*]pyrene-7,8-oxide was weakly mutagenic in *S. typhimurium* TA1538 (Wood *et al.*, 1975) and induced sister chromatid exchange in Chinese hamster ovary cells (Pal *et al.*, 1980).

Benzo[*a*]pyrene-7,8-diol

Carcinogenicity studies of benzo[a]pyrene-7,8-diol

A group of 29 female CD-1 mice, 51–65 days of age, received a single dermal application of 200 nmol [57 μg] racemic benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of skin tumours in the dosed group 30 weeks after promotion was 86% (5.0 tumours/mouse). No data were reported for skin tumours in mice treated with TPA alone (Slaga *et al.*, 1977b).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received dermal applications of 10 subdoses every other day for a total initiating application of 30 μg [136 nmol] racemic benzo[*a*]pyrene-7,8-diol in acetone followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of skin tumours in the dosed groups 21 weeks after treatment was 90% (nine tumours/mouse). No skin tumours occurred in control mice treated with TPA alone (LaVoie *et al.*, 1982).

Groups of 29–30 female CD-1 mice, 51–65 days of age, received a single dermal application of 100 nmol [29.6 μg] (–)-benzo[*a*]pyrene-7,8-diol or (+)-benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1) followed 11 days later by 16 nmol [10 μg] TPA in acetone twice a week for 21 weeks. The incidence of skin tumours in the dosed groups 22 weeks after treatment was 77% (3.8 tumours/mouse) or 23% (0.43 tumours/mouse), respectively. The incidence of skin tumours in mice treated with TPA alone was 7% (0.7 tumours/mouse) (Levin *et al.*, 1977a).

Groups of 29 female C57BL/6J mice, 56–63 days of age, received dermal applications of 25, 50 or 100 nmol [7.2, 14.5 or 29 μg] racemic benzo[*a*]pyrene-7,8-diol in acetone:ammonium hydroxide (1000:1) every two weeks for 60 weeks. The incidence of squamous-cell carcinomas in the dosed groups at 60 weeks after treatment was 22% (seven tumours), 76% (24 tumours) or 92% (28 tumours), respectively. No skin tumours occurred in mice treated with acetone:ammonium hydroxide (1000:1) alone (Levin *et al.*, 1977b).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-diol (total dose, 28 nmol) [8 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 28 weeks of age. In 62 male and female mice, pulmonary adenomas were observed in 66% of the surviving mice (1.77 tumours/mouse). In 67 male and female mice treated with DMSO alone, pulmonary tumours were observed in 12% of the surviving mice (0.13 tumours/mouse) (Kapitulnik *et al.*, 1978a).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic benzo[*a*]pyrene-7,8-diol (total dose, 1.4 μmol) [400 μg] in

DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 18 male and female mice, pulmonary tumours developed in 100% of the surviving mice (59.1 tumours/mouse) and malignant lymphomas in 78% of the surviving mice. Under the same experimental conditions, benzo[*a*]pyrene (1.4 μmol) [353 μg] produced pulmonary tumours in 74% of the surviving mice (4.13 tumours/mouse). In 48 male and female mice treated with DMSO alone, pulmonary tumours were observed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphomas (Kapitulnik *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (–)-benzo[*a*]pyrene-7,8-diol (total dose, 140, 700 or 1400 nmol) [40, 200 or 400 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 46, 29 or two male and female mice, pulmonary tumours developed in 98, 100 or 100% of the surviving mice (9.28, 32.2 or 5 tumours/mouse) and malignant lymphomas in 4, 70 or 83% of the surviving mice, respectively. In 48 male and female mice treated with DMSO alone, pulmonary tumours developed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphomas (Kapitulnik *et al.*, 1978b).

Groups of newborn BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-benzo[*a*]pyrene-7,8-diol (total dose, 140, 700 or 1400 nmol) [40, 200 or 400 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 17 weeks of age. In 38, 35 or 47 male and female mice, pulmonary tumours developed in 16, 54 or 94% of the surviving mice (0.16, 2.34 or 18.5 tumours/mouse) and malignant lymphomas in 0, 6 or 0% of the surviving mice, respectively. In 48 male and female mice treated with DMSO alone, pulmonary tumours developed in 10% of the surviving mice (0.1 tumours/mouse) and no mice had malignant lymphoma (Kapitulnik *et al.*, 1978b).

Groups of 20 female Ha:ICR mice, 87 days of age, received oral intubations of racemic benzo[*a*]pyrene-7,8-diol in tricaprylin three times a week for 6 weeks (total dose, 18 μmol) [5153 μg] and were killed at 41 weeks of age. In 14 surviving mice at 32 weeks, tumour incidence was 100% for forestomach papilloma tumours (4.6 tumours/mouse), 30.6 pulmonary adenomas/mouse and 50% for lymphomas (almost all thymic in origin). In 19 surviving control mice that received vehicle alone, tumour incidence was 11% for forestomach papilloma tumours (0.1 tumours/mouse), 0.7 pulmonary adenomas/mouse and no lymphomas (Wattenberg *et al.*, 1979).

Groups of 18–19 male C3H/fCum mice, 28–35 days of age, received a single subcutaneous injection of 900 nmol [258 μg] racemic benzo[*a*]pyrene-7,8-diol in DMSO. The mice were killed after 18 months. The incidence of fibrosarcomas in the dosed group at 18 months after treatment was 42%. There were no tumours in mice treated with DMSO alone (Kouri *et al.*, 1980).

Metabolism of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol was metabolized by control, 3-methylcholanthrene- or phenobarbital-induced rat liver microsomes to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Thakker *et al.*, 1976). (–)-Benzo[*a*]pyrene-7,8-diol was metabolized by purified induced rabbit liver cytochromes to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide; the *anti* isomer predominated with 5,6-benzoflavone- and phenobarbital-induced forms (Deutsch *et al.*, 1978). Racemic benzo[*a*]pyrene-7,8-diol was metabolized to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide in epidermis after topical administration to mouse skin (Melikian *et al.*, 1987). (+)-Benzo[*a*]pyrene-7,8-diol was metabolized in mouse skin by two pathways: by CYP to (+)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide and by peroxy radicals to (–)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Pruess-Schwartz *et al.*, 1989). Hamster tracheal and human bronchus explants metabolized racemic benzo[*a*]pyrene-7,8-diol to both *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide; the *anti* isomer predominated. Prostaglandin H synthetase co-oxygenation in the human bronchus explants produced the *anti* isomer (Reed *et al.*, 1984).

DNA adducts of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol forms four DNA adducts following topical treatment of mouse skin: two *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts and two *syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts (Rojas & Alexandrov, 1986).

Genotoxicity of benzo[a]pyrene-7,8-diol

Racemic benzo[*a*]pyrene-7,8-diol induced mutations in *S. typhimurium* strain TA100 in the presence of intact hepatocytes, homogenized hepatocytes or homogenized hepatocytes with an NADPH-generating system from rats pretreated with Aroclor 1254 (Glatt *et al.*, 1981) and in TA98 in the presence of a reconstituted cytochrome P448 system (Wood *et al.*, 1977c). Racemic benzo[*a*]pyrene-7,8-diol induced mutations in Chinese hamster V79 cells (8-azaguanine and ouabain resistance) in the presence of 3-methylcholanthrene-induced rat liver preparations (Kuroki *et al.*, 1979), in V79 cells (6-thioguanine resistance) that express rat CYP1A1 (Dogra *et al.*, 1990) and in V79 cells (ouabain resistance) co-cultured with human bronchus explants (Hsu *et al.*, 1978). Racemic benzo[*a*]pyrene-7,8-diol induced mutations in Chinese hamster ovary cells (6-thioguanine resistance) supplemented with an Aroclor 1254-induced rat liver preparation (Recio & Hsie, 1987). It induced morphological cell transformation in C3H10T½Cl8 cells (Gehly *et al.*, 1982). Each enantiomer of benzo[*a*]pyrene-7,8-diol induced malignant transformation of cultured rat hepatocytes (Heintz *et al.*, 1980).

Benzo[*a*]pyrene-7,8-diol-9,10-oxide in animals

Carcinogenicity studies of benzo[a]pyrene-7,8-diol-9,10-oxide in animals

Groups of 29 female CD-1 mice, 49–63 days of age, received a single dermal application of 200 nmol [60 μg] racemic *anti*-benzo[*a*]pyrene-7β,8α-diol-9α,10α-oxide or *syn*-benzo[*a*]pyrene-7β,8α-diol-9β,10β-oxide in anhydrous DMSO:acetone (1:3), follo-

wed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 30 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 30 weeks after promotion was 69 or 7% (1.5 or 0.07), respectively. No data were reported on skin tumours in mice treated with TPA alone (Slaga *et al.*, 1977b).

Groups of 29–30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [30 μg] (+)-*anti*-benzo[*a*]pyrene-7β,8α-diol-9α,10α-oxide, (–)-*syn*-benzo[*a*]pyrene-7β,8α-diol-9β,10β-oxide, (–)-*anti*-benzo[*a*]pyrene-7α,8β-diol-9β,10β-oxide or (+)-*syn*-benzo[*a*]pyrene-7α,8β-diol-9α,10α-oxide in anhydrous tetrahydrofuran, followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 24 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 24 weeks after promotion were 75% (2.0), 13% (0.13), 3% (0.03) or 10% (0.01), respectively. The incidence of skin tumours (tumours/mouse) in mice treated with TPA alone was 10% (0.1). In comparison, the incidence of skin tumours (tumours/mouse) in mice treated with benzo[*a*]pyrene (100 nmol) [25 μg] was 86% (2.8) (Slaga *et al.*, 1979).

Groups of newborn Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide or racemic *syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (total dose, 28 nmol) [8.5 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 28 weeks of age. In 64 or 21 male and female mice, pulmonary adenomas developed in 86 or 10% of the surviving mice (4.42 or 0.14 tumours/mouse), respectively. In 67 male and female mice treated with DMSO alone, pulmonary tumours occurred in 12% of the surviving mice (0.13 tumours/mouse) (Kapitulnik *et al.*, 1978a).

Groups of Swiss Webster BLU:Ha (ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, (–)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, (+)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide or (–)-*syn*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (total dose, 7 or 14 nmol) [2 or 4 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 34–37 weeks of age. Only mice treated with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide had a significant incidence of tumours. In 54 or 79 male and female mice treated with (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, pulmonary tumours (adenomas and adenocarcinomas) developed in 100 or 71% of the surviving mice (7.67 or 1.72 tumours/mouse), respectively. In 98 male and female mice treated with DMSO alone, pulmonary tumours (adenomas and adenocarcinomas) occurred in 11% of the surviving mice (0.12 tumours/mouse) (Buening *et al.*, 1978).

*DNA adducts of benzo[*a*]pyrene-7,8-diol-9,10-oxide*

Benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been measured in various mammalian cells and tissues exposed to benzo[*a*]pyrene-7,8-diol-9,10-oxide. An *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was detected in Chinese hamster V79 cells (Sundberg *et al.*, 2002), in mouse skin DNA after topical treatment with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide (Chen *et al.*, 1996), in human A549

epithelial lung carcinoma cells (Dreij *et al.*, 2005), in human lymphoblasts *in vitro* (Vähäkangas *et al.*, 1985) and in human fibroblasts (Hanelt *et al.*, 1997).

Genotoxicity of benzo[a]pyrene-7,8-diol-9,10-oxide

Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104, induced SOS response in *E. coli* (SOS chromotest in strain PQ37) and were mutagenic in Chinese hamster V79 cells (6-thioguanine resistance) (Glatt *et al.*, 1991). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced chromosomal aberrations (predominantly single chromatid breaks) in cultures of lymphocytes from normal individuals *in vitro* (Wei *et al.*, 1996). Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced sister chromatid exchange in Chinese hamster ovary cells; the *anti* isomer was 10-fold more active than the *syn* isomer (Pal *et al.*, 1980). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide induced DNA strand breaks and *HPRT* gene mutation (6-thioguanine resistance) in human fibroblast MRC5CV1 cells (Hanelt *et al.*, 1997).

(+)-Benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide induced mutations in the coding region of the *HPRT* gene in Chinese hamster V79 cells. Mutations at GC base pairs exceeded those at AT base pairs (Wei *et al.*, 1993). Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide morphologically transformed C3H/10T½ cells in culture (Krolewski & Little, 1985) and transformed human fibroblast MSU-1.1 cells into cell strains that formed colonies in agarose and formed sarcomas when injected into athymic mice (Yang *et al.*, 1992). (+)-Benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide also induced mutations, mainly at G:C base pairs (GC→TA), frameshift mutations and large deletion mutations in the *supF* gene of pUB3 in an *E. coli* plasmid (Rodriguez & Loechler, 1993).

DNA repair of adducts: quantitative repair

Benzo[*a*]pyrene-diol epoxide–DNA adducts in C57BL/6 mouse skin were repaired within 1 week after a single dose of benzo[*a*]pyrene (Bjelogrlic *et al.*, 1994).

In human A549 lung carcinoma cells, about 45% of the benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts formed by incubation with 50 nM (+)-*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide for 2 h were repaired within 8 h after treatment (Schwerdtle *et al.*, 2003).

Conclusion

There is strong evidence that benzo[*a*]pyrene is metabolically activated to the diol epoxide at the bay region in mouse skin and mouse lung carcinogenesis. Benzo[*a*]pyrene was metabolized by mouse and rat liver microsomes, rat hepatocytes, mouse epidermal tissues, hamster and mouse embryo cells, rat and hamster trachea, mouse skin and mouse lung tissues, human liver microsomes, human bronchial explants and epithelial cells, and human epidermal keratinocytes and fibroblasts to benzo[*a*]pyrene-7,8-diol. This metabolism proceeded through to two oxides: the major form, (+)-benzo[*a*]pyrene-7*R*,8*S*-oxide, and the minor form, (–)-benzo[*a*]pyrene-7*S*,8*R*-oxide, which, upon the action of epoxide hydrolase, gave (–)-benzo[*a*]pyrene-7*R*,8*R*-diol and (+)-benzo[*a*]pyrene-7*S*,8*S*-

diol, respectively. Upon further oxidation, (–)-benzo[*a*]pyrene-7*R*,8*R*-diol was metabolized to the major *anti* diol epoxide form, *anti*-(+)-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide, and the minor *syn* diol epoxide form, *syn*-(–)-benzo[*a*]pyrene-7*R*,8*S*-diol-9*R*,10*S*-oxide; (+)-benzo[*a*]pyrene-7*S*,8*S*-diol was metabolized to the minor *anti* diol epoxide form, *anti*-(–)-benzo[*a*]pyrene-7*S*,8*R*-diol-9*R*,10*S*-oxide, and the major *syn* diol epoxide form, *syn*-(+)-benzo[*a*]pyrene-7*S*,8*R*-diol-9*S*,10*R*-oxide. Racemic benzo[*a*]pyrene-7,8-oxide was genotoxic in bacteria and mammalian cells. (+)-Benzo[*a*]pyrene-7*R*,8*S*-oxide was more active than (–)-benzo[*a*]pyrene-7*S*,8*R*-oxide as a tumour initiator in mouse skin and as a pulmonary carcinogen in newborn mice. Racemic benzo[*a*]pyrene-7,8-diol was genotoxic in bacteria and in mammalian cells in the presence of various metabolic activation systems. (–)-Benzo[*a*]pyrene-7*R*,8*R*-diol was more active than (+)-benzo[*a*]pyrene-7*S*,8*S*-diol as a tumour initiator in mouse skin, as a pulmonary carcinogen in newborn mice and in the induction of malignant lymphomas in newborn mice. In mice, racemic benzo[*a*]pyrene-7,8-diol induced forestomach papillomas after treatment by gavage, malignant skin tumours after repeated treatment of the skin and fibrosarcomas after subcutaneous treatment.

Both racemic *syn*- and *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide were genotoxic in bacteria and mammalian cells. Racemic *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide was more active that the *syn* isomer as a tumour initiator in mouse skin and as a pulmonary carcinogen in mice and (+)-*anti*-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide had the greatest activity in both bioassay systems. Quantitatively, (+)-*anti*-benzo[*a*]pyrene-7*R*,8*S*-diol-9*S*,10*R*-oxide was more active as a tumour initiator in mouse skin or pulmonary carcinogen in newborn mice than (–)-benzo[*a*]pyrene-7*R*,8*R*-diol which was more carcinogenic than benzo[*a*]pyrene. The most common benzo[*a*]pyrene diol epoxide–DNA adduct detected in multiple bioassay systems was the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct. This adduct was formed *in vitro* and *in vivo* after treatment with benzo[*a*]pyrene, benzo[*a*]pyrene-7,8-diol or *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide, and is consistent with proto-oncogene mutations in tumours induced by benzo[*a*]pyrene. In addition, *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts are preferentially formed at lung cancer mutational hotspots in human p53 (see below (viii)).

(vi) *Evidence for other metabolic activation mechanisms*

9-Hydroxybenzo[*a*]pyrene was metabolized by an Aroclor 1254-induced rat liver preparation to an intermediate that formed a single DNA adduct in calf thymus DNA and in Chinese hamster V79 lung cell DNA (Sebti & Baird, 1984). *In vivo*, intraperitoneal administration of 9-hydroxybenzo[*a*]pyrene to rats produced this adduct in lung and peripheral blood lymphocytes (Ross *et al.*, 1990). It was identified as a 9-hydroxybenzo[*a*]pyrene-4,5-oxide adduct of deoxyguanosine (Vigny *et al.*, 1980; Fang *et al.*, 2001), and was detected *in vivo* after intraperitoneal exposure to benzo[*a*]pyrene of mouse lung (Mass *et al.*, 1993) and of rat lung and peripheral blood lymphocytes (Ross *et al.*, 1990). The significance of this adduct is questionable since 9-hydroxybenzo[*a*]pyrene

was not carcinogenic on mouse skin (Kapitulnik *et al.*, 1976), and was a weak tumour initiator (Slaga *et al.*, 1978b).

(vii) *Epigenetic effects of benzo[a]pyrene and its metabolites*

Effects on cell proliferation

Benzo[*a*]pyrene increased cell proliferation in primary human mammary epithelial cells (Tannheimer *et al.*, 1997) and in the spontaneously immortalized, non-tumorigenic growth factor-dependent human mammary epithelial cell line, MCF-10A (Tannheimer *et al.*, 1998). In human mammary epithelial cells, benzo[*a*]pyrene increased the level of intracellular Ca^{2+} after 2 h, but the effect was maximal at 18 h (Tannheimer *et al.*, 1997). Under the same conditions, no increase in calcium was seen with benzo[*e*]pyrene or anthracene. When benzo[*a*]pyrene was given at concentrations of 1, 3 and 10 μM the increase in Ca^{2+} after 18 h was dose-dependent (Tannheimer *et al.*, 1999) and dependent on metabolism, because benzo[*a*]pyrene-7,8-diol and benzo[*a*]pyrene diol epoxide were more effective than the parent compound and the increase in Ca^{2+} was inhibited by α-naphthoflavone, a CYP1A and CYP1B inhibitor. *N*-Acetylcysteine, an antioxidant, did not inhibit the increase in Ca^{2+}, which indicates that oxidative damage by benzo[*a*]pyrene is not responsible. The increase in Ca^{2+} seemed to be due to the influx of extracellular Ca^{2+}, which may be caused by the effects of benzo[*a*]pyrene on cell membranes where it can perturb the physical organization of phosphatidylcholine membranes (Jiménez *et al.*, 2002). Free intracellular Ca^{2+} is important for the activation of protein kinase C (PKC) pathways, which again is associated with tumour promotion (Rasmussen *et al.*, 1995).

1,6- and 3,6-Benzo[*a*]pyrene quinones produce superoxide anion and hydrogen peroxide in MCF-10A cells. They also increased epidermal growth factor receptor (EGFR), serine/threonine kinase Akt and extracellular signal-regulated kinase (ERK) activity, which led to increased cell numbers in the absence of epidermal growth factor. The benzo[*a*]pyrene quinone-induced EGFR activity and associated cell proliferation were attenuated by the EGFR inhibitor AG1478, as well as by the antioxidant *N*-acetylcysteine (Burdick *et al.*, 2003).

Low concentrations of benzo[*a*]pyrene (1, 3 or 10 μM) [0.25, 0.75 or 2.5 μg/mL] induce proliferation of cultured rat osteoblasts, the human osteosarcoma cell line, MG-63, as well as the human breast carcinoma cell line, MCF-7 (Tsai *et al.*, 2004). This effect could be inhibited by antagonists of estrogen receptors and inhibitors of ERK/mitogen-activated PK (MAPK) and phosphatidylinositol-3-kinase (PI3K), but not by α-naphthoflavone (AhR antagonist) or the p38 MAPK inhibitor. Benzo[*a*]pyrene induced phosphorylation of ERK1/2 and protein kinase B (Akt) (PI3K downstream effector). Other proteins that were increased in benzo[*a*]pyrene-treated osteoblasts were proliferating cell nuclear antigen, cyclooxygenase 2 (COX-2) (but not COX-1) and prostaglandin E_2. COX-2 inhibitors also inhibited benzo[*a*]pyrene-induced osteoblast proliferation. Proliferation at lower concentrations (up to 1 μM [0.25 μg/mL]), but cell death at higher concentrations of benzo[*a*]pyrene (10 μM) [2.5 μg/mL] were reported in

MCF-7 cells. Benzo[*a*]pyrene also induced p53 protein and a partial S-phase arrest (Pliskova *et al.*, 2005).

Benzo[*a*]pyrene diol epoxide increased Cdc25B (which regulates cell cycle progression and genetic stability) mRNA and protein levels in terminal squamous differentiated human bronchial epithelial cells and lung cancer cells but not in undifferentiated bronchial cells. With chronic exposure, the growth rate of lung cancer cells was increased significantly (Oguri *et al.*, 2003).

In the human trophoblastic JEG-3 cell line, benzo[*a*]pyrene inhibits proliferation in a dose-dependent manner by causing cell-cycle arrest at the G2/M phase, with a marked increase in p53 phosphorylation at serine 15 which is known to activate p53. However, no evidence of apoptosis was noted (Drukteinis *et al.*, 2005).

Effects on apoptosis

Benzo[*a*]pyrene has been shown to induce apoptosis in many types of murine and human cells, e.g. rat Sertoli cells (Raychoudhury & Kubinski, 2003), mouse hepatoma Hepa1c1c7 cells (Ko *et al.*, 2004) and human HepG2 cells (Chen *et al.*, 2003). There are many ways to apoptosis and benzo[*a*]pyrene may affect different apoptotic pathways in different cell types. For instance, benzo[*a*]pyrene-induced apoptosis has been reported to be Jun N-terminal kinase (JNK)-dependent in HeLa cells, but independent in Hepa1c1c7 cells (Yoshii *et al.*, 2001; Solhaug *et al.*, 2005).

Using mouse Hepa1c1c7 cells, Chen, S. *et al.* (2003) showed that the effect of benzo[*a*]pyrene-7,8-diol is AhR-dependent, but that of benzo[*a*]pyrene diol epoxide is not. Mouse embryo fibroblasts null for p38 were resistant to the apoptotic effect of the latter. In Hepa1c1c7 cells, Ko *et al.* (2004) showed that catalytic activation of caspases 3 and 9 by benzo[*a*]pyrene was associated with cytosolic release of cytochrome c, a decrease in B-cell lymphoma protein 2 (Bcl-2) to Bcl-2-antagonist X protein (Bax) ratio and ser-15 phosphorylation of p53. Benzo[*a*]pyrene and its 7,8-dihydrodiol and diol epoxide metabolites induced apoptosis and phosphorylation of p53 in mouse hepatoma Hepa1c1c7 cells, while benzo[*a*]pyrene-4,5-dihydrodiol did not. While Bcl-xl, Bad and Bid proteins were down-regulated, the anti-apoptotic phosphor-βad was up-regulated (Solhaug *et al.*, 2005). In rat liver cells, Na^+/H^+ exchange has been implicated as an early target of benzo[*a*]pyrene-induced apoptosis (Huc *et al.*, 2004).

At high concentrations (10, 50 or 100 μg/mL, equivalent to about 50, 200 or 400 μM), benzo[*a*]pyrene inhibits growth and is apoptotic in human mammary carcinoma MCF-7 cells (Ogba *et al.*, 2005). In A549 cells, BAX expression was increased at 24 h by a 25-μM [6.3 μg/mL] concentration of benzo[*a*]pyrene (Zhu *et al.*, 2005). Benzo[*a*]pyrene induced apoptosis in the human lung fibroblast cell line, MRC-5, via JNK1/FasL and JNK/p53 pathways (Chen *et al.*, 2005). According to studies in human embryonal kidney 293T cells and HeLa cells, p21 PK interacting factor accelerates benzo[*a*]pyrene-induced apoptosis through activation of JNK1 pathway kinases (Yoshii *et al.*, 2001). In human HepG2 cells, both benzo[*a*]pyrene-7,8-diol and benzo[*a*]pyrene diol

epoxide induced apoptosis and increased the phosphorylation of both MAPK p38 and ERK1/2 (Chen *et al.*, 2003).

In primary human haematopoietic CD34+ stem cells, benzo[*a*]pyrene induced the expression of the cleaved forms of caspases 3 and 9 and reduced mitochondrial membrane potential, and increased annexin V-positive cells indicative of apoptosis (van Grevenynghe *et al.*, 2005).

Effects on cell cycle

Exposure of A549 cells to a 25-μM concentration of benzo[*a*]pyrene for up to 72 h did not result in substantial phase-specific cell-cycle effects, but induced a distinctive pattern of gene expression: CYP1B1 was up-regulated at 6–24 h and many cell-cycle regulatory genes were down-regulated at 48–72 h (Zhu *et al.*, 2005).

A lower concentration (1 μM) [0.25 μg/mL] of benzo[*a*]pyrene induced an increase in p53 protein in MCF-7 and A549 cells that activate benzo[*a*]pyrene to benzo[*a*]pyrene diol epoxide–DNA adducts (Rämet *et al.*, 1995). A similar effect was seen in the skin of C57BL/6 mice *in vivo* (Bjelogrlic *et al.*, 1994; Tapiainen *et al.*, 1996). There is a positive correlation between the amount of induced p53 and benzo[*a*]pyrene diol epoxide–DNA adducts as measured by synchronous fluorescence spectrophotometry. Since these original studies, benzo[*a*]pyrene-induced, benzo[*a*]pyrene-diol epoxide–DNA-associated induction of p53 protein has been described in many experimental systems (e.g. in human lymphocytes exposed to benzo[*a*]pyrene *in vitro*) (Godschalk *et al*., 2001).

Gap-junctional intercellular communication

Gap-junctional communication is important in cell proliferation, differentiation and apoptosis and has been suggested to be important for the promotion of carcinogenesis (see Blaha *et al.*, 2002). Of 35 PAHs tested for inhibition of gap-junctional communication in WB-F344 rat liver epithelial cells, 12, including benzo[*a*]pyrene, were found to be strong but transient inhibitors (Blaha *et al.*, 2002).

Upham *et al.* (1994) compared the effect of a selection of methylated and non-methylated PAHs on gap-junction intercellular communication of rat liver epithelial cells with a scrape-loading/dye transfer assay. Methylated PAHs inhibited cell–cell communication to a greater extent than their non-methylated counterparts, and three-ringed PAHs to a greater extent than four- or five-ringed PAHs. Benzo[*a*]pyrene reduces the attachment of cultured human endometrial cells to Matrigel-coated membranes in culture, which is also relevant to the effect of benzo[*a*]pyrene on cell–cell communication (McGarry *et al.*, 2002). This was associated with decreased localization of EGFR protein along the cell membrane. A similar effect on EGFRs has been caused by benzo[*a*]pyrene in cell lines of placental origin and by cigarette smoking in the placenta (Zhang *et al*., 1995). *In vitro*, the physical organisation of phosphatidylcholine membranes was disturbed by benzo[*a*]pyrene (Jiménez *et al.*, 2002). Benane *et al.* (1999) demonstrated that multiple concentrations of benzo[*a*]pyrene and dibenzo[*a,l*]pyrene failed to induce any alterations in gap-junction intercellular communication in clones of normal rat

hepatocytes. However, DNA from the exposed cells was shown to contain PAH–DNA adducts, demonstrating that the cells were able to take up and metabolize both PAHs.

(viii) *Relevance of the diol epoxide mechanism for benzo[*a*]pyrene to human cancer*

General

Pathways of benzo[*a*]pyrene metabolism that possibly induce mutations include the formation of bulky benzo[*a*]pyrene-diol epoxide–DNA adducts, or the formation of radical cations, reactive oxygen species and *ortho*-quinones (see below). Both experimental and human data link bulky benzo[*a*]pyrene diol epoxide–DNA adducts with mutations in oncogenes and tumour-suppressor genes in human lung cancer.

The processes that metabolize benzo[*a*]pyrene to its active diol epoxides are present in human tissues, and humans exposed to benzo[*a*]pyrene metabolically activate it to benzo[*a*]pyrene diol epoxides that form DNA adducts. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts have been measured in populations exposed to benzo[*a*]pyrene in complex mixtures including coke-oven workers (Rojas *et al.*, 1995; Pavanello *et al.*, 1999a) and chimney sweeps (Pavanello *et al.*, 1999a) (as mentioned above in (ii)).

Studies on the ras gene

Experimental studies

anti-Benzo[*a*]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. The mutations (G→T transversions) in the K-*ras* proto-oncogene in lung tumours from benzo[*a*]pyrene-treated mice are associated with *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts (Mass *et al.*, 1993). Although this is not a direct correlation, similar mutations in the Ki-*ras* proto-oncogene were found in lung tumours from nonsmokers exposed to PAH-rich coal combustions (known to contain benzo[*a*]pyrene as well as many other PAHs) (DeMarini *et al.*, 2001).

Low doses of benzo[*a*]pyrene (8 nmol) [2 μg/mL] or benzo[*a*]pyrene combined with ultraviolet A (UVA) radiation induced G→A transitions in H-*ras* codon 12 or 13 in SKH-1 mouse skin, while no *TP53* mutations were found. Only the second guanosine of the codon was changed, and the *ras* mutations were already found in non-tumorous skin of all the mice after 10 weeks of treatment with benzo[*a*]pyrene (Wang *et al.*, 2005).

Benzo[*a*]pyrene induces G:C→T:A mutations in the murine *ras* gene (Lehman & Harris, 1994). While in some studies the majority of K-*ras* mutations in smoking-associated human lung cancer have been G→T transversions, discrepant results occur in the literature (Vähäkangas *et al.*, 2001 and references therein). Codons 12 and 14 of K-*ras* in genomic DNA from normal human bronchial epithelial (NHBE) cells are targeted *in vitro* by benzo[*a*]pyrene-7,8-diol-9,10-epoxide (2 μM [0.5 μg/mL], which is a high dose) (Hu *et al.*, 2003). In NHBE cells, benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts are preferentially formed within K-*ras* codon 12 (Feng *et al.*, 2002). Feng *et al.* (2002) also found that the repair of benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts formed at codon 14 was significantly faster than that of adducts at codon 12.

Human studies

There is a clear difference between the exact localization of K-*RAS* codon 12 mutations in colon cancer (not linked to smoking) and lung cancer (associated with smoking) (see Kelley & Littman, 2002). While in colon cancer 80% of K-*RAS* codon 12 mutations occur at position 2, in lung cancer, 50% occur at position 1, which is the same position where benzo[*a*]pyrene-7,8-diol-9,10-epoxide–DNA adducts are preferentially formed in NHBE cells.

Benzo[*a*]pyrene adducts (7-(benzo[*a*]pyren-6-yl)guanine) have been found in the urine of nonsmoking women exposed to smoky coal (Casale *et al*., 2001). Lung cancers from such women have a high percentage of GC→TA transversions of the *TP53* (76%) and K-*RAS* (86%) mutations (DeMarini *et al*., 2001). Smoky coal emissions, cigarette-smoke condensate and benzo[*a*]pyrene induce similar mutation spectra in *Salmonella* base substitution strain TA100: primarily GC→TA transversions (Granville *et al*., 2003). In a recent study (Le Calvez *et al.*, 2005) based on the analysis of primary lung tumours from 64 (mainly) heavy smokers, 27 former smokers and 40 never smokers, K-*RAS* mutations were more frequent in former smokers than in never or current smokers.

H-*RAS*-Gene mutations correlated with the N7-guanine and N7-adenine depurinating adducts (Colapietro *et al.*, 1993; Chakravarti *et al.*, 1995). Occupational exposures to PAHs, benzo[*a*]pyrene and gasoline have a weak association with K-*RAS* mutations in pancreatic cancer (Alguacil *et al.*, 2003).

Studies on TP53

The importance of p53

p53 Protein is of interest in connection with benzo[*a*]pyrene because it is in the centre of cellular defence against DNA damage (for recent reviews, see e.g. Meek, 2004; Harris & Levine, 2005). p53 Protein is involved in the regulation of cell cycle and apoptosis, as well as DNA repair, mainly by acting as a transcription factor for cellular key proteins involved in these processes. The significance of p53 protein as a tumour suppressor is demonstrated by studies on p53-deficient mice that develop tumours (predominantly lymphomas) spontaneously by the age of 6 months (reviewed by Hoogervorst *et al.*, 2005). $p53^{-/-}$ Mice are viable except for some female embryos that die before birth of neural tube defects. About 50% of $p53^{+/-}$ mice develop predominantly osteosarcomas and soft-tissue sarcomas by the age of 18 months. This phenotype mimics partially human patients with Li-Fraumeni syndrome, who, in addition to sarcomas, also develop breast and brain tumours that are rarely observed in p53-deficient mice. Importantly for chemical carcinogenesis, p53-deficient mice show enhanced tumour response among other tissues (in lung and skin), when treated with known chemical carcinogens.

Experimental studies of TP53 mutations

Experimental findings on benzo[*a*]pyrene-induced lesions should account for the mutations seen in *TP53* in patients with lung cancer, e.g. mutational pattern (G→T transversions), mutational spectrum (mutations at specific codons 157,158, 245, 248,

273), preference for methylated CpG islands and the bias for the non-transcribed strands. Two experimental paradigms could account for these lesions: (i) site-specific adduction of hotspots by *anti*-benzo[*a*]pyrene diol epoxide or (ii) random mutagenesis by reactive oxygen species coupled with biological selection. Most of the existing data support the former hypothesis (Pfeifer & Hainaut, 2003). Metabolic activation can account for both of these paradigms: CYP activation of benzo[*a*]pyrene to *anti*-benzo[*a*]pyrene diol epoxide or AKR activation of benzo[*a*]pyrene-7,8-diol to benzo[*a*]pyrene-dione which is redox active.

Incorporation of a single (+)- or (–)-*trans*-*anti*-benzo[*a*]pyrene diol epoxide–DNA adduct at the second position of codon 273 ($^{5'}$CGT) of the human *TP53* gene in Simian kidney COS-7 cells resulted in predominant mutations by both stereoisomers being G→T transversions, with some G→A transitions (Dong *et al.*, 2004). When the cytosine 5′ to deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide was replaced by 5-methylcytosine, the mutational frequencies of (+)-*trans*-deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide and (–)-*trans*-deoxyguanosine-N^2-benzo[*a*]pyrene diol epoxide were reduced, while the mutational specificity remained unchanged. Thus, the mutational hotspot at codon 273 in *TP53* may reflect either sequence-specific reactivity of benzo[*a*]pyrene diol expoxide and/or inefficient repair of its DNA adducts positioned at this site.

Denissenko *et al.* (1996), Pfeifer and Denissenko (1998) and Smith *et al.* (2000) have shown that codons 157, 248 and 273 in the *TP53* gene are most mutated in lung cancer, are also targets for DNA adduct formation by benzo[*a*]pyrene and are more prone to mutations by it. Slow repair of these adducts (Denissenko *et al.*, 1998) and the preferential formation of benzo[*a*]pyrene diol epoxide adduct at methylated CpG sites (Denissenko *et al.*, 1997; Tang *et al.*, 1999) probably contribute to the mutation spectrum in smoking-related lung cancer, where G→T mutations at CpG sites are the most common single type of mutation (Hainaut & Pfeifer, 2001). The same group (Smith *et al.*, 2000) has also studied other PAH compounds and found a similar preference for adduct formation in codons 157, 158, 245, 248 and 273 in human bronchial epithelial BEAS-2B cells. In support for these findings, Hussain *et al.* (2001) demonstrated a high dose-dependent G:C to T:A transversion rate of *TP53* codons 157 and 248 by benzo[*a*]pyrene diol epoxide in the same BEAS-2B cell line.

Smoky coal combustion induced GC→TA transversions in *Salmonella* to a similar extent as cigarette-smoke condensate and benzo[*a*]pyrene (Granville *et al.*, 2003). Liu *et al.* (2005) showed that, in murine embryonic fibroblasts from human *TP53* knock-in (Hupki) mice, benzo[*a*]pyrene induced mutations similar to those found in smoking-related lung cancer: predominance of G→T mutations, unequivocal strand bias of the transversions and mutational hotspots at codons 157 and 158.

Human studies of TP53 mutations

Data from lung cancer patients exposed to both smoky coal and cigarette smoke provide evidence that PAHs, especially benzo[*a*]pyrene, are involved in human lung carcinogenesis caused by these exposures. Benzo[*a*]pyrene depurinating adducts N7-

guanine and N7-adenine have been found in the urine of cigarette smokers and women exposed to household smoke (Casale *et al.*, 2001).

In the *TP53* gene, G→T transversions are more common in lung cancer than in any other human cancer: over 30% versus 10% (except for hepatocellular cancer associated with exposure to aflatoxin) (Hainaut & Pfeifer, 2001; Pfeifer & Hainaut, 2003). The G→T transversions are found in all histological types of lung cancer of smokers and support their common origin as the direct result of DNA damage. In other organs, differences in *TP53* mutation types occur depending on the histology. There is a statistically significant difference in the frequency of G:C→T:A transversions in lung cancer of smokers (higher) compared with nonsmokers (Hainaut & Pfeifer, 2001; Pfeifer & Hainaut, 2003). In smoking-related lung cancer, the frequency of these mutations increases with increasing smoking (dose–response relationship) (Bennett *et al.*, 1999). Codons 157, 248 and 273 in the *TP53* gene that are most mutated in lung cancer are also targets for DNA adduct formation by benzo[*a*]pyrene and are more prone to its mutations (Denissenko *et al.*, 1996; Pfeifer & Denissenko, 1998; Smith *et al.*, 2000).

However, the final proof for a specific *TP53* mutation spectrum related to smoking requires the comparison of the mutation spectrum in lung cancers from smokers with that in nonsmokers. Such studies are difficult to carry out because of the rarity of lung cancer in nonsmokers. A further difficulty is that most of the smokers and smoking-related lung cancers are found in men and most nonsmokers are women. Consequently, the possible gender difference, as well as the possible geographical difference (Bennett *et al.*, 1999) have to be taken into account in such studies. The few existing studies of nonsmokers support the hypothesis that smoking-related *TP53* mutations are more frequent in smokers than in nonsmokers (Vähäkangas *et al.*, 2001 and references therein; Le Calvez *et al.*, 2005). In the study by Le Calvez *et al.* (2005) on never (n = 40), former (n = 27) and current heavy smokers (n = 64), GC→TA transversions and AT→GC transitions were associated with smoking while GC→AT transitions were associated with never smoking.

Nearly 90% of the G→T mutations occur on the non-transcribed (coding) strand of the gene, as indicated by the overrepresentation of G→T compared with C→A. On the transcribed strand, G→T mutation would result in C→A on the coding, non-transcribed strand according to the Watson-Crick rule for base-pairing. A possible reason for the strand bias is slow repair of bulky benzo[*a*]pyrene diol epoxide–DNA adducts along the non-transcribed strand of human *TP53* (Denissenko *et al.*, 1998). A similar preferential repair of benzo[*a*]pyrene diol epoxide in the *HPRT* gene of diploid human fibroblasts has been described (Chen *et al.*, 1992).

An alternative hypothesis for the origin of specific *TP53* mutations in lung cancer has been put forward by Rodin and Rodin (2005), who propose that repetitive exposure to tobacco components drives clonal expansion of cells with a pre-existing endogenous *TP53* mutation. However, no data are available that would support the idea that mutant proteins encoded by genes with a G→T transversion would give a better growth advantage than other types of mutation, which would explain the overrepresentation of these mutants in lung cancer (Pfeifer & Hainaut, 2003).

Epigenetic modification of C in a CpG sequence to 5-methylcytosine makes the C prone to mutations (Rideout *et al*., 1990; Tornaletti & Pfeifer, 1995). All CpGs are methylated in the central region of the *TP53* gene that codes for the DNA-binding region of the protein. Methylated CpGs in human lung cancers are the preferred sites for G→T mutations (over 50% of such mutations in lung cancer) (Yoon *et al*., 2001; Pfeifer & Hainaut, 2003). In cells that have reporter genes that contain the methylated CpG, these are targets of G→T mutations after exposure to benzo[*a*]pyrene diol epoxide (Yoon *et al*., 2001). In contrast, transitions (C→T and G→A at CpG sites) occur in excess in cancers that are associated with inflammatory diseases, where oxidative stress is a more probable cause of *TP53* mutations. The fact that transition mutations rather than transversions are very common in the *TP53* gene in cancers related to inflammatory responses argues against a role of oxidative stress in *TP53* mutations of smoking-associated lung cancers. Furthermore, there is a discrepancy about whether 8-oxo-deoxyguanosine, which is caused by oxidative stress, is repaired by strand-specific transcription-coupled repair (Pfeifer & Hainaut, 2003).

In China, smoky coal is used for cooking and the indoor air levels of benzo[*a*]pyrene can reach levels comparable with those experienced by workers in old-type coking plants (Lan *et al.*, 2000). The high incidence of lung cancer among nonsmoking Chinese women is clearly associated with the use of smoky coal and the increase in risk is dose-dependent. This association was substantially larger and achieved statistical significance among patients with sputum samples that were positive for p53 overexpression (Lan *et al.*, 1993, 2000, 2001). This is in accordance with the higher number of benzo[*a*]pyrene–DNA adducts in the bronchoalveolar-lavage cells of these women than in non-exposed controls (Gallagher *et al.*, 1993). DeMarini *et al.* (2001) found that the *TP53* mutations in the lung cancer tissue of these women clustered in codons 153–158; most of the mutations were G→T and all G→T transversions were found on the non-transcribed strand. A hotspot at the *TP53* codon 154, which is a hotspot for PAH adducts (Smith *et al.*, 2000) but is not found in lung cancers from smokers (Hainaut & Pfeiffer, 2001), was identified.

Individual susceptibility to benzo[*a*]pyrene-induced mutations is well-illustrated by the study of Hussain *et al.* (2001) that shows that non-tumorous tissue of lung cancer patients contained *TP53* codon 157 G:C→T:A mutations while lung tissue from non-cancer patients did not, regardless of the fact that the majority of the controls were also smokers. The whole chain of events from exposure to lung cancer is thus covered by the current evidence. Many of the details, however, still remain to be solved. Studies in silico (computational tools that translate raw data into workable models or simulations) using the genetic algorithm, a molecular dynamics-based protocol have revealed, for instance, how a single adduct ((+)-*anti*-benzo[*a*]pyrene diol epoxide) may give rise to different mutations by adopting different conformations (Kozack & Loechler, 1999 and references within).

Conclusions

The evidence strongly supports benzo[*a*]pyrene in smoky coal and in cigarette smoke as the cause of *TP53* mutations in lung cancer. This is based on the following:

1. Metabolic activation of benzo[*a*]pyrene and the formation of its 7,8-diol-9,10-oxide and benzo[*a*]pyrene diol epoxide–DNA adducts occur in human bronchial cells and tissue explants.
2. Benzo[*a*]pyrene diol epoxide–DNA adducts are found in PAH-exposed people, with a higher level of the adducts in the lung and bronchial tissue from smokers than in those from nonsmokers.
3. G→T transversions in the TP53 gene are more common in lung cancers from smokers than in any other cancer type.
4. A high frequency of G→T transversions is found in lung cancers from women exposed to smoky coal combustion at either the K-*RAS* or *TP53* gene.
5. The formation of DNA adducts by benzo[a]pyrene in vitro (including in cells with a human *TP53* sequence) targets the same hotspots in the *TP53* gene as those where G→T mutations are found in cigarette smoke- and smoky coal-associated lung cancer.
6. Exposure to both cigarette smoke and smoky coal is associated with a dose-dependent increase in G→T transversions in the *TP53* gene.
7. A similar strand bias, with an overrepresentation of G→T mutations, occurs in the cigarette smoke- and smoky coal-associated *TP53* mutation pattern in human lung cancer and in vitro in benzo[*a*]pyrene-treated mouse cells that contain the human *TP53* sequence (in accordance with slow repair of bulky benzo[*a*]pyrene diol epoxide–DNA adducts on the non-transcribed strand).
8. A preference for G→T transversions in the methylated CpG dinucleotides in human lung tumours is in accordance with in-vitro studies that show the same dinucleotide as a target of benzo[*a*]pyrene diol epoxide.

Cyclopenta[*cd*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of cyclopenta[*cd*]pyrene has been documented in a number of studies in rat liver, human liver and liver microsomes. There are two potential centres for biochemical oxidation of cyclopenta[*cd*]pyrene ─ the cyclopenta-ring (C3–C4) and the K-region (C9–C10) ─ and both are involved in this process. 3-Methylcholanthrene- and phenobarbital-induced rat liver microsomes metabolized cyclopenta[*cd*]pyrene to the cyclopenta-ring diol, *trans*-3,4-dihydroxy-3,4-dihydro-cyclopenta[*cd*]pyrene (cyclopenta-[*cd*]pyrene-3,4-diol) (Gold & Eisenstadt, 1980). 3-Methylcholanthrene- and phenobarbital-induced rat liver microsomes and reconstituted cytochrome P448 and CYP systems metabolized cyclopenta[*cd*]pyrene to the K-region diol, *trans*-9,10-dihydroxy-9,10-dihydro-cyclopenta[*cd*]pyrene (cyclopenta[cd]pyrene-9,10-diol) (Eisenstadt *et al.*, 1981). Uninduced mouse liver and human liver microsomes metabolized cyclopenta-[*cd*]pyrene to cyclopenta[*cd*]pyrene-3,4-diol, *cis*-cyclopenta[*cd*]pyrene-3,4-diol, 4-hydroxy-

3,4-dihydro-cyclopenta[*cd*]pyrene, 4-oxo-3,4-dihydro-cyclopenta[*cd*]pyrene and two diastereomeric *trans,trans*- 3,4,9,10-tetrahydro-cyclopenta[*cd*]pyrene-3,4,9,10-tetrols (Sahali *et al.*, 1992). Human CYP1A1, CYP1A2 and CYP3A4 metabolized cyclopenta[*cd*]pyrene at the cyclopenta double bond to give cyclopenta[*cd*]pyrene-3,4-diol. Human CYP1A2 metabolized cyclopenta[*cd*]pyrene at the K-region to give cyclopenta[*cd*]pyrene-9,10-diol as indirectly demonstrated by the triols and tetrols formed (Kwon *et al.*, 1992). Cyclopenta[*cd*]pyrene is presumed to be converted to cyclopenta[*cd*]pyrene-3,4-oxide by a 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE)/haematin system (Reed *et al.*, 1988). A 15-HPETE/haematin system, a prostaglandin H synthetase system or a sulfite auto-oxidation system converted cyclopenta[*cd*]pyrene to *cis* and *trans* isomers of cyclopenta[*cd*]pyrene-3,4-diol and 4-keto-(3*H*)-cyclopenta[*cd*]pyrene, presumably through cyclopenta[*cd*]pyrene-3,4-oxide (Reed & Ryan, 1990).

(ii) *Formation of DNA adducts*

Cyclopenta[*cd*]pyrene formed DNA adducts in a rat liver microsomal calf thymus DNA system using livers from untreated, Aroclor 1254-treated, β-naphthoflavone-treated and phenobarbital-treated rats. Cyclopenta[*cd*]pyrene formed DNA adducts in the liver, lung, heart and white blood cells of Sprague-Dawley rats. The rat lung adducts were identified as cyclopenta[*cd*]pyrene-3,4-oxide–deoxyguanosine and cyclopenta[*cd*]pyrene-3,4-oxide–deoxyadenosine adducts. These two DNA adducts were formed in the skin of NIH Swiss mice and in the lungs of $B6C3F_1$ mice treated with cyclopenta[*cd*]pyrene (Beach & Gupta, 1994).

(iii) *Genotoxicity of cyclopenta[*cd*]pyrene*

In a previous monograph, cyclopenta[*cd*]pyrene was reported to induce mutations in *S. typhimurium* TA1537, TA98 and TM677, in mouse lymphoma L5178Y cells and in human lymphoblastoid HH-4 cells *in vitro* and to induce morphological cell transformation in C3H10T½CL8 mouse embryo cells (IARC, 1983). It was mutagenic in *S. typhimurium* TA98 after activation by systems that generate peroxyl radicals, by prostaglandin H synthetase and arachidonic acid, by 15-HPETE and haematin or by the autoxidation of the sulfite ion (Reed *et al.*, 1988). Cyclopenta[*cd*]pyrene was mutagenic in Chinese hamster V79 cells (6-thioguanine resistance or ouabain resistance) co-incubated with X-irradiated golden hamster embryo fibroblasts (Raveh *et al.*, 1982), in human cells that express CYP1A1 such as MCL-3 cells (thymidine kinase resistance) (Lafleur *et al.*, 1993), human B lymphoblastoid MCL-5 cells (6-thioguanine resistance) (Keohavong *et al.*, 1995) and in human B lymphoblastoid H1A1v2 cells (thymidine kinase locus) (Durant *et al.*, 1996). Cyclopenta[*cd*]pyrene induced sister chromatid exchange in C3H10T½Cl8 cells in culture (Krolewski *et al.*, 1986).

(iv) *Cyclopenta[*cd*]pyrene-induced mutations in proto-oncogenes*

Cyclopenta[*cd*]pyrene induced two major classes of Ki-*ras* codon 12 mutations in lung adenomas from cyclopenta[*cd*]pyrene-treated strain A mice: GGT→CGT (50%),

GGT→GTT (15%), GGT→TGT (25%) and GGT→GAT (10%) which indicated that guanine was a primary target for this PAH (Nesnow *et al.*, 1994a, 1995, 1998a).

(v) *Other effects of cyclopenta[*cd*]pyrene*

Cyclopenta[*cd*]pyrene induced apoptosis in mouse hepatoma Hepa1c1c7 cells, both apoptotic (DNA-dependent PK, ataxia telangiectasia-mutated (ATM) and/or ATM-related kinase, p53, and p38-MAPK) and anti-apoptotic (Akt and ERK) signals and the formation of the active form of caspase-3, cleavage of PARP and DNA fragmentation (Solhaug *et al.*, 2004a,b).

(vi) *Evidence for cyclopenta-ring oxidation as a mechanism of carcinogenesis*

DNA adducts of cyclopenta[*cd*]pyrene-3,4-oxide

Cyclopenta[*cd*]pyrene-3,4-oxide forms primarily deoxyguanosine DNA adducts in calf thymus DNA (Beach *et al*., 1993; Hsu *et al*., 1997); the major adducts observed were diastereoisomers of *cis*-3-(deoxyguanosin-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]-pyrene (Hsu *et al.*, 1997). Cyclopenta[*cd*]pyrene-3,4-oxide-2′–deoxyguanosine adducts have been detected in lung DNA from strain A/J mice treated with cyclopenta[*cd*]pyrene (Nesnow *et al.*, 1995).

Genotoxicity of cyclopenta[*cd*]pyrene-3,4-oxide

Cyclopenta[*cd*]pyrene-3,4-oxide was mutagenic in *E. coli* and induced G→T and A→T mutations (Eisenstadt *et al.*, 1982) and in *S. typhimurium* strains TA98, TA100, TA1537 and TA1538 in the absence and presence of a phenobarbital- or a 3-methyl-cholanthrene-induced rat liver preparation (Gold & Eisenstadt, 1980). Cyclopenta-[*cd*]pyrene-3,4-oxide was mutagenic in mouse lymphoma L5178Y cells (thymidine kinase resistance) and induced morphological cell transformation in C3H10T½CL8 mouse embryo cells in culture (Gold *et al.*, 1980).

DNA adducts of cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydro-cyclopenta[*cd*]pyrene

Cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene were bound to calf thymus DNA in the presence of rodent liver cytosol and 3′-phos-phoadenosine-5′-phosphosulfate, suggesting a SULT-mediated activation mechanism (Surh *et al.*, 1993). The adducts formed with cyclopenta[*cd*]pyrene-3,4-diol were iden-tified as *cis*-3-(deoxyguanosine-N^2-yl)-4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (Hsu *et al*., 1999). SULT activation of 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene with deoxyguanosine or reaction of 4-sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene with deoxy-guanosine gave the same DNA adduct (Hsu *et al.*, 1999).

Genotoxicity of cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene (4-sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene)

Cyclopenta[*cd*]pyrene-3,4-diol was mutagenic to *S. typhimurium* TM677 in the presence of a rat liver cytosol and 3′-phosphadenosine-5′-phosphosulfate (Surh *et al.*, 1993).

4-Hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene was mutagenic to *S. typhimurium* TM677 in the presence of a rat liver cytosol and 3′-phosphadenosine-5′-phosphosulfate. 4-Sulfooxy-3,4-dihydrocyclopenta[*cd*]pyrene was a direct-acting mutagen in *S. typhimurium* TM677 (without metabolic activation) (Surh *et al.*, 1993).

Conclusion

Cyclopenta[*cd*]pyrene is metabolically activated by the cyclopenta-ring mechanism. The evidence for this mechanism is based on the detection of the cyclopenta-ring hydroxylated metabolites, cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*] pyrene, in microsomal systems from rat, mouse and human liver and by peroxy radical-mediated activation systems. The formation of cyclopenta[*cd*]pyrene-3,4-oxide is inferred by the detection of cyclopenta[*cd*]pyrene-3,4-diol as a metabolite. Cyclopenta[*cd*]pyrene-3,4-oxide is genotoxic in bacteria and mammalian cells and forms DNA adducts with deoxyguanosine. 4-Hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene–DNA adducts are formed from SULT-activated 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene. In mouse lung, cyclopenta[*cd*]pyrene forms cyclopenta[*cd*]pyrene-3,4-oxide–deoxyguansine adducts and induces mutations in tumours at guanine in codon 12 of the Ki-*ras* oncogene. Cyclopenta[*cd*]pyrene-3,4-diol and 4-hydroxy-3,4-dihydrocyclopenta[*cd*]pyrene have not been evaluated for tumour initiation in mice.

Dibenz[*a,h*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of dibenz[*a,h*]anthracene has been documented in a number of studies in rat microsomes, recombinant human liver CYPs and human liver microsomes. Two equivalent potential centres of metabolism and metabolic activation are situated on the two benzo rings (or two bay regions) at the 1–2 and 3–4 bonds and the 10–11 and 12–13 bonds. Dibenz[*a,h*]anthracene was metabolized by 3-methylcholanthrene-induced rat liver preparations to a compound supposed to be 5,6-dihydroxy-5,6-dihydrodibenz-[*a,h*]anthracene (dibenz[*a,h*]anthracene-5,6-diol) (Selkirk *et al.*, 1971) and by Aroclor 1254-induced rat liver microsomes or a reconstituted cytochrome P448 system to 1,2-dihydroxy-1,2-dihydrodibenz[*a,h*]anthracene (dibenz[*a,h*]anthracene-1,2-diol), the major metabolite, 3,4-dihydroxy-3,4-dihydrodibenz[*a,h*]anthracene (dibenz[*a,h*]anthracene-3,4-diol), trace amounts of dibenz[*a,h*]anthracene-5,6-diol and two unidentified phenols (Nordqvist *et al.*, 1979). Dibenz[*a,h*]anthracene formed more than 30 metabolites after incubation with Aroclor 1254-induced rat liver microsomes. Among those identified were dibenz-[*a,h*]anthracene-5,6-oxide, dibenz[*a,h*]anthracene-1-, -2-, -3-, -4-, -5- and -6-phenols, *trans-trans*-3,4:12,13-tetrahydroxy-3,4:12,13-tetrahydrodibenz[*a,h*]anthracene, *r*1,*t*2,*t*3,*c*4-

tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene, *r*1,*t*2,*c*3,*t*4-tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene, dibenz[*a*,*h*]anthracene-3,4-catechol and a dibenz[*a*,*h*]-anthracenephenol dihydrodiol derived from the 2-phenol (Platt & Reischmann, 1987). 3-Methylcholanthrene-induced microsomes metabolized dibenz[*a*,*h*]anthracene to a bis-diol, *trans-trans*-3,4:10,11-tetrahydroxy-3,4:10,11-tetrahydrodibenz[*a*,*h*]anthracene (dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol), and the related hexols 1,2,3,4,8,9-hexahydroxy-1,2,3,4,8,9-hexahydrodibenz[*a*,*h*]anthracene, 1,2,3,4,10,11-hexahydroxy-1,2,3,4,10,11-hexahydrodibenz[*a*,*h*]anthracene and the related tetrol, 1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene (Lecoq *et al.*, 1989, 1991a). Dibenz[*a*,*h*]anthracene was stereospecifically metabolized by Aroclor 1254-induced rat liver microsomes to the *trans* diols, dibenz[*a*,*h*]anthracene-1,2-diol, dibenz[*a*,*h*]anthracene-3,4-diol and dibenz[*a*,*h*]-anthracene-5,6-diol, each of which is highly enriched in *R*,*R* enantiomers (Platt *et al.*, 1990). Microsomes from untreated, 3-methylcholanthrene-treated, phenobarbital-treated and Aroclor 1254-treated rats metabolized dibenz[*a*,*h*]anthracene to dibenz[*a*,*h*]-anthracene-5,6-oxide highly enriched with the dibenz[*a*,*h*]anthracene-5*S*,6*R*-oxide form (Mushtaq *et al.*, 1989). Dibenz[*a*,*h*]anthracene was metabolized by recombinant human CYP enzymes CYP1A2, 2B6, 2C8, 2C9, 2E1, 3A3, 3A4 and 3A5 expressed in hepatoma G2 cells and by 14 different human liver microsomes to dibenz[*a*,*h*]anthracene-1,2-diol, dibenz[*a*,*h*]anthracene-3,4-diol, dibenz[*a*,*h*]anthracene-5,6-diol and several phenols. CYP1A2 and CYP2C9 were the most active and CYP2B6 was moderately active in the rate of total dibenz[*a*,*h*]anthracene metabolism. Human liver microsomes gave metabolic patterns similar to those generated from CYP1A2 (Shou *et al.*, 1996a). Rat liver microsomal UGT mediated the glucuronidation of the 1-, 2-, 4-, 5-, 6- and 7-phenols of dibenz[*a*,*h*]anthracene and the UGT activities were affected by the inducers 3-methylcholanthrene and phenobarbital (Lilienblum *et al.*, 1987).

(ii) *Formation of DNA adducts*

Dibenz[*a*,*h*]anthracene formed 14 DNA adducts in mouse liver DNA *in vitro* in the presence of 3-methylcholanthrene-induced rat liver microsomes. The same pattern of adducts was obtained in mouse liver DNA after incubation *in vitro* with dibenz[*a*,*h*]anthracene-3,4-diol. One adduct was identified as a *trans* adduct of *anti*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene-1,2-oxide (*anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide) and the other as a *trans* adduct of *syn*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenz[*a*,*h*]anthracene-1,2-oxide (*syn*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide) (Lecoq *et al.*, 1991b). Dibenz[*a*,*h*]anthracene formed eight DNA adducts with calf thymus DNA incubated with Aroclor 1254-induced rat liver microsomes. The major bay-region diol epoxide adduct was identified as *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine. Other DNA adducts were *anti*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyguanosine, *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine, *anti*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyadenosine, *syn*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide–deoxyadenosine and *syn*-dibenz[*a*,*h*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide–deoxyadenosine. Unidentified, highly polar adducts were also

observed (Mlcoch *et al.*, 1993). The highly polar dibenz[*a*,*h*]anthracene calf thymus DNA adducts originated from the bis-diols, 3*R*,4*R*,10*R*,11*R*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene and 3*R*,4*R*,10*S*,11*S*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]-anthracene. Quantitatively, the highly polar bis-diol DNA adducts represented 38% of the adducts and the dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide bay-region diol epoxide adducts represented 25% (Fuchs *et al.*, 1993a). Calf thymus DNA adducts of 3*R*,4*R*,10*R*,11*R*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene and 3*R*,4*R*,10*S*,11*S*-tetrahydroxy-3,4,10,11-tetrahydrodibenz[*a*,*h*]anthracene were found after incubation of racemic dibenz-[*a*,*h*]anthracene-3,4:10,11-bis-diol with Aroclor 1254-induced rat liver microsomes (Fuchs *et al.*, 1993b). Dibenz[*a*,*h*]anthracene formed DNA adducts in mouse embryo C3H10T½CL8 cells, most of which were also found in C3H10T½CL8 cells treated with dibenz[*a*,*h*]anthracene-3,4-diol or *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide. Two of these were *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts. Several additional polar dibenz[*a*,*h*]anthracene–DNA adducts were derived from racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol (Nesnow *et al.*, 1994b). Dibenz[*a*,*h*]anthracene formed DNA adducts on mouse skin *in vivo*. A similar pattern of adducts was observed after topical treatment of mouse skin with dibenz[*a*,*h*]anthracene-3,4-diol, but only four of these adducts were observed after topical treatment with *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide and none after treatment with *syn*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide. The authors suggested that most of the DNA adducts formed in dibenz[*a*,*h*]-anthracene-treated mouse skin proceeded through the related dibenz[*a*,*h*]anthracene-3,4-diol and that only some were formed following the conversion of this diol to the bay-region *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide (Lecoq *et al.*, 1991c). Further analyses of the polar dibenz[*a*,*h*]anthracene DNA adducts in mouse skin indicated that one of them was from the further metabolism of the intermediate, dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol. A bis-diol epoxide, dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol-1,2-oxide, was proposed as the ultimate DNA-binding intermediate (Carmichael *et al.*, 1993). Human skin or mouse skin treated in short-term organ culture with dibenz[*a*,*h*]anthracene gave an adduct profile that was qualitatively similar to that obtained from treatment of mouse skin *in vivo*. Mouse skin treated with dibenz[*a*,*h*]anthracene-3,4-diol *in vivo* and in short-term organ culture gave similar DNA adduct profiles (Lecoq *et al.*, 1992). Dibenz[*a*,*h*]anthracene binds at different levels to the DNA in the epidermis of Cyp1a2$^{-/-}$, Cyp1b1$^{-/-}$ and Ahr$^{-/-}$ knockout mice *in vivo*, with the least binding in the Ahr$^{-/-}$ mice; this suggests that CYP1A1 is involved in the bioactivation of dibenz[*a*,*h*]anthracene (Kleiner *et al.*, 2004).

Dibenz[*a*,*h*]anthracene induced five DNA adducts in strain A/J mouse lung, three of which resulted from metabolism via dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol and two via dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide (Ross *et al*., 1995).

(iii) *Genotoxicity of dibenz[*a,h*]anthracene*

In a previous monograph, dibenz[*a*,*h*]anthracene was reported to induce DNA damage and mutations in bacteria, DNA damage in rodent and human cells in culture,

mutations and chomosomal damage in rodent cells in culture and morphological cell transformation (IARC, 1983).

Dibenz[*a,h*]anthracene was mutagenic in *S. typhimurium* TA100 in the presence of a phenobarbital-induced mouse liver preparation (Platt *et al.*, 1982), 3-methylcholanthrene-induced rat liver microsomes or a metabolic activation system (Lecoq *et al.*, 1989), in Chinese hamster V79 cells co-cultivated with human hepatoma HepG2 cells (Diamond *et al.*, 1984) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996). Dibenz[*a,h*]anthracene induced sister chromatid exchange and micronucleus formation in lung cells cultured *in vitro* that were taken from rats treated with dibenz[*a,h*]anthracene by intratracheal instillation (Whong *et al.*, 1994). Dibenz[*a,h*]anthracene induced micronucleus formation in bone marrow and spleen polychromatic erythrocytes from rats treated *in vivo* by intratracheal instillation (Zhong *et al.*, 1995). It induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Metabolism of dibenz[*a,h*]anthracene-3,4-diol

Dibenz[*a,h*]anthracene-3,4-diol formed more than 13 metabolites when metabolized by Aroclor 1254-induced rat liver microsomes. The major metabolites were *anti*-dibenz-[*a,h*]anthracene-3,4-diol-1,2-epoxide, *trans,trans*-3,4:8,9-tetrahydroxy-3,4,8,9-tetrahydro-dibenz[*a,h*]anthracene, dibenz[*a,h*]anthracene-3,4:10,11-bis-diol and *trans,trans*-3,4:12,13-tetrahydroxy-3,4,12,13-tetrahydrodibenz[*a,h*]anthracene (Platt & Schollmeier, 1994).

Carcinogenicity studies of dibenz[*a,h*]anthracene-3,4-diol

Groups of 29 or 30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [31 μg] racemic dibenz[*a,h*]anthracene-3,4-diol followed 7 days later by 3.25 nmol [2 μg] TPA in acetone twice a week for 15 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 16 weeks after treatment was 37% (0.7). Skin tumours developed in 6% (0.1 tumours/mouse) of the mice treated with TPA alone (Slaga *et al.*, 1980).

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 10, 40 or 160 nmol [3.1, 12.4 or 50 μg] racemic dibenz[*a,h*]-anthracene-3,4-diol in acetone:ammonium hydroxide (1000:1) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 31 (0.69), 50 (1.17) and 57% (1.52), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Buening *et al.*, 1979b).

Groups of newborn Swiss Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic dibenz[*a,h*]anthracene-3,4-diol (total dose, 70 or 420 nmol) [22 or 130 μg] in DMSO. Mice were weaned at 23 days of age, separated by sex and killed at 25–29 weeks of age. In 42 or 40 female mice, pulmonary

tumours developed in 81 or 100% (2.5 or 33.4 tumours/mouse), respectively, but no liver tumours. In 27 or 45 male mice, pulmonary tumours developed in 85 or 98% (2.3 or 23.8 tumours/mouse), and liver tumours in 0 or 19%, respectively. In 40 female mice treated with DMSO alone, pulmonary tumours developed in 12.5% (1.2 tumours/mouse), but no liver tumours. In 36 male mice treated with DMSO alone, pulmonary tumours developed in 11% (1.25 tumours/mouse), but no liver tumours (Buening *et al.*, 1979b).

DNA adducts of dibenz[*a,h*]anthracene-3,4-diol

Dibenz[*a,h*]anthracene-3*R*,4*R*-diol produced four DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. The same adducts were also observed in calf thymus DNA incubated with dibenz[*a,h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes. One adduct was identified as *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine and two others were derived from dibenz[*a,h*]anthracene-3,4:10,11-bis-diol. Dibenz[*a,h*]-anthracene-3*S*,4*S*-diol gave two DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. These adducts were also observed in calf thymus DNA incubated with dibenz[*a,h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes. Two adducts were derived from dibenz[*a,h*]anthracene-3,4,10,11-bis-diol (Fuchs *et al.*, 1993b).

Dibenz[*a,h*]anthracene-3,4-diol formed 14 DNA adducts in mouse liver DNA *in vitro* in the presence of 3-methylcholanthrene-induced rat liver microsomes. One of these adducts was an adduct of *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide and the other was an adduct of *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide. It was estimated that 50% of the adducts from dibenz[*a,h*]anthracene may be formed though the dibenz[*a,h*]-anthracene-3,4-diol and *syn*- and *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide pathways (Lecoq *et al.*, 1991b). Dibenz[*a,h*]anthracene-3*S*,4*S*-diol and dibenz[*a,h*]anthracene-3*R*,4*R*-diol formed six and seven DNA adducts in calf thymus DNA, respectively, after incubation with Aroclor 1254-induced rat liver microsomes. The major bay-region diol epoxide adduct was identified as *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine (Mlcoch *et al.*, 1993). Dibenz[*a,h*]anthracene-3,4-diol formed a number of DNA adducts in mouse embryo C3H10T½CL8 cells, two of which were *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts; one polar adduct identical to a polar dibenz[*a,h*]anthracene–DNA adduct was derived from racemic dibenz[*a,h*]anthracene-3,4:10,11-bis-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a,h*]anthracene-3,4-diol

Racemic dibenz[*a,h*]anthracene-3,4-diol induced mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation or a purified monoxygenase system (Wood *et al.*, 1978; Platt & Schollmeier, 1994). Both dibenz[*a,h*]-anthracene-3*R*,4*R*-diol and dibenz[*a,h*]anthracene-3*S*,4*S*-diol induced mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver fraction; the *R,R* enantiomer was the stronger mutagen (Platt *et al.*, 1990). Racemic dibenz[*a,h*]anthracene-

3,4-diol induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994b).

Carcinogenicity study of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

Groups of 29 or 30 female Sencar mice, 51–65 days of age, received a single dermal application of 100 nmol [33 μg] racemic *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide, followed 7 days later by 3.25 nmol [2 μg] TPA in acetone twice a week for 15 weeks. The incidence of tumours in the dosed groups 16 weeks after treatment was 7% (0.1 tumours/mouse). Skin tumours developed in 6% (0.1 tumours/mouse) of the mice treated with TPA alone (Slaga *et al.*, 1980). [The Working Group noted that it is possible that the diol epoxide was not stable in the solvent used for its application to the backs of mice.]

DNA adducts of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

anti-Dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide and *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide each form a number of DNA adducts in mouse liver DNA *in vitro* (Lecoq *et al.*, 1991b). In calf thymus DNA, *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2 oxide formed *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine, *anti*-dibenz[*a,h*]-anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyguanosine, *anti*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine and *anti*-dibenz[*a,h*]anthracene-3*R*,4*S*-diol-1*S*,2*R*-oxide–deoxyadenosine adducts. In calf thymus DNA, *syn*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide formed *syn*-dibenz[*a,h*]anthracene-3*S*,4*R*-diol-1*S*,2*R*-oxide–deoxyadenosine and *syn*-dibenz[*a,h*]anthracene-3*R*,4*S*-diol-1*R*,2*S*-oxide–deoxyadenosine adducts (Mlcoch *et al.*, 1993). *anti*-Dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide formed DNA adducts in mouse embryo C3H10T½CL8 cells, two of which were *anti*-dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts and one polar adduct identical to a polar dibenz[*a,h*]-anthracene–DNA adduct was derived from racemic dibenz[*a,h*]anthracene-3,4,10,11-bis-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide

Racemic dibenz[*a,h*]anthracene-3,4-diol-1,2-oxide induced morphological cell transformation in C3H10T½CL8 mouse embryo fibroblasts (Nesnow *et al.*, 1994b).

Conclusion

Dibenz[*a,h*]anthracene is activated metabolically by the diol epoxide mechanism. It was metabolized to a diol that was further metabolized in mouse and rat tissues to a bay-region diol epoxide that then formed DNA adducts and induced mutations. Dibenz[*a,h*]anthracene was metabolized to the dibenz[*a,h*]anthracene-3,4-diol by rat liver preparations, human liver CYPs, human liver microsomes, mouse skin and human skin in organ culture and mouse skin *in vivo*. The *R,R*-diol enantiomer was the predominant rat liver microsomal form. Dibenz[*a,h*]anthracene-3,4-diol is mutagenic to bacteria, morphologically transforms cells in culture, initiates tumours in mouse skin and is carcinogenic in newborn mouse lung. Dibenz[*a,h*]anthracene-3,4-diol was metabolized to

two bay-region diol epoxides, *syn*- and *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide, by rat liver preparations and mouse skin and to *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide by mammalian cells in culture. There is a consistent pattern of formation of *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide–DNA adducts in calf thymus DNA and in mouse skin by dibenz[*a*,*h*]anthracene, dibenz[*a*,*h*]anthracene-3,4-diol and *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide, and an *anti*-dibenz[*a*,*h*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine bay-region diol epoxide adduct is common to all in-vivo treatments. In the only tumour initiation study in mouse skin, *anti*-dibenz[*a*,*h*]anthracene-3,4-diol-1,2-oxide was inactive; however, this result could have been due to the instability of the diol epoxide in the solvent used to apply the agent.

(v) *Evidence for bis-diol epoxide metabolic activation as a mechanism of carcinogenesis*

DNA adducts of dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol

Racemic dibenz[*a*,*h*]anthracene-3,4,10,11-diol gave two DNA adducts in calf thymus DNA after metabolic activation by Aroclor 1254-induced rat liver microsomes. These adducts were also observed in calf thymus DNA incubated with dibenz[*a*,*h*]anthracene-3*R*,4*R*-diol or dibenz[*a*,*h*]anthracene after metabolic activation by Aroclor 1254-induced rat liver microsomes (Fuchs *et al.*, 1993a,b). Topical application of racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol to the skin of mice gave two adducts which were also observed in dibenz[*a*,*h*]anthracene- or dibenz[*a*,*h*]anthracene-3,4-diol-treated mouse skin (Carmichael *et al.*, 1993). Racemic dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol gave two polar dibenz[*a*,*h*]anthracene–DNA adducts in C3H10T½CL8 cells in culture. One of these DNA adducts was observed in C3H10T½CL8 cells treated with dibenz[*a*,*h*]anthracene or dibenz[*a*,*h*]anthracene-3,4-diol (Nesnow *et al.*, 1994b).

Genotoxicity of dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol

Dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol was mutagenic in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Platt & Schollmeier, 1994).

Conclusion

Dibenz[*a*,*h*]anthracene is metabolically activated to bis-diol epoxides. Dibenz[*a*,*h*]anthracene was metabolized to a diol that was then metabolized to a bis-diol and further converted to intermediates that formed DNA adducts in mouse and rat tissues. Dibenz[*a*,*h*]anthracene-3,4:10,11-bis-diol was a rat liver metabolite of both dibenz[*a*,*h*]-anthracene and dibenz[*a*,*h*]anthracene-3,4-diol. It was mutagenic in bacteria and formed highly polar calf thymus DNA adducts when activated by rat liver preparations, and highly polar DNA adducts in mouse embryo cells in culture and in mouse skin. The same highly polar DNA adducts were also observed in calf thymus DNA incubated with dibenz[*a*,*h*]anthracene-3,4-diol and rat liver preparations and in mouse skin and human skin treated topically with dibenz[*a*,*h*]anthracene or dibenz[*a*,*h*]anthracene-3,4-diol. Human skin treated in short-term organ culture with dibenz[*a*,*h*]anthracene gave an

adduct profile that was qualitatively similar to the profiles obtained from the treatment of mouse skin with dibenz[*a,h*]anthracene *in vivo*. Dibenz[*a,h*]anthracene-3,4:10,11-bis-diol has not been evaluated for tumour initiation in mice.

Dibenz[*a,j*]anthracene

(i) *Metabolism and metabolic activation*

The metabolism of dibenz[*a,j*]anthracene has been documented in a number studies in rat microsomes and mouse keratinocyes in culture. Two equivalent potential centres of metabolism and metabolic activation are situated on the two benzo rings (or two bay regions) at the 1–2 and 3–4 bonds or the 10–11 and 12–13 bonds. 3-Methylcholanthrene-induced microsomes metabolized dibenz[*a,j*]anthracene to the bis-diol, *trans-trans*-3,4:8,9-tetrahydroxy-3,4:8,9-tetrahydrodibenz[*a,j*]anthracene (Lecoq *et al.*, 1989, 1991a). Dibenz[*a,j*]anthracene is metabolized in primary cultures of mouse keratinocytes to *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene (dibenz[*a,j*]anthracene-3,4-diol), *trans*-5,6-dihydroxy-5,6-dihydrodibenz[*a,j*]anthracene (dibenz[*a,j*]anthracene-5,6-diol) and several other unidentified metabolites. Both diols were also conjugated to glucuronic acid (Nair *et al.*, 1992).

(ii) *Formation of DNA adducts*

Dibenz[*a,j*]anthracene forms DNA adducts in mouse epidermis after topical application. Eleven adducts were detected and seven were identified as deoxyguanosine and deoxyadenosine adducts formed from the *anti*- and *syn*-bay-region diol epoxides of dibenz[*a,j*]anthracene, *anti-trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene-1,2-oxide (*anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide) and *syn-trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a,j*]anthracene-1,2-oxide (*syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide). The major bay-region diol epoxide–DNA adduct formed was *anti-trans*-dibenz[*a,j*]-anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyguanosine and substantial amounts of an *anti-trans*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–deoxyadenosine adduct were produced. In addition, a K-region adduct, dibenz[*a,j*]anthracene-5,6-oxide–deoxy-adenosine, was identified. Unidentified, more polar covalent DNA adducts in epidermal DNA samples from dibenz[*a,j*]anthracene-treated mice were also detected (Baer-Dubowska *et al.*, 1995). Topical application of dibenz[*a,j*]anthracene and its metabolites to mouse skin gave at least 23 DNA adducts, including four less polar (derived from *syn*- and *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide; 23% of total adducts) and 19 highly polar (derived primarily from the dibenz[*a,j*]anthracene-3,4:10,11-bis-diol; 77% of total adducts) DNA adducts (Vulimiri *et al.*, 1999). Dibenz[*a,j*]anthracene forms DNA adducts in primary cultures of mouse keratinocytes that were identified as (+)-*anti*-dibenz[*a,j*]-anthracene-3,4-diol-1,2-oxide–deoxyguanosine adducts, (+)-*anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine adducts (the deoxyadenosine adducts were predominant), *trans* and *cis* adducts with deoxyguanosine and deoxyadenosine of (+)-*syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide and the major adduct, dibenz[*a,j*]anthracene-5,6-oxide–deoxyadenosine (Nair *et al.*, 1991).

(iii) *Genotoxicity of dibenz[*a,j*]anthracene*

In a previous monograph, dibenz[*a,j*]anthracene was reported to induce mutations in *S. typhimurium* TA100 (IARC, 1983). Dibenz[*a,j*]anthracene was mutagenic in *S. typhimurium* TA100 in the presence of 3-methylcholanthrene-induced rat liver microsomes or preparations (Lecoq *et al.*, 1989) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Dibenz[a,j]anthracene-induced mutations in proto-oncogenes*

Dibenz[*a,j*]anthracene induced $A^{182} \rightarrow T$ tranversion mutations in Ha-*ras* codon 61 in mouse skin papillomas obtained from a mouse skin initiation–promotion study of dibenz[*a,j*]anthracene (Gill *et al.*, 1992).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenz[*a,j*]anthracene-3,4-diol

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 or 800 nmol [124 or 248 μg] racemic dibenz[*a,j*]anthracene-3,4-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours in the dosed groups 22 weeks after treatment was 97 (2.38 tumours/mouse) and 90% (3.17 tumours/mouse), respectively. Skin tumours developed in 16% (0.16 tumours/mouse) of the mice treated with TPA alone. Similar results were obtained in a repeat study in which dibenz[*a,j*]anthracene-3,4-diol was administered in tetrahydrofuran (Sawyer *et al.*, 1988).

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 nmol [124 μg] racemic dibenz[*a,j*]anthracene-3,4-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 16 weeks. In the dosed group, 3.48 tumours/mouse were observed 18 weeks after treatment. No data were given on the control mice treated with TPA alone (Vulimiri *et al.*, 1999).

DNA adducts of dibenz[*a,j*]anthracene-3,4-diol

Dibenz[*a,j*]anthracene-3,4-diol formed DNA adducts in mouse epidermis after topical application. Eleven adducts were formed and five were identified as (–)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine, (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (major adduct), (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine and two *syn*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide adducts (Baer-Dubowska *et al.*, 1995).

Carcinogenicity study of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 or 600 nmol [124 or 248 μg] racemic *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 22 weeks after treatment was 86 (3.55) and 80% (3.7), respectively. Skin tumours developed in 16%

(0.16 tumours/mouse) of the mice treated with TPA alone. Similar results were obtained in a repeat study in which dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide was administered in tetrahydrofuran (Sawyer *et al.*, 1988).

DNA adducts of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

(+)-*anti*-Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide formed three DNA adducts in mouse epidermis after topical application: (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine, (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–adenosine (major adduct) and (+)-*anti-cis*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine (Baer-Dubowska *et al.*, 1995). Racemic *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide formed DNA adducts with calf thymus DNA. The major adducts were (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyguanosine and (+)-*anti-trans*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide–deoxyadenosine (major adduct) (Nair *et al.*, 1989, 1991).

Genotoxicity of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide

(+)-*anti*-Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide primarily induced point mutations in the *supF* gene in SOS-induced mutants of *E. coli* ES87. The specific mutations were: GC→AT (37%), GC→TA (21%) and GC→CG (8.6%) (Gill *et al.*, 1993).

Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide-induced mutations in proto-oncogenes

Dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide induced A^{182}→T tranversion mutations in Ha-*ras* codon 61 in mouse skin papillomas from a tumour initiation–promotion study of dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide (Gill *et al.*, 1992).

Conclusion

Dibenz[*a,j*]anthracene is activated metabolically by a diol epoxide mechanism. Dibenz[*a,j*]anthracene is metabolized to a diol that is further metabolized to a bay-region diol epoxide in mouse tissues and then forms DNA adducts and induces mutations. In primary cultures of mouse skin keratinocytes, dibenz[*a,j*]anthracene is metabolized to the bay-region diol, dibenz[*a,j*]anthracene-3,4-diol. In mouse skin and in mouse skin kerotinocytes, dibenz[*a,j*]anthracene forms DNA adducts primarily from the bay-region diol epoxide, (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide with both deoxyguanosine and deoxyadenosine. Dibenz[*a,j*]anthracene-3,4-diol is a tumour initiator in mouse skin and forms (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–DNA adducts with both deoxyguanosine and deoxyadenosine in mouse skin. *anti*-Dibenz[*a,j*]-anthracene-3*S*,4*R*-diol-1,2-oxide is mutagenic in bacteria and initiates tumours in mouse skin. In mouse skin, it forms (+)-*anti*-dibenz[*a,j*]anthracene-3*S*,4*R*-diol-1*R*,2*S*-oxide–DNA adducts with both deoxyguanosine and deoxyadenosine. Tumours induced by both dibenz[*a,j*]anthracene and *anti*-dibenz[*a,j*]anthracene-3,4-diol-1,2-oxide have A^{182}→T tranversion mutations in Ha-*ras* codon 61 which suggests that deoxyadenosine is a major target.

(vi) *Evidence for bis-diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity study of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol

Groups of 30 female Sencar mice, 51–65 days of age, received dermal applications of 400 nmol [124 μg] racemic dibenz[*a,j*]anthracene-3,4:10,11-bis-diol in acetone, followed 2 weeks later by 3.4 nmol [2.1 μg] TPA in acetone twice a week for 16 weeks. No tumours were observed in the dosed group 18 weeks after treatment. No data were given on the control mice treated with TPA alone (Vulimiri *et al.*, 1999).

DNA adducts of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol

Topical application of dibenz[*a,j*]anthracene-3,4:10,11-bis-diol to mouse skin *in vivo* produced nine adducts, seven of which were found in the skin of mice treated with dibenz[*a,j*]anthracene and five of which were detected in the skin of mice treated with dibenz[*a,j*]anthracene-3,4-diol (Vulimiri *et al.*, 1999).

Conclusion

The data are insufficient to demonstrate a mechanism of bis-diol epoxide metabolic activation. Bis-diols are mouse skin metabolites of dibenz[*a,j*]anthracene, the major one of which is dibenz[*a,j*]anthracene-3,4:10,11-bis-diol. Dibenz[*a,j*]anthracene-3,4:10,11-bis-diol forms DNA adducts in mouse skin; however, the contribution of this metabolite to the tumour-initiating activity of dibenz[*a,j*]anthracene appears to be small on the basis of its inability to initiate tumours in mouse skin.

Dibenzo[*a,e*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a,e*]pyrene was metabolized by 3-methylcholanthrene-induced rat liver microsomes. The major metabolites were the proximate bay-region diol, 3,4-dihydroxy-3,4-dihydrodibenzo[*a,e*]pyrene, and 3-, 7- and 9-hydroxydibenzo[*a,e*]pyrene (Devanesan *et al.*, 1990).

(ii) *Formation of DNA adducts*

Dibenzo[*a,e*]pyrene applied topically to the skin of male mice formed unidentified DNA adducts in both skin and lungs (Hughes & Phillips, 1990).

(iii) *Genotoxic effects of dibenzo[*a,e*]pyrene*

In a previous monograph, dibenzo[*a,e*]pyrene was reported to induce mutations in *S. typhimurium* TA100 (IARC, 1983). Dibenzo[*a,e*]pyrene is mutagenic in *S. typhimurium* TA98, TA100 and TM677 (8-azaguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation (Devanesan *et al.*, 1990; Busby *et al.*, 1995). It was mutagenic in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996) and in human B-lymphoblastoid MCL-5 cells that contain five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

No data were available to the Working Group.

Dibenzo[*a,h*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a,h*]pyrene was metabolized to a bay-region diol, *trans*-1,2-dihydroxy-1,2-dihydrodibenzo[*a,h*]pyrene (dibenzo[*a,h*]pyrene-1,2-diol), and *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[*a,h*]pyrene by an Aroclor 1254-induced rat liver preparation (Hecht *et al.*, 1981).

(ii) *Formation of DNA adducts*

Dibenzo[*a,h*]pyrene formed two major DNA adducts in the skin of topically treated mice (Hughes & Phillips, 1990). Laser-excited fluorescence analyses suggested that the adducts resulted from metabolism on either or both terminal benzo rings (Marsch *et al.*, 1992).

(iii) *Genotoxic effects of dibenzo[a,h]pyrene*

In a previous monograph, dibenzo[*a,h*]pyrene was reported to induce mutations in *S. typhimurium* TA98 and TA100 (IARC, 1983). Dibenzo[*a,h*]pyrene was very weakly mutagenic in human B-lymphoblastoid MCL-5 cells that contain activity for five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995). It was mutagenic in human B-lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1999) and in Chinese hamster V79 cells (ouabain and 6-thioguanine resistance) co-cultivated with golden hamster embryo cells (Hass *et al.*, 1982).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,h*]pyrene-1,2-diol

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [17, 67 or 202 μg] racemic dibenzo[*a,h*]pyrene-1,2-diol in DMSO:tetrahydrofuran (1:10) followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol. The incidence of tumours (tumours/mouse) in the dosed group 17 weeks after treatment was 39 (0.96), 57 (2.73) and 80% (4.4), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,h*]-1,2-diol was 79% (2.96), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intra-peritoneally on days 1, 8 and 15 of life with racemic dibenzo[*a,h*]pyrene-1,2-diol (total dose, 87.5 nmol) [30 μg] in DMSO. Mice were weaned at 25 days of age, separated by

sex and killed at 49–54 weeks of age. In 28 female mice, at 54 weeks pulmonary tumours developed in 96% of the surviving mice (15.82 tumours/mouse) and liver tumours in 7% (0.07 tumours/mouse). In 17 male mice, pulmonary tumours developed in 100% of the surviving mice (19 tumours/mouse) and liver tumours in 41% (3.76 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,h*]pyrene-1,2-diol

Dibenzo[*a*,*h*]pyrene-1,2-diol was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Wood *et al.*, 1981).

Carcinogenicity studies of dibenzo[*a,h*]pyrene-1,2-diol-3,4-oxide

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [18, 71 or 212 μg] racemic *anti-trans*-1,2-dihydroxy-1,2,3,4-tetrahydrodibenzo[*a*,*h*]pyrene-3,4-oxide (*anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide) in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with of 50 nmol. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 33 (0.43), 70 (1.87) and 50% (1.83), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a*,*h*]-1,2-diol-3,4-oxide was 60% (one tumour/mouse), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In 18 female mice, pulmonary tumours developed in 94% of the surviving mice (5.72 tumours/mouse) and liver tumours in 6% (0.06 tumours/mouse). In 19 male mice, pulmonary tumours developed in 95% of the surviving mice (5.37 tumours/mouse) and liver tumours in 26% (1.37 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,h*]pyrene-1,2-diol-3,4-oxide

anti-Dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide was mutagenic in *S. typhimurium* TA98 and TA100 in the absence of exogenous metabolic activation and in Chinese hamster V79 cells in culture (8-azaguanine resistance) (Wood *et al.*, 1981)**.**

Conclusion

Dibenzo[*a*,*h*]pyrene can be metabolically activated by the diol epoxide pathway. Dibenzo[*a*,*h*]pyrene is metabolized to the proximate bay-region diol, dibenzo[*a*,*h*]pyrene-1,2-diol, by rat liver preparations. Dibenzo[*a*,*h*]pyrene-1,2-diol was mutagenic to bacteria and was a tumour initiator in mouse skin. Dibenzo[*a*,*h*]pyrene-1,2-diol induced pulmonary and hepatic tumours in newborn mice. Although *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide–DNA adducts have not been characterized directly, synthetic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide is genotoxic in bacteria and mammalian cells in culture. Synthetic *anti*-dibenzo[*a*,*h*]pyrene-1,2-diol-3,4-oxide is a tumour initiator in mouse skin and induces pulmonary and hepatic tumours in newborn mice.

Dibenzo[*a*,*i*]pyrene

(i) *Metabolism and metabolic activation*

Dibenzo[*a*,*i*]pyrene was metabolized to a proximate bay-region diol, *trans*-3,4-dihydroxy-3,4-dihydrodibenzo[*a*,*i*]pyrene (dibenzo[*a*,*i*]pyrene-3,4-diol), and *trans*-1,2-dihydroxy-1,2-dihydrodibenzo[*a*,*i*]pyrene by an Aroclor 1254-induced rat liver preparation (Hecht *et al.*, 1981).

(ii) *Formation of DNA adducts*

Dibenzo[*a*,*i*]pyrene formed two unidentified DNA adducts in the lungs of rats dosed by intratracheal instillation (Whong *et al.*, 1994) and in the skin of mice treated topically (Hughes & Phillips, 1990).

(iii) *Genotoxic effects of dibenzo[*a,i*]pyrene*

In a previous monograph, dibenzo[*a*,*i*]pyrene was reported to induce DNA damage in bacteria and mutations in *S. typhimurium* TA98 and TA100 but not unscheduled DNA synthesis in primary rat hepatocytes (IARC, 1983). Dibenzo[*a*,*i*]pyrene was mutagenic in *S. typhimurium* TM677 in the presence of an Aroclor 1254-induced rat liver preparation (Busby *et al.*, 1995), in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1999) and in Chinese hamster V79 cells (ouabain and 6-thioguanine resistance) co-cultivated with golden hamster embryo cells (Hass *et al.*, 1982). It induced cell transformation of rat epithelial cells *in vivo* after intracheal instillation and in treated isolated cultures of rat epithelial cells *in vitro* (Ensell *et al.*, 1998). The rat epithelial cells transformed *in vivo* were shown to exhibit anchorage-independent growth and to produce squamous-cell carcinomas when injected into nude mice (Ensell *et al.*, 1999). Dibenzo[*a*,*i*]pyrene induced micronucleus formation in bone marrow and spleen polychromatic erythrocytes in rats treated *in vivo* (Zhong *et al.*, 1995). It induced sister chromatid exchange and micronucleus formation in lung cells cultured *in vitro* taken from rats dosed by intratracheal instillation (Whong *et al.*, 1994).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,i*]pyrene-3,4-diol

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [17, 67 or 202 μg] racemic dibenzo[*a,i*]pyrene-3,4-diol in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol only. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 37 (0.6), 66 (3.03) and 81% (5.0), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,i*]-3,4-diol was 60% (2.14), and no skin tumours developed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic dibenzo[*a,i*]pyrene-3,4-diol (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In 30 female mice, pulmonary tumours developed in 100% of the surviving mice (32.2 tumours/mouse) and liver tumours in 6% (0.1 tumours/mouse). In 21 male mice, pulmonary tumours developed in 100% of the surviving mice (35 tumours/mouse) and liver tumours in 67% (4.48 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,i*]pyrene-3,4-diol

Dibenzo[*a,i*]pyrene-3,4-diol was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Wood *et al.*, 1981)**.**

Carcinogenicity studies of dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide

Groups of 30 female Charles River CD-1 mice, 51–58 days of age, received a single dermal application of 50, 200 or 600 nmol [18, 71 or 212 μg] racemic *anti-trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenzo[*a,i*]pyrene-1,2-oxide (*anti*-dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide) in DMSO:tetrahydrofuran (1:10), followed 7 days later by 16 nmol [10 μg] TPA in acetone twice a week for 16 weeks, or 24 weeks for another group treated with 50 nmol only. The incidence of tumours (tumours/mouse) in the dosed groups 17 weeks after treatment was 20 (0.2), 43 (0.9) and 67% (2.03), respectively. Skin tumours developed in 0 or 10% (0 or 0.1 tumours/mouse) of the mice treated with TPA alone. The incidence of tumours (tumours/mouse) 25 weeks after treatment in the group dosed with 50 nmol dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide was 53% (0.9) and no skin tumours were observed in mice treated with TPA alone (Chang *et al.*, 1982).

Groups of newborn Swiss-Webster BLU:Ha(ICR) mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide (total dose, 87.5 nmol) [31 μg] in DMSO. Mice were weaned at 25 days of age, separated by sex and killed at 49–54 weeks of age. In seven female mice, pulmonary tumours developed in 100% of the surviving mice (1.57 tumours/mouse) but no liver tumours. In eight male mice, pulmonary tumours developed in 62% of the surviving mice (3.13 tumours/mouse) and liver tumours in 25% (0.25 tumours/mouse). In 39 female mice treated with DMSO alone, pulmonary tumours developed in 28% of the surviving mice (0.44 tumours/mouse) but no liver tumours. In 32 male mice treated with DMSO alone, pulmonary tumours developed in 22% of the surviving mice (0.8 tumours/mouse) but no liver tumours (Chang *et al.*, 1982).

Genotoxicity of dibenzo[*a,i*]pyrene-3,4-diol-1,2-oxide

anti-Dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide was mutagenic in *S. typhimurium* TA98 and TA100 in the absence of exogenous metabolic activation and in Chinese hamster V79 cells in culture (8-azaguanine resistance) (Wood *et al.*, 1981).

Conclusion

There is some evidence that dibenzo[*a*,*i*]pyrene can be activated metabolically by the diol epoxide mechanism. Dibenzo[*a*,*i*]pyrene was metabolized to the proximate bay-region diol, dibenzo[*a*,*i*]pyrene-3,4-diol, by rat liver preparations. Dibenzo[*a*,*i*]pyrene-3,4-diol was mutagenic to bacteria and was a tumour initiator in mouse skin. It induced pulmonary and hepatic tumours in newborn mice. Although DNA adducts from *anti*-dibenzo[*a*,*i*]pyrene-3,4-diol-1,2-oxide have not been identified, synthetic *anti*-dibenzo-[*a*,*i*]pyrene-3,4-diol-1,2-oxide was genotoxic in bacteria and mammalian cells in culture, was a tumour initiator in mouse skin and induced pulmonary and hepatic tumours in newborn mice.

Dibenzo[*a,l*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of dibenzo[*a*,*l*]pyrene has been documented in a number of studies in rat liver microsomes, recombinant human CYPs, cells that express human CYPs and human liver and lung microsomes. One primary potential centre of metabolism and metabolic activation of dibenzo[*a*,*l*]pyrene leads to the fjord-region diol epoxide at the 11–12 and 13–14 bonds. Dibenzo[*a*,*l*]pyrene is metabolized by 3-methylcholanthrene-induced rat liver microsomes to 11,12-dihydroxy-11,12-dihydro-dibenzo[*a*,*l*]pyrene (dibenzo[*a*,*l*]pyrene-11,12-diol), 8,9-dihydroxy-8,9-dihydro-dibenzo[*a*,*l*]pyrene (dibenzo-[*a*,*l*]pyrene-8,9-diol), 7-hydroxydibenzo[*a*,*l*]pyrene and a dibenzo[*a*,*l*]pyrene-dione (Devanesan *et al.*, 1990). Dibenzo[*a*,*l*]pyrene was metabolized by human recombinant CYPs and human lung and liver microsomes to 13,14-dihydroxy-13,14-dihydro-dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*l*]pyrene-11,12-diol, dibenzo[*a*,*l*]pyrene-8,9-diol, 7-hydroxy-dibenzo[*a*,*l*]pyrene and a dibenzo[*a*,*l*]pyrene-dione. Of all of the recombinant CYPs

evaluated, CYP1A1 was the most active in the metabolism of dibenzo[*a*,*l*]pyrene. The order of activity in the formation of dibenzo[*a*,*l*]pyrene-11,12-diol was CYP1A1 > CYP2C9 > CYP1A2 > CYP2B6 > CYP3A4 (Shou *et al.*, 1996b). The two major CYPs involved in the metabolism of dibenzo[*a*,*l*]pyrene are CYP1A1 and CYP1B1. In human lung, both CYP1A1 (in smokers) and CYP1B1 proteins are expressed (Hukkanen *et al.*, 2002). Dibenzo[a,l]pyrene was metabolized by Chinese hamster V79 cells that express human CYP1A1 or CYP1B1 to dibenzo[a,l]pyrene-11,12-diol, 7-hydroxydibenzo-[a,l]pyrene, dibenzo[a,l]pyrene-11,12-diol-phenol and 11r,12t,13t,14c-tetrahydroxy-11,12,13,14,-tetrahydrodibenzo[a,l]pyrene. Chinese hamster V79 cells that express human CYP1B1 also formed *cis*-dibenzo[*a*,*l*]pyrene-11,12-diol. Those that express either of the human CYP enzymes stereospecifically catalysed the formation of (–)-dibenzo-[*a*,*l*]pyrene-11*R*,12*R*-diol from dibenzo[*a*,*l*]pyrene (Luch *et al.*, 1999a).

(ii) *Formation of DNA adducts*

The formation of dibenzo[*a*,*l*]pyrene–DNA adducts has been studied in calf thymus DNA, in rodent and human mammalian cells in culture and in rodents. Dibenzo[*a*,*l*]pyrene forms DNA adducts in calf thymus DNA in the presence of liver microsomes from β-naphthoflavone- or Aroclor 1254-treated rats. These adducts were identified as deoxyadenosine and deoxyguanosine adducts of both *anti*- and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides in the following order: *anti*-dibenzo[*a*,*l*]-pyrene-11,12-diol-13,14-oxide–deoxyadenosine, *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine >> *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguano-sine, *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (Arif & Gupta, 1997). Dibenzo[*a*,*l*]pyrene forms adducts in calf thymus DNA in the presence of 3-methylcholanthrene-induced rat liver microsomes. These adducts were identified as a *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, an *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, a *syn-trans*-dibenzo-[*a*,*l*]pyrene-11,12,-diol-13,14-oxide–doxyadenosine adduct, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and an *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adduct. Equal amounts of deoxyguanosine and deoxy-adenosine adducts were formed (Devanesan *et al.*, 1999). Dibenzo[*a*,*l*]pyrene formed DNA adducts in mouse embryo fibroblast C3H10T½Cl8 cells that were derived from the metabolism of dibenzo[*a*,*l*]pyrene to its fjord-region diol epoxides through dibenzo-[*a*,*l*]pyrene-11,12-diol. The predominant adduct was *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. Other major adducts were *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine with smaller amounts of *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine (Nesnow *et al.*, 1997).

Chinese hamster V79 cells that express human CYP1B1 produced six DNA adducts exclusively derived from dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide (Luch *et al.*, 1998b). These adducts were similar to those found after the treatment of human mammary carcinoma MCF-7 cells in culture, which were identified as four (–)-*anti*-

dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide adducts and two (+)-*syn*-dibenzo[*a*,*l*]-pyrene-11*S*,12*R*-diol-13*S*,14*R*-oxide adducts. The three major adducts found in MCF-7 cells were identified as a (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine and two (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adducts. This indicated that, in these cells, the metabolism of dibenzo[*a*,*l*]pyrene proceeds through dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol (Ralston *et al.*, 1995, 1997). Chinese hamster V79 cells that express human or rat CYP1A1 formed many of the same dibenzo[*a*,*l*]pyrene adducts as cells that express CYP1B1, as well as several unidentified highly polar DNA adducts (Luch *et al.*, 1998b). Embryonic fibroblasts from wild-type mice (mixed genetic background of C57B6 and 129/Sv mice) were exposed to dibenzo[*a*,*l*]pyrene. Those isolated from *CYP1B1*-null mice failed to bind dibenzo[*a*,*l*]pyrene to DNA. In contrast, those from wild-type mice produced a series of five DNA adducts derived from both *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides, the *anti* form of which predominated. The same pattern of dibenzo[*a*,*l*]pyrene–DNA adducts was observed in dibenzo[*a*,*l*]pyrene-exposed Chinese hamster V79 cells that express murine CYP1B1 (Buters *et al.*, 2002).

Female Sprague-Dawley rats injected intramammillary with dibenzo[*a*,*l*]pyrene formed DNA adducts in mammary tissues as well as distal tissues (lung, heart, bladder and pancreas), which were identified as deoxyadenosine and deoxyguanosine adducts of both *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides (Arif *et al.*, 1997, 1999). Dibenzo[*a*,*l*]pyrene binds at different levels to the DNA in the epidermis of *Cyp1a2*$^{-/-}$, *Cyp1b1*$^{-/-}$ and *Ahr*$^{-/-}$ knockout mice *in vivo*; the least binding occurs in the *Cyp1a2*$^{-/-}$ and *Cyp1b1*$^{-/-}$ mice, which suggests that CYP1A2 and CYP1B1 and possibly CYP1A1 are involved in the bioactivation of dibenzo[*a*,*l*]pyrene (Kleiner *et al.*, 2004).

Dibenzo[*a*,*l*]pyrene forms three major and four minor DNA adducts after topical application to mouse skin. One of the major adducts is (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine (with some possible contribution of a (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine). A second major adduct is primarily (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (with some possible contribution of (+)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA). The third major adduct is (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. The minor adducts are (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and deoxyadenosine adducts of (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide. Quantitatively, the majority of dibenzo[*a*,*l*]pyrene–DNA adducts in mouse skin are (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosines (Jankowiak *et al.*, 1998).

Strain A/J mice injected intraperitoneally with dibenzo[*a*,*l*]pyrene produced dibenzo[*a*,*l*]pyrene–DNA adducts in the lung which were deoxyadenosines and deoxyguanosines derived from both *anti*- and *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide; the major adduct was *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine (Prahalad *et al.*, 1997). C57BL/6 mice exposed to dibenzo[*a*,*l*]pyrene by gavage formed a number of DNA adducts in their lungs, the majority of which were

derived from *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide and one from *syn*-dibenzo-[*a*,*l*]pyrene-11,12-diol-13,14-oxide (Mahadevan *et al.*, 2005a).

(iii) *Genotoxicity of dibenzo[*a,l*]pyrene*

Dibenzo[*a*,*l*]pyrene is mutagenic in *S. typhimurium* TA98, TA100 and TM677 (8-azaguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation (Devanesan *et al.*, 1990; Busby *et al.*, 1995). It was mutagenic in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996) and in human B-lymphoblastoid MCL-5 cells that contain five human liver CYPs and microsomal epoxide hydrolase (Busby *et al.*, 1995). Chinese hamster V79 cells (6-thioguanine resistance) co-cultivated with irradiated human mammary carcinoma MCF-7 cells were mutated by dibenzo[*a*,*l*]pyrene (Ralston *et al.*, 1997). Dibenzo[*a*,*l*]-pyrene induced morphological cell transformation in mouse embryo fibroblast C3H10T½Cl8 cells and was more active than benzo[*a*]pyrene (Nesnow *et al.*, 1997). Dibenzo[*a*,*l*]pyrene induced mutations in the *cII* locus of Big-Blue mouse embryonic fibroblasts (Yoon *et al.*, 2004).

(iv) *Dibenzo[*a,l*]pyrene-induced mutations in ras gene*

Dibenzo[*a*,*l*]pyrene induced Ki-*ras* codon 12 and codon 61 mutations in lung adenomas from treated strain A/J mice. In codon 12, the following mutations were observed: GGT→TGT (28%), GGT→GTT (11%) and GGT→CGT (6%). In codon 61, the following mutations were observed: CAA→CTA (22%), CAA→CGA (17%), CAA→CAT (11%) and CAA→CAC (6%) (Prahalad *et al.*, 1997). Dibenzo[*a*,*l*]pyrene induced Ha-*ras* codon 61 mutations in mouse skin papillomas with CAA→CTA mutations being predominant (Chakravarti *et al.*, 1995; Khan *et al.*, 2005). It induced A→G mutations in the Ha-*ras* proto-oncogene in early preneoplastic skin (Chakravarti *et al.*, 2000).

(v) *DNA damage and repair of dibenzo[*a,l*]pyrene*

In comparison with benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene induces 10–100 times more DNA adducts (Binkova *et al.*, 2000; Melendez-Colon *et al.*, 2000; Binkova & Sram, 2004), which are more resistant to nucleotide excision repair, probably because they distort DNA to a lesser degree (Dreij *et al.*, 2005). Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide reacts preferably with deoxyadenosine while benzo[*a*]pyrene reacts more readily with deoxyguanosine (Ralston *et al.*, 1997; Jankowiak *et al.*, 1998; Smith *et al.*, 2001). These differences also apply more generally to fjord-region and bay-region diol epoxides (see references in Dreij *et al.*, 2005). However, the preferred adduction of dibenzo[*a*,*l*]-pyrene-11,12,-diol-13,14-oxide to deoxyadenosine was challenged (Todorovic *et al.*, 2005). The repair of DNA adducts by (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide is significantly quicker than that of adducts by (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide (Yoon *et al.*, 2004). Accordingly, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide adducts are the major adducts in Chinese hamster cells V79 that express

CYP1B1 (Luch *et al.*, 1998b) and in the lungs of orally dosed mice (Mahadevan *et al.*, 2005a).

(vi) *Apoptosis and cellular pathways of dibenzo[*a,l*]pyrene*

In many ways, benzo[*a*]pyrene and dibenzo[*a*,*l*]pyrene seem to have a similar profile in cellular effects that differ from many other PAH compounds (See Section 4.2.1.(*b*) **Benzo[*a*]pyrene** (vii)). Both are strong mutagens and induce apoptosis and cell-cycle arrest (Chramostova *et al.*, 2004), increase greatly the percentage of S-phase cells and induce phosphorylation of extracellular signal-regulated kinases (Andrysik *et al.*, 2005), and the number of their DNA adducts correlates with the p53 response to both compounds in cells (Rämet *et al.*, 1995; Luch *et al.*, 1999b). (–)-*anti*-Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide induced DNA adducts and cell-cycle arrest in human diploid fibroblast cultures (Mahadevan *et al.*, 2001).

(vii) *Changes in gene expression induced by dibenzo[*a,l*]pyrene*

Very few data exist on the effect of dibenzo[*a*,*l*]pyrene on gene expression. In colon carcinoma Caco cells, dibenzo[*a*,*l*]pyrene induces CYP1A1, CYP1B1, UGT and multi drug resistance 1 (MDR-1) genes, with no effect on epoxide hydrolase or SULTs (Lampen *et al.*, 2004). In MCF-7 cells, however, benzo[*a*]pyrene, but not dibenzo-[*a*,*l*]pyrene, induces CYP1A1 and CYP1B1 (Mahadevan *et al.*, 2005b).

(viii) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of dibenzo[*a,l*]pyrene-11,12-diol

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 4, 20 or 100 nmol [1.3, 7 or 34 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 21 days later by 3.24 nmol [2 μg] TPA in acetone three times a week for 24 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after treatment was 100 (7.08), 96 (6.96) and 87% (2.5), respectively. The numbers of squamous-cell carcinomas/number of tumour bearing mice were 6/3, 1/1 and 1/1, respectively. A group of mice treated with 100 nmol [34 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol only without TPA promotion had a 12% tumour incidence (0.17 tumours/mouse) with two squamous-cell carcinomas in two mice. In an initiation–promotion study, the parent compound dibenzo[*a*,*l*]pyrene (4 nmol) [1.2 μg] induced a skin tumour incidence of 92% with 6.96 tumours/mouse and three squamous-cell carcinomas in two mice. There were no skin tumours in control mice treated with TPA only (Cavalieri *et al.*, 1991).

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 0.25 or 1 nmol [0.084 or 0.336 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 7 days later by 2.16 nmol [1.33 μg] TPA in acetone twice a week for 27 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 27 weeks after

treatment was approximately 0 (0) and 18% (0.17), respectively. The responses in control mice treated with TPA alone were not stated (Higginbotham *et al.*, 1993).

Groups of 23–25 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.3 or 4 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice weekly for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 72 (2.84), 76 (4.36) and 78% (4.65), respectively. The numbers of squamous-cell carcinomas/number of tumour-bearing mice were 2/2, 3/2 and 3/2, respectively. The responses in control mice treated with TPA alone were not given. In the same study, dibenzo[*a*,*l*]pyrene (1.33 nmol) [0.5 μg] gave a 70% tumour incidence and 5.22 tumours/mouse (Gill *et al.*, 1994).

Groups of 16 female outbred NMRI mice, 49 days of age, received a dermal application of 40 nmol [13.5 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed group 19 weeks after treatment was 100% (5.8). In the same study, dibenzo[*a*,*l*]pyrene (40 nmol) [13 μg] gave a skin tumour incidence of 94% with 6.5 tumours/mouse. No skin tumours were observed in control mice treated with TPA alone (Luch *et al.*, 1999c).

Groups of 16 female outbred NMRI mice, 49 days of age, received dermal applications of 10, 20 or 40 nmol [3.4, 6.8 or 13.6 μg] (+)-dibenzo[*a*,*l*]pyrene-11*S*,12*S*-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 19 weeks after treatment was 0 (0), 13 (0.1) or 13% (0.1), respectively. No skin tumours were observed in control mice treated (Luch *et al.*, 1999c).

Groups of 16 female outbred NMRI mice, 49 days of age, received dermal applications of 10, 20 or 40 nmol [3.4, 6.8 or 13.6 μg] (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol in acetone, followed 7 days later by 10 nmol [6.2 μg] TPA in acetone twice a week for 18 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 19 weeks after treatment was 93 (4.7), 88 (3.6) and 80% (1.7), respectively. No skin tumours occurred in control mice treated with TPA alone (Luch *et al.*, 1999c).

Groups of 22–27 female Swiss mice, 56 days of age, received dermal applications of 1 or 4 nmol [0.34 or 1.3 μg] racemic dibenzo[*a*,*l*]pyrene-11,12-diol in acetone twice weekly for 40 weeks and the surviving mice were killed at 48 weeks. In 23 or 22 mice, skin tumours occurred in 9 or 36% of the surviving mice with a mean latency of 45 or 33 weeks. The major tumours identified were squamous papillomas and sebaceous gland tumours in the 4-nmol treatment group and sebaceous gland tumours in the 1-nmol treatment group. In the same study, dibenzo[*a*,*l*]pyrene (4 nmol) [1.2 μg] gave a higher tumour incidence (83%) and the majority of tumours were squamous-cell carcinomas. Mice treated with acetone alone developed no tumours (Higginbotham *et al.*, 1993).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic dibenzo[*a*,*l*]pyrene-11,12-diol (total dose, 40 or 100 nmol) [13.6 or 34 μg] in DMSO. Mice were weaned at 3–4 weeks

of age, separated by sex and killed at 26 or 55 weeks of age. In 12 or 13 female mice, pulmonary tumours developed in 83.3 or 84.6% of the surviving mice (5.92 or 5.69 tumours/mouse), liver tumours in 25 or 23.1% (0.42 or 0.23 tumours/mouse) and other tumours in 41.7 or 30.8% (0.5 or 0.38 tumours/mouse), respectively. In 13 or seven male mice, pulmonary tumours developed in 100 or 71.4% of the surviving animals (8.85 or 3.14 tumours/mouse), liver tumours in 92.3 or 28.6% (9.77 or 2.29 tumours/mouse) and other tumours in 23.1 or 42.9% (0.54 or 0.43 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours developed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Metabolism of dibenzo[*a,l*]pyrene-11,12-diol

Dibenzo[*a,l*]pyrene-11,12-diol is metabolized by Aroclor 1254-induced rat or mouse liver microsomes to a series of diastereomeric tetraols formed through intermediary *syn*- and *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxides; the *anti* isomer predominated (90:10) (Luch *et al.*, 1997, 1998b).

DNA adducts of dibenzo[*a,l*]pyrene-11,12-diol

Racemic and enantiomeric dibenzo[*a,l*]pyrene-11,12-diol formed calf thymus DNA adducts in the presence of Aroclor 1254-induced liver microsomes from Sprague-Dawley rats or CD-1 mice. Larger quantities of these adducts were formed by the (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol compared with the (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Luch *et al.*, 1997). Chinese hamster V79 cells that express human recombinant CYP1A1 produced both non-polar and polar DNA adducts from (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol (Luch *et al.*, 1998b). Of the four non-polar DNA adducts, one was identified specifically as (–)-*anti*-dibenzo[*a,l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide–deoxyadenosine (Ralston *et al.*, 1995) and the other three were all (–)-*anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide–DNA adducts (Luch *et al.*, 1998b). These four non-polar adducts were also formed exclusively in V79 cells that express human CYP1B1 after exposure to (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol. Chinese hamster V79 cells that express human recombinant CYP1A1 produced fewer adducts than those that express CYP1B1 (Luch *et al.*, 1998b).

Genotoxicity of dibenzo[*a,l*]pyrene-11,12-diol

Chinese hamster V79 cells (6-thioguanine resistance) co-cultivated with irradiated human mammary carcinoma MCF-7 cells were mutated by racemic dibenzo[*a,l*]pyrene-11,12-diol and (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol, but not by (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Ralston *et al.*, 1997). Chinese hamster V79 cells (6-thioguanine resistance) in the presence of an Aroclor 1254-induced rat liver preparation were mutated by racemic dibenzo[*a,l*]pyrene-11,12-diol and each enantiomer. The (–)-dibenzo[*a,l*]pyrene-11*R*,12*R*-diol was more mutagenic than the racemic mixture which was more active than the (+)-dibenzo[*a,l*]pyrene-11*S*,12*S*-diol (Luch *et al.*, 1997).

Carcinogenicity studies of dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide

Groups of 24 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.4 or 4.3 μg] racemic *syn*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice a week for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 54 (1.87), 50 (1.54) and 58% (1.79), respectively. The numbers of squamous-cell carcinomas/number of tumour-bearing mice were 5/3 in the 1.33-nmol [0.5-μg] dose group only. The responses in control mice treated with TPA alone were not given (Gill *et al.*, 1994).

Groups of 23–25 female SENCAR mice, 56 days of age, received dermal applications of 1.33, 4 or 12 nmol [0.5, 1.4 or 4.3 μg] racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide in acetone, followed 1 week later by 1.62 nmol [1 μg] TPA in acetone twice a week for 28 weeks. The incidence of skin tumours (tumours/mouse) in the dosed groups 29–30 weeks after treatment was 44 (0.68), 44 (0.68) and 68% (1.96), respectively. The responses in control mice treated with TPA alone were not given (Gill *et al.*, 1994).

Groups of female Charles River CD rats, 30 days of age, were injected twice with a total dose of 1200 nmol [425.3 μg] *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide dissolved in DMSO under the three nipples on the left side. The DMSO control was injected under the nipples on the right side. At 41 weeks, 88% of the 16 rats had mammary tumours (adenomas, adenocarcinomas, sarcomas, poorly differentiated tumours and carcinosarcoma) after a mean latent period of 15.1 weeks. In the DMSO groups, 11% of the 19 rats had mammary tumours (adenomas, adenocarcinomas) after a mean latent period of 39 weeks. The differences between the treated and control group were statistically significant (Amin *et al.*, 1995b).

Groups of newborn Hsd:ICR mice were injected intraperitoneally on days 1, 7 and 15 of life with racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide (total dose, 1 nmol) [0.35 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 20 female mice, pulmonary tumours occurred in 95.8% of the surviving mice (3.83 tumours/mouse) and liver tumours in 29.2% (0.83 tumours/mouse). In 27 male mice, pulmonary tumours occurred in 92.8% of the surviving mice (3.39 tumours/mouse) and liver tumours in 71.4% (6.29 tumours/mouse). In 17 female mice treated with DMSO alone, pulmonary tumours occurred in 5.9% of the surviving mice (0.059 tumours/mouse) and liver tumours in 5.9% (0.059 tumours/mouse). In eight male mice treated with DMSO alone, no pulmonary tumours and liver tumours occurred in 25% of the surviving mice (0.25 tumours/mouse). The differences in pulmonary tumours between the treated and control groups of males and females were statistically significant (Amin *et al.*, 1995a).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide (total dose, 0.2 or 0.4 nmol) [71 or 142 μg] in DMSO. Mice were weaned at 3–4 weeks of age, separated by sex and killed at 55 weeks of age. In 20 or six female mice, pulmonary tumours were observed in 35 or 50% of the surviving mice (0.45

or 0.50 tumours/mouse), liver tumours in 5 or 16.7% (0.05 or 0.17 tumours/mouse) and other tumours in 10 or 33.3% (0.1 or 0.33 tumours/mouse), respectively. In 22 or nine male mice, pulmonary tumours occurred in 18.2 or 11.1% of the surviving mice (0.23 or 0.33 tumours/mouse), liver tumours in 9.1 or 66.7% (0.77 or 2.33 tumours/mouse) and other tumours in 0 or 11.1% (0 or 0.11 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours developed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Groups of newborn Charles River Crl:CD®-1(ICR)BR mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide (total dose, 0.2 or 0.4 nmol) [77 or 142 μg] in DMSO. Mice were weaned at 3–4 weeks of age, separated by sex and killed at 55 weeks of age. In 27 or 16 female mice, pulmonary tumours developed in 11.1 or 12.5% of the surviving mice (0.11 or 0.13 tumours/mouse), liver tumours in 7.4 or 12.5% (0.11 or 0.38 tumours/mouse) and other tumours in 7.4 or 12.5% (0.07 or 0.13 tumours/mouse), respectively. In 26 or 26 male mice, pulmonary tumours were observed in 15.4 or 15.4% of the surviving mice (0.15 or 0.19 tumours/mouse), liver tumours in 7.7 or 23.1% (0.12 or 0.35 tumours/mouse) and other tumours in 7.7 or 3.8% (0.08 or 0.04 tumours/mouse), respectively. In 27 female mice treated with DMSO alone, pulmonary tumours developed in 37% of the surviving mice (0.67 tumours/mouse) but no liver or other tumours. In 35 male mice treated with DMSO alone, pulmonary tumours were observed in 31.4% of the surviving mice (0.54 tumours/mouse) but no liver or other tumours (Platt *et al.*, 2004).

Metabolism of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

syn- and *anti*-Dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide were hydrolysed to diasteromeric tetraols (Jankowiak *et al.*, 1997). Of human recombinant α-class GSTs, GSTA1-1 was highly active for conjugating dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxides and was seven times more active against the (+)-*syn* than the (–)-*anti*-isomer (Dreij *et al.*, 2002).

DNA adducts of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide formed adducts with calf thymus DNA that were identified as *anti-cis*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide–deoxyguanosine, *anti-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine and *anti-cis*- dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine. Racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12,-diol-13,14-oxide formed adducts with calf thymus DNA that were identified as two *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adducts, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyguanosine adduct, a *syn-cis*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adduct and two *syn-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–deoxyadenosine adducts. For both *syn-*

and *anti-trans*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxides, deoxyguanosine adducts were 1.4-fold more frequent than deoxyadenosine adducts (Devanesan *et al.*, 1999).

Genotoxicity of dibenzo[*a,l*]pyrene-11,12,-diol-13,14-oxides

Racemic *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were mutagenic in *S. typhimurium* TA97, TA98, TA100 and TA104 and in Chinese hamster V79 cells in the absence of exogenous metabolic activation. In *S. typhimurium* TA104, the *anti* isomer was more active than the *syn* isomer and produced the highest number of revertants/nmol (1 030 000) ever recorded (Luch *et al.*, 1994; Seidel *et al.*, 1994).

Conclusion

Dibenzo[*a*,*l*]pyrene is activated metabolically by a diol epoxide mechanism. Dibenzo[*a*,*l*]pyrene was stereospecifically metabolized by CYP1A1 and CYP1B1 of both rodent and human liver microsomes, by cells that express these CYPs and by mouse skin to give two diols: the major diol, (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol, and the minor diol, (+)-dibenzo[*a*,*l*]pyrene-11*S*,12*S*-diol. (–)-Dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was then further metabolized by rodent or human CYP1B1 or rodent CYP1A1 to the fjord-region diol epoxide, (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide, while (+)-dibenzo-[*a*,*l*]pyrene-11*S*,12*S*-diol was metabolized to (+)-*syn*-dibenzo[*a*,*l*]pyrene-11*S*,12*R*,-diol-13*S*,14*R*-oxide. Human CYP1A1 metabolizes (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol to dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and highly polar unidentified DNA adducts. Dibenzo[*a*,*l*]pyrene-11,12-diol was genotoxic in mammalian cells, and (–)-dibenzo-[*a*,*l*]pyrene-11*R*,12*R*-diol was the most active form. Racemic dibenzo[*a*,*l*]pyrene-11,12-diol initiated skin tumours in SENCAR and NMRI mice with an activity approximately equal to that of the parent dibenzo[*a*,*l*]pyrene. In NMRI mice, (–)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was slightly less active than dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and (+)-dibenzo[*a*,*l*]pyrene-11*R*,12*R*-diol was inactive. Racemic dibenzo-[*a*,*l*]pyrene-11,12-diol was carcinogenic and induced benign skin tumours in mice but with lower activity at the same dose than the parent dibenzo[*a*,*l*]pyrene, which induced malignant skin tumours in Swiss mice. These studies on mouse skin are complicated by the concurrent toxicity of all of these agents which confounds the interpretation of the quantitative results. Racemic dibenzo[*a*,*l*]pyrene-11,12-diol also induced lung tumours in newborn mice. Racemic *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were genotoxic in bacteria, and the *anti* isomer was the most active mutagen ever measured. Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide was slightly less active than racemic *syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide as a skin tumour initiator in SENCAR mice. Racemic *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide induced benign and malignant mammary tumours when injected into rats and lung and liver tumours in newborn mice. The DNA adducts formed by dibenzo[*a*,*l*]pyrene, dibenzo[*a*,*l*]pyrene-11,12-diol and *syn*- and *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide were similar. In mammalian cells, the major adducts were derived from both (–)-*anti*-dibenzo[*a*,*l*]pyrene-11*R*,12*S*-diol-13*S*,14*R*-oxide and (+)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide and

were adducts of deoxyadenosine and deoxyguanosine. These DNA adducts are consistent with the mutations observed in *ras* proto-oncogenes in skin or lung tumours from mice treated with dibenzo[*a*,*l*]pyrene.

(ix) *Relevance of stable dibenzo[*a,l*]pyrene-11,12-diol-13,14-oxide adducts*

There are divergent views on the relevance of stable dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide adducts and unstable depurinating dibenzo[*a*,*l*]pyrene adducts in the metabolic activation mechanism of dibenzo[*a*,*l*]pyrene. A number of investigators have studied this in different test systems, such as isolated DNA, mammalian cells in culture and mammals *in vivo*. When the quantity of stable dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA adducts was compared with apurinic site formation in either isolated DNA from Chinese hamster ovary B11 cells or in Chinese hamster ovary B11 cells treated directly with (+)-*syn*- or (–)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide, stable adducts could be measured (3 or 10 per 14 kb DNA fragment, respectively), while the levels of apurinic sites were below the detection limits (0.1 apurinic site in a 14 kb DNA fragment). When human mammary carcinoma MCF-7 cells were treated with dibenzo-[*a*,*l*]pyrene, only stable adducts and no apurinic sites were detected. These results suggested a predominant role for stable DNA adducts in the carcinogenic activity of dibenzo[*a*,*l*]pyrene (Melendez-Colon *et al.*, 1997). Human leukaemia HL-60 cells (that contain high peroxidase activity and lack CYP enzymes responsible for activation of PAH to diol epoxide) or human mammary carcinoma MCF-7 cells (that contain CYP activity but no peroxidase activity) were exposed to dibenzo[*a*,*l*]pyrene in a effort to discern the relative proportions of stable and depurinating DNA adducts (measured as apurinic sites). Human HL-60 cells produced no detectable levels of either stable DNA adducts or apurinic sites while MCF-7 cells produced stable adducts and low levels of apurinic sites that were not increased by dibenzo[*a*,*l*]pyrene. The levels of stable adduct increased 100-fold after exposure for 24 h, while the levels of apurinic sites remained low. These results indicated that metabolic activation of dibenzo[*a*,*l*]pyrene in human MCF-7 cells by CYP enzymes proceeds through diol epoxides that form stable DNA adducts with little formation of apurinic sites. Human HL-60 cells that contain high levels of peroxidase form neither stable DNA adducts nor apurinic sites. However, in these studies no measurement of unstable adducts was reported (Melendez-Colon *et al.*, 1999a, 2000).

The proportions of stable diol epoxide dibenzo[*a*,*l*]pyrene–DNA adducts and dibenzo[*a*,*l*]pyrene-induced apurinic sites formed in epidermal DNA were compared after topical application of dibenzo[*a*,*l*]pyrene to the skin of female SENCAR mice. Regardless of time after treatment and dose of dibenzo[*a*,*l*]pyrene applied, the levels of stable DNA adducts were significantly (from 32- to 86-fold) higher than those of apurinic sites (Melendez-Colon *et al.*, 1999b). The authors suggested that stable DNA adducts rather than apurinic sites were responsible for tumour initiation by dibenzo[*a*,*l*]pyrene in mouse skin.

(x) *Relevance of the diol epoxide metabolic activation mechanism for dibenzo[*a,l*]pyrene to human cancer*

The metabolic processes that metabolize dibenzo[*a*,*l*]pyrene to its active diol epoxides are present in human tissues (CYP1A1 and CYP1B1) and in human mammary cells in culture, which suggests the strong possibilty that humans exposed to dibenzo[*a*,*l*]pyrene would metabolically activate the compound to dibenzo[*a*,*l*]pyrene diol epoxides that could form DNA adducts and induce mutations and other genotoxic damage.

The mutations (G→T and A→T transversions) in the K-*ras* proto-oncogene codon 12 and 61 in mouse lung tumours from dibenzo[*a*,*l*]pyrene-treated mice are associated with *anti*-dibenzo[*a*,*l*]pyrene-11,12-diol-13,14-oxide–DNA adducts (Prahalad *et al.*, 1997). Although this is not a direct correlation, similar mutations in the Ki-*RAS* proto-oncogene were found in lung tumours from nonsmokers exposed to PAH-rich emissions from coal combustion (known to contain dibenzo[*a*,*l*]pyrene as well as many other PAHs) (DeMarini *et al.*, 2001).

Indeno[1,2,3-*cd*]pyrene

(i) *Metabolism and metabolic activation*

The metabolism of indeno[1,2,3-*cd*]pyrene has been documented in a number studies in rat liver microsomes and in mouse epidermis *in vivo*. Indeno[1,2,3-*cd*]pyrene is metabolized by Aroclor 1254-induced rat liver preparations to the K-region diol, *trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene (indeno[1,2,3-*cd*]pyrene-1,2-diol), 8-, 9- and 10-hydroxy-indeno[1,2,3-*cd*]pyrene, 8- and 9-hydroxy-*trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene and indeno[1,2,3-*cd*]pyrene-1,2-quinone (Rice *et al.*, 1985). Topical application of indeno[1,2,3-*cd*]pyrene to mouse epidermis *in vivo* produced 8-hydroxy-indeno[1,2,3-*cd*]pyrene as the primary metabolite, and 9-hydroxy-indeno[1,2,3-*cd*]pyrene and *trans*-1,2-dihydroxy-1,2-dihydro-indeno[1,2,3-*cd*]pyrene as major metabolites. Several minor metabolites were also identified: *trans*-1,2-dihydro-1,2,8-trihydroxy-indeno[1,2,3-*cd*]pyrene, *trans*-1,2-dihydro-1,2,9-trihydroxy-indeno[1,2,3-*cd*]pyrene, indeno[1,2,3-*cd*]pyrene-1,2-quinone and 10-hydroxy-indeno[1,2,3-*cd*]pyrene (Rice *et al.*, 1986).

(ii) *Formation of DNA adducts*

Indeno[1,2,3-*cd*]pyrene formed a single unidentified DNA adduct in mouse skin epidermis *in vivo* (Weyand *et al.*, 1987). Studies of DNA adducts in mouse epidermis *in vivo* using fluorine probes suggested that indeno[1,2,3-*cd*]pyrene undergoes metabolic activation at carbons 7–10 either alone or in conjunction with dihydrodiol formation at the 1,2 carbons (Rice *et al.*, 1990).

(iii) *Genotoxic effects of indeno[1,2,3-*cd*]pyrene*

In a previous monograph, indeno[1,2,3-*cd*]pyrene was reported to induce mutations in *S. typhimurium* in the presence of an exogenous metabolic activation system (IARC, 1983). Indeno[1,2,3-*cd*]pyrene was mutagenic in *S. typhimurium* TA100 in the presence

of an Aroclor 1254-induced rat liver preparation (Rice *et al.*, 1985) and in human B lymphoblastoid h1A1v2 cells (thymidine kinase locus) that express CYP1A1 (Durant *et al.*, 1996).

(iv) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity study of indeno[1,2,3-*cd*]pyrene-1,2-diol

Groups of 25 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of indeno[1,2,3-*cd*]pyrene-1,2-diol in acetone every other day (total dose, 3226 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours in the dose groups 21 weeks after treatment was 80% with 1.68 tumours/mouse. The response in control mice treated with TPA alone was 0.04 tumours/mouse. Quantitatively, indeno[1,2,3-*cd*]pyrene-1,2-diol produced fewer tumours/mouse than the parent indeno[1,2,3-*cd*]pyrene (Rice *et al.*, 1986).

Genotoxicity of indeno[1,2,3-*cd*]pyrene-1,2-diol

Indeno[1,2,3-*cd*]pyrene-1,2-diol did not induce mutations in *S. typhimurium* TA100 in the presence of an Aroclor 1254-induced rat liver preparation (Rice *et al.*, 1985).

Carcinogenicity study of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of indeno[1,2,3-*cd*]pyrene-1,2-oxide in acetone every other day (total dose, 3425 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dose group 21 weeks after treatment was 80% (1.60). The response in control mice treated with TPA alone was 0.04 tumours/mouse. Quantitatively, indeno[1,2,3-*cd*]pyrene-1,2-oxide produced fewer tumours/mouse than the parent indeno[1,2,3-*cd*]pyrene (1.60 versus 2.83) (Rice *et al.*, 1986).

DNA adducts of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Indeno[1,2,3-*cd*]pyrene-1,2-oxide forms one major DNA adduct with calf thymus DNA (King *et al.*, 1994).

Genotoxicity of indeno[1,2,3-*cd*]pyrene-1,2-oxide

Indeno[1,2,3-*cd*]pyrene-1,2-oxide induced mutations in *S. typhimurium* TA100 in the absence of an exogenous metabolic activation system (Rice *et al.*, 1985).

Carcinogenicity study of 8-hydroxy-indeno[1,2,3-*cd*]pyrene

Groups of 25 female Charles River CD-1 mice, 50–55 days of age, received skin applications of 10 subdoses of 8-hydroxy-indeno[1,2,3-*cd*]pyrene in acetone every other day (total dose, 3425 nmol) [1 mg], followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours in the dose group 21

weeks after treatment was not stated; however, there were 0.48 tumours/mouse. The response in control mice treated with TPA alone was 0.04 tumours/mouse (Rice *et al.*, 1986).

Genotoxicity of 8-hydroxy-indeno[1,2,3-*cd*]pyrene

8-Hydroxy-indeno[1,2,3-*cd*]pyrene induced mutations in *S. typhimurium* TA100 in the absence of an exogenous metabolic activation system (Rice *et al.*, 1985).

Conclusion

No data are available that support a diol epoxide mechanism for indeno[1,2,3-*cd*]pyrene. While indeno[1,2,3-*cd*]pyrene-1,2-oxide is an ultimate bacterial mutagenic metabolite of indeno[1,2,3-*cd*]pyrene and forms a single DNA adduct *in vitro*, it is not the ultimate tumorigenic metabolite on mouse skin based on quantitative tumour formation. 8-Hydroxy-indeno[1,2,3-*cd*]pyrene is mutagenic to bacteria but exhibited only weak tumour-initiating activity. These results indicate that the principal metabolic activation pathways associated with the mutagenic activity of indeno[1,2,3-*cd*]pyrene are not related to its tumour-initiating activity in mouse skin. In mouse skin, indeno[1,2,3-*cd*]pyrene appears to undergo metabolic activation at carbons 7–10 either alone or in conjunction with dihydrodiol formation at the 1,2 carbons. The route of metabolic activation of indeno[1,2,3-*cd*]pyrene in mouse skin has yet to be determined.

5-Methylchrysene

(i) *Metabolism and metabolic activation*

The metabolism of 5-methylchrysene has been documented in a number studies in rat and mouse liver microsomes, in mouse epidermis *in vivo* and in human liver and lung microsomes. Two potential centres of metabolism and metabolic activation are situated on the two benzo rings, the pseudo-fjord-region (1–2 and 3–4 bonds) and the bay-region (7–8 and 9–10-bonds). Control, Aroclor-treated and 3-methylcholanthrene-induced rat liver preparations metabolized 5-methylchrysene to 1,2-dihydro-1,2-dihydroxy-5-methylchrysene (5-methylchrysene-1,2-diol), 7,8-dihydro-7,8-dihydroxy-5-methylchrysene (5-methylchrysene-7,8-diol), 9,10-dihydro-9,10-dihydroxy-5-methylchrysene (5-methylchrysene-9,10-diol), 3,4-dihydro-3,4-dihydroxy-5-methylchrysene (5-methylchrysene-3,4-diol), 9-hydroxy-5-methylchrysene, 7-hydroxy-5-methylchrysene, 1-hydroxy-5-methylchrysene and 5-hydroxymethylchrysene (Hecht *et al.*, 1978; Amin *et al.*, 1985b). Phenobarbital-induced rat liver microsomes metabolized 5-methylchrysene to two K-region diols, 5,6-dihydroxy-5,6-dihydro-5-methylchrysene and 11,12-dihydroxy-11,12-dihydro-5-methylchrysene (Bao & Yang, 1986). 5-Methylchrysene was stereoselectively metabolized to the *R*,*R* dihydrodiol enantiomers, 5-methylchrysene-1*R*,2*R*-diol and 5-methylchrysene-7*R*,8*R*-diol, *in vitro* by 3-methylcholanthrene-induced mouse liver preparations and human liver microsomes, and *in vivo* in mouse epidermis (Melikian *et al.*, 1983; Amin *et al.*, 1987; Koehl *et al.*, 1996). Mouse skin epidermis *in vivo* produced the metabolites 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-

9,10-diol and 5-hydroxymethylchrysene and their glucuronide and sulfate conjugates (Melikian *et al.*, 1983). Human liver microsomes metabolized 5-methylchrysene to 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-9,10-diol (5-methylchrysene-3,4-diol), 9-hydroxy-5-methylchrysene, 7-hydroxy-5-methylchrysene, 1-hydroxy-5-methylchrysene and 5-hydroxymethylchrysene. Human lung microsomes metabolized 5-methylchrysene to 5-methylchrysene-1,2-diol, 5-methylchrysene-7,8-diol, 5-methylchrysene-9,10-diol, 5-methylchrysene-3,4-diol and 1-hydroxy-5-methylchrysene. CYP1A1 was shown to play a major role in the metabolic activation of 5-methylchrysene in human lung microsomes. Purified recombinant human CYP1A1 had high activity for the formation of 5-methylchrysene-1,2-diol and 5-methylchrysene-7,8-diol and CYP3A4 was active for methyl hydroxylation (Koehl *et al.*, 1996).

(ii) *Formation of DNA adducts*

5-Methylchrysene forms a major DNA adduct in mouse skin after dermal application and in the human MCF-7 mammary carcinoma cell line (Shiue *et al.*, 1987; Kuljukka-Rabb *et al.*, 2001). It forms several bay-region diol epoxide–DNA adducts after application to mouse skin *in vivo*, which were identified as *anti*-1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydro-5-methylchrysene (*anti*-5-methylchrysene-1,2-diol-3,4-oxide)–DNA and *anti*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-5-methylchrysene–DNA in the ratio of 2.7:1. This indicated that both 5-methylchrysene-1,2-diol and 5-methylchrysene-7,8-diol were metabolized to their corresponding diol epoxides *in vivo* (Melikian *et al.*, 1982). The adducts were further identified as *trans*-N^2-deoxyguanosine adducts at the benzylic carbon of the epoxide ring of each diol epoxide (Melikian *et al.*, 1984). The *anti*-5-methylchrysene-1,2-diol-3,4-oxide–DNA adduct was further characterized as a 1*R*,2*S*,3*S*-trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene adduct (Melikian *et al.*, 1988), which was detected in the lungs of newborn mice injected with 5-methylchrysene (Melikian *et al.*, 1991) and as one of six DNA adducts observed in lung tissues of mice treated with 5-methylchrysene (You *et al.*, 1994).

(iii) *Genotoxicity of 5-methylchrysene*

In a previous monograph, 5-methylchrysene was reported to induce mutations in *S. typhimurium* TA100 and to induce unscheduled DNA synthesis in primary rat hepatocytes (IARC, 1983). 5-Methylchrysene was a gene mutagen in *S. typhimurium* TA100 in the presence of Aroclor 1254-induced rat liver metabolic activation (Cheung *et al.*, 1993). It produced G:C→A:T (31.2%) transitions, G:C→T:A (15.3%) transversions and A:T→T:A (37.2%) transversions in a *lacZ* reversion assay in *E. coli* in the presence of an Aroclor 1254-induced rat liver preparation (Garganta *et al.*, 1999). Human CYP1A1 and CYP1B1 enzymes have higher activity for the metabolism of 5-methylchrysene-1,2-diol than for the parent compound 5-methylchrysene to DNA-damaging forms using cDNA-based recombinant (*E. coli* or *T. ni*) systems that express these forms of CYP (Shimada *et al.*, 2001a).

(iv) *5-Methylchrysene-induced mutations in proto-oncogenes*

5-Methylchrysene induced three major classes of Ki-*ras* codon 12 mutations in lung adenomas from intraperitoneally treated strain A/J mice — GGT→TGT (50%), GGT→GTT (23%) and GGT→CGT (27%) — which indicates that deoxyguanosine was a primary target for this PAH in mouse lung (You *et al.*, 1994; Nesnow *et al.*, 1998b). Tumours from mice treated by skin application with racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide (the ultimate metabolite of 5-methylchrysene) had very low numbers of Ha-*ras* codon 12, 13 or 61 mutations (Hecht *et al.*, 1998).

(v) *Evidence for diol epoxide metabolic activation as a mechanism of carcinogenesis*

Carcinogenicity studies of 5-methylchrysene-1,2-diol

Groups of 20 female Ha(ICR) Swiss mice, 50–55 days of age, received dermal applications of 10 subdoses of racemic 5-methylchrysene-1,2-diol [total dose, 109 nmol] (30 μg) in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 95% (7.3). No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1980).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [9 μg] racemic 5-methylchrysene-1,2-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks after treatment was 80% (2.5). Skin tumours developed in 5% (0.5 tumours/mouse) of the mice treated with TPA alone. The tumour incidence in the treatment and control groups was statistically different (Amin *et al.*, 1985b).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [9 or 27.5 μg] racemic 5-methylchrysene-1,2-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 85 (9.9) and 100% (12.7), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 10 or 33 nmol [2.75 or 9 μg] 5-methylchrysene-1*S*,2*S*-diol or 5-methylchrysene-1*R*,2*R*-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was: 5-methylchrysene-1*S*,2*S*-diol, 60 (0.9) and 85% (3.0); 5-methylchrysene-1*R*,2*R*-diol, 100 (9.4) and 85% (7.9), respectively. No skin tumours developed in mice treated with TPA alone. These results indicated a higher reactivity of the *R*,*R* diol compared with the *S*,*S* diol (Amin *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic 5-methylchrysene-1,2-diol (total dose, 56 nmol) [15 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In

43 female mice, pulmonary tumours occurred in 12% of the surviving mice (0.14 tumours/mouse) and liver tumours in 7% (0.23 tumours/mouse). In 44 male mice, pulmonary tumours occurred in 11% of the surviving mice (0.18 tumours/mouse) and liver tumours in 25% (0.52 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

DNA adducts of 5-methylchrysene-1,2-diol

1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with 5-methylchrysene-1*R*,2*R*-diol (Melikian *et al.*, 1991).

Genotoxicity of 5-methylchrysene-1,2-diol

Human CYP1A1 and CYP1B1 enzymes metabolically activated 5-methylchrysene-1,2-diol to DNA-damaging forms in cDNA-based recombinant (*E. coli* or *T. ni*) systems that express different forms of the CYP (Shimada *et al.*, 2001a).

Carcinogenicity studies of 5-methylchrysene-1,2-diol-3,4-oxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [10 or 29.5 μg] racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 65 (1.3) and 80% (4.4), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [10 μg] 5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide, *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide or racemic *syn*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 80 (3.2), 20 (0.4) or 5% (0.1), respectively. No skin tumours developed mice treated with TPA alone (Hecht *et al.*, 1987).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33, 100 or 400 nmol [10, 29.5 or 118 μg] racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone twice a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 21 weeks after treatment was 90 (3.1), 100 (7.5) and 100% (9.1), respectively. Skin tumours developed in 5% (0.05 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1998).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-5-methylchrysene-1,2-diol-3,4-oxide or racemic *syn*-5-methyl-

chrysene-1,2-diol-3,4-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 48 or 41 female mice, pulmonary tumours were observed in 81 or 29% of the surviving mice (5.6 or 0.34 tumours/mouse) and liver tumours in 4 or 7% (0.13 or 0.46 tumours/mouse), respectively. In 38 or 49 male mice, pulmonary tumours were observed in 82 or 6% of the surviving mice (3.3 or 0.06 tumours/mouse) and liver tumours in 34 or 14% (2.6 or 0.2 tumours/mouse), respectively. In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide or *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 20 or 25 female mice, pulmonary tumours developed in 85 or 56% of the surviving mice (19.4 or 0.84 tumours/mouse), respectively, but no liver tumours. In 24 or 40 male mice, pulmonary tumours developed in 88 or 18% of the surviving mice (8.4 or 0.23 tumours/mouse) and liver tumours in 46 or 8% (1.38 or 0.1 tumours/mouse), respectively. In 28 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) but no liver tumours. In 31 male mice treated with DMSO alone, pulmonary tumours developed in 3% of the surviving mice (0.13 tumours/mouse) but no liver tumours. The numbers of pulmonary tumours/mouse in both male and female mice and hepatic tumours/mouse in male mice were significantly different from those in the controls for *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide and those of pulmonary tumours/mouse in female mice for *anti*-5-methylchrysene-1*S*,2*R*-diol-3*R*,4*S*-oxide (Hecht *et al.*, 1987).

DNA adducts of 5-methylchrysene-1,2-diol-3,4-oxide

anti-5-Methylchrysene-1,2-diol-3,4-oxide and *anti*-5-methylchrysene-7,8-diol-9,10-oxide formed DNA adducts with calf thymus DNA (Melikian *et al.*, 1984; King *et al.*, 1994). These adducts result from the addition of the exocyclic amino group of deoxyguanosine to the benzylic carbon of the epoxide ring of the dihydrodiol epoxide (Melikian *et al.*, 1984). One *anti*-5-methylchrysene-1,2-diol-3,4-oxide–calf thymus DNA adduct was further characterized as 1*R*,2*S*,3*S*-trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene (Reardon *et al.*, 1987; Melikian *et al.*, 1988). Other *anti*-5-methylchrysene-1,2-diol-3,4-oxide–calf thymus DNA adducts have been identified as *cis* and *trans* deoxyadenosine (Reardon *et al.*, 1987; Melikian *et al.*, 1988). Both *syn* and *anti* forms of 5-methylchrysene-1,2-diol-3,4-oxide form *cis* and *trans* adducts with calf thymus DNA (Szeliga *et al.*, 1999). 1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with *anti*-5-methylchrysene-1,2-diol-3,4-oxide (Melikian *et al.*, 1991).

Genotoxicity of 5-methylchrysene-1,2-diol-3,4-oxide

The *R,S,S,R* enantiomer of *anti*-5-methylchrysene-1,2-diol-3,4-oxide was mutagenic in *S. typhimurium* TA100 (Melikian *et al.*, 1988). *anti*-5-Methylchrysene-1,2-diol-3,4-oxide produced the base-specific mutations GC→TA (62%), GC→CG (23%), GC→AT (9%) and AT→TA (4%) and *syn*-5-methylchrysene-1,2-diol-3,4-oxide produced the base-specific mutations GC→TA (43%), GC→CG (22%), GC→AT (14%) and AT→TA (12%) in *supF* DNA using a SV40-based shuttle vector system (Bigger *et al.*, 2000). In the *supF* gene of the pSP189 vector, *syn*-5-methylchrysene-1,2-diol-3,4-oxide gave transversion mutations at GC pairs, predominantly G→T and G→C, which showed the preference for reaction with deoxyguanosine residues in DNA (Page *et al.*, 1996). Both *syn*- and *anti*- 5-methylchrysene-1,2-diol-3,4-oxide were mutagenic at *Hprt* gene of Chinese hamster V79 (6-thioguanine resistance) cells, and the *anti* form was the most mutagenic (Brookes *et al.*, 1986).

In normal human bronchial epithelial cells, *anti*-5-methylchrysene-1,2-diol-3,4-oxide formed significant levels of guanine adducts within codon 158 of exon 5, codons 237, 245 and 248 of exon 7 and codon 273 of exon 8 in the human *TP53* tumour-suppressor gene. These codons were also hotspots for mutations in the *TP53* gene of lung cancer patients (Smith *et al.*, 2000). (+)-*anti*-5-Methylchrysene-1,2-diol-3,4-oxide was conjugated to GSH to a greater extent than (–)-*anti*-5-methylchrysene-1,2-diol-3,4-oxide by human hGSTP1-1 (I104, A113) allele, the most frequent allele in human populations (Hu *et al.*, 1998).

Other effects of 5-methylchrysene-1,2-diol-3,4-oxide

anti-5-Methylchrysene-1,2-diol-3,4-oxide induces activator protein-1 through PI3K- and Akt-dependent and $p70^{SK6}$-independent pathways in mouse epidermal Cl41 cells suggesting that oxidative stress, such reactive oxygen species may be involved (Li *et al.*, 2004a). *anti*-5-Methylchrysene-1,2-diol-3,4-oxide also resulted in the activation of MAPKs as well as the activation of inhibitory subunit kappa-B phosphorylation and degradation involved in the activation of nuclear factor (NF) kappaB. The authors suggested that *anti*-5-methylchrysene-1,2-diol-3,4-oxide may play a role in the tumour promotion effects of its parent compound 5-methylchrysene or of itself (Li *et al.*, 2004b). In human breast carcinoma MCF-7 cells, p53 was stabilized in response to DNA damage by *anti*-5-methylchrysene-1,2-diol-3,4-oxide. However, the protective mechanism of G1 arrest of the cells was not induced. Therefore, this DNA damage escapes the p53-mediated cellular defence mechanism of G1 arrest (Khan *et al.*, 1999).

Carcinogenicity studies of 5-methylchrysene-7,8-diol

Groups of 20 female Ha/ICR Swiss mice, 50–55 days of age, received 10 subdoses by dermal application of racemic 5-methylchrysene-7,8-diol (total dose, 109 nmol) [30 μg] in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed group 21 weeks

after treatment was 50% (1.1). No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1980).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [9 or 27.5 μg] racemic 5-methylchrysene-7,8-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 30 (0.3) and 75% (1.3), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [9 μg] 5-methylchrysene-7*S*,8*S*-diol or 5-methylchrysene-7*R*,8*R*-diol in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 20 weeks. The incidence of papillomas (papillomas/mouse) in the dosed groups 21 weeks after treatment was: 5-methylchrysene-7*S*,8*S*-diol, 30% (0.4); 5-methylchrysene-7*R*,8*R*-diol, 75% (2.3). No skin papillomas developed in mice treated with TPA alone. These results indicated a higher reactivity of the *R*,*R* diol compared with the *S*,*S* diol (Amin *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic 5-methylchrysene-7,8-diol (total dose, 56 nmol) [15 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 45 female mice, pulmonary tumours developed in 18% of the surviving mice (0.24 tumours/mouse) and liver tumours in 11% (0.49 tumours/mouse). In 46 male mice, pulmonary tumours developed in 13% of the surviving mice (0.13 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Carcinogenicity studies of 5-methylchrysene-7,8-diol-9,10-epoxide

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 or 100 nmol [10 or 29.5 μg] racemic *anti*-5-methylchrysene-7,8-diol-9,10-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] TPA in acetone three times a week for 25 weeks. The incidence of tumours (tumours/mouse) in the dosed groups 26 weeks after treatment was 5 (0.1) and 0% (0), respectively. Skin tumours developed in 10% (0.1 tumours/mouse) of the mice treated with TPA alone (Hecht *et al.*, 1985).

Groups of 20 female Charles River CD-1 mice, 50–55 days of age, received a single dermal application of 33 nmol [10 μg] racemic *syn*-5-methylchrysene-7,8-diol-9,10-oxide, *anti*-5-methylchrysene-7*R*,8*S*-diol-9*S*,10*R*-oxide or *anti*-5-methylchrysene-7*S*,8*R*-diol-9*R*,10*S*-oxide in acetone, followed 10 days later by 4 nmol [2.5 μg] PA in acetone three times a week for 20 weeks. The incidence of tumours (tumours/mouse) in the dosed

groups 21 weeks after treatment was 21 (0.3), 5 (0.1) or 16% (0.2), respectively. No skin tumours developed in mice treated with TPA alone (Hecht *et al.*, 1987).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with racemic *anti*-5-methylchrysene-7,8-diol-9,10-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 50 female mice, pulmonary tumours developed in 6% of the surviving mice (0.06 tumours/mouse) and liver tumours in 4% (0.04 tumours/mouse). In 49 male mice, pulmonary tumours developed in 18% of the surviving mice (0.18 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 41 female mice treated with DMSO alone, pulmonary tumours developed in 7% of the surviving mice (0.07 tumours/mouse) and liver tumours in 2% (0.02 tumours/mouse). In 48 male mice treated with DMSO alone, pulmonary tumours developed in 4% of the surviving mice (0.04 tumours/mouse) and liver tumours in 2% (0.04 tumours/mouse) (Hecht *et al.*, 1985).

Groups of newborn ICR/Ha mice were injected intraperitoneally on days 1, 8 and 15 of life with 5-methylchrysene-7*R*,8*S*-diol-9*S*,10*R*-oxide or 5-methylchrysene-7*S*,8*R*-diol-9*R*,10*S*-oxide (total dose, 56 nmol) [16.5 μg] in DMSO. Mice were weaned at 21 days of age, separated by sex and killed at 35 weeks of age. In 34 or 23 female mice, pulmonary tumours developed in 38% or 17% of the surviving mice (0.47 or 0.83 tumours/mouse) and liver tumours in 0 or 4% (0 or 0.04 tumours/mouse), respectively. In 26 or 38 male mice, pulmonary tumours developed in 35% or 0% of the surviving mice (0.23 or 0 tumours/mouse) and liver tumours in 19% or 3% (0.23 or 0.03 tumours/mouse), respectively. In 28 female mice treated with DMSO alone, pulmonary tumours occurred in 7% of the surviving mice (0.07 tumours/mouse) and no liver tumours. In 31 male mice treated with DMSO alone, pulmonary tumours occurred in 3% of the surviving mice (0.13 tumours/mouse) and no liver tumours (Hecht *et al.*, 1987).

Genotoxicity of 5-methylchrysene-7,8-diol-9,10-oxide

The *R,S,S,R* enantiomer of *anti*-5-methylchrysene-7,8-diol-9,10-oxide was mutagenic in *S. typhimurium* TA100 (Melikian *et al.*, 1988). Both *syn*- and *anti*-5-methylchrysene-7,8-diol-9,10-oxide were mutagenic in the *Hprt* gene of Chinese hamster V79 cells (6-thioguanine resistance) (Brookes *et al.*, 1986).

Conclusion

5-Methylchrysene is activated metabolically by the diol epoxide mechanism at the pseudo bay region in mouse skin carcinogenesis. 5-Methylchrysene was stereoselectively metabolized to the *R,R* enantiomer, 5-methylchrysene-1*R*,2*R*-diol, in mouse epidermis *in vivo* and with human liver and lung microsomes. 5-Methylchrysene-1*R*,2*R*-diol was a tumour initiator in mouse skin and was more active than the *S,S* enantiomer. *anti*-5-Methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide was the most active 5-methylchrysene diol epoxide in mouse skin. The major diol epoxide adduct from treatment of mouse skin with 5-methylchrysene was *anti*-5-methylchrysene-1,2-diol-3,4-oxide–deoxyguanosine. In the

newborn mouse model, 5-methylchrysene-1,2-diol and *anti*-5-methylchrysene-1*R*,2*S*-diol-3*S*,4*R*-oxide were pulmonary carcinogens. 1*R*,2*S*,3*S*-Trihydroxy-4*R*-(N^2-deoxyguanosyl)-1,2,3,4-tetrahydro-5-methylchrysene was detected in the DNA of lungs of newborn mice injected with 5-methylchrysene, 5-methylchrysene-1*R*,2*R*-diol and *anti*-5-methylchrysene-1,2-diol-3,4-oxide. This DNA adduct was also observed in the lungs of juvenile mice administered 5-methylchrysene, and its formation was related to the detection of Ki-*ras* mutations in 5-methylchrysene-induced tumours. *anti*-5-Methylchrysene-1,2-diol-3,4-oxide–DNA adducts were formed *in vitro* within human *TP53* codons that were also hotspots for mutation in the *TP53* gene of lung cancer patients.

The bay-region diol, 5-methylchrysene-7*R*,8*R*-diol, was formed in mouse skin where it was a tumour initiator. It was a weak carcinogen in the newborn mouse model. *anti*- and *syn*-5-Methylchrysene-7,8-diol-9,10-oxide were either weak or not active as carcinogens in mouse skin or in newborn mice. The role of the bay-region diol, 5-methylchrysene-7,8-diol, and 5-methylchrysene-7,8-diol-9,10-oxide in chemical carcinogenesis remains to be determined.

4.2.2 *Mechanism via formation of Radical cations*

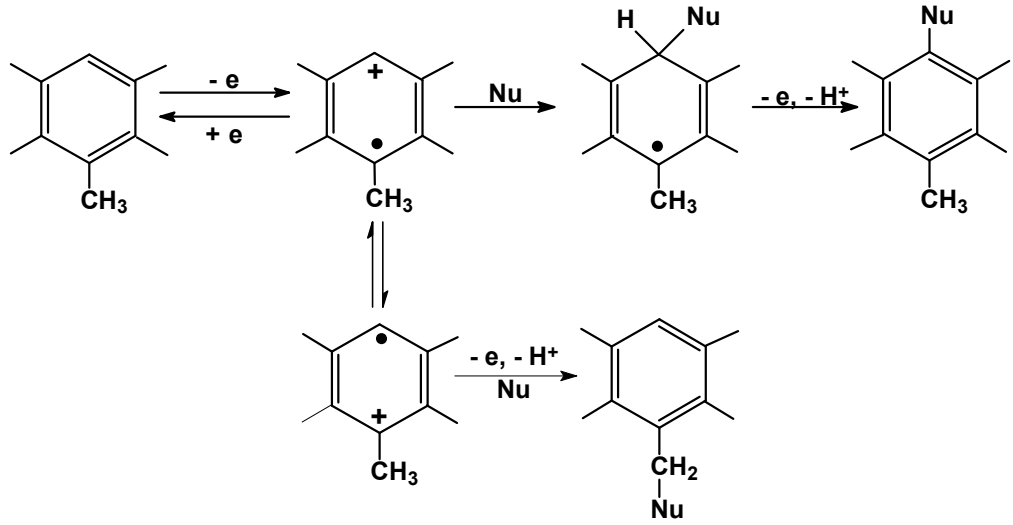
(a) Chemistry and physical chemistry of PAH radical cations

Removal of one electron from the π system of a polycyclic aromatic compound generates a radical cation, in which the positive charge is usually localized at an unsubstituted carbon atom or adjacent to a methyl group. Nucleophilic attack at the position of highest charge density at an unsubstituted carbon atom produces an intermediate radical that is further oxidized to an arenium ion to complete the substitution reaction. When the charge is localized adjacent to the methyl group, the latter becomes electrophilic and subsequently reacts with a nucleophile (Figure 4.4).

Development of the chemistry of PAH radical cations has provided evidence that these intermediates can play a role in the process of tumour initiation of several potent PAHs (Cavalieri & Rogan, 1985, 1992). Radical cations of unsubstituted and methyl-substituted PAHs have been generated by iodine oxidation (Hanson *et al*., 1998), manganic acetate oxidation (Cremonesi *et al*., 1992) and electrochemical oxidation (RamaKrishna *et al*., 1993a,b), with subsequent binding to nucleophiles. Furthermore, radical cations of benzo[*a*]pyrene and its derivatives have been isolated after oxidation with iodine in the presence of silver perchlorate (Stack *et al*., 1995).

The notion that radical cations play an important role in the metabolic activation of some PAHs derives from certain features that are common to several carcinogenic PAHs such as benzo[*a*]pyrene, dibenzo[*a,l*]pyrene, DMBA and 3-methylcholanthrene. These characteristics are: first, a relatively low ionization potential, which allows the removal of one electron and the formation of a relatively stable radical cation; second, a charge localization in the radical cation that renders this intermediate specifically and efficiently reactive toward nucleophiles; and third, an optimal geometric configuration that allows

Figure 4.4. Nucleophilic trapping in PAH radical cations at unsubstituted and methylsubstituted sites



Adapted from Cavalieri & Rogan (1985)
Nu, Nucleophile; PAH, polycyclic aromatic hydrocarbon

the formation of appropriate intercalating radical-cation complexes with DNA, and thus favours the formation of covalent DNA adducts.

(i) *Ionization potential*

The formation and relative stability of PAH radical cations are dependent on their ionization potential. Above a certain potential, activation by one-electron oxidation becomes improbable due to the more difficult removal of one electron by the oxidant, CYP enzymes or peroxidases. A cut-off ionization potential above which one-electron oxidation will probably not occur was estimated to be about 7.35 eV, based on the level of horseradish peroxidase-catalysed or prostaglandin H synthase-catalysed covalent binding to DNA of a series of PAHs with ionization potentials ranging from 8.19 eV (phenanthrene) to 6.68 eV (6,12-dimethylanthanthrene) (Cavalieri *et al*., 1983; Devanesan *et al*., 1987). Therefore, the ionization potential of a PAH can serve as an indicator to predict whether or not one-electron oxidation can play a role in its metabolic activation.

(ii) *Charge localization*

A relatively low ionization potential is a necessary but not a sufficient prerequisite for the activation of a PAH by one-electron oxidation. Another important factor that must be considered is the localization of the charge in the PAH radical cation. PAHs have positions of unequal charge localization and the extent of this unequal distribution depends on the symmetry of the condensed benzene ring. In benzo[*a*]pyrene, C6 is the

position with the greatest electron density and the highest reactivity with electrophiles in the neutral molecule (Cavalieri & Calvin, 1971). In the radical cation, C6 has the lowest electron density and the highest reactivity with nucleophiles (Cremonesi *et al*., 1989). Therefore, the relatively low ionization potential of 7.23 eV for benzo[*a*]pyrene (Cavalieri *et al*., 1983) and the charge localization at C6 render this molecule receptive to activation by one-electron oxidation.

For dibenzo[*a*,*l*]pyrene, the charge in the radical cation is mainly localized at C10 (Cremonesi *et al*., 1992). Nucleophilic substitution occurs regiospecifically at this position with subsequent formation of an intermediate radical. The intermediate is rapidly oxidized to an arenium ion with loss of a proton to complete the substitution reaction.

(iii) *Optimal geometric configuration*

An important factor that determines the carcinogenic activity of PAHs is related to the geometry of the molecule. In general, activity is found in PAHs that contain three to seven condensed rings (Boström *et al.*, 2002). A more precise requirement related to the geometric characteristics of PAHs is the presence of an angular ring, for example, in the benz[*a*]anthracene series. Optimal geometric configuration of PAHs is essential for the arrangement of the appropriate intercalation complexes with DNA, which are a prerequisite for the formation of a covalent bond with nucleophiles (Lesko *et al*., 1968). Strong evidence for the formation of these intercalation complexes is demonstrated by the fact that unstable adducts that generate apurinic sites upon release from DNA (hereafter called unstable adducts) are formed from the reaction of radical cations of benzo[*a*]pyrene, DMBA or dibenzo[*a*,*l*]pyrene with double-stranded DNA but not with single-stranded DNA or RNA (Devanesan *et al*., 1993; Rogan *et al*., 1993; Li *et al*., 1995).

(b) Enzymology of PAH activation

PAHs are activated by both peroxidases and CYPs to form ultimate carcinogenic metabolites that bind to DNA. One-electron oxidation of PAHs can be catalysed by both types of enzyme (Devanesan *et al*., 1987; Cavalieri *et al*., 1990), but only CYPs can carry out the initial epoxidation of PAHs, which leads to the proximate dihydrodiol metabolites in the diol epoxide pathway of activation (Sims *et al*., 1974; Conney, 1982). CYP is present in both the endoplasmic reticulum and the nuclear membrane of mammalian cells. Enzymes in the nuclear membrane catalyse the formation of radical cation adducts with DNA because radical cations formed in the endoplasmic reticulum react with other cellular nucleophiles before they can reach the nucleus to react with DNA. There is evidence that the CYP isoforms in the nucleus are similar to those in the endoplasmic reticulum (Bresnick *et al.*, 1977; Mukhtar *et al.*, 1979).

(c) Pathways of PAH activation: radical cations and diol epoxides

Based on several lines of evidence discussed in this section, it has been postulated that PAHs are activated via two main pathways: one-electron oxidation to form radical cations

and mono-oxygenation to yield diol epoxides. The two types of reactive intermediate, radical cations and diol epoxides, can bind to DNA to form adducts that presumably initiate the process of tumour formation. Some PAHs are activated exclusively to diol epoxides, for example 5-methylchrysene and benzo[*c*]phenanthrene, whereas several others, such as benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene, DMBA and 3-methylcholanthrene, are activated by the formation of both radical cations and diol epoxides.

Adenine and guanine are the two DNA bases that are most susceptible to the nucleophilic attack of activated PAHs, whereas thymine and cytosine are less reactive. Covalent binding to the endocyclic positions *N*3 and *N*7 of adenine and *N*7, and sometimes C8, of guanine produces unstable adducts that generate apurinic sites upon release from DNA. These adducts are sometimes called 'depurinating adducts' or 'depurination adducts'. In contrast, adduct formation at the exocyclic 2-amino group of guanine and the 6-amino group of adenine results in stable adducts that remain in DNA unless removed by repair.

The main approach to demonstrating that PAHs are indeed activated primarily by these two mechanisms has been the identification and quantification of PAH–DNA adducts formed *in vitro* and *in vivo*. The unstable PAH–DNA adducts formed at endocyclic positions of adenine and guanine have been identified by a combination of high-performance liquid chromatography (HPLC) and fluorescence line-narrowing spectroscopy (Jankowiak & Small, 1998). The mechanism of activation, the types of DNA damage formed and their biological significance in initiating the tumour process are supported by these studies.

(i) *Benzo[*a*]pyrene*

Benzo[*a*]pyrene–DNA adducts were among the first PAH–DNA adducts to be identified. Important evidence for the diol epoxide activation pathway was the identification of a stable adduct formed *in vitro* and *in vivo* by the reaction of benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide with the exocyclic 2-amino group of guanine (Sims *et al*., 1974; Koreeda *et al*., 1978). The formation of adducts by one-electron oxidation was demonstrated by the identification of an unstable benzo[*a*]pyrene–6-*N7*-guanine adduct after in-vitro activation of benzo[*a*]pyrene by horseradish peroxidase or CYP in the presence of DNA (Rogan *et al*., 1988). Subsequently, complete profiles of the two types of benzo[*a*]pyrene–DNA adduct formed after activation of benzo[*a*]pyrene by CYP *in vitro* and in mouse skin *in vivo* were determined (Chen *et al.*, 1996). Qualitative and quantitative comparisons of the adducts were made by treating mouse skin *in vivo* with 200 nmol each of tritiated benzo[*a*]pyrene, benzo[*a*]pyrene-7,8-dihydrodiol (its proximate carcinogenic metabolite) or *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (an ultimate carcinogenic metabolite) in 50 μL acetone. The comparison comprised assessment of the relative amounts of stable and unstable adducts and the relative abundance of unstable adducts at guanine and adenine.

Benzo[*a*]pyrene formed both stable (29%) and unstable (71%) adducts in mouse skin (Chen *et al*., 1996). The unstable adducts were 8-(benzo[*a*]pyrene-6-yl)guanine (34%), 7-

(benzo[*a*]pyren-6-yl)guanine (10%), 7-(benzo[*a*]pyren-6-yl)adenine (22%), 10-(guanin-7-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (2%) and 10-(adenin-7-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (3%), whereas the stable 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene constituted 23% and the remaining 6% were unidentified stable adducts. Most of the adducts found after treatment with benzo[*a*]pyrene were unstable adducts formed by one-electron oxidation (66%), whereas stable adducts were mostly formed by the diol epoxide, the major one of which is 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene.

Benzo[*a*]pyrene-7,8-dihydrodiol formed more stable adducts (63%) than unstable adducts (37%) in mouse skin (Chen *et al*., 1996), but the absolute amount of unstable adducts (1.2 μmol/mol DNA-Phosphate) was very similar to that formed after treatment with benzo[*a*]pyrene (1.5 μmol/mol DNA-Phosphate). After treatment with *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, fewer unstable adducts (0.6 μmol/mol DNA-Phosphate) were formed than with the precursors benzo[*a*]pyrene and benzo[*a*]-pyrene-7,8-dihydrodiol. Among the stable adducts formed by benzo[*a*]pyrene, benzo[*a*]-pyrene-7,8-dihydrodiol and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide, the main adduct is 10-(guanin-2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, and the ratio of the amount of stable adducts formed by these three agents is 1:3:80, respectively. The relative tumorigenicity of these three compounds corresponds to the amount of unstable adducts formed: benzo[*a*]pyrene and benzo[*a*]pyrene-7,8-dihydrodiol exhibit similar potency in mouse skin and *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide is less active (Slaga *et al*., 1976; Levin *et al*., 1977b; Slaga *et al*., 1977b). In contrast, carcinogenicity is not related to the amount of stable adducts, since the less carcinogenic *anti*-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide forms much higher levels of such adducts (Chen *et al*., 1996).

(ii) *Dibenzo[*a,l*]pyrene*

Dibenzo[*a*,*l*]pyrene is the most potent of the known carcinogenic PAHs (Cavalieri *et al*., 1989; LaVoie *et al*., 1993). Comparative carcinogenicity studies in mouse skin and rat mammary gland indicated that dibenzo[*a*,*l*]pyrene is slightly more potent than its 11,12-dihydrodiol and much more potent than its 11,12-dihydrodiol-13,14-epoxide derivatives (Higginbotham *et al*., 1993; Gill *et al*., 1994). Identification and quantification of the adducts formed in the skin of mice treated with dibenzo[*a*,*l*]pyrene revealed that 99% are unstable (Cavalieri *et al*., 2005; Todorovic *et al*., 2005). Most of these adducts are formed by one-electron oxidation, with 63% at adenine and 12% at guanine. The remainder are formed by the diol epoxide, with 18% at adenine and 6% at guanine. When mouse skin is treated *in vivo* with dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol, unstable adducts comprise 80% of the total and are formed mainly with adenine (69%). Treatment of mouse skin with (±)-*syn*-dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol-13,14-epoxide results in 32% unstable adducts, primarily at adenine (25%), whereas treatment with (±)-*anti*-dibenzo[*a*,*l*]pyrene-11,12-dihydrodiol-13,14-epoxide produces 97% stable adducts (Cavalieri *et al*., 2005). Comparison of the relative tumorigenicity of dibenzo[*a*,*l*]pyrene and its metabolites with

the amount of DNA adducts formed in mouse skin suggests that the unstable adducts at adenine play a major role in the initiation of tumours by dibenzo[*a*,*l*]pyrene.

(iii) *7,12-Dimethylbenz[*a*]anthracene*

When DMBA is bound to DNA after activation with CYP *in vitro* or in mouse skin *in vivo*, 99% of the adducts are formed by one-electron oxidation. Of these, 79–82% have the 12-methyl group of DMBA linked to *N*7 of adenine, whereas 20–17% have the 12-methyl group linked to the *N*7 of guanine. Stable adducts arising from the bay-region diol epoxide represent less than 1% of the adducts formed in mouse skin (RamaKrishna *et al*., 1992; Devanesan *et al*., 1993).

The 12-methyl group of DMBA plays an important role in radical cation formation and DNA binding. When the two methyl groups are substituted with two ethyl groups, the resulting 7,12-diethylbenz[*a*]anthracene was not carcinogenic when administered to Long-Evans rats by subcutaneous injection (Pataki & Balick, 1972), which is consistent with the absence of nucleophilic substitution at the benzylic methylene group of the radical cation of an ethyl-substituted PAH (Cavalieri & Roth; 1976; Tolbert *et al*., 1990). Furthermore, 7-methyl-12-ethylbenz[*a*]anthracene is a much weaker carcinogen than DMBA, whereas 7-ethyl-12-methylbenz[*a*]anthracene displays a carcinogenic activity similar to that of DMBA (Pataki & Balick, 1972). These data clearly suggest that the 12-methyl group plays a major role in the carcinogenic activity of DMBA.

Although less potent than DMBA, 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene is a strong carcinogen despite being fully saturated in the angular ring and, thus, unable to be activated by the diol epoxide pathway (DiGiovanni *et al*., 1982b). Electrochemical oxidation of 1,2,3,4-tetrahydro-7,12-dimethylbenz[*a*]anthracene in the presence of deoxyguanosine or deoxyadenine yields numerous adducts that include those at the 12-methyl and 7-methyl groups that are similar to those obtained from DMBA (Mulder *et al*., 1996).

In conclusion, based on the several lines of evidence described above, DMBA initiates tumours by forming radical cations at the 12-methyl group that bind specifically to the *N*7 of adenine. Although adducts may also be formed via the diol epoxide mechanism, these adducts are expected to make at most only a minor contribution.

4.2.3 *Mechanism via formation of ortho-quinones and generation of reactive oxygen species*

(a) Description

PAHs with a terminal benzo-ring in a bay region are complete carcinogens in experimental animals (Cook *et al*., 1932, 1933; Bachmann *et al*., 1937). They are metabolically activated by CYP1A1/1B1 enzymes to form arene oxides of defined stereochemistry, which are hydrated by epoxide hydratase to form non-K region *R,R-trans*-dihydrodiols (Gelboin, 1980; Shimada *et al*., 1996). These *trans*-dihydrodiols are proximate carcinogens that undergo further mono-oxygenation by CYP1A1/1B1 to form

predominantly bay-region *anti*-diol epoxides as described in Section 4.2.1. The formation of *trans*-dihydrodiols represents a branch point in PAH metabolism (Figure 4.5).

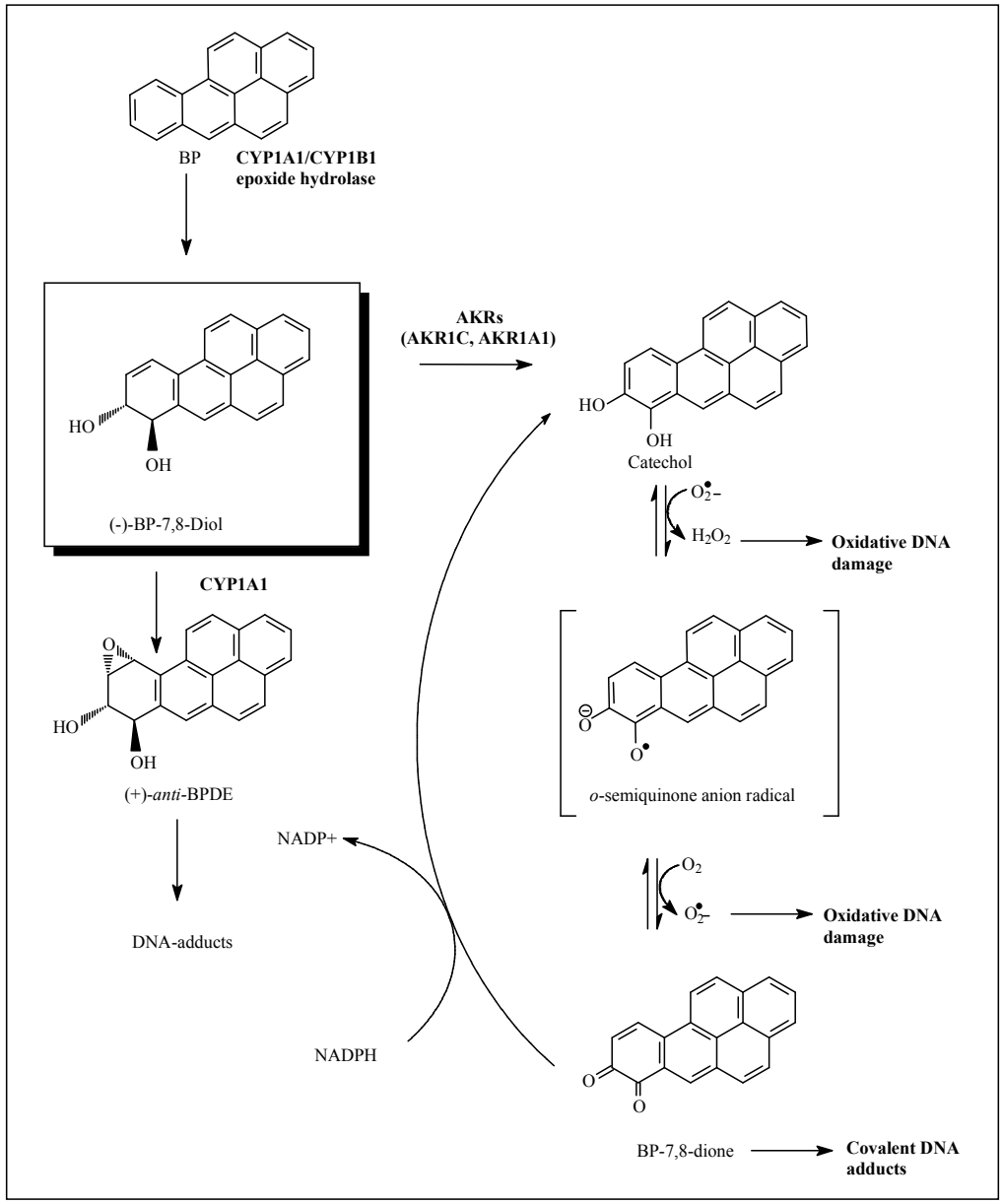
Non-K region *trans*-dihydrodiols also undergo a $NAD(P)^+$-dependent dehydrogenation that is catalysed by monomeric cytosolic oxidoreductases of the AKR superfamily to yield ketols, which spontaneously rearrange to yield catechols. Catechols are extremely air-sensitive and undergo two sequential one-electron auto-oxidation steps to yield the corresponding reactive PAH *ortho*-quinones (Smithgall *et al*., 1986, 1988a). An intermediate in this auto-oxidation is the corresponding *ortho*-semiquinone anion radical. Each one-electron oxidation event (either catechol → *ortho*-semiquinone anion radical or *ortho*-semiquinone anion radical → *ortho*-quinone) yields reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl radical). For benzo[*a*]pyrene, this reaction sequence would comprise dehydrogenation of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to form 7,8-dihydroxybenzo[*a*]pyrene (catechol) and auto-oxidation to yield benzo[*a*]pyrene-7,8-dione (see Figure 4.5) (Penning *et al*., 1996).

The resulting PAH *ortho*-quinone is a highly reactive Michael acceptor that can undergo 1,4- or 1,6-Michael addition reactions with cellular nucleophiles (e.g. L-cysteine, GSH) to yield conjugates (Murty & Penning, 1992a,b; Sridhar *et al*., 2001) or with macromolecules (e.g. protein, RNA and DNA) to yield adducts (Shou *et al*., 1993; McCoull *et al*., 1999; Balu *et al*., 2004). PAH *ortho*-quinones can also be reduced back to the catechol, either non-enzymatically by the addition of $2H^+ + 2e^-$ from cellular reducing species (e.g. NADPH) or in two sequential one-electron steps catalysed by NADPH:CYP reductases (Flowers-Geary *et al*., 1992, 1995). Once re-formed, the catechol can undergo further auto-oxidation to create a futile redox-cycle in which each round of auto-oxidation forms reactive oxygen species which continues until the reducing agent is exhausted. This leads to oxidative stress and a pro-oxidant state. The PAH *ortho*-quinones and the reactive oxygen species that they generate may form mutagenic lesions in DNA (initiation) or act as electrophilic and pro-oxidant signals that may affect cell growth (see below). In this manner, the pathway may contribute to the complete carcinogenicity of the parent PAH. An evaluation of this mechanism of PAH carcinogenesis requires an understanding of the human enzymes involved and the genotoxic and non-genotoxic properties of PAH *ortho*-quinones.

(i) *Aldo-keto reductase isoforms*

In humans, five AKR isoforms catalyse the oxidation of non-K region *trans*-dihydrodiols to *ortho*-quinones; these are AKR1C1, AKR1C2, AKR1C3, AKR1C4 and AKR1A1 (Burczynski *et al*., 1998, 1999a,b; Palackal *et al*., 2001a,b, 2002a,b). AKR1C1 isoforms are induced by bi- and monofunctional inducers, e.g. 3-methylcholanthrene and *tert*-butylhydroquinone, respectively, which is consistent with regulation via an anti-oxidant-response element (Burczynski *et al*., 1999a,b). These enzymes have a substrate preference for non-K region *trans*-dihydrodiols of bay-region methylated PAHs, oxidize both the *R,R* and *S,S* stereoisomers and are overexpressed in lung adenocarcinoma (A549) cells (Palackal et al., 2002a,b). Two independent studies have validated the elevated

Figure 4.5. Formation of DNA-reactive metabolites of benzo[*a*]pyrene via formation of 7,8-dihydrodiol and (a) diol epoxide or (b) catechol and *ortho*-quinone



Modified from Penning 2004
AKR, aldo-keto reductase; BP, benzo[a]pyrene; CYP, cytochrome P450; DE, diol epoxide; NAD(P)+/NAD(P)H, nicotinamide adenine dinucleotide (phosphate)

expression of AKR1C1 in lung cancer tissue of patients with non-small-cell lung carcinoma where it was an indicator of poor prognosis (Hsu *et al*., 2001; Fukumoto *et al*., 2005). In contrast, AKR1A1 is ubiquitously and endogenously expressed since it is a major metabolic enzyme; it is stereospecific for non-K region *R,R-trans*-dihydrodiols (Palackal *et al*., 2001a,b).

(ii) *Quinone-mediated DNA lesions and mutation*

PAH *ortho*-quinones produced by AKRs can form stable adducts, e.g., benzo[*a*]-pyrene-7,8-dione–N^2-deoxyguanosine (Shou *et al*., 1993; Balu *et al*., 2004), and unstable adducts, e.g., benzo[*a*]pyrene-7,8-dione–*N*7-guanine (McCoull *et al*., 1999). In addition, the reactive oxygen species generated by PAH *ortho*-quinones can attack DNA bases to produce oxidized purines (8-oxo-7,8-dihydro-2′–deoxyguanosine; 8-oxo-dGuo) (Park, J.-H. *et al*., 2005). Further oxidation can take place to yield highly mutagenic spiroiminodihydantoin or guanidinohydantoin adducts (Luo *et al*., 2001; Henderson *et al*., 2003). Reactive oxygen species can also abstract the C4-hydrogen from deoxyribose to form base propenals, which can lead to formation of a malondialdehyde–deoxyguanosine adduct. Alternatively, they can attack polyunsaturated fatty acids to yield lipid hydroperoxides that decompose to 4-hydroperoxy-2-nonenal, 4-hydroxy-2-nonenal and 4-oxo-2-nonenal, each of which can react with bases in DNA to yield potentially mutagenic lesions (Burcham, 1998; Pollack *et al*., 2003). However, most of the lesions derived from reactive oxygen species are generally not detected by the traditional methods used to analyse covalent PAH–DNA adducts.

In the initiation phase of PAH-induced carcinogenesis, critical proto-oncogenes, e.g., K-*ras*, and tumour-suppressor genes, e.g., *p53*, are mutated to undergo a change in function. One of the most prominent mutations observed are G→T transversions which occur at so-called hotspots, e.g. the K-*ras* codons 12 and 61 and the *p53* codons 157, 248 and 273 (Rodenhuis, 1992; Hainaut & Pfeifer, 2001). Two of the DNA lesions produced by PAH *ortho*-quinones provide straightforward routes to these G→T transversions. First, benzo[*a*]pyrene-7,8-dione–*N*7-guanine adducts are unstable and lead to formation of abasic sites which, if unrepaired, result in the incorporation of A opposite the lesion and, upon replication of the daughter strand lead to, a G→T transversion (Sagher & Strauss, 1983). Second, 8-oxo-dGuo, if unrepaired, undergoes a base-pair mismatch with A and, upon replication of the daughter strand, a G→T transversion would again occur (Breen & Murphy, 1995). Under redox-cycling conditions, individual PAH *ortho*-quinones produce substantial amounts of 8-oxo-dGuo (Park, J.-H. *et al*., 2005) and introduce G→T transversions in the *p53* gene, which results in a loss of transcriptional competency (Yu *et al*., 2002a,b). The pattern of G→T transversions is similar to that seen in patients with lung cancer. The formation of these mutations can be prevented by scavengers of reactive oxygen species, which suggests that 8-oxo-dGuo is the responsible lesion (Yu *et al*., 2002a,b). It is noteworthy that 100% of small-cell lung cancers and 50% of non-small-cell lung cancers have lost one allele of the gene *hOOG1*, which encodes the key *N*-glycosylase/AP lyase in the base-excision repair pathway responsible for the removal of

8-oxo-dGuo (Lu *et al*., 1997). Thus deficient base-excision repair of oxidative lesions is a risk factor in human lung cancer.

For a complete and more detailed review of the mechanism involved in the formation of PAH *ortho*-quinones and reactive oxygen species the reader is referred to Penning *et al*. (1999).

(b) Key relevant data

(i) *Benz[*a*]anthracene*

Enzymatic data

Homogeneous rat liver dihydrodiol dehydrogenase (AKR1C9) and AKR1C1–AKR1C4 oxidize both the (–)-3*R*,4*R*- and (+)-3*S*,4*S*-entantiomers of benz[*a*]anthracene-3,4-dihydrodiol to yield benz[*a*]anthracene-3,4-dione (Smithgall *et al*., 1988a; Palackal *et al*., 2002a). AKR1A1 was found to be stereospecific and to oxidize only the relevant (–)-3*R*,4*R*-benz[*a*]anthracene-3,4-diol to yield benz[*a*]anthracene-3,4-dione (Palackal *et al*., 2001a).

Cell-based data

No studies have been performed to show that benz[*a*]anthracene-3,4-dione is a cellular metabolite. However, the formation of DMBA-3,4-dione has been demonstrated by liquid chromatography/mass spectrometry (LC/MS) in cell lysates from human lung A549 adenocarcinoma cells, which constitutively overexpress AKR1C enzymes. In this instance, DMBA-3,4-dione was trapped as both a mono-thioether (1,6-addition product) and a bis-thioether conjugate (Palackal *et al*., 2002a).

Chemical reactivity

Benz[*a*]anthracene-3,4-dione is highly reactive with cellular thiols including GSH, L-cysteine and *N*-acetyl-L-cysteine, with a bi-molecular rate constant for GSH of 1.8×10^4 min^{-1} M^{-1} (Murty & Penning, 1992a). Reactions with deoxyribonucleosides have not been investigated.

Cytotoxicity

Benz[*a*]anthracene-3,4-dione (20 μM) had no effect on cell viability but reduced cell survival (>50%) of H4-II-e rat hepatoma cells following a single treatment for up to 4 h (Flowers-Geary *et al*., 1996).

Mutagenicity (see Table 4.2)

Benz[*a*]anthracene-3,4-dione was not mutagenic in the absence of redox-cycling in the Ames test using *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA104 (Flowers-Geary *et al*., 1996). In the presence of rat liver metabolic activation and an NADPH-generating system, no mutagenic effect was found either, perhaps due to sequestration of the quinone by protein. Benz[*a*]anthracene-3,4-dione was a poor direct-acting mutagen in a yeast reporter-gene assay that detects p53 mutations. In the same assay,

Table 4.2. Genetic and related effects of polycyclic aromatic hydrocarbon *ortho*-quinones

Test system	Result[a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Benz[*a*]anthracene-3,4-dione				
Salmonella typhimurium TA97a, TA98, TA100, TA102	–	–	–[1]	Flowers-Geary *et al.* (1996)
Saccharomyces cerevisiae, *p53* reporter gene assay	–	+[2]	31 nM	Shen *et al.* (2006)
8-oxo-dGuo formation *in vitro*	–	+[3]	160 nM	Park, J.H. *et al.* (2005)
Benzo[*a*]pyrene-7,8-dione				
Salmonella typhimurium TA97a, TA98, TA100	+	–	70 nmol/plate	Flowers-Geary *et al.* (1996)
Salmonella typhimurium TA102, TA104	+	–	35 nmol/plate	Flowers-Geary *et al.* (1996)
Saccharomyces cerevisiae, *p53* reporter gene assay	–	+[2]	125 nM	Shen *et al.* (2006)
8-oxo-dGuo formation *in vitro*	–	+[3]	160 nM	Park, J.H. *et al.* (2005)
DNA single-strand breaks (plasmid)	–	+[4]	10 μM	Flowers *et al.* (1997)
Gene mutation, A549 cells, *p53* gene, *in vitro*	+	NT	10 μM	Penning *et al.* (2004)
5-Methylchrysene-1,2-dione				
Salmonella typhimurium TA97a	+	–	70 nmol/plate	Flowers-Geary *et al.* (1996)
Salmonella typhimurium TA100	+	–	35 nmol/plate	Flowers-Geary *et al.* (1996)

[a] +, positive; –, negative; NT, not tested
[b] the lowest effective dose (LED) is given
[1] None of the tester strains was responsive (dose range, 0–100 μM)
[2] 1 nM NADPH plus 100 μM $CuCl_2$
[3] 180 μM NADPH plus 10 μM $CuCl_2$
[4] 1 mM NADPH plus 10 μM $CuCl_2$

it was highly mutagenic under redox-cycling conditions in the presence of copper chloride and NADPH: single point mutations were observed, and the mutation pattern showed a high preference (>42%) for the formation of G→T transversions (Field *et al*., 2005; Shen *et al*., 2006).

(ii) *Benzo[a]pyrene*

Enzymatic data

Purified dihydrodiol dehydrogenase of rat liver oxidizes both the (–)-7*R*,8*R*- and (+)-7*S*,8*S*-entantiomers of the benzo[*a*]pyrene-7,8-*trans*-dihydrodiol to yield benzo[*a*]pyrene-7,8-dione (Smithgall *et al*., 1986, 1988b). Benzo[*a*]pyrene-7,8-dione was trapped as a thioether conjugate with 2-mercaptoethanol and the product was identified by electron-impact mass spectrometry and nuclear magnetic resonance (NMR) spectrometry (Smithgall *et al*., 1988b). During this oxidation of benzo[*a*]pyrene-7,8-diol, consumption of molecular oxygen was observed and the formation of a superoxide anion radical was quantified wiht the spin-trapping agent, 5,5-dimethyl-1-pyrroline-*N*-oxide, which provided evidence for the generation of reactive oxygen species (Penning *et al*., 1996). Human recombinant AKR1C1–AKR1C4 each oxidized both stereoisomers of (±)-benzo[*a*]pyrene-7,8-diol in the following rank order AKR1C2 >AKR1C1 >AKR1C4 >AKR1C3. Each AKR1C isoform consumed the entire racemic benzo[*a*]pyrene-diol mixture, which indicated that both the minor (+)-*S,S*- and major (–)-*R,R*-stereoisomers formed *in vivo* are substrates for these enzymes. The quinone products of the reactions were trapped as either glycine or thioether conjugates, which were identified by co-elution with authentic synthetic standards (Burczynski *et al*., 1998, 1999a). AKR1A1 was found to be stereospecific and to oxidize only the metabolically relevant (–)-7*R*,8*R*-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to benzo[*a*]pyrene-7,8-dione, which was identified by use of LC/MS (Palackal *et al*., 2001b).

Cell-based data

Formation of benzo[*a*]pyrene-7,8-dione has been observed in rat hepatocytes (Flowers-Geary *et al*., 1995), in MCF-7 human breast carcinoma cells stably transfected with AKR1C9 (Tsuruda *et al*., 2001) and in human bronchoalveolar H-358 cells stably transfected with AKR1A1 (Jiang *et al*., 2005b). Formation of benzo[*a*]pyrene-7,8-dione and reactive oxygen species in rat hepatocytes was blocked by the AKR1C9 inhibitor, indomethacin, which showed that their formation was AKR1C9-dependent (Flowers-Geary *et al*., 1995).

Chemical reactivity

Benzo[*a*]pyrene-7,8-dione is highly reactive with cellular thiols including GSH, L-cysteine and *N*-acetyl-L-cysteine. For GSH, the bi-molecular rate constant is 1.3×10^3 min^{-1} M^{-1} (Murty & Penning, 1992a). The thioether conjugates of benzo[*a*]pyrene-7,8-dione have been characterized by two-dimensional NMR as the 1,4-Michael addition

products (Murty & Penning, 1992b). Reactions with deoxyribonucleosides are described below.

Cytotoxicity

Benzo[*a*]pyrene-7,8-dione (20 μM) reduced cell viability (by up to 40% after 4 h), had no effect on cell survival and depleted reduced GSH in H4-II-e rat and Hep-G2 human hepatoma cells (Flowers-Geary *et al*., 1993, 1996).

Genotoxicity

Benzo[*a*]pyrene-7,8-dione forms stable deoxyguanine and deoxyadenine adducts in reactions with deoxyribonucleosides and in calf-thymus DNA. Reactions of [^{3}H]benzo[*a*]pyrene-7,8-dione with calf thymus DNA or plasmid DNA followed by digestion led to the isolation of a single nucleoside adduct that co-eluted with a standard that was synthesized by reaction of benzo[*a*]pyrene-7,8-dione with oligo-deoxyguanosine. No adducts were observed upon reaction with oligomers of deoxythymidine, deoxycytidine or deoxyadenosine (Shou *et al.*, 1993). Benzo[*a*]pyrene-7,8-dione–deoxyguanosine and –deoxyadenosine adducts were also obtained by reaction with deoxyribonucleosides. Analysis of the benzo[*a*]pyrene-7,8-dione–N^2-deoxyguanosine adducts shows that they exist as either hydrated 1,4-Michael addition products or as cyclized hydrated 1,6-Michael addition products. The structure of the deoxyadenosine adduct provides evidence for a hydrated cyclized 1,4-Michael addition product involving the *N*1 position of adenine. A number of different stereoisomers of each adduct are possible (Balu *et al.*, 2004).

Upon incubation with deoxyguanosine under acidic conditions, benzo[*a*]pyrene-7,8-dione also forms the unstable adduct benzo[*a*]pyrene-7,8-dione-*N*7–guanine, which has been characterized by LC/MS (McCoull *et al.*, 1999). Under redox-cycling conditions in the presence of copper chloride and NADPH, nanomolar concentrations of benzo[*a*]-pyrene-7,8-dione can produce significant amounts of 8-oxo-dGuo (>60 adducts/10^5dGuo, a 30-fold increase over background) as measured by HPLC with electrochemical detection (ECD). In this case, the oxidant is singlet oxygen (Park, J.-H. *et al*., 2005). Under the same redox-cycling conditions, low micromolar concentrations of benzo[*a*]-pyrene-7,8-dione caused strand scission of plasmid-DNA and oligonucleotides; the damaging species was the hydroxyl radical. The corresponding *ortho*-semiquinone did not cause strand scission (Flowers *et al.*, 1997).

Mutagenicity (see Table 4.2)

Benzo[*a*]pyrene-7,8-dione was found to be a direct-acting mutagen in *S. typhimurium* strains TA97a, TA98, TA100, TA102 and TA104 at concentrations of 35 and 70 nmol/plate, and was more mutagenic than the positive control used for each tester strain. The majority of the mutations observed were frameshifts. No increase in mutation efficiency was noted in the presence of a metabolic activation system containing rat liver microsomes and an NADPH-generating system when tester strains sensitive to oxidative mutagens (TA102 and TA194) were used (Flowers-Geary *et al*., 1996).

Benzo[*a*]pyrene-7,8-dione was found to be a poor direct-acting mutagen in a yeast reporter-gene assay that can detect *p53* mutations. In the same assay, benzo[*a*]pyrene-7,8-dione was found to be highly mutagenic at concentrations of 125 nM under redox-cycling conditions (copper chloride and NADPH). In contrast, (±)-*anti*-benzo[*a*]pyrene diol epoxide was only mutagenic at concentrations greater than 10 μM. Benzo[*a*]pyrene-7,8-dione produced single point mutations in *p53* and the mutation pattern showed a high preference for G→T transversions, which occurred preferentially at the same hotspots that were mutated in patients with lung cancer. The mutations were not found when the assay was conducted in the presence of scavengers of reactive oxygen species and the enzymes superoxide dismutase and catalase, which suggested that the superoxide anion and the hydroxyl radical were the responsible mutagens (Yu *et al*., 2002b). In human lung adenocarcinoma A549 cells, benzo[*a*]pyrene-7,8-dione was found to mutate *p53*, which led to predominately unstable A→G (T→C) transitions following acute treatment (Penning *et. al*., 2004).

(iii) *5-Methylchrysene*

Enzymatic data

5-Methylchrysene can be metabolically activated in mouse skin to yield 1,2-*trans*-dihydroxy-1,2-dihydro-5-methylchrysene and 7,8-*trans*-dihydroxy-7,8-dihydro-5-methylchrysene and their respective 3,4- and 9,10-epoxides (Melikian *et al*., 1982; Hecht *et. al*., 1985). The former is the more relevant metabolite. With regard to stereospecificity, it is probable that rat liver dihydrodiol dehydrogenase oxidizes (+)-*1S,2S*-dihydroxy-1,2-dihydro-5-methylchrysene and (–)-*7R,8R-trans*-dihydroxy-7,8-dihydro-5-methylchrysene to yield the presumptive quinone (Smithgall *et al*., 1986). Each of the human AKR1C enzymes (AKR1C1–AKR1C4) oxidizes the racemic 5-methylchrysene-7,8-*trans*-dihydrodiol but the stereochemistry of the reaction has not been elucidated. The reaction preference was AKR1C4 >AKR1C2 >AKR1C1 >AKR1C3 (Burczynski *et al*., 1999b; Palackal *et al*., 2002a,b). Human AKR1A1 also oxidizes racemic 5-methylchrysene-7,8-*trans*-dihydrodiol, which is the *trans*-dihydrodiol substrate with the highest V_{max}/K_m utilization ratio identified for this enzyme to date (Palackal *et al*., 2001a,b).

Cytoxicity

5-Methylchrysene-7,8-dione significantly reduced the survival of H4-II-e cells (Flowers-Geary *et al*., 1996).

Mutagenicity (see Table 4.2)

5-Methylchrysene-7,8-dione was found to be a direct-acting mutagen in *S. typhimurium* TA97a and TA100 at concentrations of 70 and 35 nmol/plate, respectively, and was more mutagenic than the positive control used for each tester strain (Flowers-Geary *et. al*., 1996). The mutagenicity of this compound has not been examined in other assays.

Data gaps

There are gaps in our knowledge concerning the possible role of the PAH *ortho*-quinone pathway and reactive oxygen species in carcinogenesis: (i) not all the relevant PAHs have been examined; (ii) human AKRs other than AKR1A1 and AKR1C1–AKR1C4 may be involved in the metabolic activation of PAH *trans*-dihydrodiols, e.g., AKR1B10, which is highly elevated in non-small-cell lung carcinomas (Fukumoto *et al*., 2005); (iii) there is little information to address the competing roles of CYP- versus AKR-mediated activation of PAHs; (iv) covalent DNA adducts (stable or unstable) or oxidative lesions that can be assigned to the AKR pathway have yet to be detected in cells or animals; the successful detection of these adducts requires the development of appropriate analytical chemical methods with sufficient sensitivity; (v) the mutagenicity of PAH *ortho*-quinones in mammalian cells has not been completely addressed; (vi) the transforming potential of the PAH *ortho*-quinones has not been measured; and (vii) the tumorigenicity of PAH *ortho*-quinones as initiators, promoters or both has not been examined systematically.

4.2.4 *Mechanism via meso-region biomethylation and benzylic oxidation*

The role of mechanisms that involve meso-region biomethylation and benzylic oxidation in the carcinogenesis of PAHs is based on the methylation of unsubstituted PAHs and the subsequent metabolic activation of the methyl group to electrophilic moieties. The meso-region of PAHs (also known as the L-region) has been purported to be a region of high reactivity either in an aromatic nucleus or on a side chain (Flesher *et al.*, 2002, 2004). According to this theory, the chemical and biochemical activation pathways of both unsubstituted and meso-substituted PAHs are essentially the same, since unsubstituted PAHs are converted to generally more carcinogenic meso-methyl-substituted PAHs in the metabolic activation process. For example, DMBA is more carcinogenic than 7-methylbenzo[*a*]anthracene which is more carcinogenic than benzo[*a*]anthracene (see review by Dipple *et al.*, 1984). In a series of three biochemical transformation reactions, the first is the aralkylation (methylation) of unsubstituted PAHs at a meso-centre of high reactivity (Myers & Flesher, 1991). This conversion is mediated by the methyl donor, *S*-adenosyl methionine (Flesher *et al.*, 1986). The second step is the hydroxylation of a meso-region methyl group by CYP isozymes (Sims, 1970); more recently, a chemical one-electron oxidation process has also been proposed at this stage (Flesher *et al.*, 2004; Lehner *et al.*, 2004). The third step is the formation of a reactive ester (e.g. sulfuric acid ester) via 3′-phosphoadenosine-5′-phosphosulfate (Chou *et al.*, 1998). Sulfooxymethyl esters generate a highly reactive benzylic carbonium ion that may form DNA adducts (Lehner *et al.*, 2004; Ravi Kumar *et al.*, 2005), and some have been found to be mutagenic (Watabe *et al.*, 1986) and carcinogenic (Surh *et al.*, 1991; Flesher *et al.*, 1997a,b).

*(a) Benzo[*a*]pyrene*

(i) *Biomethylation to 6-methylbenzo[a]pyrene*

Benzo[*a*]pyrene was converted to 6-methylbenzo[*a*]pyrene using rat liver microsomal preparations (Flesher *et al.*, 1990). *S*-Adenosylmethionine was identified as a carbon donor in this biotransformation (Flesher *et al.*, 1982).

(ii) *DNA adducts of benzo[*a*]pyrene-related methylated intermediates*

Weanling female Sprague-Dawley rats injected subcutaneously with benzo[*a*]pyrene produced two groups of adduct profile: one that resulted from alkyl substitution and the other from ring oxidation. One major and two minor aralkyl–DNA adducts were detected in subcutaneous tissues. The total levels of diol epoxide adducts were 15–50 times higher than those of aralkyl adducts. Rats injected with 6-methylbenzo[*a*]pyrene, 6-hydroxymethylbenzo[*a*]pyrene and 6-acetoxymethylbenzo[*a*]pyrene produced the same aralkyl–DNA adducts as those obtained from benzo[*a*]pyrene. The predominant in-vivo aralkyl–DNA adduct was a deoxyguanosine adduct while the second major adduct was a deoxyadenosine adduct (Stansbury *et al.*, 1994). 6-Hydroxymethylbenzo[*a*]pyrene is converted to 6-sulfooxymethylbenzo[*a*]pyrene by 3′-phosphoadenosine-5′-phosphosulfate sulfotransferase *in vitro* (Surh *et al.*, 1990).

(iii) *Genotoxicity of 6-methylbenzo[*a*]pyrene and related metabolites*

6-Methylbenzo[*a*]pyrene and 6-sulfooxymethylbenzo[*a*]pyrene were mutagenic to *S. typhimurium* TA100 (Santella *et al.*, 1982; Rogan *et al.*, 1986) and formed DNA adducts in the liver of infant mice (Surh *et al.*, 1990).

(iv) *Carcinogenicity of 6-methylbenzo[*a*]pyrene, 6-hydroxymethylbenzo[a]pyrene and 6-sulfooxymethylbenzo[a]pyrene*

The carcinogenic activities of 6-substituted benzo[*a*]pyrenes have been reviewed to some extent (Dipple *et al.*, 1984; Harvey, 1991). 6-Methylbenzo[*a*]pyrene and 6-hydroxymethylbenzo[*a*]pyrene were weak initiators of skintumours in CD1 mice, with five and eight fold lower activity, respectively, than that of benzo[*a*]pyrene (Slaga *et al.*, 1978c) and similar results were found for 6-methylbenzo[*a*]pyrene in SENCAR mice (Iyer *et al.*, 1980). Based on studies that involved inhibition of CYP, it was concluded that methylation and hydroxymethylation of benzo[*a*]pyrene were not important pathways in the initiation of mouse skin tumours (Slaga *et al.*, 1978c). However, 6-methyl- and 6-hydroxymethylbenzo[*a*]pyrene induced malignant skin carcinomas after repeated application on mouse skin; 6-methylbenzo[*a*]pyrene was the more active. The sodium salt of 6-sulfooxymethylbenzo[*a*]pyrene was also tumorigenic on mouse skin (Cavalieri *et al.*, 1978). 6-Methyl-, 6-hydroxymethyl- and 6-sulfooxymethylbenzo[*a*]pyrene induced sarcomas at the injection site when given subcutaneously to rats (Flesher *et al.*, 1997a),

and 6-sulfooxymethylbenzo[*a*]pyrene was also tumorigenic in the liver of infant mice (Surh *et al.*, 1990).

In conclusion, there is no direct evidence that this mechanism contributes to the tumorigenic activity of benzo[*a*]pyrene in mouse skin.

*(b) Benzo[*a*]anthracene*

(i) *Biomethylation to methylbenz[*a*]anthracenes*

Qualitative studies of the metabolism of benz[*a*]anthracene *in vitro* with rat liver cytosol preparations fortified with *S*-adenosyl-L-methionine showed the formation of 7-methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene, which were further methylated to DMBA (Flesher *et al.*, 1984). Subcutaneous injection of benz[*a*]anthracene into rats confirmed the formation of 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene and DMBA as well as that of hydroxymethylated benz[*a*]anthracene metabolites. No bioalkylation was detected after injection of six non-carcinogenic PAHs. (Flesher & Myers, 1990).

(ii) *Benzylic oxidation and formation of reactive intermediates*

In in-vitro studies with rat liver cytosol, 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene and DMBA were metabolized to their hydroxymethyl metabolites. No oxidation was detected at the ring positions (Flesher & Myers, 1985). *In vivo*, DMBA was metabolized in rat subcutaneous tissues to the corresponding hydroxyalkyl metabolites, 7 hydroxymethylbenz[*a*]anthracene, 7-hydroxymethyl-12-methylbenz[*a*]anthracene and 7,12-dihydroxymethylbenz[*a*]anthracene (Myers & Flesher, 1989). These hydroxymethylated benz[*a*]anthracenes were further conjugated to sulfate to form reactive and mutagenic sulfooxymethylated benz[*a*]anthracene species (Watabe *et al.*, 1985, 1986). Subcutaneous injection of either 7-hydroxymethyl-12-methylbenz[*a*]anthracene or 7-sulfooxymethyl-12-methylbenz[*a*]anthracene into rats produced adduct patterns in DNA of subcutaneous tissue that were similar to those observed after injection of DMBA, with a 2:1 ratio of DMBA diol epoxide–DNA adducts to benzylic adducts (Ravi Kumar *et al.*, 2005).

(iii) *Carcinogenicity of methylated benz[*a*]anthracene and its metabolites*

There is extensive literature on the carcinogenic activities of methylated benz[*a*]anthracenes. DMBA is carcinogenic and induces lung, skin and liver tumours in mice and tumours of the subcutaneous tissue and mammary gland in rats. 7-Methylbenz[*a*]anthracene and 12-methylbenz[*a*]anthracene are also carcinogenic in mice and rats (see Dipple *et al.*, 1984). 7-Hydroxymethyl-12-methylbenz[*a*]anthracene and 7-sulfooxymethyl-12-methylbenz[*a*]anthracene were tumorigenic when injected subcutaneously into rats (Flesher *et al.*, 1997b). However, it was considered improbable that DMBA would act via metabolic activation to 7-sulfooxymethyl-12-methylbenz[*a*]anthracene to induce hepatomas in male B6C3F_1 mice and lung adenomas in A/J mice,

to initiate mouse skin tumours, to induce injection-site sarcomas in rats or to initiate preneoplastic enzyme-altered foci in rat liver. In all these systems, the sulfate ester was not more carcinogenic than DMBA itself (Surh *et al.*, 1991).

In conclusion, in subcutaneous tissues of rats, benz[*a*]anthracene was biomethylated to DMBA which was metabolized to a series of hydroxymethylated and sulfooxymethylated methylbenz[*a*]anthracenes, some of which formed benzylic DNA adducts. However, for DMBA, there is disagreement in the literature on the role of this biomethylation mechanism in the formation of subcutaneous tumours in rats, and there is no evidence that this mechanism explains the tumorigenic activities of benzo[*a*]anthracene in mouse skin and lung.

*(c) Dibenz[*a,h*]anthracene*

Qualitatively, rat liver cytosol fortified with *S*-adenosyl-L-methionine transformed dibenz[*a*,*h*]anthracene to methylated and hydroxymethylated metabolites (Flesher *et al.*, 1986). No quantitative results were available. Both 7-methyl- and 7,14-dimethyldibenz[*a*,*h*]anthracene were carcinogenic (see review by Dipple *et al.*, 1984), while the biological activities of hydroxymethylated dibenz[*a*,*h*]anthracenes are unknown. There is no evidence of genotoxicity of these intermediates or of DNA adducts of methylated dibenz[*a*,*h*]anthracene in rodent or human tissues.

There are no adequate data to support a role for the biomethylation mechanism in the carcinogenicity of dibenz[*a*,*h*]anthracene.

(d) 5-Methylchrysene

5-Methylchrysene was converted *in vitro* by induced rat liver microsomal preparations and *in vivo* in rat dorsal subcutaneous tissues to the metabolites 5-hydroxymethylchrysene and 4,5-methylenechrysene (Myers & Flesher, 1991). 5-Hydroxymethylchrysene was a metabolite of 5-methylchrysene after incubation with induced rat liver and human liver microsomes. Further metabolism of 5-hydroxymethylchrysene by Aroclor 1254-induced rat-liver preparations produced the 1,2-diol and the 7,8-diol (Amin *et al.*, 1981). 5-Hydroxymethylchrysene, after activation to its sulfate conjugate, formed DNA adducts in calf thymus DNA (Okuda *et al*., 1986). 4,5-Methylenechrysene, a cyclization product of 5-methylchrysine, caused skin tumours in mice (Rice *et al.*, 1988).

5-Methylchrysene administered to strain A/J mice induced six different DNA adducts in the lung, none of which co-migrated with the adduct formed by sulfotransferase-mediated activation of 5-hydroxymethylchrysene (Ross *et al*., 1995).

(i) *Carcinogenicity study of 5-hydroxymethylchrysene*

Groups of Charles River CD-1(ICR)BR mice received 10 daily dermal applications of 5-hydroxymethylchrysene (total dose, 116 or 39 nmol) in acetone followed 10 days later by 4 nmol TPA in acetone three times a week for 20 weeks. At 21 weeks, the incidence of skin tumours (tumours/mouse) in the two dose groups was 90%

(9.5 tumours/mouse) and 45% (2.6), respectively. Skin tumours developed in 5% (0.1 tumours/mouse) of the mice treated with TPA alone (Amin *et al.*, 1981).

(ii) *Genotoxicity of 5-hydroxymethylchrysene*

Rat-liver hydroxysteroid sulfotransferase converted 5-hydroxymethylchrysene to its sulfate ester, 5-sulfooxymethylchrysene (Ogura *et al.*, 1990), which bound *in vitro* to purine bases of calf thymus DNA to form N^6-[(chrysen-5-yl)methyl]adenosine and N^2-[(chrysen-5-yl)methyl]guanosine (Okuda *et al.*, 1989). This 5-hydroxymethylchrysene sulfate is a strong bacterial mutagen in the presence of a 3′-phosphoadenosine 5′-phosphosulfate-generating system (Okuda *et al*, 1986)

In conclusion, 5-methylchrysene was metabolized in mouse skin to 5-hydroxymethylchrysene which acted as a skin carcinogen. *In vitro*, 5-hydroxymethylchrysene was conjugated to sulfate and this conjugate formed DNA adducts. However, 5-hydroxymethylchrysene–DNA adducts have not been detected after treatment of mouse skin with 5-methylchrysene.

4.2.5 *Receptor-mediated mechanism*

Several of the biological effects of PAHs, such as induction of xenobiotic metabolizing enzymes, immunosuppression, teratogenicity and carcinogenicity, are thought to be mediated by activating AhR signalling. This receptor is widely distributed and has been detected in most cells and tissues. There is also evidence that AhR signals act through a variety of pathways, and more recently, cross-talk with other nuclear receptors has been demonstrated to enable cell type- and tissue-specific control of gene expression. In addition, high-affinity ligands for AhR such as benzo[*a*]pyrene and 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) differ in their biological responses. Furthermore, translocation of activated AhR may require threshold concentrations of the ligand and involves a variety of cellular responses. AhR induces phase I and II metabolism; additional responses include lipid peroxidation and the production of arachidonic acid-reactive metabolites, decreased levels of serum thyroxine and vitamin A, persistent activation of thyroid hormone receptor and cross-talk with steroid hormone receptors. Responses to altered AhR signalling may therefore be designated as adaptive or toxic and/or as perturbations of endogenous pathways. Some basic information on AhR-mediated mechanisms in relation to biochemical and toxicological effects is discussed below.

(a) Basic principles of AhR

AhR is a ligand-activated transcription factor that mediates responses to a variety of toxins, including halogenated aromatic toxins such as TCDD, PAHs and combustion products and numerous phytochemicals such as flavonids and indole-3-carbinol (Nebert *et al.*, 2004 ; Chang *et al.*, 2005). This receptor plays an essential role in the regulation of the metabolism of xenobiotics (phase I/phase II enzymes; also termed AhR signalling in

the adaptive response pathway) and the initiation of homeostatic responses (also termed AhR signalling in endogenous pathways) upon exposure to xenobiotics.

Despite many attempts to identify AhR endogenous ligands, the nature of these ligands remains enigmatic. However, Adachi *et al.* (2001) identified the tryptophan analogues, indirubin and indigo in human urine, and these activate AhR.

The greatest source of AhR ligands is food. AhR ligands may activate signalling pathways in a manner similar to that of growth factors, hormones, neurotransmitters and extracellular signals and can therefore act like classical mitogenic factors. Little information is available on receptor binding and AhR activity of individual PAHs. In the unligated state, AhR is complexed with chaperone proteins, i.e. the heat-shock protein 90 dimer. Upon ligand binding, the chaperones dissociate, which enables AhR to translocate into the nucleus (Schmidt & Bradfield, 1996). Therefore, AhR is regulated primarily through receptor occupancy and through de-novo synthesis of the AhR repressor (AhRR), a protein which was first identified in mice (Mimura *et al.*, 1999). AhRR is closely related to AhR in the primary sequence of the basic helix-loop-helix (bHLH) and Period (Per)-AhR nuclear translocator (Arnt)-Single-minded protein (Sim) (PAS)-A domain (N-terminal half of the PAS domain) but is highly divergent from AhR in the PAS-B domain (ligand binding domain). AhRR proteins typically do not bind AhR ligands and inhibit AhR signalling by competing for binding to xenobiotic or dioxin response element (XRE/DRE) recognition sites usually occupied by AhR-Arnt (Mimura *et al.*, 1999).

In addition, PKC is of critical importance for AhR activity. Inhibiton of PKC by staurosporine prevented ligand-induced DNA binding to XRE/DRE recognition sites of targeted genes (Delescluse *et al.*, 2000). Furthermore, phosphorylation of AhR and Arnt threonine residues has been reported, and threonine kinases probably modify the activity of AhR (Puga *et al.*, 2002). Notably, Vaziri *et al.* (1996) demonstrated the induction of AhR expression by the tyrosine kinase v-src (a viral oncoprotein) and by the platelet-derived growth factor receptor basic fibroblast growth factor.

Early observations suggest that the role of AhR was confined to adaptive responses to exposure to PAHs that mediated toxicity upon activation by halogenated dioxins, polychlorinated biphenyls (PCBs) and other halogenated aromatic hydrocarbons. More recently, however, AhR was shown to play a wider role in cell-cycle control (Elferink, 2003) and seems to act as an environmental check-point that senses exposure to environmental toxicants. AhR may inhibit the cell cycle by displacing p300 from early region 2 binding factor-dependent promoters and repressing S phase-specific gene expression (Marlowe *et al.*, 2004). Furthermore, AhR interacts with the retinoblastoma suppressor protein (pRb) by the control of $p27^{kip1}$ expression which in turn inhibits cell-cycle progression by the repression of CDK2 activity which prevents pRb hyperphosphorylation. This enables cell-cycle arrest in the G1 phase (Marlowe et *al.*, 2004).

(b) Control of gene expression by AhR

The nuclear AhR complex consists of a heterodimer of AhR and Arnt. Unligated AhR is almost exclusively located in the cytoplasm whereas Arnt is found in the nucleus. AhR can still translocate to the nucleus in the absence of Arnt as shown in Arnt-deficient mice. Both AhR and Arnt are member of the bHLH-PAS family of transcription factor proteins which have essential roles in development (differentiation, neurogenesis, myogeneisis, B-cell differentiation, sex determination) and in various signalling pathways. The PAS domain is required for binding to DNA and the bHLH motif is localized in the N-terminal region where it codes for two α helices that are separated by a non-helical loop. AhR and Arnt proteins were shown to contact the DRE or XRE sequence of regulated genes. The thymine position within a half-site of an E-box element (5′-GTG-3′) is specifically contacted by Arnt whereas AhR contacts a thymine outside of the E-box element (Bacsi *et al.*, 1995). It is of considerable importance that E-box elements are recognized by many bHLH transcription factors. Thereafter, binding to the DRE or XRE leads to activation of a large number of genes, including cell-cycle genes, and xenobiotic metabolizing enzymes. Furthermore, in-depth studies over the past years have revealed many details of the mechanism of action of the AhR transcription factor and the activation of promotors of targeted genes.

Transcriptional activation of targeted genes in response to AhR are not only species- and tissue-specific, but are also ligand-specific. This is of importance since AhR activation by certain PAHs did not produce the same spectrum of toxic effects as those observed with TCDD. AhR-mediated toxic responses may therefore be ligand-dependent and differences in biological responses may also be linked to the resistance of halogenated hydrocarbons to metabolism and may thereby increase AhR occupancy. Furthermore, there is evidence that the ligand-dependent recruitment of additional transcription factors upon activation of AhR results in combinatorial interactions of networked transcription factors with consequent alterations in signalling that perturb several metabolic pathways. Recently, a genetic algorithm was developed to study promoters of AhR-regulated genes. This method enabled the identification of transcription factor-binding sites of AhR-regulated genes (Kel *et al.*, 2004). Promoters and long-distance regulatory regions of AhR-responsive genes were analysed by the genetic algorithm and this computational approach was applied to currently unknown AhR-regulated genes. In essence, the method was able to predict in silico novel gene candidates, which were confirmed experimentally using cultures of human and rat hepatocytes. Mathematical models are therefore promising for the prediction of novel gene targets of AhR by interrogating promoter and regulatory sequences for further consideration in risk assessment. This includes, among others, the identification of tumour-suppressor and proto-oncogenes regulated by activated AhR.

(c) Genetic models to investigate AhR signalling

Genetic studies and the application of gene targeting technologies provided valuable insight into AhR signalling pathways. Studies with AhR knockout mice provided clear evidence for the need of a functional receptor to obtain a xenobiotic response after exposure to halogenated aromatic hydrocarbons, PAHs and related chemicals (Fernandez-Salguero *et al.*, 1995). Specifically, AhR is required to induce toxicity or carcinogenicity after exposure to TCDD or benzo[*a*]pyrene, respectively (Fernandez-Salguero *et al.*, 1995; Mimura *et al.*, 1997; Peters *et al.*, 1999; Shimizu *et al.*, 2000). Furthermore, Andersson *et al.* (2002) showed that a constitutively active AhR reduced the lifespan of transgenic mice and induced tumours in the stomach. This further demonstrates the oncogenic potential of AhR.

The various AhR knockout strains, however, differ in phenotypes as a result of targeting mutations either in the first exon by deletion of the first methionine and a portion of the basic region (Fernandez-Salguero *et al.*, 1995) or by deletion of exon 2 which encodes bHLH–DNA binding and the dimerization domain (Schmidt *et al.*, 1996). Targeting exon 2 resulted in a frameshift with no synthesis of AhR protein. However, deletion of exon 1 resulted in 50% neonatal mortality and the inflammation of several major organs. Furthermore, surviving mice had decreased liver weights and portal fibrosis similar to that seen in mice with the exon 2 deleted, although the phenotypes appeared to be more severe upon targeting of exon 1. In addition, reduced liver size in AhR-null mice was associated with the incidence of apoptosis by a currently unknown mechanism (Zaher *et al.*, 1998).

(d) Cross-talk of AhR with steroid and other nuclear receptors

AhR plays a much broader role than that initially assumed and there is evidence for inhibitory and additive cross-talk of AhR with other nuclear receptors (Pocar *et al.*, 2005).

TCDD induces hepatocellular carcinogenesis in female but not male Sprague-Dawley rats and the tumorigenic response in females has been reported to be estrogen-dependent (Safe, 2001). Cross-talk of AhR with the steroid receptor has been studied in human breast cancer and endometrial carcinoma cell lines. Inhibitory XRE/DRE were identified in the promotors of the estrogen-inducible *pS2* breast cancer coding genes, in cathepsin D and c-*fos* genes (Safe *et al.*, 2000). AhR–ARNT complexes competitively inhibited the binding of estrogen receptor (ER)-α to imperfect ER element sites. In T47D human breast cancer cells, a rapid proteasome-mediated degradation of AhR and ERα by activated AhR was observed, but TCDD elicited stronger effects than benzo[*a*]pyrene or 6-methyl-1,3,8-trichlorodibenzofuran (Wormke *et al.*, 2000). In MCF-7 cells, the direct interaction of activated AhR with the hypophosphorylated tumour suppressor *RB* was independent of Arnt and did not require an intact AhR–ARNT complex to contact XRE/DRE in order to mediate transcriptional activity (Carlson & Perdew, 2002). In contrast, a strong association of ARNT splice variants with ER-negative breast cancer and poor prognosis was reported. A truncated ARNT may affect AhR signalling and receptor cross-talk (Qin

et al., 2001). AhR–ER cross-talk affects multiple estrogen-dependent pathways with the induction of CYP monooxygenases by activated AhR that results in rapid metabolism and depletion of 17β-estradiol *in vitro*; however, this was not seen *in vivo* (Safe, 2001).

Comparisons of AhR-sensitive and AhR knockout mice revealed thymic athrophy in AhR-sensitive strains as a result of T-cell apopotosis (Fernandez-Salguero *et al.*, 1995; Kamath *et al.*, 1997). Several AhR ligands, including DMBA, benzo[*a*]pyrene and TCDD, have been reported to induce apoptosis in various cell types of non-reproductive tissues (Miller *et al.*, 1996; Lei *et al.*, 1998; Jyonouchi *et al.*, 1999). In human folicular granulosa cells, the AhR ligand TCDD also induced apoptosis in a dose- and time-dependent manner by disturbing steroid metabolism (Heimler *et al.*, 1998). Matikainen *et al.* (2001) studied AhR-dependent activation of the pro-apoptotic *bax* encoding gene. In murine oocytes, DMBA but not TCDD induced bax protein expression and subsequent apopotosis. When, however, guanine or cytosine was exchanged for adenine three bases downstream of the core XRE/DRE recognition site, TCDD also induced the bax protein. Therefore, ligand-dependent and single base-pair discrimination of flanking AhR recognition sites in promotors of targeted genes may be an important mechanism to rationalize selective responses in the control of gene expression. Furthermore, in-utero exposure to the AhR ligand TCDD induced cleft clitoris and vaginal threads of mesenchymal tissue in female rat offspring, which suggested an imbalance of proliferation and apoptosis in the development of female sexual organs. AhR may act as a novel regulator of ovulation, and the ovulatory gonadotropin surge has been shown to induce expression of AhR-regulated genes (Chaffin *et al.*, 1999). In summary, AhR appears to play a prominent role in female reproduction, and complex interactions occur between AhR–Arnt and sex steroid receptors.

Furthermore, pleiotropic responses to AhR signalling are probably tissue- and may be ligand-dependent and induce transcriptional activation of genes, the expression of which is normally restricted or even prevented. Selective and de-novo activation of additional genes, as well as repression of constitutively expressed genes, impacts cellular phenotype. There is clear evidence for tissue-specific regulation of certain genes with the induction for instance of transforming growth factor and plasminogen activator inhibitor-2 in human keratinocytes but not in rat hepatocytes (Vanden Heuvel *et al.*, 1994). Cell type-specific responses may also depend on the ability to recruit co-activator proteins selectively. Taken collectively, this may provide a molecular mechanism for tissue specificity and sensitivity to responses to AhR activation.

(e) Ligand-independent AhR regulation

Other regulatory mechanisms of AhR signalling may be active during inflammation when exposure to cytokines is increased. Indeed, various interleukins (iL) and interferons can provoke the expression of NF-κB, signal transducers and activators of transcription as well as CCAAT enhancer-binding proteins. Networking of AhR with these additional transcription factors may alter its signalling pathways. For instance, AhR and NF-κB interact physically and modulate each other transcriptionally (Tian *et al.*, 1999, 2002).

Divergent signalling of AhR may also be operable through activation by cyclic adenosine monophosphate in the absence of an AhR ligand (Oesch-Bartlomowicz *et al.*, 2005). Regulation of xenobiotic metabolizing enzymes by nuclear receptors other than AhR has been studied extensively. The pregnane X receptor, the retinoid x-receptor and the constitutive androstane receptor have been identified and play pivotal roles in induction of *CYP* monooxygenase gene families 2 and 3 (Xu *et al.*, 2005). Other nuclear receptors involved in the regulation of xenobiotic metabolizing CYP1 forms include the glucocorticoid receptor and ER, both of which potentiate the induction of CYP1 (Honkakoski & Negishi, 2000).

The orphan nuclear receptor hepatic nuclear factor 4 is of critical importance in regulating the expression of CYP monooxygenase families 2 and 3. Cross-talk of AhR with these nuclear receptors remains uncertain and the importance of genetic polymorphism in xenobiotic metabolizing enzymes has been adressed elsewhere (Section 4.1).

(f) Regulation of xenobiotic metabolizing enzymes by antioxidant/electrophile response elements

An electrophile response element has been identified in the promotors of some AhR-regulated genes and provides an AhR-independent means to control gene expression. Examples include CYP1A1 and other members of the AhR gene battery. Specifically, for phase II metabolizing enzymes, a number of inducers have been identified such as butylated hydroxyanisole, *tert*-butylhydroquinone, green tea phenol, (–)-epicatechin-3-gallate and the isothiocyanates. Upon metabolic activation, electrophiles are produced and cause cellular stress. This, in turn, activates MAPK pathways and results in the activation of the basic leucine zipper transcription factor, Nrf2, which dimerizes with the basic zipper transcription factors, Mafs. The heterodimeric complex then binds to an antioxidant/electrophile response element which has been identified in many xenobiotic metabolizing (phase I/II) and other cellular defence enzymes, of which thioredoxins and haemeoxygenase-1 are examples (Kong *et al.*, 2001). Therefore, cellular stress can regulate xenobiotic metabolizing enzymes with the aim of removing and detoxifying harmful intermediates such as reactive oxygen species.

(g) Genetic variability in AhR

Genetic variability in the coding sequences of *AhR* accounts for marked strain and species differences in sensitivity to AhR ligands when responsiveness to AhR ligands depends on different alleles. To date, relatively few polymorphisms have been reported in humans and, in the case of the murine *AhR* gene, approximately 2200 mutations have been identified in 13 inbred strains (Thomas *et al.*, 2002). Although variation in the human *AhR* gene exists, the relevance of this to risk for cancer remains uncertain. Indeed, a >12-fold variation in CYP1A1 activity in 3-methylcholanthrene-treated lymphocytes from 47 unrelated individuals has been reported, but none of the *AhR* gene

polymorphisms could explain the observed variability (Harper *et al.*, 2002). In contrast, AhR-induced expression of CYP1 enzymes impacts the metabolism of PAHs and results in genotoxicity, mutations and tumour initiation (Nebert *et al.*, 2000). Individual risk for cancer may be attributed to metabolic activation of PAHs but the balance between detoxification and metabolic potentiation depends on many factors (Nebert *et al.*, 2004) and loosely or tightly coupled phase I and II metabolic reactions may be influential factors for risk of toxicity and cancer (see Sections 4.1.2 (b) and 4.3 on genetic variability in PAH-metabolizing enzymes).

4.2.6 *Immunological and haematological mechanisms*

(a) Introduction

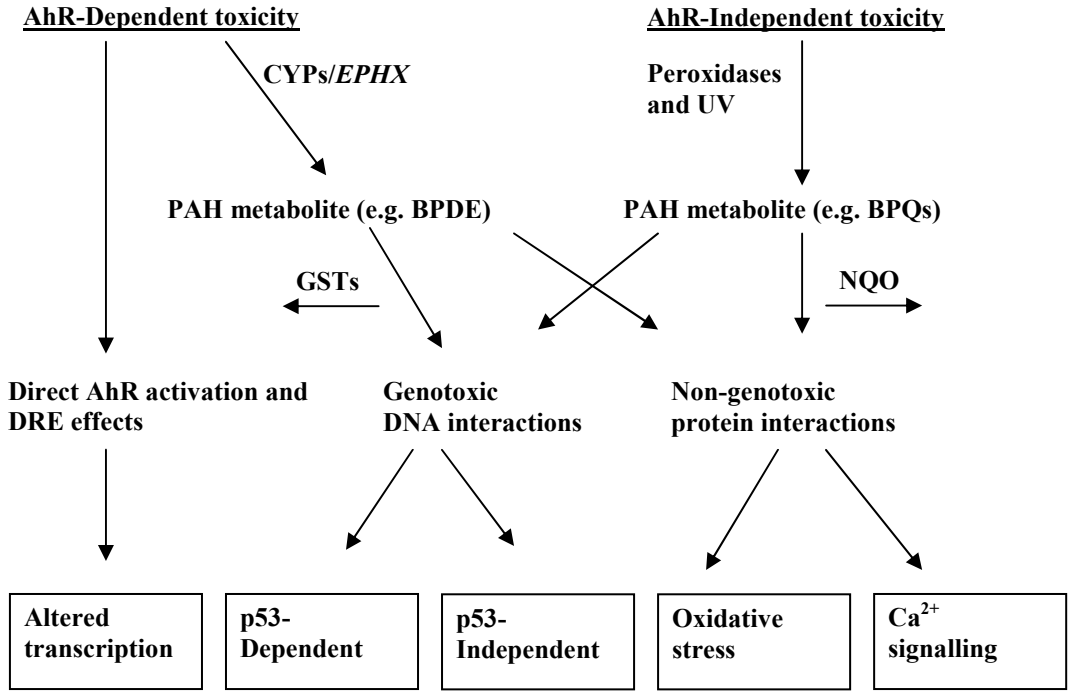
Innate and adaptive immune responses appear to play important roles in the protection of mammalian host organisms from cancer (Dupont, 2002). Therefore, a decrease in immune surveillance by agents such as PAHs might have negative consequences for cancer in humans. A significant number of studies have demonstrated that PAHs are immunosuppressive in animal models and following in-vitro exposure of human leukocytes. In animals, the concentrations of PAHs that are required to produce immunosuppression are generally quite high compared with those required to produce cancer. There are limited human epidemiological data to show that PAHs are immunosuppressive following environmental exposures. There are also extremely limited or no immunotoxicity data on many of the PAHs or complex mixtures that contain PAHs, with the exception of benzo[*a*]pyrene, on the immune system.

The biological and toxicological actions of PAHs on the immune and haematopoietic systems represent a complicated interplay between the ability of a specific PAH to bind to endogenous AhR and induce CYPs in central and peripheral organs which results in the formation of oxidative and electrophilic metabolites and the removal of reactive molecules via secondary metabolic processes. Therefore, as with many tissues, the toxicity of PAHs to the immune system is dependent upon the exposure of cells and tissues to circulating parent compounds and metabolites, their expression of AhR and their propensity to form bioactive versus detoxified metabolites. The dose and route of exposure to PAHs are important determinants of immunotoxicity in animals and humans. In general, the total cumulative dose of exposure to PAHs appears to correlate with immunotoxicity in mice. It should be noted that PAHs have been observed in several studies to produce biphasic dose–response curves, whereby low doses stimulate immune responses and high doses produce inhibition (Burchiel & Luster, 2001; Booker & White, 2005). An explanation for this finding may relate to signalling properties, which are discussed below.

The overall effects of PAHs on the immune and haematopoietic systems result from activation of both genotoxic and non-genotoxic (epigenetic) pathways (Figure 4.6). Because of the heterogeneity of lymphoid and myeloid cell populations and the complex interplay between different types of cells and secreted products, the mechanisms of action

of PAHs have been difficult to assess. Many PAHs clearly exert effects on the developing as well as the mature immune system in many mammalian species, and some correlation exists between the carcinogenicity of PAHs and their ability to produce immunosuppression. An understanding of the mechanisms of action for both carcinogenicity and immunotoxicity may help to evaluate potential risks of untested agents or complex mixtures.

Figure 4.6 Polycyclic aromatic hydrocarbon (PAH) immunotoxicity



AhR, aryl hydrocarbon receptors; BPDE, benzo[*a*]pyrene diol epoxide;BPQ, benzo[*a*]pyrene quinone; CYP, cytochrome P450; DRE, dioxin response element; EPHX, epoxide hydrolase enzyme; GST, glutathione *S*-transferase; NQO, nicotinamide adenine dinucleotide phosphate quinone oxidoreductase; UV, ultraviolet light

(b) Aromatic hydrocarbon receptors

(i) *Immunosuppressive PAHs as AhR ligands*

In general, PAHs are semi-volatile compounds that are quite lipophilic and exert both specific and non-specific effects on membranes. Highly specific structure–activity relationships have been observed for the effects of PAHs on cells and tissues, including the immune system. Specific effects generally relate to the expression of AhR and the ability of 'bay-region' PAHs to bind to AhR and to activate AhR-dependent gene

promoter regions referred to as DREs. AhR appears to play an important role in normal development of the immune system in mice (Fernandez-Salguero *et al.*, 1995). The biological and toxicological activities of PAHs are largely dependent on their ability to interact with AhRs present in many mammalian cells and tissues (Nebert *et al.*, 1993; Hankinson, 1995; Schmidt & Bradfield, 1996; Whitlock, 1999; Nebert *et al.*, 2004).

The mechanism whereby the activation of AhR leads to immunotoxicity is not known for AhR agonists, although certain effects have become better understood in recent years. Because many PAHs and their metabolites are moderate to strong (high-affinity) AhR ligands, it is difficult to distinguish between the action of a parent compound, such as benzo[*a*]pyrene, and that of metabolites that are formed in response to AhR binding and the induction of metabolic enzymes. However, certain AhR ligands that are poorly metabolized, such as TCDD (or dioxin) and some PCBs, have been studied extensively for their immunotoxicity. AhR-dependent processes are activated by TCDD and some PCBs through binding to AhR and accessory molecules (such as Arnt), which leads to immune effects via XRE/XDREs. XRE/XDREs are also activated by many PAH AhR ligands, and it appears that their activation is well correlated with immunotoxicity for halogenated aromatic hydrocarbons and many PAHs. Several lymphoid cell lines have been found to express AhR in mammalian species, including humans, although T lymphocytes may require activation of phytohaemagglutinin before significant levels of CYP1A1 can be induced (Whitlock *et al.*, 1972; Germolec *et al.*, 1996). TCDD suppresses numerous B- and T-cell responses in rodents (Vecchi *et al.*, 1983) and produces thymic atrophy at higher concentrations (Camacho *et al.*, 2005; Nohara *et al.*, 2005). It may also induce immunosuppressive factors or prevent growth factors from being released from cells (Jensen *et al.*, 2003; Boverhof *et al.*, 2004). Current evidence suggests that direct activation of AhR in T cells may play a major role in the immunosuppressive effects of TCDD on cell-mediated and perhaps humoral immunity (Kerkvliet *et al.*, 2002; Funatake *et al.*, 2004; Temchura *et al.*, 2005). The affinity of AhR in various mouse strains is correlated with the potency of immunosuppression and thymic atrophy (Nebert *et al.*, 1993).

(ii) *PAH metabolism in the immunotoxicity of benzo[*a*]pyrene and DMBA*

CYP metabolism occurs in central (liver, lung) and peripheral lymphoid tissues (lymph nodes, spleen and bone marrow) and is very important in the immunotoxicity of PAHs. The local metabolism of PAHs is generally much lower than that in the liver and is modified by the expression of AhR and constitutive levels of CYPs such as CYP1B1. CYP1A1 is highly inducible in some white blood cells. In murine spleen cells, the highest levels of CYP activity have been detected in monocytes and macrophages, and low levels have been detected in B and T cells (Kawabata & White, 1989; Ladics *et al.*, 1992a,b,c). Most human and murine B- and T-cell lines used for in-vitro modelling studies have little CYP activity. Human peripheral blood T cells demonstrate an increase in levels of CYP1A1 following treatment with phytohaemagglutinin and PAHs (Whitlock *et al.*,

1972). PAHs are metabolized by CYP-dependent and -independent pathways, which are referred to as phase I metabolic pathways. The CYP-dependent pathways, most notably CYP1A1, CYP1A2 (liver only) and CYP1B1, are induced following binding of AhR and activation of DREs.

In general, benzo[*a*]pyrene and many other bay-region PAHs, including DMBA, are metabolized by CYP and other enzymes to form oxidative metabolites, such as epoxides. Although DMBA is not a naturally occurring compound, knowledge of its mechanism of action on the immune system has helped investigators to understand PAH pathways more generally. In the presence of microsomal epoxide hydrolase, epoxides are converted to dihydrodiols, which, in the presence of CYP1A1 or CYP1B1, can then undergo further metabolism to form diol epoxides, including benzo[*a*]pyrene-7,8-diol-9,10-epoxide and DMBA-3,4-diol-1,2-epoxide. These diol epoxides and other metabolites of benzo[*a*]-pyrene (such as benzo[*a*]pyrene quinones) and DMBA (such as DMBA quinones) are strong electrophiles that trigger the induction of secondary phase 2 metabolizing enzymes through the Keap-1/Nrf-2 redox sensing system and the anti-oxidant/electrophilic response element signalling systems (Primiano *et al*., 1997; Nguyen *et al.*, 2005). Phase II enzymes such as GSTs, NQO1, UGTs, epoxide hydrolase, aldehyde dehydrogenases and others are mainly expressed in the liver and lung, although low levels of GSTs are detected in human lymphocytes. Because lymphoid cells have limited expression of phase II detoxification enzymes, they may be somewhat sensitive to the formation of the phase I metabolites. Electrophilic metabolites of benzo[*a*]pyrene, most notably benzo[*a*]pyrene-7,8-diol-9,10-epoxide and perhaps 7,8-benzo[*a*]pyrene quinone, bind covalently to DNA which leads to genetic mutations that are responsible for tumour initiation. In addition, the formation of benzo[*a*]pyrene diol epoxide–DNA adducts leads to the induction of the p53 pathway, which may trigger cell-cycle arrest and apoptotic pathways in target cells. Therefore, the same general genotoxic mechanisms that lead to mutagenicity may also play a role in immunosuppression for PAHs that can be bioactivated.

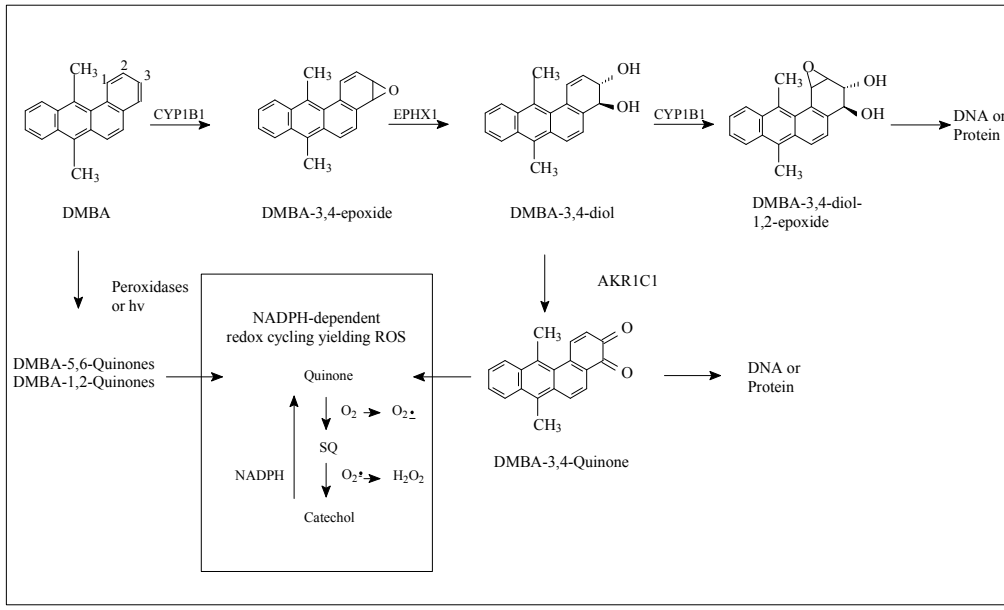
Several oxidative metabolites of PAHs, such as benzo[*a*]pyrene quinones, are known to redox-cycle and lead to the production of reactive oxygen species (Zhu *et al*., 1995). PAH quinones are formed via CYP-dependent and -independent reactions, such as by peroxidases or by ultraviolet light (Reed *et al*., 2003). PAH quinones redox-cycle and thereby form reactive oxygen species, including superoxide anion and hydrogen peroxide. Through reactive oxygen species, PAH quinones may exert both genotoxic and non-genotoxic effects, as shown by their ability to form 8-hydroxydeoxyguanosine on DNA as well as to alter signalling pathways in cells. Redox-cycling requires the reduction of equivalents generally supplied by NADPH, and thus benzo[*a*]pyrene quinones and related agents interact with the mitochondrial electron transport chain that leads to oxidative stress and ATP depletion. The immunotoxicological effects of benzo[*a*]pyrene quinones have not been fully evaluated.

Support for an important role of CYP metabolism in the immunotoxicity of PAHs has been obtained from studies that demonstrate that the AhR antagonist and CYP1A1/CYP1B1, inhibitor, α-naphthoflavone, prevents the immunotoxicity of benzo-

[*a*]pyrene and DMBA in murine spleens cells (Kawabata & White, 1987; Ladics *et al*., 1991), human peripheral blood T cells (Davila *et al*., 1996) and murine bone marrow (Dertinger *et al*., 2001). The putative CYP1A1 and CYP1B1 metabolite responsible for murine spleen cell and human T-cell immunotoxicity due to benzo[*a*]pyrene is probably its diol epoxide (Davila *et al.*, 1996). In addition, in these studies, the rank order of PAH immunotoxicity was found to be benzo[*a*]pyrene >DMBA >dibenz[*a*,*c*]anthracene >dibenz[*a*,*h*]anthracene >dimethylanthracene, benzo[*e*]pyrene >benz[*a*]anthracene >anthracene. The latter three PAHs had minimal immunotoxicity.

An important role of metabolism in the toxicity of DMBA in the bone marrow of mice has been established through the use of CYP1B1-null (knockout) mice. DMBA-induced pre-B cell toxicity was nearly totally abolished in mice that did not express CYP1B1 (Heidel *et al*., 2000). The splenic immunotoxicity of DMBA has been shown to be dependent on the expression and activity of CYP1B1 (Gao *et al*., 2005a), and it has recently been reported that microsomal epoxide hydrolase is also required for this spleen cell immunotoxicity. Thus, it is probable that DMBA-3,4-diol-1,2-epoxide is responsible for the immunotoxicity of DMBA in mice (Figure 4.7).

Figure 4.7. 7,12-dimethylbenz[*a*]anthracene (DMBA) metabolism that yields genotoxic and non-genotoxic metabolites



CYP, cytochrome P450; EPHX1, epoxide hydrolase enzyme; NADPH, nicotinamide adenine dinucleotide phosphate

(c) Correlation between carcinogenicity and immunotoxicity for some PAHs

Activation of AhR may be linked to tumour promotion in various cells, since TCDD has been shown to increase the proliferation of epithelial cells through the stimulation of cell proliferation, inhibition of apoptosis and production of growth factors (Davis *et al*., 2000, 2003). TCDD is a suspected tumour promoter in several tissues. The role of TCDD in promoting human leukaemia and lymphomas is controversial, but studies suggest that continual activation of AhR in human T cells leads to adult T-cell leukaemia (Hayashibara *et al*., 2003). Many PAHs are complete carcinogens and have the ability both to initiate and promote cancers. Tumour initiation pathways are associated with DNA binding and mutagenesis.

Carcinogenic PAHs have been found to suppress the immune system of animals (White & Holsapple, 1984; Wojdani & Alfred, 1984; Wojdani *et al*., 1984). Initial studies showed that benzo[*a*]pyrene, DMBA and 3-methylcholanthrene suppressed humoral immunity, and later studies showed that many immune cells are targets of PAH. The humoral immune response to T-dependent antigens is considered to be a sensitive indicator of immune suppression. Suppression of T- and B-cell proliferation has been observed at similar exposure levels, but proliferation is considered to be a somewhat less sensitive indicator of immunotoxicity than T-dependent antibody responses. In general, there is some correlation between the carcinogenicity of a PAH and its immunotoxicity, which is probably due to requirements for AhR-binding activity and metabolic activation. (White *et al*., 1985). In a detailed analysis of more than 50 selected chemicals, many of which were PAHs, Luster *et al.* (1992) found that immunotoxicity in rodents was probably correlated with carcinogenicity; however, examples of immunotoxic chemicals that had unknown carcinogenicity status were found, and therefore the converse relationship might not be true.

Mechanistically, the immunosuppression produced by benzo[*a*]pyrene and DMBA has both similarities and differences. Benzo[*a*]pyrene is a moderate to strong AhR ligand, whereas DMBA is a weak AhR ligand. In addition, as discussed above, much of the immunotoxicity of benzo[*a*]pyrene is largely due to CYP1A1-dependent metabolism. CYP1A1 is expressed only at low levels in lymphocytes until AhRs are activated. In the case of DMBA, the major metabolizing enzyme is CYP1B1, which is expressed in many tissues constitutively, but can also be induced through AhR and antioxidant response element mechanisms.

(d) Targets of PAH toxicity in the immune system

(i) *Bone marrow*

Mammalian bone-marrow cells, and especially bone-marrow stromal cells, express AhR. PAHs that bind to and/or are activated by CYP are toxic to mouse and human stem cells (Fine *et al*., 1989; Murante & Gasiewicz, 2000; van Grevenynghe *et al*., 2005), and myeloid (Luster *et al*., 1985; Laupeze *et al*., 2002; van Grevenynghe *et al*., 2003) and

lymphoid progenitors (Luster *et al*., 1988; Yamaguchi *et al*., 1997a,b; Near *et al*., 1999; Thurmond *et al*., 2000). Both DMBA and benzo[*a*]pyrene exert important effects on bone marrow that alter the formation of B cells. The mechanism of pre-B cell bone-marrow suppression appears to be CYP1B1-dependent and may be caused by pre-B cell apoptosis (Heidel *et al*., 1999; Mann *et al*., 1999; Heidel *et al*., 2000; Allan *et al*., 2003; Galvan *et al*., 2003, 2005).

(ii) *Thymus*

Overactivation by TCDD of AhR in the thymus causes thymic atrophy (Nohara *et al*., 2005). Most PAHs do not produce significant thymic atrophy until very high exposure levels are reached; however, DMBA is an exception and thymic atrophy is observed at low doses (Burchiel *et al.,* 1992). The effects of DMBA on the thymus have not been found to correlate with AhR phenotype (Thurmond *et al*., 1987; Holladay & Smith, 1995).

(iii) *Spleen*

Many studies have been performed with spleens obtained from PAH-treated mice, in which both DMBA and benzo[*a*]pyrene have been found to be immunosuppressive for humoral (Ward *et al*., 1984; White & Holsapple, 1984; Ward *et al*., 1986; Thurmond *et al*., 1987; Burchiel *et al*., 1988) and cell-mediated (Dean *et al*., 1986; House *et al*., 1989) immunity. DMBA was also found to be immunosuppressive following exposure *in vitro* (Thurmond *et al*., 1988). In general, the most sensitive target cells of PAHs appear to be B- and T-helper cells for humoral immunity and cytotoxic T cells for cell-mediated immunity, although macrophages and antigen-presenting cells have also been implicated (Myers *et al.*, 1987; Blanton *et al.*, 1988; Myers *et al.*, 1988). Splenic natural killer cells have also been shown to be suppressed by DMBA (Dean *et al*., 1985, 1986).

(iv) *Peripheral lymphoid tissues*

A few peripheral lymphoid tissues have been examined for the effects of PAHs following intragastric administration of benzo[*a*]pyrene to rats; lymphoid organ weights were decreased at high doses (90 mg/kg) of benzo[*a*]pyrene (De Jong *et al*., 1999). It was found that the antibody response of lung-draining lymph nodes was suppressed in rats exposed intratracheally to benzo[*a*]pyrene (Bice *et al*., 1979). In mice, DMBA suppressed the response of murine mesenteric lymph nodes and Peyer's patches following intragastric administration (Burchiel *et al*., 1990; Davis *et al*., 1991). The concentrations of PAHs required to suppress humoral and cell-mediated immunity in mice are extremely high, typically in the range of 10–50 mg/kg benzo[*a*]pyrene. In general, DMBA produced more cytotoxicity in peripheral lymphoid organs than benzo[*a*]pyrene.

(v) *Skin*

The skin is not considered to be a primary or secondary lymphoid tissue; however, there are significant immune responses that occur in this tissue, many of which fall into the category of hypersensitivity responses. PAHs, such as DMBA, have been shown to

enhance cell-mediated hypersensitivity reactions in the skin of mice that may play a role in immune surveillance (Casale *et al.*, 2000).

(e) Potential mechanisms of immunosuppression by PAHs

(i) *Genotoxic mechanisms of PAH metabolite-induced immunotoxicity*

The mechanisms by which PAHs produce immunotoxicity are divided into two general categories: genotoxic (DNA targets) and non-genotoxic (protein targets). Genotoxic and epigenetic effects are seen for many complete carcinogens, such as PAHs that both initiate and promote tumours. Many PAHs are bioactivated to reactive metabolites that bind to DNA and exert mutagenicity and genotoxicity. The mechanism by which genotoxic chemicals produce immunosuppression is probably p53-dependent and may result from stable adduct formation in lymphoid cells. Bulky PAH–DNA adducts induce p53 which in turn inhibits cell cycling and induces apoptosis in many cells (Vogelstein & Kinzler, 1992).

DMBA has been found to produce immunotoxicity in bone marrow through the induction of apoptosis (Page *et al*., 2002), and recent studies showed that p53 knockout mice are resistant to the suppressive effects of DMBA on bone marrow (Heidel *et al*., 2000; Page *et al*., 2003). Many genotoxic chemicals that induce p53 are also immunosuppressive. Therefore, p53 is probably an important pathway for the immunotoxicity of numerous agents. In some studies, there is good agreement between the functional immunotoxicity observed and changes in immunophenotypic cell-surface markers (Burchiel *et al*., 1988), while in others, there has been a lack of agreement (House *et al*., 1987). These differences probably relate to the degree of apoptosis and cell death produced by PAHs, as it is improbable that functional changes would be detected simply by immunophenotypic analysis of spleen cells. Thus, immunophenotyping is probably not a sensitive marker for immunotoxicity at non-cytotoxic concentrations of xenobiotic compounds.

(ii) *Non-genotoxic (epigenetic) mechanisms of PAH immunotoxicity*

Several studies have shown that PAHs can activate or interfere with lymphocyte signalling pathways in both murine and human B and T cells. The ability of PAHs to signal through various receptor and oxidative stress pathways may correlate with tumour promotion. Many xenobiotic compounds produce biphasic effects on immune responses: low concentrations stimulate and high doses inhibit responses. Agents that mimic or alter signalling pathways may manifest these characteristics.

AhR ligands

Generally, a positive correlation is seen between the carcinogenicity and immunotoxicity of a PAH. This correlation probably exists because both carcinogenicity and immunotoxicity are largely dependent on AhR binding, increased CYP expression and the formation of bioactive metabolites (White *et al*., 1985; Burchiel & Luster, 2001).

Direct AhR-dependent immunosuppression has been reported in both T (Kerkvliet *et al*., 2002) and B cells (Sulentic *et al*., 2000) for the pure AhR agonist, TCDD. TCDD has been shown to produce persistent changes in immunosuppressive cytokine production in monkeys (Rier *et al*., 2001), although the prevention of tumour necrosis factor -α activity has not been found to restore the T-dependent antibody responses in mice (Moos & Kerkvliet, 1995). Nevertheless, AhR-dependent altered cytokine production is a potential mechanism for PAH-induced immunosuppression.

Calcium signalling

The antigen receptor signalling pathways of lymphocytes are linked to changes in intracellular Ca^{2+} (reviewed in Davila *et al*., 1995; Burchiel & Luster, 2001). The structure–activity relationships for elevated of Ca^{2+} have largely been determined *in vitro*, and there appear to be PAH metabolism-dependent and -independent mechanisms that rapidly increase levels of Ca^{2+}. DMBA produces a rapid increase in intracellular Ca^{2+} (Burchiel *et al*., 1991). Elevation of Ca^{2+} appears to be an inositol-1,4,5-triphosphate (IP_3)-dependent process (Archuleta *et al*., 1993). However, further studies revealed that many PAHs also produce a rapid increase in intracellular Ca^{2+} that did not follow any structure–activity relationships relating to AhR binding (Davila *et al*., 1999). Sustained elevations in intracellular Ca^{2+} appeared to correlate with human T-cell signalling (Krieger *et al*., 1994). Benzo[*a*]pyrene increased intracellular Ca^{2+} in human peripheral blood mononuclear B and T cells and monocytes (Mounho *et al*., 1997). Benzo[*a*]pyrene diol epoxide appeared to be responsible for this elevation of Ca^{2+}, perhaps due to the activation of protein tyrosine kinase (PTK) detected in a human B-cell line (Mounho *et al*., 1997). Thus, there appear to be several potential mechanisms of elevation of Ca^{2+} by PAHs in murine and human lymphocytes. The significance of altered Ca^{2+} signalling in PAH-induced immunosuppression may be increased in the presence of cyclosporine A, which is a potent immunosuppressive drug that is used in the prevention of transplant rejection and autoimmunity, and which interferes with Ca^{2+} signalling (Ruggenenti *et al*., 1993).

Inhibition of sarcoplasmic–endoplasmic reticulum calcium–ATPase

Initial studies showed that the ability of PAHs to increase intracellular Ca^{2+} in human T cells correlated with an inhibition of sarcoplasmic–endoplasmic reticulum Ca^{2+}–ATPase (SERCA) activity (Krieger *et al*., 1995). However, PAHs failed to inhibit cloned rat SERCA enzymes (transiently expressed in human embryonic kidney cells), which suggested that metabolism might be required for this activity (Zhao *et al*., 1996). A well known SERCA inhibitor, thapsigargin, has been classified as a tumour promoter (Thastrup *et al*., 1990), which suggests that inhibition of SERCA by PAHs may be a potential mechanism of tumour promotion.

Increased PTK activity

PAHs have been found to increase PTK activity in lymphocytes leading to Ca^{2+}-dependent signalling in B and T cells in mice and humans (for a review, see Davila *et al*.,

1995; Burchiel & Luster, 2001). DMBA activates human T cells directly (Burchiel *et al*., 1991; Archuleta *et al*., 1993; Davis & Burchiel, 1992), whereas benzo[*a*]pyrene requires metabolism to its diol epoxide to activate human B cells (Mounho & Burchiel, 1998). In-vitro studies with PAHs have shown that non-specific components are involved in PTK activation (Davila *et al*., 1999). The precise mechanism whereby PAHs activate PTK appears to be through the inhibition of protein tyrosine phosphatases. There is evidence that UV light increases PTK activity via this mechanism, as well as numerous oxidant chemicals and some metals (Schieven *et al*., 1994; Rhee *et al*., 2005; Tonks, 2005). Therefore, the mechanism of inhibition of protein tyrosine phosphatases by PAHs may be due to oxidative changes in the protein. Overactivation of PTK pathways and receptor tyrosine kinase pathways is associated with cancer in many cell types.

Activation of Ca^{2+} channels: IP_3 and ryanodine receptors

Phospholipase Cγ (Cγ1 in T cells and Cγ2 in B cells) is activated by PAHs in human and murine T and B cells by PTKs. Phospholipase Cγ is responsible for the cleavage of membrane phosphatidylinositol-4,5-biphosphate and the release of IP_3 and diacylglycerol. IP_3 diffuses into the cytoplasm and binds to IP_3 receptors that are located on the endoplasmic reticulum. IP_3 receptors control release of Ca^{2+} into the endoplasmic reticulum and their activation leads to a rapid increase in intracellular Ca^{2+}. Benzo[*a*]pyrene diol epoxide has been shown to activate B cell PTKs that are associated with the formation of IP_3 (Mounho *et al*., 1989), and this activation may be dependent on protein tyrosine phosphatase. Therefore, one mechanism of PAH-induced elevation of Ca^{2+} is via the activation of phospholipase Cγ.

A novel mechanism, ryanodine receptors that control Ca^{2+} refilling of endoplasmic reticulum stores, was discovered in the endoplasmic reticulum of lymphocytes (Sei *et al*., 1999; Schwarzmann *et al*., 2002). Gao *et al*. (2005b) found that a novel metabolite, 7,8-benzo[*a*]pyrene-quinone, produced a rapid increase in intracellular Ca^{2+} via the ryanodine receptor in human peripheral blood leukocytes and murine splenic B and T cells.

Consequences of increased Ca^{2+} signalling in lymphocytes

PAHs have the potential to activate numerous isoforms of PKC in lymphocytes and other immune cells. During the activation of PTKs, diacylglycerol and Ca^{2+} are produced and activate these isoforms of PKC, which are defined on the basis of their sensitivities to these two substances (Parker & Murray-Rust, 2004). Activation of PKC has been associated with tumour promotion by phorbol esters in many cells and tissues (Weinstein, 1991). It is unclear which of the isoforms of PKC have immunological consequences. Because of altered B- and T-cell Ca^{2+} signalling, inappropriate activation may lead to such phenomena as persistent immunosuppression and tolerance (Ward *et al*., 1986; Burchiel *et al*., 1988). In T and B cells, excess Ca^{2+} signalling has been associated with tolerance (Schwartz, 2003). One of the consequences of helper T-cell tolerance would be the lack of production of IL-2, which is a key cytokine for both humoral and cell-mediated immunity. DMBA prevents the formation of IL-2 and exogenously added IL-2 can

partially overcome the immunosuppression produced by this agent (House *et al*., 1987; Pallardy *et al*., 1989).

Oxidative stress

In many cells, PAHs are known to cause oxidative stress (Nebert *et al*., 2000), which is associated with the activation of the AhR (Senft *et al*., 2002). Although few studies have been carried out on oxidative stress produced by benzo[*a*]pyrene quinones in lymphocytes, previous studies in bone-marrow cells demonstrated that these redox-cycling agents may also lead to GSH depletion (Zhu *et al*., 1995; Romero *et al*., 1997). Direct exposure of murine and human lymphocytes to 7,8-benzo[*a*]pyrene quinone (but not 1,6-, 3,6- or 6,12-benzo[*a*]pyrene quinone) led to rapid elevation of Ca^{2+} (Gao *et al*., 2005b), whereas treatment with 1,6-, 3,6-, and 6,12-benzo[*a*]pyrene quinone increased free intracellular Ca^{2+} only after prolonged (12–18 h) exposures *in vitro*, presumably due to mitochondrial redox-cycling, depletion of ATP and Ca^{2+} overload. Other PAH quinones have also been shown to produce immunotoxicity. For example, treatment of murine spleen cells *in vitro* with 1,4-naphthaquinone produced immunotoxicity in mice, whereas exposure to naphthalene produced no adverse immune effects (Kawabata & White, 1990). In-vitro exposure of murine spleen cells to benzo[*a*]pyrene quinones produced a strong proliferative signal in T cells, but not B cells (Burchiel *et al*., 2004). Thus, the effects of PAHs that induce oxidant stress on murine spleen cells appear to be mixed.

(f) Complex mixtures

Humans are exposed to complex mixtures of PAHs via the diet, air and skin contact (Rothman *et al*., 1993; Schoket, 1999; Scherer *et al*., 2000; Arrieta *et al*., 2003; Oh *et al*., 2005). Low concentrations of high-molecular-weight PAHs, such as benzo[*a*]pyrene, are found in diesel exhaust and higher levels are found in woodsmoke (Burchiel *et al*., 2005). Occasional dermal exposure may also occur and is usually associated with occupational exposures to tars, soots and vapours. Exposures to PAHs can be monitored using major urinary metabolites (Wu *et al*., 2002). Some epidemiological evidence indicates that complex mixtures containing PAHs produce immunosuppression under conditions of environmental and industrial exposures in humans (Szczeklik *et al.*, 1994; Winker *et al.*, 1997; Karakaya *et al*., 1999; Biro *et al.*, 2002; Karakaya *et al.*, 2004; Oh *et al*., 2005).

In animal models, recent data demonstrate that diesel exhaust, which contains an abundance of low-molecular-weight PAHs (such as naphthalene, anthracene and phenanthrene), and woodsmoke (which contains measurable concentrations of benzo[*a*]pyrene) are immunosuppressive to murine spleen cells following chronic (6 months) inhalation (Burchiel *et al*., 2004, 2005). It is unclear which components of diesel exhaust and woodsmoke are responsible for this suppression, but PAHs may play a role. The particulate fraction of diesel exhaust contains many potential immuno-suppressive chemicals, including PAHs and metals, and important interactions may occur between oxidant-generating PAHs and metals. In addition, because it is well known that

diesel exhaust causes inflammation in the lung and generates significant inflammatory and chemo-attractant cytokine production, it is possible that immune modulation may be caused secondary to altered cytokine production.

(g) Summary

PAHs exert many important effects on the immune system of many species. The dose and route of exposure determine the nature of the effect of specific and adaptive immune responses. Studies with pure PAHs suggest that AhRs play a critical role in the activation of immunotoxic PAHs, such as benzo[*a*]pyrene, via diol epoxide mechanisms which lead to DNA interactions that cause genotoxicity and suppress immunity by p53-dependent pathways. Benzo[*a*]pyrene diol epoxide may also affect protein targets and modulate lymphocyte signalling pathways via non-genotoxic (epigenetic) mechanisms. Certain oxidative PAHs, such as benzo[*a*]pyrene quinones, may be formed via CYP-dependent and -independent (peroxidase) pathways. Redox-cycling PAH quinones may exert oxidative stress in lymphoid cells. Human exposures to PAHs are usually in the form of complex mixtures, and it is difficult to attribute the relative contributions of individual PAHs to the overall immunotoxic effects. Some evidence suggests that environmental exposures to PAH may produce immunotoxicity, but further epidemiological studies are needed.

4.2.7 *Genotoxic and epigenetic effects of mixtures*

(a) Aluminium production

Filter extracts of airborne particles from a Söderberg pot-room and an anode paste plant were mutagenic in *S. typhimurium* TA98 and TA100 after metabolic activation, and positive results were obtained without metabolic activation in strain TA98 (Krokje *et al.*, 1985). Several studies examined the levels of aromatic DNA adducts in peripheral blood lymphocytes of aluminium workers and showed mixed results. In 172 Hungarian aluminium plant workers, increased levels of aromatic DNA adducts were measured compared with controls (Schoket *et al.*, 1999). Other studies have observed aromatic DNA adducts in peripheral blood lymphocytes of Hungarian aluminium workers at different factories, at different times of the year and in different job categories (Schoket *et al.*, 1993a,b, 1995). A significant linear correlation was observed between total aromatic DNA adducts in white blood cells and urinary 1-hydroxypyrene levels in Hungarian pot-room workers with the GSTM1-nul genotype (Schoket *et al.*, 2001).

Other populations of aluminium plant workers had detectable levels of aromatic DNA adducts (Kriek *et al.*, 1993; Ovrebo *et al.*, 1995; van Schooten *et al.*, 1995). Ninety-eight Swedish pot-room workers were examined for aromatic DNA adducts and polymorphisms. No significant differences were observed in the levels of total or individual DNA adducts between pot-room workers and controls (Tuominen *et al.*, 2002). Only one sample from the lymphocytes of 30 aluminium plant workers was found to contain benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts (Vähäkangas *et al.*, 1985).

Analysis of levels in a group of 36 aluminium anode plant workers did not show a significant percentage of subjects with DNA adduct levels that exceeded the 95th percentile of the control value (Pavanello *et al.*, 1999a,b). Serum antibodies to benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA were detected in 13.3% of 105 aluminium plant workers (Galati *et al.*, 2001). Urine samples from pot workers and anode paste workers in a Swedish aluminium plant were not mutagenic in *S. typhimurium* (Krokje *et al.*, 1988). The lymphocytes of 42 Italian aluminium plant workers were examined for micronuclei and DNA damage (single-cell gel electrophoresis assay). While none of the workers showed significant changes in micronucleus formation, significant increases in DNA damage were noted (Crebelli *et al.*, 2002). Ninety-eight Swedish pot-room workers and 55 controls were examined for the effect of genetic polymorphisms of biotransformation enzymes on gene mutations, DNA strand breaks and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine. No correlations were found between any of the genotoxicity biomarkers and any of the exposure measures, length of employment in the pot-room, 1-hydroxypyrene in urine or PAH–DNA adducts in peripheral lymphocytes, even when genotypes for biotransformation enzymes were considered (Carstensen *et al.*, 1999).

(b) Chimney sweeps

The frequencies of micronuclei in peripheral B or T lymphocytes and whether genetic polymorphisms in metabolic activating enzymes could explain some of the variation in micronucleus formation was studied in 71 Swedish chimney sweeps. The sweeps did not have higher frequencies of micronuclei in either cell type when the results were adjusted for age and tobacco smoking and there was no association between duration of employment and micronuclei formation (Carstensen *et al.*, 1993). The same cohort was further studied for the presence of aromatic DNA adducts and micronuclei and was genotyped for *CYP1A1* and *GST1*. While no specific DNA adducts were identified, the sweeps had higher but not significantly increased total DNA adduct levels in white blood cells. There were no systematic differences in DNA adduct patterns between sweeps and controls. DNA adducts in sweeps were moderately but statistically significantly correlated with micronuclei in both T and B lymphocytes. This correlation between adduct levels and micronuclei was most marked in the T lymphocytes of individuals who lacked the *GST1* gene (Ichiba *et al.*, 1994).

The lymphocytes of 45 five Swedish chimney sweeps and 49 controls were investigated for micronucleus formation after stimulation with phytohaemagglutinin and pokeweed mitogen and analysis of lymphocyte subgroups and neutrophilic leukocytes. There was a statistically significant effect among sweeps with respect to both micronucleus variables and neutrophilic leukocytes. The effect on lymphocyte micronuclei was more significant in pokeweed mitogen-stimulated cells, which may imply that T4 lymphocytes were preferentially damaged by the occupational exposure (Holmen *et al.*, 1994). Analysis of benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels in a group of 19 chimney sweeps showed that the percentage of subjects with adduct levels that

exceeded the 95th percentile of the control value was significantly high in chimney sweeps (Pavanello *et al.*, 1999a), and these higher levels were associated with the lack of GSTM1 activity (Pavanello *et al.*, 1999b).

(c) Creosote

Creosote induced mutations in *S. typhimurium* TA1537, TA1538, TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Bos *et al.*, 1983, 1985). A portion of the mutagenicity was attributed to benzo[*a*]pyrene, benz[*a*]anthracene and fluoranthene in the complex mixture (Bos *et al.*, 1984a, 1987). Four creosotes used in Finland were mutagenic in *S. typhimurium* TA98 , TA100, YG1021 and YG1024 in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Nylund *et al.*, 1992). The urine from rats treated with creosote by intraperitoneal administration was mutagenic in *S. typhimurium* TA98 and TA100 in the presence of an Aroclor 1254-induced rat liver homogenate supplemented with β-glucuronidase (Bos *et al.*, 1984b). Using the same bioassay method, urine samples of three workers in the creosote wood-preserving industry were not mutagenic although organic extracts of wipe-test samples from the surfaces of their work environments were (Bos *et al.*, 1984a). Several creosote samples induced sister chromatid exchange in Chinese hamster ovary cells in the presence of an Aroclor 1254-induced rat liver metabolic activation system (Nylund *et al.*, 1992). Creosote applied topically to mouse skin *in vivo* or human skin in short-term organ culture produced a complex pattern of aromatic DNA adducts with similar levels in both systems (Schoket *et al.*, 1988a,b). Multiple topical treatments of mice with creosote resulted in accumulation of DNA adducts in lung tissues (Schoket *et al.*, 1988a). Extracts of soil samples from a wood-preserving waste site known to contain creosote and pentachlorophenol were topically applied to mouse skin. Aromatic DNA adducts were detected in distal organs (lung, liver, kidney and heart) as well as the skin. The *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was detected in all organs (Randerath *et al.*, 1996, 1997).

(d) Coal tar

(i) *Human data*

Urine samples from some nonsmoking psoriasis patients treated with coal tar and UV light were mutagenic in *S. typhimurium* TA98 in the presence of an Aroclor 1254-induced rat liver metabolic system (Wheeler *et al.*, 1981). The urine of all 15 nonsmoking patients who were treated with a 2% coal-tar ointment and who had avoided a high-temperature cooked meat diet was mutagenic in *S. typhimurium* YG1024 with exogenous metabolic activation. *GSTM1*-nul patients had higher levels of mutagens in their urine than *GSTM1*-positive patients (Gabbani *et al.*, 1999). The skin and white blood cells (monocytes, lymphocytes and granulocytes) of a group of eczema patients treated topically with coal-tar ointments showed the presence of aromatic DNA adducts by ^{32}P-postlabelling analysis. One of the adducts co-migrated with the benzo[*a*]pyrene-7,8-diol-9,10-oxide–

DNA adduct (Godschalk *et al.*, 1998). Analysis of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels by an HPLC/fluorescence method in a group of 26 psoriasis patients showed that the percentage of subjects with adduct levels that exceeded the 95th percentile of the control value was not significant (Pavanello *et al.*, 1999a). The white blood cells of 23 psoriasis patients who were undergoing clinical coal-tar therapy were examined for benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts by an enzyme-linked immunsorbent (ELISA) method. Although these adducts were detected and their levels decreased with time after treatment, no relationship could be ascertained between the level of exposure and the amount of adducts. Also, no difference in the level of DNA adducts was found between smoking and nonsmoking patients (Paleologo *et al.*, 1992). PAH diol epoxide–DNA adducts and *GSTM1* genotype in the white blood cells of 57 psoriasis patients and 53 controls were determined by ELISA methods and polymerase chain reaction respectively. PAH diol epoxide–DNA adducts were slightly elevated in patients compared with controls, but there was no relationship between the presence of the *GSTM1* gene and DNA adducts (Santella *et al.*, 1995). Skin biopsy samples from 12 psoriasis patients who received therapy with coal-tar ointment contained aromatic DNA adducts as measured by ^{32}P-postlabelling analysis (Schoket *et al.*, 1990). No significant effect of coal-tar treatment of psoriasis patients on the levels of benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts was detected by ^{32}P-postlabelling analyses in peripheral blood lymphocytes (Pavanello & Levis, 1994). In a study of 111 Korean coal tar-based paint workers, the levels of aromatic DNA adducts measured by ^{32}P-postlabelling analysis were slightly higher than those of 27 on-site control workers (Lee *et al.*, 2003). The lymphocytes of 49 coal-tar workers exhibited a significant increase in the frequency of chromosomal aberrations, sister chromatid exchange and satellite associations compared with controls (Yadav & Seth, 1998). Increased levels of p53 were found in skin biopsies of atopic eczema patients treated topically with coal tar. A correlation was also observed between p53 and levels of aromatic DNA adducts measured in the same tissue by ^{32}P-postlabelling analysis (Godschalk *et al.*, 2001).

(ii) *Studies in experimental systems*

In a previous monograph, coal-tar pitch and roofing-tar emissions were found to be mutagenic in bacteria (in the presence of an Aroclor 1254-induced rat liver metabolic system) and mammalian cells (in the presence and absence of an Aroclor 1254-induced rat liver metabolic system), to induce sister chromatid exchange in Chinese hamster ovary CHO cells and to enhance viral transformation in Syrian hamster embryo cells (both in the absence of an Aroclor 1254-induced rat liver metabolic system) (IARC, 1985). Coal tar applied topically to the skin of male Parkes mice *in vivo* produced a complex pattern of DNA adducts in skin and lung tissues (Schoket *et al.*, 1988a). In the skin, several groups of adducts were attributed to groups of PAHs. Coal-tar adduct spot 2 was the major adduct formed by benzo[*ghi*]perylene (Hughes *et al.*, 1993). Male B6C3F1 mice fed a diet of coal tar from manufactured gas plant residue produced a complex pattern of aromatic adducts in the liver, lung and forestomach DNA which increased with dose and

time of treatment (Culp & Beland, 1994). In lung DNA, one adduct was tentatively identified as the *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct (Culp & Beland, 1994). The identity of this adduct was confirmed by analysis of the lung DNA of female B6C3F1 mice fed a diet of coal tar from manufactured gas plant residue; however, based on the levels of this adduct, it was suggested that benzo[*a*]pyrene may contribute only a small fraction to the DNA adducts formed in the lung tissue of mice administered coal tar (Beland *et al.*, 2005). Female B6C3F1 mice were fed diets of coal tar, and DNA adduct formation, cell proliferation and mutations in tumours in the K-*ras*, H-*ras* and *p53* genes were determined. The *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct was identified in forestomach DNA. Cell proliferation was increased in the small intestines by dietary coal tar. Of the K-*ras*, H-*ras* and *p53* mutations observed in coal tar-induced tumours, the most abundant were K-*ras* mutations in forestomach and lung tumours (Culp *et al.*, 2000). B6C3F1 mice fed diets containing coal tar from manufactured gas plant residue produced a complex pattern of aromatic DNA adducts in the lung, forestomach and spleen tissues. Benzo[*a*]pyrene content alone in the coal tar could not account for the levels of aromatic DNA adducts (Weyand *et al.*, 1991). Strain A/J mice formed aromatic DNA adducts in the lungs when fed coal tar (from manufactured gas plant residue) in the diet. Three major DNA adducts were identified as being derived from benzo[*b*]fluoranthene, benzo[*a*]pyrene and benzo[*c*]-fluorene (Koganti *et al.*, 2001). Female ICR mice received topical applications of manufactured gas plant residue. Of the complex pattern of lung adducts, one was identified as being derived from 7*H*-benzo[*c*]fluorene. The quantitative results suggested that components other than 7*H*-benzo[*c*]fluorene played an important role in lung DNA adduct formation (Cizmas *et al.*, 2004). Epidermal cells but not hepatocytes isolated from C3H/Tif/hr hairless mice that received topical applications of coal tar had higher levels of DNA strand breaks as measured by the alkaline comet assay. Coal tar applied topically to lambda lacZ transgenic mice (MutaMouse) strongly increased the mutation frequency in epidermal cells but not in hepatocytes (Thein *et al.*, 2000). A retrospective comparison of tumour induction and DNA adduct formation by benzo[*a*]pyrene and coal tars in several experimental protocols indicated that tumour outcomes were not predicted by either quantitation of total DNA adducts or by DNA adducts formed by benzo[*a*]pyrene. These data suggested that benzo[*a*]pyrene content alone may not accurately predict tumour outcomes (Goldstein *et al.*, 1998).

(e) Coke ovens

As reported in a previous monograph, coke-oven materials were found to be mutagenic in bacteria in the presence or absence of an Aroclor 1254-induced rat liver metabolic system, to be mutagenic in several mammalian cell lines, to induce DNA strand breaks in Syrian hamster embryo cells, to induce sister chromatid exchange in Chinese hamster ovary CHO cells (in the presence or absence of an Aroclor 1254-induced rat liver metabolic system) and to enhance viral transformation in Syrian hamster embryo cells and morphological cell transformation of BALB/c 3T3 cells (in the absence of an Aroclor

1254-induced rat liver metabolic system) (IARC, 1984). In two early studies (Harris *et al*., 1985; Haugen *et al*.,1986), two methods were used to confirm the presence of benzo[*a*]-pyrene diol epoxide–DNA adducts in the lymphocytes of coke-oven workers. Urine samples from 31 male nonsmoking coke-oven workers were mutagenic in *S. typhimurium* YG1024 in the presence of an exogenous source of metabolic activation but not two samples from 31 male nonsmoking controls (Simioli *et al.*, 2004). A higher percentage (51%) of 39 French coke-oven workers had *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts in their lymphocytes and monocytes as measured by an HPLC/fluorescence method compared with 18% in 39 unexposed persons. Smokers in the exposed group had 3.5 times more DNA adducts than nonsmokers (Rojas *et al.*, 1995). Analysis of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels by an HPLC/fluorescence method in a group of 15 male coke-oven workers showed that the percentage of subjects with adduct levels that exceeded the 95th percentile of the control value was significantly higher (Pavanello *et al.*, 1999a), and these higher levels were associated with a lack of *GSTM1* activity (Pavanello *et al.*, 1999b). In a study of 95 male Polish coke-oven workers, those with the *GSTM1*-nul genotype had a significantly higher risk of having high levels of *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–DNA adduct as measured by an HPLC/fluorescence method than individuals with the active *GSTM1* genotype (Pavanello *et al.*, 2004). Eighty-nine male Taiwanese coke-oven workers in three exposure groups (topside workers, cokeside workers and plant office staff) were compared with 63 referents. All groups contained smokers and nonsmokers. Levels of aromatic DNA adducts in white blood cells were measured by ^{32}P-postlabelling analysis and were highest in the topside workers; smoking contributed only a small effect (Chen, M.L. *et al.*, 2003). Thirty-five Dutch coke-oven workers were compared with 37 controls for genotoxic effects and genetic polymorphisms in *GSTM1* and *GSTT1*. Occupational exposure did not cause a significant induction of sister chromatid exchange, high-frequency sister chromatid exchange, DNA strand breaks or aromatic DNA adducts (as measured by ^{32}P-postlabelling analysis) in lymphocytes or micronuclei in exfoliated urothelial cells. Smoking caused a significant increase in the incidence of sister chromatid exchange, high-frequency sister chromatid exchange and DNA adducts, but not of micronuclei or DNA strand breaks. *GSTM1* and *GSTT1* polymorphisms had no effect on any biomarker (van Delft *et al.*, 2001). Twenty-nine coke-oven workers and a control group were studied for frequencies of DNA single-strand breakage, DNA protein cross-links (alkaline filter elution assay), sister chromatid exchange and DNA adducts (measured by ^{32}P-postlabelling analyses) in lymphocytes. While the frequency of DNA strand breaks in the lymphocytes of coke-oven workers was significantly higher than that in controls, the DNA adduct rate was not significantly increased in workers (Popp *et al.*, 1997). Eighty-nine French coke-oven workers were compared with 44 power plant workers for *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels and genetic polymorphisms to *CYP1A1*, *GSTM1* and *GSTT1* genes. Higher levels of DNA adducts were detected in individuals with the combined *CYP1A1*(*1/*2 or *2A/*2A)-*GSTM1*-nul genotype (Rojas *et al.*, 2000). Non-tumorous lung tissues from 20 lung cancer patients and white blood

cells from 20 coke-oven workers were examined for levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts by HPLC/fluorescence analyses and for genetic polymorphisms. All subjects were current smokers. Subjects with the combination of homozygous mutated *CYP1A1* (MspI/MspI) and *GSTM1*0/*0* genotypes had higher levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels than those with *CYP1A1* and *GSTM1* wild-types (Rojas *et al.*, 1998). Twenty coke-oven workers, 30 graphite electrode-producing plant workers and 47 control subjects were compared for 8-oxo-deoxyguanosine levels, DNA damage by the alkaline single-cell comet assay and genetic polymorphisms in their white blood cells. Levels of 8-oxo-deoxyguanosine in white blood cells and DNA damage in lymphocytes were higher in coke-oven workers and graphite electrode-producing plant workers than in control subjects. The polymorphisms of the genes *CYP1A1*, *GSTM1*, *GSTT1* and *GSTP1* showed no association with the biomarkers of effect (Marczynski *et al.*, 2002). Urinary 8-oxo-deoxyguanosine levels were higher in 55 Taiwanese topside coke-oven workers than in 162 sideoven coke-oven workers (Wu *et al.*, 2003). The influence of four polymorphisms of nucleotide excision-repair genes and of *GSTT1* on levels of *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adducts was studied in lymphocyte and monocyte fractions of white blood cells from 67 Polish coke-oven workers by HPLC/fluorescence analyses. The increase in *anti*-benzo[*a*]pyrene-7,8-diol-9,10-oxide–DNA adduct levels was significantly related to lack of *GSTM1* activity and to the low nucleotide DNA excision-repair capacity of the *XPC-PAT*$^{+/+}$ genotype (Pavanello *et al.*, 2005). The peripheral blood lymphocytes of coke-oven workers (143) and non-coke-oven workers (50) were studied for DNA damage by the alkaline comet assay and for genetic polymorphisms of *XRCC1* and *ERCC2* DNA excision-repair genes. DNA damage was significantly greater in coke-oven workers than in non-coke-oven workers and greater in coke-oven workers with the GA genotype of the G27466A polymorphism of *XRCC1* than those with the GG genotype (Leng *et al.*, 2004a). Peripheral blood lymphocytes from 141 coke-oven workers and 66 non-coke-oven worker controls were studied for cytokinesis-block micronucleus frequencies and the effects of genetic polymorphisms. The micronucleus frequency was significantly higher in coke-oven workers than in control workers. Among the coke-oven workers, the *mEH His*^{113} variant genotype exhibited a significantly lower frequency of micronuclei than the *Tyr*^{113}*/Tyr*^{113} genotype; the low-microsomal epoxide hydrolase activity phenotype exhibited a lower frequency than the high-microsomal epoxide hydrolase activity phenotype; the *GSTP1 Val*^{105}*/Val*^{105} genotype exhibited a higher frequency than the *GSTP1 Ile*^{105}*/Ile*^{105} or *Ile*^{105}*/Val*^{105} genotypes; and a joint effect of high –microsomal epoxide hydrolase activity phenotype and *GSTM1*-null genotype on frequencies of micronuclei was also found (Leng *et al.*, 2004b). The frequency of mutants at the *HPRT* locus (6-thioguanine resistance) in lymphocytes from a population of 43 coke-oven workers was compared with that of a group of 26 non-exposed workers. A non-significant increase in frequency was observed in the exposed group compared with the non-exposed group. The percentages of the different types of gene alteration were similar in exposed and non-exposed subjects based on an analysis of mutations in 161

HPRT clones derived from exposed and non-exposed workers. Only the frequency of splice mutations in mutant clones derived from coke-oven workers was higher than that in controls (Zanesi *et al.*, 1998). No difference in the plasma levels of either p53 (mutated or wild-type) or $p21^{WAF1}$ protein was found between 66 exposed Czech coke-oven workers and 49 controls; however, significantly higher levels of p53 and $p21^{WAF1}$ protein were found in the group exposed to higher levels of carcinogenic PAHs compared with the group exposed to lower levels. Overall, a negative correlation between the levels of p53 protein and personal exposure to carcinogenic PAHs was found (Rossner *et al.*, 2003). Cytogenetic markers (chromosomal aberrations, sister chromatid exchange, cells with a high frequency of sister chromatid exchange, the heterogeneity index of sister chromatid exchange and genetic polymorphism of genotypes *GSTM1* and *NAT2*) were evaluated in the peripheral lymphocytes of 64 coke-oven workers and 34 machine worker controls from the same plant. All the cytogenetic markers and sister chromatid exchange were significantly increased in the exposed compared with the control group even when smoking status was taken into account. No effects of *GSTM1* or N-*acetyltransferase* (*NAT2*) genotypes (individually or in combination) on the cytogenetic markers were observed (Kalina *et al.*, 1998).

4.3 Potentially susceptible subpopulations

4.3.1 *Polymorphisms*

(a) Introduction

Individuals who are deficient in enzymes that activate PAHs to reactive metabolites may be at lower risk for chemical carcinogenesis (Nebert *et al.*, 1999; Bouchardy *et al.*, 2001; Kiyohara *et al.*, 2002a; Daly, 2003; Nebert, 2005b), whereas those who are deficient in enzymes that detoxicate reactive metabolites of PAHs may have a higher risk (Bartsch *et al.*, 2000; Williams, 2001; see Table 4.3).

A large number of epidemiological studies have been conducted to ascertain whether genetic polymorphisms of drug-metabolizing enzymes are related to cancer susceptibility in humans (Bartsch *et al.*, 2000; Williams & Phillips, 2000; Kiyohara *et al.*, 2002b; Daly, 2003). Some showed positive relationships between genetic polymorphisms of particular enzymes and the occurrence of cancers in selected organs (Bartsch *et al.*, 2000; Williams & Phillips, 2000), but others suggested that none of the roles of these genetic polymorphisms can be determined with respect to cancer susceptibility, even when the same combination of enzymes and sites of cancers are compared (Kiyohara *et al.*, 2002b). There are many race-related differences in genetic polymorphisms of drug-metabolizing enzymes in humans, and these phenomena may explain in part why such different results were obtained (Bartsch *et al.*, 2000; Inoue *et al.*, 2000; Williams & Phillips, 2000; Kiyohara

Table 4.3. Genetic polymorphisms in polycyclic aromatic hydrocarbon-metabolizing enzymes and susceptible subpopulations

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
AKR1C3	Aldo-keto reductase 1C3; dihydrodiol dehydrogenase	*AKR1C3*Gln5His*	Residents in Xuan Wei, China, exposed to smoky coal (113 lung cancer cases/119 controls)	*AKR1C3*Gln/Gln* genotype [1.84-fold risk for lung cancer; 95% CI, 0.98–3.45]	Lan *et al.* (2004)
SULT1A1[a]	Phenol sulfotransferase	*SULT1A1*2*	Ethnically homogeneous Han Chinese exposed to indoor cooking and tobacco smoke (805 lung cancer cases/809 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [1.85-fold risk for lung cancer; 95% CI, 1.44–2.37]	Liang *et al.* (2004)
		*SULT1A1*2*	Caucasians with lung cancer versus matched controls exposed to tobacco smoke (463 lung cancer cases/485 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [1.41-fold risk for lung cancer; 95% CI, 1.04–1.91]	Wang *et al.* (2002)
		*SULT1A1*2*	Population in Brescia, Italy, exposed to tobacco smoke (201 bladder cancer cases/214 controls)	*SULT1A1*Arg/His*+ *SULT1A1*His/His*, genotypes [0.67-fold risk for bladder cancer; 95% CI, 0.45–1.03]	Hung *et al.* (2004)
		*SULT1A1*1* (fast sulfonation)	Patients from 8 Dutch hospitals exposed to tobacco smoke (431 colorectal cancer cases/432 controls)	*SULT1A1(*1/*1)* (synonymous) combination within smoking for more than 25 years doubled risk for colorectal adenomas [4.32; 95% CI, 1.59–11.77 for more than 25 years smoking compared with [1.64; 95% CI, 0.69–3.90 for never smokers]	Tiemersa *et al.* (2004)
UGT1A1	UDP-glucuronosyltransferase	*UGT1A1*28* (non-coding)	Population in Shanghaî, China (1047 breast cancer cases/1083 controls)	*UGT1A1*28* [1.7; 95% CI, 1.0–2.7]	Adegoke *et al*. (2004)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
NQO1	NAD(P)H quinone oxido-reductase 1	*NQO1*2*	Patients from bronchoscopy clinics in the UK (82 lung cancer cases/145 controls)	*NQO1**2 (heterozygote); one variant allele gave 4-fold increase of small-cell lung cancer [3.80; 95% CI, 1.19–12.1]	Lewis *et al.* (2001)
		*NQO1*2/*2*	Lung cancer patients in Nanjing, China, and Chinese controls in Sweden (84 lung cancer cases/84 controls)	No association with lung cancer	Yin *et al.* (2001)
		*NQO1*2/*2*	Non-Hispanic white colorectal cancer patients (725 colorectal cancer cases/729 controls)	*NQO1*2/**2 with variant *CYP1A1* increased risk for adenoma in smokers [17.4; 95% CI, 7.8–79.8]	Hou *et al.* (2005)
		*NQO1*2/*2*	Lung cancer patients and controls from Denmark (265 lung cancer patients/272 controls)	No association with lung cancer	Sorensen *et al.* (2005)
		*NQO1*2*	Lung cancer patients and controls from Sweden (524 lung cancer patients/530 controls)	With variant *CYP1A1*, in smokers, increased risk of squamous cell carcinoma [3.54; 95% CI, 0.88–14.3]	Alexandrie *et al.* (2004)
CYP1A1	Cytochrome P450 1A1	*CYP1A1*2A* *CYP1A1*2C*	Monozygous rare allele in Asians	Increased risk for lung cancer for patients with genotype C [odds ratio, 7.31]	Kawajri & Fujii-Kuriyama (1991)
		*CYP1A1*3*	African–Americans; 15–20% frequency	Lung adenocarcinoma	Taîolì *et al.* (1995a)
CYP1B1	Cytochrome P450 1B1	*CYP1B1*2*	Japanese and Caucasian populations (336 breast cancer cases/330 lung cancer cases/324 controls)	Increased susceptibility to breast and lung carcinoma with a higher penetrance in Caucasians	Watanabe *et al.* (2000)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
EH	Epoxide hydrolase	*EH3* *EH4*	French Caucasian population (150 lung cancer cases/172 controls)	*EH3 His/His* genotype; decreased risk for lung cancer	Benhamou *et al*. (1998)
			Meta-analysis (2078 lung cancer cases/3081 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.98; 95% CI, 0.72–1.35] *EH4 Arg/Arg* genotype; no risk for lung cancer [1.00; 95% CI, 0.71–1.41]	Lee *et al*. (2002)
			Pooled analysis (986 lung cancer cases/1633 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.70; 95% CI, 0.51–0.96]	
			Lung cancer patients and controls from Austria (277 lung cancer patients/496 controls)	*EH3 His/His* genotype; decreased risk for lung cancer [0.38; 95% CI, 0.20–0.75]	Gsur *et al*. (2003)
			Caucasian with breast cancer versus matched controls (267 breast cancer cases/293 controls	No association with breast cancer	de Assis *et al*. (2002)
			Caucasian patients with lung cancer (182 lung cancer patients/365 controls)	Increased risk for lung cancer [2.3; 95% CI, 1.2–4.3]	Park, J.Y. *et al*. (2005)
			Patients with advanced adenoma in the distal colon (772 colon cancer patients/777 controls)	Increased risk for colorectal adenoma $_{113}$*Tyr/Tyr* genotype [1.5; 95% CI, 1.0–2.2]; $_{139}$*Arg/Arg* genotype [1.4; 95% CI, 0.8–2.5]	Huang *et al*. (2005)

Table 4.3 (contd)

Gene	Enzyme	Polymorphism	Subpopulation and exposure to PAH	Associated risk	Reference
GST	Glutathione *S*-transferase	*GSTM1* *GSTP1* *GSTT1*	Patients with breast cancer (2048 breast cancer cases/1969 controls)	No risk for breast cancer: [0.98; 95% CI, 0.86–1.12] with the *GSTM1* null; [1.01; 95% CI, 0.79–1.28] with *GSTP1* heterozygous mutants; [0.93; 95% CI, 0.62–1.38] with *GSTP1* homozygous mutants	Vogl *et al*. (2004)
		GSTM1	Patients with epithelial ovarian cancer (285 ovarian cancer cases/299 controls)	*GSTM1* null genotype; increased risk of ovarian cancer [2.04; 95% CI, 1.01–4.09]	Spurdle *et al*. (2001)
			Patients with prostate cancer (206 prostate cancer cases/194 controls)	*GSTM1* null genotype; decreased risk of prostate cancer [0.64; 95% CI, 0.43–0.95]	Kidd *et al*. (2003)

[a]*SULT1A1*1*, name of gene for which the nucleotide in original coding sequence is synonymous; *SULT1A1*2*, name of gene for which the nucleotide in the original sequence is non-synonymous; *SULT1A1*2/*2*, name of gene for which the nucleotide in the original sequence and its allele are non-synonymous.
Note: Susceptibilities due to multi-gene polymorphisms were not considered.
CI, confidence interval; NAD(P)H, nicotinamide adenine dinucleotide phosphaste; UDP, uridine 5′-diphosphate

et al., 2002b). Other factors such as age, sex, race, tobacco smoking, alcohol drinking and several environmental factors that modulate (induce or inhibit) drug-metabolizing activities affect the differences in individual susceptibilities towards cancer (Daly, 2003) and indicate that complex interactions occur between multiple genes and the environment.

Individuals have different and unique combinations of drug-metabolizing enzymes in their organs (Nebert *et al.*, 1999; Daly, 2003). For example, some people have enzymes that are defective for the activation of PAHs and normal enzymes that detoxicate PAHs, whereas others have normal enzymes that activate PAHs and enzymes that are defective for the detoxication of reactive PAH metabolites (Garte *et al.*, 2001; Murata *et al.*, 2001; Yin *et al.*, 2001; Kiyohara *et al.*, 2002a; Hung *et al.*, 2004; Lodovici *et al.*, 2004; Masson *et al.*, 2005). In these cases, it is difficult to interpret from epidemiological studies which polymorphisms of drug-metabolizing enzymes are involved in susceptibility to human cancer. In many cases, individuals have different combinations of polymorphic enzymes that activate and/or detoxicate PAHs and metabolites (Le Marchand *et al.*, 1998; Quiñones *et al.*, 1999; Grzybowska *et al.*, 2000; Nerurkar *et al.*, 2000; Olshan *et al.*, 2000; Kiyohara *et al.*, 2002b; Hashibe *et al.*, 2003; Sarmanova *et al.*, 2004; Masson *et al.*, 2005).

(b) Polymorphism of CYP1A1

CYP1A1 is mainly expressed in extrahepatic organs and participates in the activation of carcinogenic PAHs including benzo[*a*]pyrene (Pelkonen & Nebert, 1982; Kawajiri & Fujii-Kuriyama, 1991; Shimada *et al.*, 1992).

Of 11 alleles of *CYP1A1* genetic polymorphisms reported to date (see http://www.imm.ki.se/CYPalleles), four types of genetic polymorphism of *CYP1A1* have been reported to be related to susceptibility to cancer (Kawajiri & Fujii-Kuriyama, 1991). Two genetically linked polymorphisms of the *CYP1A1* gene — the *Msp*I polymorphism (*CYP1A1*2A*) located in the 3'-flanking region and the *Ile-Val* polymorphism (*CYP1A1*2C*) at amino acid residue 462 in the haeme-binding region — were first reported to be associated with susceptibility to tobacco smoking-associated squamous-cell carcinoma of the lung in Japanese populations (Hayashi *et al.*, 1991; Nakachi *et al.*, 1993; Kawajiri *et al.*, 1996). Three genotypes of the *Msp*I polymorphism were found, namely genotype A (dominant homozygous allele), genotype B (heterozygote) and genotype C (homozygous rare allele). Type C was found at high levels among lung cancer patients in Japanese and Asian populations, but not among those in non-Asian populations (Kawajiri & Fujii-Kuriyama, 1991; Kiyohara *et al.*, 2002b). A lower incidence of *CYP1A1* variants in the latter populations has been reported (London *et al.*, 1995; Ishibe *et al.*, 1998; London *et al.*, 2000).

A *CYP1A1*3* polymorphism has been reported, with a single base change (adenine to guanine) in the 3′-noncoding region (Crofts *et al.*, 1994; London *et al.*, 1995; Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002a). No linkage between *CYP1A1*3* and either *CYP1A1*2A* (*Msp*I) or *CYP1A1*2C* (*Ile-Val*) polymorphisms was observed (London *et al.*, 2000). This race-specific polymorphism in African–Americans, with frequencies of 15–20% for

*CYP1A1*2*, is associated with an increased risk for adenocarcinoma (Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002b), although contrasting results have been obtained (Taioli *et al.*, 1995a; Kiyohara *et al.*, 2002b; Nagata & Yamazoe, 2002; Daly, 2003). Rare cases of this polymorphism have been reported in Japanese and Caucasians (Inoue *et al.*, 2000; Kiyohara *et al.*, 2002b). The *CYP1A1*5* polymorphism (*Thr461Asn*) is reported to be unrelated to susceptibility to lung cancer (Cascorbi *et al.*, 1996).

Race-related differences in several types of polymorphism in the *CYP1A1* gene have been reported (Hayashi *et al.*, 1991; Kelsey *et al.*, 1994; Taioli *et al.*, 1995b; Inoue *et al.*, 2000). Inoue *et al*. (2000) showed that the occurrence of *Msp*I (*CYP1A1*1* polymorphism) and *Ile-Val* (*CYP1A1*2* polymorphism) types of *CYP1A1* genetic polymorphism was more frequent in Japanese populations than in Caucasians. No cases of *CYP1A1*4* polymorphism were found in Japanese populations, whereas two of 45 Caucasians had a heterozygous m4-mutant (C4887A at exon 7) (Inoue *et al.*, 2000). The *CYP1A1*3*-type polymorphism, a type specific for black Africans, was not detected in either Japanese or Caucasian populations (Crofts *et al.*, 1993; Cascorbi *et al.*, 1996; Aynacioglu *et al.*, 1998; Inoue *et al.*, 2000).

(c) Polymorphism of CYP1B1

Among 26 CYP1B1 alleles determined (see http://www.imm.ki.se/CYPalleles), different *CYP1B1* polymorphisms with amino acid changes at *Arg48Gly*, *Ala119Ser*, *Leu432Val*, *Ala443Gly* and *Asn453Ser* have been studied in relation to susceptibility to cancer (Stoilov *et al.*, 1997; Bailey *et al.*, 1998; Fritsche *et al.*, 1999; Bejjani *et al.*, 2000; Watanabe *et al.*, 2000; Ko *et al.*, 2001; Aklillu *et al.*, 2002; Sørensen *et al.*, 2005). Several combinations of these polymorphisms lead to various halotypes — *CYP1B1*2* (*Arg48Gly*, *Ala119Ser*), **3* (*Leu432Val*), **4* (*Asn453Ser*), **5* (*Arg48Gly*, *Leu432Val*), **6* (*Arg48Gly, Ala119Ser, Leu432Val*) and **7* (*Arg48Gly, Ala119Ser, Leu432Val*, *Ala443Gly*) (Aklillu *et al.*, 2002; Nagata & Yamazoe, 2002).

Watanabe *et al*. (2000) studied the frequency of *CYP1B1*2* (*Ala-Ser*) and *CYP1B1*3* (*Leu-Val*) polymorphisms in 336 breast and 330 lung cancer patients and 324 randomly selected healthy controls in a Japanese population. Their results showed that the former polymorphism is associated with genetically determined susceptibility to cancer of the breast or squamous-cell carcinoma of the lung but that the *Leu-Val* polymorphism did not show any association with the cancers.

Several studies have reported the relationship between *CYP1B1* polymorphisms and risk for breast cancer (Zheng *et al.*, 2000; Rylander-Rudqvist *et al.*, 2003), squamous-cell neck cancer (Ko *et al.*, 2001) and lung cancer (in combination with *GST1*, *NQO1* and *NAT2*) (Inoue *et al.*, 2000; Kiyohara *et al.*, 2002b; Sørensen *et al.*, 2005) in various ethnic populations. Rylander-Rudqvist *et al*. (2003) found no association between *CYP1B1*1*, **2*, **3* or **4* alleles and risk for breast cancer in a study of 1521 cases and 1498 controls. No association of polymorphisms of *CYP1B1* or other genes (including *GST*, *NQO1* and *NAT 2*) and risk for lung cancer was reported (Sørensen *et al.*, 2005).

Inoue *et al*. (2000) showed that the incidence of the genetic polymorphism in the *CYP1B1* gene is more frequent in Caucasians than in Japanese populations. The frequency of the *CYP1B1*2*- and *CYP1B1*3*-type polymorphisms was expressed by 28.9% and 37.5%, respectively, in Caucasian and by 14.1% and 21.8%, respectively, in Japanese. No *CYP1B1*4*-type polymorphisms were detected in the Japanese population, whereas 24% of Caucasians had this allele mutant (Inoue *et al*., 2000).

(d) CYP1A2, CYP2C9 and 3A4

CYP1A2 is mainly expressed in the liver (Guengerich & Shimada, 1991; Shimada *et al.*, 1994). This is a major enzyme involved in the activation of aryl- and heterocyclic amines to carcinogenic products (Shimada *et al.*, 1989a,b; Shimada & Guengerich, 1991; Lang *et al.*, 1994). CYP1A2 also catalyses the oxidation of PAHs, although usually at much slower rates than CYP1A1 and 1B1 (Shimada *et al.*, 1989b; Shimada & Guengerich, 1991; Shimada *et al.*, 2001a).

Little is known about the relationships between *CYP1A2* polymorphisms and susceptibility to cancer (Chida *et al.*, 1999; Huang *et al.*, 1999; Aklillu *et al.*, 2003; Murayama *et al.*, 2004; Li *et al.*, 2006).

Other CYP enzymes, including CYP2C9 and 3A4, have been shown to play a role in the metabolism of PAHs, particularly in the liver where these enzymes are expressed at high levels (Yun *et al.*, 1992; Shimada *et al.*, 2001a). The implications of polymorphisms of these CYPs in susceptibility to cancer in humans are not clear at present (Garcia-Martin *et al.*, 2002a,b; Plummer *et al.*, 2003).

(e) Epoxide hydrolase

Microsomal epoxide hydrolase (mEH) plays two roles in the metabolism of PAHs: one is detoxication of various reactive PAH metabolites and the other is activation of PAH epoxides to form proximate PAH diols that are finally activated to ultimate carcinogenic metabolites by CYP (Jerina & Dansette, 1977; Lu & West, 1979; Pelkonen & Nebert, 1982; Hassett *et al.*, 1994; Hecht, 2002b).

Two genetic polymorphisms, a *Tyr113His* substitution in exon 3 (*EH3*) and a *His139Arg* substitution in exon 4 (*EH4*), have been reported in relation to susceptibility to cancer (Omiecinski *et al.*, 2000; Kiyohara *et al.*, 2002b; Lee *et al.*, 2002; Park, J.Y. *et al.*, 2005). The exon 3 polymorphism of the *mEH* gene has been reported to be associated with a significantly decreased risk for lung cancer (Benhamou *et al.*, 1998; Lee *et al.*, 2002; Gsur *et al.*, 2003). However, there was no significant association of *mEH* polymorphisms with susceptibility to breast cancer in Caucasian populations (de Assis *et al.*, 2002). A significant increase in risk for lung cancer has been reported with the exon 4 genotype (Park, J.Y. *et al.*, 2005). Reports also indicate that the polymorphisms of *mEH* at exon 3 and 4 are associated with colorectal adenoma (Huang *et al.*, 2005).

(f) Glutathione S-*transferase*

The role of genetic polymorphisms of *GST* genes in influencing susceptibility to cancer is complicated (Strange *et al.*, 2001). GST enzymes participate in the detoxication of reactive metabolites of PAHs and other xenobiotics (Strange *et al.*, 2001). Three types of genetic polymorphism of *GSTM1*, *T1* and *P1* have been examined in relation to susceptibility to cancer in humans (Strange *et al.*, 2001; Wang *et al.*, 2003). A large study with 1969 controls and 2048 cases of breast cancer showed that none of the single gene *GST* polymorphisms (e.g. *GSTM1*, *T1* or *P1*) confer a substantial risk for breast cancer to its carriers (Vogl *et al.*, 2004), although several studies have reported that one or two alleles of *GST* polymorphisms are related to susceptibility to cancer at other sites (Spurdle *et al.*, 2001). Deletion of *GSTM1* may result in an increase in the incidence of ovarian cancer (Spurdle *et al.*, 2001), a decrease in the incidence of prostate cancer (Kidd *et al.*, 2003) and enhance susceptibility to cancer induced by air pollution from indoor coal combustion emissions (Lan *et al.*, 2000).

(g) Sulfotransferase

SULTs play an important role in the conjugation of many endogenous and exogenous substrates (Raftogianis *et al.*, 1999; Wang *et al.*, 2002; Daly, 2003). Relationships between the *SULT1A1* polymorphism (*Arg213His*) and susceptibility to cancer have been reported (Raftogianis *et al.*, 1999; Moreno *et al.*, 2005). The association between this single-nucleotide polymorphism (G→A) and risk for lung cancer was assessed in 805 patients with lung cancer and 809 controls in a hospital-based case–control study in the Chinese Han population. Compared with the *GG* genotype, the variant *SULT1A1* genotype (*638GA* or *AA*) was associated with a significantly increased overall risk for lung cancer [odds ratio, 1.85; 95% CI, 1.44–2.37] (Liang *et al*., 2004). Stratification analysis showed that the increased risk for lung cancer related to the variant *SULT1A1* genotypes was more pronounced in younger subjects and limited to smokers rather than nonsmokers. The risk for lung cancer for the variant genotypes was increased significantly with cumulative dose of smoking (Liang *et al*., 2004). In a second study, the association between the variant A-allele and lung cancer was examined in 463 Caucasian lung cancer cases and 485 frequency-matched Caucasian controls (Wang *et al*., 2002). It was found that there was an overall significant difference between cases and controls when adjusted by sex and smoking status (adjusted odds ratio, 1.41; 95% CI, 1.04–1.91) and was higher in women (adjusted odds ratio, 1.64; 95% CI, 1.06–2.56) than in men (adjusted odds ratio, 1.23; 95% CI, 0.80–1.88). In both these studies, the *SULT1A1*2* (low sulfonation phenotype) was associated with an increased risk which indicated the role of this enzyme in detoxication. In a third study, the *SULT1A1*1* synonymous single-nucleotide polymorphism (fast sulfonation phenotype) was modestly associated with an increased risk for colorectal carcinoma in smokers, which was increased in the presence of the *NAT2* slow-acetylator phenotype which indicated that this enzyme can also lead to carcinogen activation (Tiemersma *et al*., 2004). In another study, the association of

polymorphisms in *GSTM1, GSTT1, NAT, SULT1A1* and *CYP1B1*with tobacco smoking and the incidence of urinary bladder cancer in Bresica, northern Italy, was examined. The *SULT1A1*2* (low sulfonation phenotype) showed a modest protective effect consistent with a role in aromatic amine activation (Hung *et al*., 2004) .

(h) UDP-Glucuronosyltransferases

Of the hepatic UGTs, only UGT1A1 and UGT1A9 exhibit activity against the 7*R*,8*R-trans*-dihydrodiol of benzo[*a*]pyrene, the precursor to the highly mutagenic (+)-*anti*-benzo[*a*]pyrene diol epoxide (Fang *et al*., 2002; Fang & Lazarus, 2004). The *UGT1A1*28* allelic variant contains an additional (TA) dinucleotide repeat in the 'TATTA' box [(TA)6 >(TA)7] of the UGT1A1 promoter which has been linked to decreased expression of the *UGT1A1* gene. Significant decreases in UGT1A1 protein and bilirubin-conjugating enzyme activity were observed in normal liver microsomes from subjects with the homozygous polymorphic *UGT1A1(*28/*28)* genotype variant compared with subjects homozygous for the wild-type *UGT1A1*1* allele. Significant decreases in the glucuronidation of the 7*R*,8*R-trans*-dihydrodiol of benzo[*a*]pyrene were observed in subjects who had the *UGT1A1 (*28/*28*) genotype compared with subjects who had a wild-type *UGT1A1 (*1/*1)* genotype (Fang & Lazarus, 2004). An association of this genotype with an increased risk for cancer induced by benzo[*a*]pyrene was suggested.

(i) *Aldo-keto* reductase

The genotic variability of AKR had been presented previously (see section 4.1.2b). The association between *AKR1C3*Gln5H* is single-nucleotide polymorphism and risk for lung cancer was assessed in a population-based case–control study of 119 cases and 113 controls in Xuan Wei, China, an area that has one of the highest rates of lung cancer in China due to the use of smoky coal for heating and cooking. The *AKR1C3*Gln/Gln* genotype was associated with a 1.84 fold [95% CI, 0.98–3.45] increased risk for lung cancer (Lan *et al*., 2004).

(ii) *NAD(P)H quinone oxidoreductase 1*

The homozygous *NQO1*2/*2* allele is essentially a null phenotype and provides a tool to assess the potential chemoprotective role of the enzyme against xenobiotics (Ross *et al*., 2000). Only one study has been performed on the association of the *NQO1*2/*2* allele with the incidence of lung cancer (Lewis *et al*., 2001). The *NQO1* genotype was related to risk for lung cancer in patients who attended a Manchester (United Kingdom) bronchoscopy clinic; those who carried at least one variant allele were found to have an almost fourfold increased risk for developing small-cell lung cancer (adjusted odds ratio, 3.80; 95% CI, 1.91–12.1). No association between *NQO1* genotypes and risk for non-small-cell lung cancer was found. Furthermore, the excess risk for small-cell lung cancer associated with non-wild-type *NQO1* genotypes was only apparent in heavy smokers who had a >10-fold increased risk (adjusted odds ratio, 12.5; 95% CI, 2.1–75.5). These results

suggest that *NQO1* is involved in the detoxication of those carcinogens that are associated with the development of small-cell lung carcinoma (Lewis *et al*., 2001).

In other studies aimed at identifying susceptible populations, genetic polymorphisms in *NQO1* were scored together with those in other enzymes that affect PAH metabolism/activation. In one study, the association of the *NQO1*2/*2*, *CYP1A1*2A*, *HYL1*2* (epoxide hydrolase) and *HYL1*3* alleles were not associated with the risk for lung cancer in Najing Province in China (Yin *et al*., 2001). However, when the data were stratified, it was found that smokers carrying the *HYL1** allele had a higher relative risk for lung cancer. In a second study, the association of cigarette smoking with the risk for advanced colorectal adenoma in relation to *CYP1A1 Val462* and *NQO1*2/*2* polymorphic variants was investigated in 725 non-Hispanic Caucasian cases with advanced colorectal adenoma of the distal colon and 729 gender- and ethnicity-matched controls. Subjects who carried either allele had a weakly associated risk for colorectal adenoma; however, those who carried both alleles had an increased risk versus nonsmokers, particularly among recent and current smokers (odds ratio, 17.4; 95% CI, 3.8–79.8; p for interaction = 0.02) (Hou *et al*., 2005). A population-based cohort study investigated the occurrence of lung cancer in relation to polymorphisms in *CYP1B1*, *GSTA1*, *NQO1* and *NAT2*. Among 54 200 Scandinavian cohort members, 265 lung cancer cases were identified and a subcohort that comprised 272 individuals was used for comparison. No overall associations were found between the polymorphisms and risk for lung cancer. The *NAT2* fast-acetylator genotype seemed to be protective against lung cancer in light smokers but not in heavy smokers (>20 cigarettes/day) (Sørenson *et al*., 2005). In a fourth study, the modifying influence of *CYP1A1*, *GSTM1*, *GSST1* and *NQO1* polymorphisms on risk for lung cancer was studied in 524 lung cancer cases and 530 control subjects. No evidence of an influence of genetic polymorphisms on risk for lung cancer was found. In smokers, however, there was a suggestion that the variant *CYP1A1 Val462* and *NQO1**2 genotypes may confer an increased risk for squamous-cell carcinoma (Alexandrie *et al*., 2004).

4.3.2 *Age-related susceptibility*

No data were available in humans on age-dependent susceptibility to cancer that results from exposure to PAHs.

Studies in animals indicate that exposure early in life to individual PAHs, including benzo[*a*]pyrene and DMBA, may lead to higher rates of tumour formation later in life. Vesselinovitch *et al*. (1975) showed that a significantly higher incidence of liver hepatomas was observed in male $B6C3F_1$ mice that were treated intraperitoneally with 75 and 150 μg/g bw benzo[*a*]pyrene on postnatal days 1, 15 or 42. Lung tumours were more prevalent in male and female $B6C3F_1$ mice treated with benzo[*a*]pyrene at birth than in animals treated at 15 or 42 days of age. Similar results were found for the incidence of liver tumours in $C3AF_1$ mice under the same experimental conditions. Walters (1966) observed a significantly higher incidence of lung tumours when 15 μg

DMBA were administered subcutaneously to newborn (1-day-old) BALB/c mice compared with suckling (2–3 weeks of age) and adult mice. Pietra *et al*. (1961) observed an increased incidence of lymphomas and lung tumours when 30–900 μg DMBA were administered subcutaneously to newborn mice compared with adult (8-week-old) mice.

The age-dependent susceptibility to benzo[*a*]pyrene and DMBA may be dependent on their ability to act as mutagens. An analysis by the Environmental Protection Agency (2005) indicated that there can be greater susceptibility for the development of tumours as a result of exposures to mutagenic chemicals early in life compared with later stages of life (see also Barton *et al*., 2005). In general, there are limited data to elucidate the mode(s) of action that leads to differences in tumour incidence following exposure early in life or later. Differences in the capacity to metabolize and clear chemicals at different ages can result in larger or smaller internal doses of the active agent(s), which either increases or decreases risk (Ginsberg *et al*., 2002). Several studies have shown increased susceptibility of weanling animals to the formation of DNA adducts following exposure to vinyl chloride (Laib *et al*., 1989; Morinello *et al*., 2002a,b), and in-vivo transplacental micronucleus assays have indicated that fetal tissues are more sensitive than maternal tissues to the induction of micronuclei by mutagenic chemicals (Hayashi *et al*., 2000).

As outlined by the Environmental Protection Agency (2005), some generalized aspects that potentially lead to childhood susceptibility may apply to benzo[*a*]pyrene and other PAHs that act through a mutagenic mechanism. (*i*) More frequent cell division during development can result in enhanced fixation of mutations due to the reduced time available for repair of DNA lesions; clonal expansion of mutant cells produces a larger population of mutants (Slikker *et al*., 2004). (*ii*) Some embryonic cells, such as brain cells, lack key DNA repair enzymes. (*iii*) Some components of the immune system are not fully functional during development (Holladay & Smialowicz, 2000; Holsapple *et al*., 2003). (*iv*) Hormonal systems operate at different levels during different stages of life (Finch & Rose, 1995). (*v*) Induction of developmental abnormalities can result in a predisposition to carcinogenic effects later in life (Birnbaum & Fenton, 2003).

Studies on the carcinogenicity of DMBA indicate a potential period of increased susceptibility during pubertal periods of tissue development. An increased incidence of mammary tumours was found in 5–8-week-old rats treated with DMBA compared with older or younger rats (Meranze *et al*., 1969; Russo *et al*., 1979). This observation corresponds with pubertal development of the mammary tissue, ovarian function that begins between 3 and 4 weeks of age and mammary ductal growth and branching that occur by approximately week 5 of age, all of which are consistent with the 5–8-week susceptible period (Silberstein, 2001).

Several carcinogenicity studies have been conducted on benzo[*a*]pyrene or coal-tar mixtures following neonatal exposure without an adult comparison or in in-utero mouse models. Strain A and C57BL mice were administered single subcutaneous injections of 4 or 6 mg benzo[*a*]pyrene on gestational days 18 and 19 (Nikonova, 1977). The offspring of strain A mice developed lung tumours at a much higher incidence than those of C57BL mice. The incidence of liver tumours was increased above controls only in C57BL

offspring. Direct intraperitoneal administration of 560 nmol benzo[*a*]pyrene in DMSO to neonatal CD-1 mice within 24 h of birth and at 8 and 15 days of age resulted in an increased incidence of liver tumours in males (Wislocki *et al*., 1986), but no liver tumour formation in female mice. This treatment induced a significantly increased incidence of lung tumours in male and female mice.

Groups of 15-day-old male and female B6C3F$_1$ mice received a single intraperitoneal injection of 0.125, 0.25 or 0.375 μg benzo[*a*]pyrene or 7980 μg manufactured gas plant residue in corn oil. Twenty-six weeks after exposure, benzo[*a*]pyrene induced liver tumours in male mice in a dose- and time-dependent manner in the high-dose group. Manufactured gas plant residue induced liver tumours in male mice to a much greater extent than benzo[*a*]pyrene. There was little or no tumour formation in the lung or forestomach with either treatment (Rodriguez *et al*., 1997), using ^{32}P-postlabelling, positive responses for DNA adduct formation were observed in the liver, lung and forestomach of both sexes of mice. However, differences in the time course of adduct accumulation and decline occurred. For example, liver adducts peaked at 24 h and declined over the next 14 days, while forestomach and lung adducts peaked at higher levels at 2–3 days after exposure, then also declined over the next 14 days. Despite the difference in tumorigenicity, adduct levels were similar in male and female mice in all three tissues examined. They indicated that the inability of benzo[*a*]pyrene to induce tumour formation in the lung and forestomach of this strain of mice and in the liver of female animals could not be accounted for in terms of the lack of DNA damage as measured by adduct formation. The authors indicated that the exposure of 15-day-old B6C3F$_1$ mice to low levels of benzo[*a*]pyrene induced well-defined premalignant changes in the liver and hepatocellular carcinomas by 40 weeks after exposure, while single doses of the same carcinogen at 42 days of age did not induce lesions over 2 years after exposure.

Numerous animal studies have been conducted that indicate effects on reproductive and developmental end-points following exposure to mixtures of PAHs and individual PAHs (ATSDR, 1995; IPCS, 1998; see also Section 4.4). These studies suggest susceptibility to the toxicity of PAHs during the prenatal period, a critical time for phase I and II enzyme development. Lack of phase I enzymes may result in protection against the toxicity of benzo[*a*]pyrene, while the lack of phase II enzymes in the presence of phase I enzymes could exacerbate toxicity (see Mukhtar & Bresnick, 1976). Animals as young as 1 day of age can metabolize benzo[*a*]pyrene to a broad spectrum of metabolites (Melikian *et al*., 1989), which allows the possible conclusion that their overall metabolism might not differ significantly from that of adults. These animals have metabolic competence to enable phase I and phase II metabolism (Melikian *et al.*, 1989), although activities may differ between newborn and adult animals.

Lyte and Bick (1985) evaluated the effects of benzo[*a*]pyrene on the generation of antibody-producing cells in young (3–6 months), middle-aged (13–16 months) and old (23–26 months) male mice. Old animals were particularly susceptible to the immunosuppressive effects of benzo[*a*]pyrene.

4.4 Other toxicity

4.4.1 *Phototoxicity*

Sunlight is a complete carcinogen (IARC, 1992) and is responsible for the induction of cutaneous squamous-cell and basal-cell carcinomas in humans. Since PAHs are ubiquitous in the environment and concomitant exposure of humans to PAHs and light is inevitable, the photomutagenicity of these compounds is of considerable importance to human health. Human exposure to PAHs occurs mainly through skin absorption, inhalation or food consumption (IARC, 1983). PAH-contaminated skin may be exposed to sunlight, which is of concern for people who work outdoors and handle products that contain PAHs, such as roofers, tanners and road-construction workers. Because of the multiple aromatic ring systems in PAHs, these compounds can absorb light energy in the UVA region (320–400 nm) and, for some PAHs, also near the visible region (400–700 nm) (Dabestani & Ivanov, 1999) to form reactive species that cause damage to human cellular components. It has been shown that concomitant exposure to PAHs and light can cause DNA single-strand breaks, oxidation of DNA bases and formation of DNA adducts (Yu, 2002). In general, for those PAHs or mixtures of PAHs that exhibit carcinogenic activity in skin, these effects were found to be enhanced when the compounds were tested in combination with UV radiation under certain experimental conditions (Santamaria *et al*., 1966).

From a mechanistic point of view, two pathways result in phototoxicity: (i) dynamic phototoxicity, which involves damage to cells during phototransformation of chemical species. This process includes excited-state energy transfer to biological macromolecules, which results in electron transfer that may convert both the PAH and the biological molecule into free radicals. It also leads to the production of short-lived reactive intermediates such as singlet oxygen, the superoxide free radical and other chemically reactive species (Yu, 2002); and (ii) formation of toxic photoproducts: during photolysis, some relatively light-stable compounds such as quinones and nitro-PAHs are produced, which may be toxic both in the presence and absence of metabolic activation or light-induced activation (Sinha & Chignell, 1983).

Irradiation with UV light (>295 nm) of a mixture of the PAH anthracene and human serum albumin induced covalent binding of anthracene to the protein, which then resulted in the formation of protein–protein cross-links and cell-membrane damage via lipid peroxidation (Sinha & Chignell, 1983). Evidence of damage to cell membranes following irradiation (near UV, 290–400 nm) of benzo[*a*]pyrene, anthracene or 1-nitropyrene mixed with cells has also been reported (Kagan *et al.*, 1989; Tuveson *et al*., 1990; Zeng *et al.*, 2002, 2004). Possible DNA damage induced by the combination of light and a photoreactive chemical includes: (i) single-strand breaks, (ii) double-strand breaks, (iii) release of DNAbases (depurination/depyrimidination), (iv) oxidation of guanine to 8-hydroxy- or 8-oxoguanine, (v) induction of covalent DNA adducts, (vi) induction of DNA–DNA cross-links and (vii) induction of DNA–protein cross-links (Yu, 2002). Most of these forms of damage can be repaired without leading to mutation. To date, several

types of DNA damage caused by the combination of PAHs and light have been studied: the formation of PAH–DNA covalent adducts, DNA single-strand breaks, depurination/depyrimidination and the formation of the oxidative product, 8-hydroxyguanine.

Studies on the photomutagenicity of PAHs (see Table 4.4) indicate that these compounds can be activated by light in the absence of metabolizing enzymes. In particular, six of 16 PAHs tested, including those that are most abundant in the environment — acenaphthene, acenaphthylene, anthracene, benzo[*ghi*]perylene, fluorene and pyrene — were not mutagenic through metabolic activation but were photomutagenic in *S. typhimurium* TA102 (see references cited in Table 4.4). This result may influence the methods of risk assessment of PAHs. At present, the data are insufficient to interpret the results of in-vitro photomutagenicity studies in terms of human health. However, the possible adverse health effects due to skin contamination with PAHs and concomitant exposure to sunlight deserve further study.

Yan *et al*. (2004) determined the photomutagenicity of 16 PAHs on the Environmental Protection Agency priority-pollutant list in *S. typhimurium* TA102 with concomitant irradiation with UVA and visible light (300–800 nm). Positive photomutagenicity (+) was scored (see Table 4.4) when the number of revertant colonies per plate due to combined exposure to light and a PAH exceeded twice that of the control with light only. Strong photomutagenicity (++) was scored when the number of revertant colonies per nanomole of PAH was higher than 2000. As shown in Table 4.4, 11 of the 16 PAHs tested were found to be photomutagenic. Based on the number of revertants per nanomole of test chemical, anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*ghi*]-perylene, indeno[1,2,3-*cd*]pyrene and pyrene were identified as strongly photomutagenic, with a number of revertant colonies per nanomole of PAH in the range of 2000–20 000. The data indicated a fair correlation between observed photomutagenicity and reported carcinogenicity.

Phototoxicity, including photomutagenicity, is closely related to the photochemical reactions that generate reactive PAH intermediates and reactive oxygen species during photolysis (Yu, 2002). Certain PAHs with extended aromatic ring systems can absorb light in the UVA (320–400 nm) and visible (>400 nm) range. Usually, PAHs with three or four aromatic rings absorb UVA light and those with five or more aromatic rings as well as the hydroxyl-, amino- and nitro-substituted PAHs with three or four aromatic rings absorb visible light (Dabestani & Ivanov, 1999). Upon absorption of light energy, PAHs are excited to higher energy states (singlet or triplet) that undergo electron or energy transfer to molecular oxygen, solvents or biological molecules in the cell and generate reactive species. These reactive species or intermediates damage cellular constituents such as the cell membrane, nucleic acids or proteins. This activation pathway is usually similar to the enzymatic activation pathway, in that it converts relatively inert PAHs to reactive species. There is evidence that PAH mixtures and individual PAHs can be phototoxic towards microorganisms, plants, cells and animals. In addition, PAHs are

Table 4.4. Photomutagenicity of 16 polycyclic aromatic hydrocarbons (PAHs) on the priority list of the Environmental Protection Agency and their reported carcinogenicity and mutagenicity

PAH	Photomutagenicity[a]	Carcinogenicity[b]	Mutagenicity[c]
Acenaphthene	+	0	–[d]
Acenaphthylene	+	I	–[d]
Anthracene	++	0	–
Benz[*a*]anthracene	++	++	+
Benzo[*a*]pyrene	++	++	+
Benzo[*b*]fluoranthene	–	++	+[d]
Benzo[*ghi*]perylene	++	I	–[d]
Benzo[*k*]fluoranthene	+	++	+[d]
Chrysene	+	+	+
Dibenz[*a,h*]anthracene	–	++	+
Fluoranthene	–	0	+[d]
Fluorene	+	I	–
Indeno[1,2,3-*cd*]pyrene	++	++[e]	+[d]
Naphthalene	–	I	–
Phenanthrene	–	I	–
Pyrene	++	0	–

Adapted from Yan *et al*. (2004)

[a] PAH is defined as photomutagenic (+ or ++) when the number of revertant colonies due to concomitant exposure to light and the PAH is greater than twice that found in the light-only control. A PAH is defined as strongly photomutagenic (++) if the number of revertant colonies per nanomole of the PAH is more than 2000.

[b] Data summarized from IARC (1983), National Toxicology Program (1993), White (2002) and the Environmental Protection Agency web-site pages for acenaphthylene, naphthalene and fluorene. Carcinogenicity symbols are: (0) no evidence of carcinogenicity; (I) inadequate evidence for evaluation; (+) limited evidence of carcinogenicity in experimental animals; (++) sufficient evidence of carcinogenicity in experimental animals.

[c] Based on McCann *et al*. (1975) using *S. typhimurium* TA98 or TA100 with metabolic activation

[d] Based on Nagai *et al*. (2002) using *S. typhimurium* TA98 with metabolic activation

[e] From the Environmental Protection Agency web site for animal carcinogenicity studies (http://www.epa.gov/NCEA/iris/subst/0457.htm)

generally more toxic when they are exposed to UV light than if they are kept in the dark. For certain PAHs, the increase in toxicity can be six- to sevenfold (Swartz *et al*., 1997).*In vivo*, the formation of covalent PAH–DNA adducts through irradiation with light may compete with enzymatic activation. Indeed, irradiation of benzo[*a*]pyrene or DMBA inside the cells tends to lower the amount of covalent DNA adducts formed by enzymatic activation (Prodi *et al*., 1984), an effect that was mainly attributed to photodegradation of the PAHs.

Photo-oxidation of the unsubstituted PAHs — two-ring naphthalene (Vialaton *et al*., 1999); three-ring anthracene (Mallakin *et al*., 2000) and phenanthrene (Wen *et al*., 2002);

four-ring benz[*a*]anthracene (Dong *et al*., 2002) and pyrene (Sigman *et al*., 1998); and five-ring benzo[*a*]pyrene (Lee-Ruff *et al*., 1988) — can produce respective quinones, ring-open products or hydroxy-substituted products. In addition to quinones, hydroxy- (Koizumi *et al*., 1994), carboxy- (Zeng *et al*., 2002), hydroxymethyl- and formyl-PAHs or methyl-substituted PAHs (Wood *et al*., 1979) are also detected among the photoproducts. Quinones are known sensitizers of reactive oxygen species, but are relatively stable against photo-oxidation. There is no appreciable degradation when 7,12-benz[*a*]-anthracene-quinone is irradiated with UVA light (170 J/cm^2/h) for 60 min. However, during the irradiation, 7,12-benz[*a*]anthracene-quinone can cause DNA strand breaks (Dong *et al*., 2002). Therefore, these quinones are potentially more phototoxic.

Some commercial medicines also contain PAHs. For example, coal tar, a complex mixture of PAHs, is widely used in creams, ointments, lotions and shampoos for the treatment of psoriasis, a common skin disease (Comaish, 1987). The formation of DNA adducts in exposed skin and of chromosomal aberrations in peripheral blood lymphocytes have been shown to occur in psoriasis patients treated with coal tar (Sarto *et al.*, 1989; Schoket *et al.*, 1990; Godschalk *et al*., 1998). Topical application of coal tar to the skin followed by UV radiation, known as the Goeckerman therapy for psoriasis, has been reported to increase the risk for developing skin cancer (Stern *et al.*, 1980), although other studies have found no appreciable increase (Pittelkow *et al*., 1981). UVB radiation and coal tar have an additive effect on the induction of metabolizing enzymes and on the formation of DNA adducts in mouse skin when coal tar is applied before, but not after, irradiation (Mukhtar *et al*., 1986). These effects are difficult to interpret because both UVB and PAHs can activate metabolizing enzymes individually. Since human skin is exposed to light, it is of particular importance and significance to investigate human health risks posed by exposure to the combination of PAHs and light.

4.4.2 *Reproductive and developmental toxicity*

The available studies of human exposure to PAHs suggest that these compounds may affect the developing fetus. These studies have generally focused on the exposure of pregnant women in situations in which PAHs are a known or perceived component of diverse complex mixtures, such as tobacco smoke or ambient air pollution, e.g. in areas of the world that are highly industrialized or burn large amounts of coal (Perera *et al*., 1998, 1999; Šrám *et al*., 1999; Landrigan *et al*., 2004; Perera *et al*., 2004; Wormley *et al*., 2004; Perera *et al*., 2005; Šrám *et al*., 2005; Wolff *et al*., 2005). A lack of information on exposure concentrations, time of exposure during pregnancy and co-exposure to other chemicals potentially confounds the results of these studies. Nevertheless, a pattern of effects has emerged that shows reduced fetal birth weight and length, impaired intrauterine growth and reduced length of gestation.

Studies in experimental animals are available that indicate the reproductive and developmental toxicity of individual PAHs, including anthracene, benz[*a*]anthracene, benzo[*a*]pyrene, chrysene, dibenz[*a*,*h*]anthracene and naphthalene (for reviews, see

ATSDR, 1995; IPCS, 1998). The majority focus on the reproductive and developmental toxicity of benzo[*a*]pyrene. Benzo[*a*]pyrene has been shown to impair male and female reproductive performance and to decrease pup weight in laboratory animals exposed by the oral and inhalation routes. Exposure of pregnant female mice to benzo[*a*]pyrene affected the fertility and the histopathology of the ovaries and testes of their offspring (MacKenzie & Angevine, 1981; Kristensen *et al*., 1995). No standard developmental or two-generation reproductive studies are available on exposure to benzo[*a*]pyrene via the oral route, but several studies have evaluated the reproductive effects of in-utero exposure. The available data show that exposure of pregnant females to benzo[*a*]pyrene affects the fertility of male and female mice exposed *in utero*. Teratogenicity has not been reported based on gross examination, and none of the studies conducted detailed skeletal or visceral examinations of pups. Inhalation studies with benzo[*a*]pyrene in pregnant rats have demonstrated decreased fetal survival, which resulted from a reduction of luteotropic hormone synthesis and release (Archibong *et al*., 2002). Similar studies in male rats showed reduced reproductive capacity due to changes in sperm parameters, impaired epididymal function and disturbed hormonal regulation in the testes (Inyang *et al*., 2003).

4.4.3 *Hepatic and renal toxicity*

The available data on the effects of PAHs other than cancer are sparse except for immunological and reproductive/developmental studies (see Sections 4.2.6 and 4.4.2). No studies were found that examined hepatic or renal toxicity following exposure to PAHs in humans. Numerous animal studies (as reviewed in ATSDR, 1995) have been conducted with individual PAHs, such as benzo[*a*]pyrene, benz[*a*]anthracene, fluoranthene, acenaphthene and fluorene. These studies have shown increases in liver weight but no accompanying overt toxicity. In addition, limited data are available concerning the potential renal toxicity of PAHs (see ATSDR, 1995). Some recent studies indicate that benzo[*a*]pyrene may exhibit limited hepato- and nephrotoxic effects at high doses (De Jong *et al*., 1999; Knuckles *et al*., 2001; Kroese *et al*., 2001).

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