

Overview information for

Benzo-(alpha) pyrene

BENZO-(ALPHA) PYRENE REFERENCES

Author Name	Title	Journal	Volume	Page number(s)	Year
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Polycyclic aromatic hydrocarbons (Benzo[a]pyrene) Toxicological Overview

Key Points

Kinetics and metabolism

- PAHs are absorbed by all routes of exposure
- PAHs are distributed widely throughout the body, with fatty tissues tending to show higher amounts
- metabolites of PAH's are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion

Health effects of acute exposure

- few studies were identified that reported the effects of BaP alone in humans following acute inhalation, ingestion or dermal exposure

Health effects of chronic exposure

- chronic exposure to mixtures of PAHs in air have resulted in a range of respiratory effects, ischemic heart disease, chronic dermatitis, depressed immune system and cancer of the skin and lungs
- BaP amongst other PAHs is able to form DNA adducts which are likely key to their mutagenic potential
- complex mixtures of PAHs which include BaP are considered to be carcinogenic to humans

Summary of Health Effects

PAHs typically occur in complex mixtures and not as individual compounds, BaP is considered to be one of the most toxic PAHs and has been extensively studied. For the general public, the main route of exposure to poly aromatic hydrocarbons (PAHs) is ingestion of food. For smokers, the contribution of smoking to total PAH exposure will be similar to that of food. Inhalation and skin absorption are the main routes of occupational exposure.

PAHs are rapidly distributed throughout the body following all routes of exposure; they may be detected in most tissues minutes to hours following exposure. Benzo[a]Pyrene (BaP) is metabolised by cytochrome P450 enzymes and epoxide hydrolase resulting in a number of metabolites being formed. These metabolites include the reactive epoxide BaP 7,8 diol-9,10-epoxide (BPDE), which is believed to play a role in the carcinogenicity of BaP. Following metabolism PAHs are excreted in the urine and/or faeces depending on their molecular weight.

No data on the acute effects of BaP in humans were identified and few studies were reported in animals. Following acute oral exposure of rats to BaP, effects on the liver were observed.

Following chronic exposure to PAHs in an occupational setting a decrease in lung function was reported, as well as chest pain, respiratory irritation, cough, dermatitis and depressed immune system; although in most cases it was not possible to evaluate the contribution of BaP to such effects. Few adverse effects were observed in rats or hamsters exposed to BaP via inhalation. Following ingestion, myelotoxicity was observed in poor affinity aryl hydrocarbon-receptor (AhR) mice but not in high affinity mice. Hepatotoxicity was also reported.

BaP can cross the placenta; BaP-DNA adducts have been found in foetal cord blood and also in the sperm. Presence of these adducts has been associated with reduced sperm count in men and decreased motor development in infants. BaP was found to cause adverse developmental and reproductive effects in mice and rats. Dietary administration during gestation reduced fertility and fetal abnormalities whereas administration by gavage caused an increase in fetal death and decreased fertility.

Biomarkers of exposure to BaP are seen concurrently with biomarkers of genotoxic effect. The International Agency for Research on Cancer (IARC) has stated that BaP likely contributes to the genotoxic action of complex PAH mixtures that individuals may be exposed to occupationally.

IARC has classified BaP as carcinogenic to humans (Group 1). No epidemiological studies of exposure to BaP alone in man can be identified; however sufficient data exists for exposure to complex PAH mixtures containing BaP.

Introduction

PAHs are a large group of compounds consisting of hydrocarbons containing two or more benzene rings fused together or to other hydrocarbon rings. They are formed during the combustion of carbonaceous material at high temperatures [1]. PAHs typically occur in complex mixtures and not as individual compounds, the process generating the mixture may define its composition. BaP is considered to be one of the most toxic PAHs and has therefore been extensively studied; hence the majority of the data in this document refers to BaP. Unless otherwise specified, "PAHs" will refer to a mixture of PAH compounds (which may include BaP).

Kinetics and Metabolism

PAHs are lipophilic compounds that are readily absorbed from the lungs following inhalation, the gastrointestinal (GI) tract following ingestion and the skin following dermal exposure [2].

PAHs may adsorb onto particulate matter in air. In humans, it was reported that BaP measured in the lungs following inhalation of soot particles was much lower than expected. This may be due to the ability of the pulmonary epithelial cells to metabolise BaP thereby facilitating its absorption and clearance from the lungs [3]. Occupational studies have inferred that inhaled PAHs are absorbed, as urinary metabolites were present in workers exposed to PAHs [2]. The absorption of BaP following inhalation is highly dependent on the type of particles onto which it is adsorbed and the site of deposition in the respiratory tract [1]. Pulmonary absorption often occurs in parallel with mucociliary clearance, by which PAHs that are absorbed onto inhaled particulates are cleared out of the pulmonary tree and subsequently swallowed [3, 4].

PAHs are well absorbed in the GI tract by passive diffusion. The extent of absorption may vary depending of the bioavailability of particles, diet and PAH size (highest for smaller molecule PAHs) [1]. In rats, approximately 40% of absorption occurred through the GI tract following administration of 0.5 µg/kg BaP for 90 minutes directly into the duodenum; 38-58% absorption occurred following administration of BaP given by gavage or in the diet [2].

BaP is efficiently absorbed through the skin of animals [1]. Extensive skin absorption has been demonstrated in mice, as almost all of an applied dose of BaP appeared in the faeces following application to the skin [4]. Similarly, rapid absorption was demonstrated in rats, monkeys and guinea pigs [2].

PAHs are rapidly distributed throughout the body by all routes of exposure; they may be detected in most tissues minutes to hours following exposure [1]. Fatty tissues generally contain more PAHs than other tissues [1, 5]. However, PAHs do not accumulate in the body [1].

BaP can readily cross the placenta following oral, inhalation or dermal exposure. One study reported that when pregnant rats were exposed to BaP via inhalation, an increase in BaP and metabolites was measured in both maternal and fetal blood and tissues. Similarly, BaP

was measured in the fetus when rats were given oral BaP on day 21 of pregnancy [2]. BaP has been detected in human breast milk, particularly in smokers, however animal studies suggest that it does not accumulate in the offspring [6].

Cytochrome P450 enzymes (CYPs) and epoxide hydrolase are the main enzymes involved in PAH metabolism. Many studies have investigated the metabolism of PAHs in tissues and cells following ingestion of food containing PAHs, or inhalation or ingestion of environmental PAHs. The liver has the greatest capacity to metabolise PAHs followed by the lungs, intestinal mucosa, skin and kidneys. BaP stimulates its own metabolism by inducing CYP enzymes via the activation of the AhR. PAHs can also inhibit CYP enzymes [1].

PAHs are initially metabolised to several epoxides by CYP enzymes. The epoxides may spontaneously rearrange to phenols or are converted to dihydrodiols. PAHs are also metabolised to a number of quinones by CYPs. The dihydrodiols are further metabolised by CYPs to 4 optically active isoforms of dihydrodiol epoxides, notable amongst these is the anti-BaP 7,8 diol-9,10-epoxide (anti-BPDE). The stereoisomer (+) anti-BPDE is considered to be the most tumorigenic and predominant metabolite of BaP that forms DNA adducts in mammalian tissues. In addition, PAHs and their reactive metabolites undergo conjugation with sulphate, glutathione (GSH) and glucuronic acid [1, 7].

The route of exposure may influence the toxicity of PAHs. Following oral exposure the compound undergoes first-pass metabolism which reduces the levels of PAHs and metabolites that reach systemic circulation. Inhalation or dermal exposure may result in higher levels of PAHs reaching peripheral tissues as the compounds may bypass the first-pass effect of the liver [1, 2]. Genetic polymorphisms may affect the capacity of individuals to metabolise PAHs [1].

Studies suggest that metabolites of PAH's are generally excreted as conjugates of GSH, glucuronic acid or sulphate in the urine, faeces and via biliary excretion [7]. High molecular weight PAHs and their metabolites are mainly excreted via the faeces [1].

Sources and Route of Human Exposure

The major route of exposure to PAHs for non-smokers in the general population is food, with a minor contribution from inhalation of ambient air. For smokers, the contribution of smoking to total exposure is likely to be similar to that of food [8].

Food may become contaminated with PAHs from environmental sources, industrial preparation or during home cooking [8]. Various foods such as vegetables, meat and fish have been shown to contain PAHs, but they are largely formed due to cooking at high temperatures such as charbroiling, grilling and frying. Smoked and barbequed food are particularly important sources of exposure, although the largest contributors to PAH intake are "cereals and cereal products" and "vegetable fats and oils" [3, 4, 9-11]. The maximum estimated daily intake of BaP for a 70 kg person is 6-8 ng/kg [8, 11]. After evaluating a recent food survey, the Food Standards Agency (FSA) concluded that PAHs were typically

found in low levels in food and that consumers do not need to change their eating habits [12].

In the past, metal production and agriculture were responsible for the majority of total BaP emissions in the UK. Likely effected by the Environmental Protection Act 1990 and the ban on burning agricultural stubble, total emissions in 2011 had reduced to roughly 1/20th (now 3 tonnes) of those in 1991. Natural sources in 2011 accounted from 47.2% of the total emissions, while residential and commercial sources contributed the greatest portion of the anthropogenic emissions at 76% [13].

Annual mean air concentrations of BaP in the UK are generally below the EU target value of 1 ng/m³, with the vast majority of monitoring sites showing levels below the UK air quality objective of 0.25 ng/m³. Locations with point sources (e.g. industrial instillations and domestic solid fuel burning) are an exception to this. The main sources of BaP in the UK are domestic coal and wood burning, outdoor fires and industrial processes [14, 15].

Indoor air may be contaminated with PAH's by infiltration of outdoor air or from indoor emissions, which include smoking, cooking, and heating with fuel stoves and fireplaces and to a lesser extent from incense and candle burning. Levels of BaP within the home appear to vary seasonally, with the highest concentrations found in winter. BaP levels in European homes were found to be between 0.01 to 0.65 ng/m³ [1].

Mainstream tobacco smoke contains high concentrations of PAHs, levels in the range of 1-1.6 µg per cigarette have been measured and as such this represents a major source of exposure for smokers. BaP levels in sidestream smoke have been reported to range from 52-95 ng per cigarette, more than three times higher than that seen in mainstream smoke [5]. In a smoker's home more than 87% of total PAH's in air may be introduced by cigarette smoke; in a room heavily polluted with cigarette smoke, BaP levels may be as high as 22 ng/m³ [1].

PAHs are commonly detected in surface waters, due to urban runoff and industrial activities [3]. Contamination of drinking water with PAHs is usually associated with coal tar linings of distribution pipes. However, drinking water contributes only a minor amount to the total intake of PAHs [4, 16].

PAHs are found in the majority of surface soils due to atmospheric deposition or urban runoff. Soils near industrial sources such as coal coking often contain high concentrations of PAHs [3, 9]. BaP in English soils comprises approximately 5-7% by weight of the total PAH content [17]. The British Geological Survey defined the normal background concentrations for BaP in England and Wales to be 3.6 mg/kg in urban areas and 0.5 mg/kg in all other areas [17, 18].

Occupational exposure is largely through inhalation and skin absorption. The greatest levels of occupational exposure to BaP are in aluminium production (up to 100 µg/m³), with lesser exposure in roofing and paving (10-20 µg/m³) and lesser still in coal processing, wood impregnation, chimney sweeping and in power plants (at or below 1 µg/m³) [5].

Health Effects of Acute/Single Exposure

Human data

Inhalation

No studies were identified that reported the effects of BaP in humans following acute inhalation exposure.

Ingestion

Data on acute oral toxicity of BaP in humans are not available.

Dermal/ocular exposure

No studies were identified that reported effects of BaP in humans following acute dermal exposure.

Animal and in-vitro data

Inhalation

No studies were identified that reported effects of BaP in animals following acute inhalation exposure.

Ingestion

Exposure of rats (intragastric administration) to 100 mg/kg bw/day BaP for four days increased relative liver weight by 27% and induced aldehyde dehydrogenase. Limited evidence suggested that acute ingestion of BaP (50-150 mg/kg bw/day for 4 days) does not cause adverse GI effects in rats, although enzyme activity was altered. It was suggested that more serious effects may occur at higher concentrations [2].

Dermal/ocular exposure

BaP applied dermally caused allergen specific contact hypersensitivity reactions in mice after acute applications of 120 µg. A dose dependent contact hypersensitivity response to dermal application of BaP has been observed in guinea pigs; two applications of 0.001% BaP over 2-3 weeks gave slight hypersensitivity while a 1% dose gave a more severe response [2].

Acute topical application of BaP (concentration and duration of exposure not stated) to the backs of shaved mice suppressed sebaceous glands, although it was not possible to determine if such effects were due to the solvent or BaP, as a control group was not used [2].

Health Effects of Chronic/Repeated Exposure

Human data

Inhalation

A large number of epidemiological studies have been carried out considering a variety of occupations in which the workforce is chronically exposed to a mixture of PAHs [4]. These studies have demonstrated that such exposures result in symptoms including respiratory distress, decreased ventilatory function, chest pain, chest and throat irritation, cough, haematemesis, chronic dermatitis, depressed immune system and cancer of the skin and lungs [2, 4]. It is not possible to determine with any certainty the contribution of individual PAHs to these effects [4].

One study investigated the respiratory effects of inhaled BaP in employees working in various areas of a rubber factory. The authors reported a decrease in ventilatory function following prolonged exposure, as assessed by duration of employment, the greatest effects being observed in workers that had the highest exposure to particulate matter and BaP. No attempt was made to identify other possible chemical exposures or to separate effects due to BaP or particulates [2].

Ischemic heart disease was observed to increase in a dose dependent manner in asphalt workers exposed to BaP. Mean exposure for the cohort was 273 ng/m³ and exposure at or above this level was associated with a 1.64 fold greater risk of ischemic heart disease mortality compared to those exposed to below 68 ng/m³ [1]

Ingestion

Data on chronic oral toxicity of BaP in humans are not available.

Dermal/ocular exposure

Few data are available pertaining to BaP alone.

Regressive verrucae (warts) were reported in humans following up to 120 applications of 1% BaP over a four month period [2].

Genotoxicity

The formation of DNA adducts is believed to be a key event in the mutagenicity and carcinogenicity of PAH's; adducts may lead to misrepair and result in mutations [1]. Anti-BPDE has been demonstrated to form DNA adducts in man and as such acts as a biomarker for exposure to BaP. Molecular epidemiological studies in individuals exposed to complex mixtures of PAHs have shown that BaP adducts are seen concomitantly with biomarkers of genotoxic effect. The observed effects include chromosomal aberrations, sister chromatid exchange, DNA damage and formation of 8-oxo-deoxyguanosine. These same markers of exposure and effect are also observed in experimental animals, with association. IARC considers that BaP contributes to the genotoxic effects seen in complex

PAH mixtures; with the anti-BPDE-DNA adduct being the most mechanistically relevant adduct [19].

Smoking and diet (major sources of BaP) have been highly correlated with levels of the anti-BPDE-DNA adduct. The metabolite responsible for these adducts is seen to cause a unique array of mutations in the TP53 tumour suppressor gene, in cancers associated with smoking [19].

Evidence suggests that inhalation exposure to BaP at levels over 1 ng/m³ is predictive of greater genomic frequency of translocation, micronuclei and DNA fragmentation [1].

Carcinogenicity

No epidemiological studies on exposure to BaP alone are available for evaluation [19]. There is however extensive literature on the epidemiology of workforces exposed to complex mixtures of PAHs which include BaP. Studies include asphalt works, coke production plants, aluminium smelters and occupations where exposure to coal tar, coal tar pitches and soot occurs. Such studies clearly showed an elevated incidence of lung tumours following inhalation and skin tumours following chronic skin contact. It is difficult to assess with any confidence the contribution of BaP or any other individual PAH to such findings [3, 4, 20].

In the 2012 evaluation, IARC classified BaP as carcinogenic to humans (Group 1). It was concluded that BaP contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAHs mixtures. The robust animal evidence and consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification [19].

Estimated cumulative exposure to BaP of 100 µg/m³ (equivalent to 3.3 µg/m³ for 30 years) in the aluminium smelting industry has been associated with a 2.68 fold increase in the incidence of lung cancer [1].

Reproductive and developmental toxicity

There is evidence to suggest that exposure to PAHs may cause developmental effects in humans. This is supported by evidence in animal developmental studies.

PAH-DNA adducts have been found in fetal cord and maternal blood after maternal exposure to PAHs in ambient air; in light of this the World Health Organisation states that prenatal exposure could increase cancer risk from PAHs [1].

Studies show a dose-response relationship between exposure to PAHs during pregnancy and effects related to intrauterine growth restriction. A study of neonates showed that those with increased levels of PAH-DNA adducts had significantly lower birth weight, length and head circumference [1].

High cord blood levels of BaP-DNA adducts has been associated with decreased birth weight and a reduction in postnatal weight [6].

An association between dietary BaP intake and decreased birth weight, decreased birth length and having a small for gestational age infant was found in women with low vitamin C intake [6].

There is evidence to suggest that BaP may cause developmental neurotoxic effects. Human studies of prenatal environmental PAH exposure (determined by personal air monitoring and measuring BaP-DNA adduct levels in cord blood) have reported neurodevelopmental effects including impaired cognitive ability, impaired neuromuscular function and increased attention problems and anxious/depressed behaviour following prenatal exposure [6]. Infants born close to a coal-fired power station in China had 0.32 ± 0.14 BaP-DNA adducts per 10^8 nucleotides, this level was associated with a decreased motor development at age 2. A 0.1 unit increase in BaP DNA adducts per 10^8 nucleotides, at birth, was associated with a 2 fold greater chance of developmental delay at age 2. After closure of this plant, lower adduct levels were seen in the cord blood of a new cohort and was no longer associated with reduced motor development [1].

Evidence from human studies indicates that PAH or BaP exposure may cause reproductive toxicity in males and females. Studies in adult men exposed to PAH mixtures via occupational exposure or smoking have reported an association between higher levels of BaP-DNA adducts in sperm and male infertility [6, 21]. In a case control study in a Chinese population a strong association was reported between maternal blood BaP-DNA adducts and risk of miscarriage. In a study addressing the probability of conception in women undergoing IVF (in vitro fertilisation), follicular fluid BaP levels were significantly higher in women who did not conceive [6].

Animal and in-vitro data

Inhalation

Rats exposed to BaP dust via inhalation (7.7 mg/m^3 , 2 hours per day, 5 days per week for 4 weeks) showed no treatment related lesions in the lungs or nasal cavities. No dose-response relationship could be demonstrated as only one concentration of BaP was tested [3]. In the same study, kidney sections were also examined and no adverse effects were noted [2, 4]. Similarly, male hamsters did not show any adverse effects following exposure via inhalation to 9.8 mg/m^3 or 44.8 mg/m^3 BaP for 4.5 hours per day, five days per week for 16 weeks [3].

Ingestion

Few data on chronic oral toxicity of BaP in animals are available. Daily oral administration of 120 mg/kg bw BaP to poor affinity AhR mice (DBA/2N) for one to four weeks caused deaths due to myelotoxicity, whereas high affinity mice (C57B1/6N) remained unaffected during the 6 month treatment. Hepatotoxicity, as well as effects on liver and kidney enzymes have also been reported at this concentration [3, 4].

Rats fed $1,100 \text{ mg/kg bw/day}$ BaP in the diet for more than 100 days showed a decreased growth rate [3].

PAHs including BaP have been shown to promote atherosclerotic plaque formation in AhR responsive mice, chickens and pigeons [1].

Dermal Exposure

BaP (16, 32 or 64 g per application) was applied once a week for 29 weeks onto the skin of female mice. Dose-related epidermal thickening and a pronounced inflammatory response of the dermis, amongst other effects were reported in the first weeks of exposure in those administered the high dose, and subsequently in the lower dose groups [2].

Genotoxicity

Several PAHs are mutagenic and genotoxic and induce DNA adducts in vitro and in vivo [1, 11].

BaP has consistently been shown to be positive in in-vitro assays for point mutations in *Salmonella* and for chromosome damage in mammalian cells, in the presence of an exogenous source of metabolic activation. Indeed it is often used as a positive control in such assays [3].

An increase in the same biomarkers of genotoxic effect seen in man on exposure to complex PAH mixtures which may include BaP have also been seen in experimental animals exposed to BaP or anti-BPDE [19]. Such effects include point mutations, sister chromatid exchange, chromosomal aberrations, sperm abnormalities and somatic mutations. BaP induced mutations are notably found in tumour suppressor genes and proto-oncogenes [5].

There is strong evidence that the formation of DNA adducts by BaP is important in mouse lung tumorigenesis and that this mechanism and the formation of radical-cations by BaP is involved in mouse skin carcinogenesis [19].

There is some evidence for the role of BaP in bitumen-fume genotoxicity; in mice exposed to bitumen fume condensates, anti-BPDE–DNA adducts and BaP metabolites have been found in the lungs and urine respectively [7].

Carcinogenicity

IARC concluded that there is sufficient evidence that BaP is carcinogenic to experimental animals [19].

BaP applied directly to the skin of various strains of mice has been reported to induce malignant (and benign) skin tumours, predominantly squamous cell carcinomas. Oral administration of BaP to mice by gavage or diet has yielded an increase in tumour response in lymphoid and hematopoietic tissues and in several organs including the lung, liver oesophagus and tongue. An increase in mammary gland adenocarcinomas has been reported in rats administered BaP by gavage. Lifetime inhalation studies in hamsters gave dose-response related increases in papilloma's and squamous cell-carcinomas in the upper respiratory tract and in the upper digestive tract [19].

Skin tumour development was not observed in AhR deficient mice, however squamous-cell carcinomas of the skin were present in the wild-type mice [19].

Reproductive and developmental toxicity

BaP exposure has been shown to have effects on the development of laboratory animals following prenatal exposure. Developmental effects reported include decreased number of pups, an increase in reabsorptions, reduced pup weight and malformations [1, 2, 6]. Studies in mice exposed to BaP via oral administration during gestation suggest that intrauterine growth restriction, stillbirths and malformations following exposure may be dependent upon the AhR status of the mother and offspring [1-3].

Evidence from animal laboratory studies indicates that gestational exposure to BaP can have an effect on the reproductive function of offspring. Decreases in testis weight, sperm production, testosterone levels and fertility have been reported in male rodents. Effects observed in female mice include decreases in ovary weight, numbers of follicles, corpora lutea and fertility [1, 6].

Persistent neurodevelopmental effects have been observed in rats and mice exposed to BaP via ingestion as neonates; such effects include deficits in learning and memory, anxiety-related behaviours, sensorimotor development and neuromuscular function [6].

Trans-placental exposure to BaP (with dibenzo[a,l]pyrene) has been shown to induce lung and livers tumours in mice [1].

Reproductive effects have been reported in adult laboratory animals exposed to BaP. Reductions in sperm count, motility and production have been reported in various strains of adult male rats and mice and across routes of exposure. Hormonal changes and histological changes in the testis have also been observed in male adult animals. Reduced fertility and reductions in ovary weight have been reported in female adult animals following oral or inhalation exposure to BaP [6].

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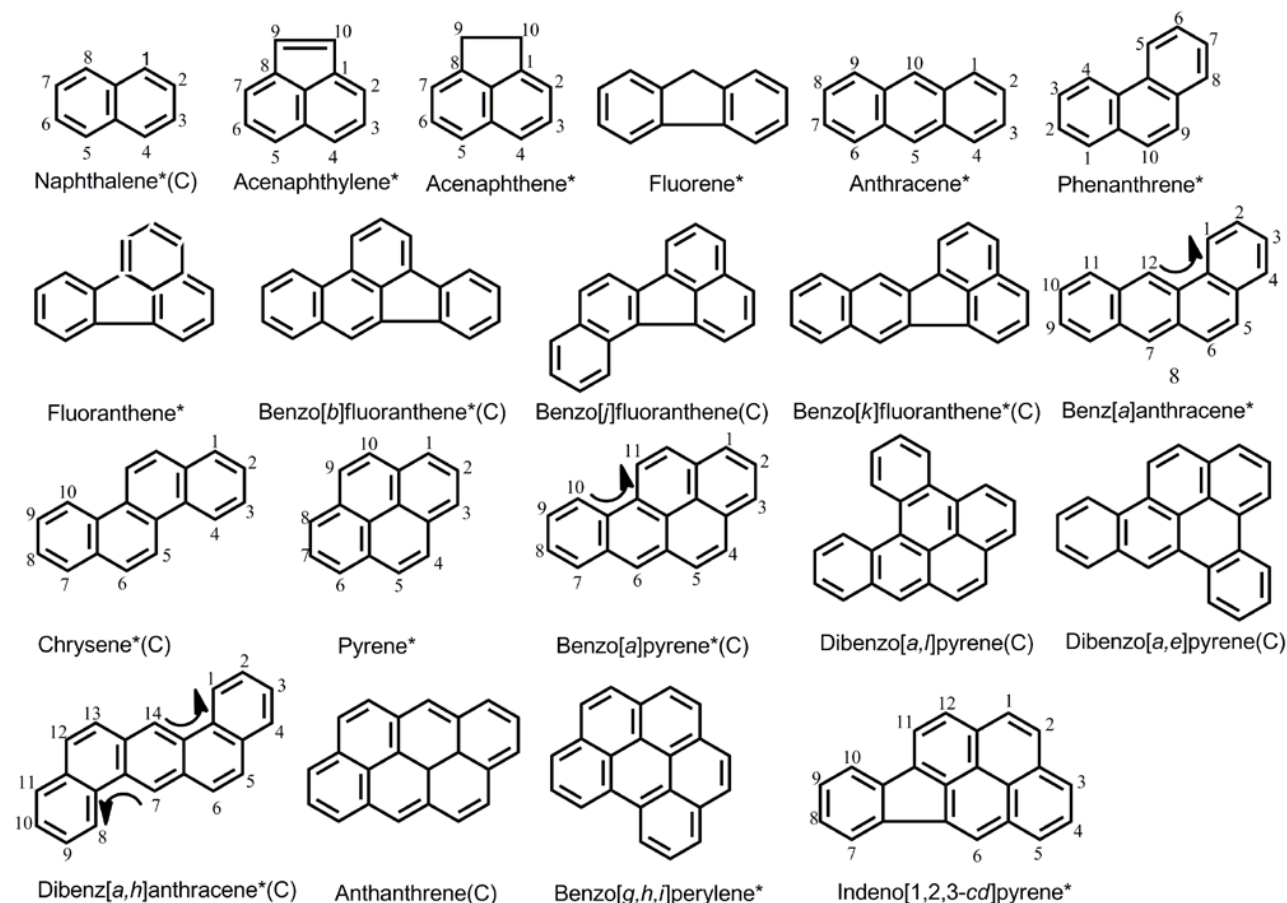
CHAPTER 7. POLYCYCLIC AROMATIC HYDROCARBONS IN AMBIENT AIR AND CANCER

Erik Dybing, Per E. Schwarze, Per Nafstad, Katarina Victorin, and Trevor M. Penning

Polycyclic aromatic hydrocarbons (PAHs), which are generated from the incomplete combustion of organic (carbonaceous) material, are ubiquitous contaminants in ambient air ([IARC, 1983](#), 1984a, 1984b, [1985](#), [2010](#); [WHO, 1998](#)). Their occurrence in the air we breathe has been substantial during the past centuries due to emissions from industrial processes and energy production, motor vehicular traffic, incineration of refuse, and residential heating.

PAHs consist of two or more fused aromatic rings made up of carbon and hydrogen atoms. The ring systems can be present in multiple configurations and may be unsubstituted or substituted. PAHs range from semivolatile molecules to molecules with high boiling points. Thus, they may be found both in the gas and the particulate phase of ambient air or in mixtures of both phases. About 500 different PAHs have been detected in air, but often the measurements focus on benzo[*a*]pyrene (B[*a*]P) as a representative of the whole PAH family ([WHO, 1998](#); [Boström et al., 2002](#)). Many of the PAHs in ambient air are carcinogenic ([IARC, 1983](#), 1984a, 1984b, [1985](#), [2010](#)) ([Figure 7.1](#)), and a recent reassessment of their carcinogenic potential led to B[*a*]P being

upgraded to a Group 1 *known human carcinogen* ([IARC, 2010](#)). Thus there is considerable concern about the relationship between PAH exposure in the ambient air and the potential to contribute to human cancer incidence. The United States Environmental Protection Agency (EPA) monitors 16 priority PAHs in air due to health concerns: naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]-perylene, and dibenz[*a,h*]anthracene (in order of number of aromatic rings per structure) ([Figure 7.1](#)). Of particular note is that several PAHs (naphthalene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, B[*a*]P, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene and dibenzo[*a,l*]pyrene, and anthanthrene) have been found to be carcinogenic in experimental animals after inhalation or intratracheal ingestion, increasing concern about the levels of these carcinogens in ambient air ([Figure 7.1](#)).

Fig 7.1 PAHs in ambient air.

An asterisk denotes a United States Environmental Protection Agency priority pollutant. (C) indicates that the compound is carcinogenic by inhalation or intratracheal administration in experimental animals. Source: [Park and Penning \(2008\)](#); reproduced with permission from John Wiley & Sons.

PAH emissions in ambient air

A recent global atmospheric emission inventory of PAHs ([Zhang and Tao, 2009](#)) showed that the emission from the 16 priority PAHs listed by the EPA was 520 000 tonnes per year. Anthropogenic sources of total PAHs in ambient air emissions are greater than those that come from natural events such as forest fires and volcanic eruptions.

Apart from localized risk at or near the source of emission, PAHs can be dispersed regionally and intercontinentally through atmospheric long-range transport. For example, PAHs

emitted from East Asia are transported to the west coast of the USA, and PAHs emitted in the Russian Federation influence atmospheric PAH concentrations in the Arctic ([Zhang and Tao, 2009](#)). The annual PAH emission from Asian countries is 290 000 tonnes (55% of the total); the amounts from China (114 000 tonnes per year) and India (90 000 tonnes per year) are the major contributors. The USA is the third largest emitter of PAHs, at 32 000 tonnes per year. By contrast, European countries account for only 9.5% of the total PAH emissions annually ([Zhang and Tao, 2009](#)). The contribution of the various anthropogenic sources of PAHs to the total emission

profile can vary by country and region. The global sources of PAH emissions are shown in [Table 7.1](#), and the main sources of PAHs in six European countries are shown in [Table 7.2](#).

The largest emission of PAHs globally comes from incomplete combustion of organic material, and the largest single source is from the combustion of biofuels. Biofuel is a single type of primary solid biomass (e.g. animal dung or peat) ([Zhang and Tao, 2009](#)). Burning biomass fuels such as wood on indoor open-pit stoves is common in developing areas, leading to harmful exposures to particulate matter $< 2.5 \mu\text{m}$ in diameter ($\text{PM}_{2.5}$), carbon monoxide (CO), and PAHs, which can be significantly reduced by the introduction of modern stoves ([Li et al., 2011](#)). Anthropogenic sources include PAHs that come from incomplete combustion processes (especially biofuels) and those that are made commercially, are by-products of industrial processes, or are generated from vehicle emissions, cooking, food preservation, and first- and second-hand cigarette smoke.

Anthropogenic sources of PAHs in ambient air

Commercial production

PAHs produced commercially include naphthalene, acenaphthene, phenanthrene, fluoranthene, and pyrene; however, only naphthalene is used directly without further processing, as a moth repellent.

Industrial processes

Many PAHs are released into the atmosphere during industrial processes such as coal coking and petroleum refining. It is estimated that coal coking was responsible for the release of thousands of tonnes of PAHs per year in different countries during the 1980s and early 1990s. Reduced coke production and technical

improvements have led to reductions in PAH emissions from this source. Little is known about the composition of these PAH emissions ([WHO, 1998](#)). In petroleum refining, most of the emissions consist of smaller two- and three-ring compounds (94–99%, depending on the process studied) ([IARC, 1989](#)). Thus, the composition of PAHs from combustion (pyrogenic) versus the composition of PAHs from petroleum refining (petrogenic) can be widely different. Other industrial sources with significant PAH emissions are carbon black plants, wood preservation (creosote) plants, the asphalt and bitumen industry, aluminium production (Söderberg electrodes), iron and steel production, foundries, tyre production, power plants, waste incinerators, and stubble burning ([WHO, 1998](#)). Further restrictions may lead to lower PAH emissions from these industries ([CORINAIR, 1997](#)).

Estimation of the PAH emissions for six European countries indicates that the industrial sources contribute PAHs in the same range as mobile sources ([Table 7.2](#); data from [CORINAIR, 1997](#)).

Residential sources

Domestic heating with oil and wood stoves leads to considerable PAH emissions in northern European countries, and especially in Scandinavia ([Boström et al., 2002](#)). In Sweden, the emissions from wood-fired domestic heating are estimated to be about 100 tonnes per year, with minor contributions from oil combustion. Environmental tobacco smoke is also a considerable source of indoor air pollution and contamination within the home ([Hoh et al., 2012](#)).

Motor vehicle emissions

The amount of PAHs released into the air from vehicles has been reduced considerably by the introduction of three-way converters. However, older diesel and gasoline cars with a

Table 7.1 Main sources of emission for the United States Environmental Protection Agency 16 priority PAHs in China, India, and the USA

Source	Global	China	India	USA
Biofuel	56.7%	66.4%	92.5%	9.1%
Wild fire	17.0%	0%	0%	3.3%
Consumer product use	6.9%	0.9%	0.6%	35.1%
Traffic oil	4.8%	2.0%	IS	23.0%
Domestic coal	3.7%	10.7%	1.3%	IS
Coke production	3.6%	14.4%	IS	IS
Petroleum refining	2.4%	1.0%	IS	8.7%
Waste incineration	1.9%	IS	IS	9.5%
Aluminium electrolysis	1.4%	IS	IS	1.9%
Open straw burning	IS	2.0%	3.2%	IS
Gasoline distribution	IS	IS	IS	3.0%
Aerospace industry	IS	IS	IS	2.5%
Other	1.5%		2.7%	3.9%
Tonnes in thousands	530	114	90	32

IS, insignificant.

Compiled from [Zhang and Tao \(2009\)](#).

catalytic converter of outmoded design have 5–10 times higher PAH emissions than modern cars. In addition, cold start at temperatures below the standardized cold start (23 °C), and especially at temperatures below 0 °C, results in a several-fold increase in PAH emissions. Several other technical variations lead to varying emissions, for example spark ignition engines ([WHO, 1998](#)). The total amounts of PAHs emitted from vehicles vary between countries; in the USA this can be as high as 6000 tonnes per year, and in six European countries the amount is about 400 tonnes per year ([Table 7.1](#) and [Table 7.2](#)).

As might be expected, not all PAHs contribute equally to the emissions into ambient air. [Table 7.3](#) lists a typical PAH profile in ambient air arising from different sources.

Human exposure

PAHs may be found in the gas and particulate phases (see Chapter 1). The levels given below frequently reflect the levels of discrete PAHs in the particulate phase and are often given as the sum of a limited number of PAH components.

B[a]P is the traditional marker for PAH exposure. Several additional PAH components have been proposed as emission markers, for example fluoranthene, B[a]P, and benzo[b]fluoranthene. [Boström *et al.* \(2002\)](#) suggested the use of the following set of PAHs as emission and effect markers for monitoring air pollution: B[a]P, fluoranthene, phenanthrene, methylanthracenes/phenanthrenes, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, indeno[1,2,3-*cd*]pyrene, benzo[*g,h,i*]-perylene, dibenz[*a*]anthracene, and dibenzo[*a,l*]pyrene. This list is quite similar to the 16 priority PAHs listed by the EPA ([Figure 7.1](#)). In some studies, the total PAH exposure is given as B[a]P toxic equivalency concentrations. In this approach, individual components are measured and ranked relative to B[a]P in terms of carcinogenicity. For example, chrysene has 1/1000th of the carcinogenicity of B[a]P and has a toxic equivalency concentration of 0.001. These calculations are used to estimate human health risk and can be used to calculate incremental lifetime cancer risk (ILCR). $ILCR = \text{exposure } (\mu\text{g/kg/day}) \times \text{cancer slope factor } (\mu\text{g/kg/day})$. The ILCR is considered negligible when it is less than 1 in 10^5 .

Table 7.2 Main source sectors for PAHs in 1994 in six European countries (Austria, Denmark, Germany, Luxembourg, Norway, and the United Kingdom)

Sector	PAH emissions	
	Amount (tonnes per year)	Percentage of total
Combustion of energy and transformation industries	6.1	0.3
Non-industrial combustion plants plus wood burning	1120	60
Combustion in manufacturing industry	63	3.4
Production processes	248	13
Road transport	383	20
Other mobile sources	10	0.5
Waste incineration	30	1.6
Agriculture and forestry	1	< 0.1
Natural sources	8	0.4
Total (approximately)	1900	

Reproduced from [Boström *et al.* \(2002\)](#).

(less than 1 additional cancer case per 100 000 persons), and the cancer slope factor is based on the extrapolation of a dose–response curve for tumorigenicity seen at high dose in experimental animals.

Background levels of PAHs in remote locations have been measured between 0.01 ng/m³ and 0.1 ng/m³ for individual PAH components ([WHO, 1998](#)). In rural districts the levels were approximately 10 times higher, whereas in city streets levels may amount to 50 ng/m³ or more of the more abundant individual PAHs ([Boström *et al.*, 2002](#)). Total PAHs in the centre of Stockholm, Sweden, ranged from below 100 ng/m³ to 200 ng/m³. The most abundant PAH was phenanthrene. In other cities higher levels of individual PAHs have been measured ([WHO, 1998](#); [Binková *et al.*, 2003](#)). PAH was measured in the gas and particulate phase over summer and winter sampling periods in Kocaeli, Turkey. Σ_{13} PAH in the gas and particulate phases ranged from 6.2 ng/m³ dibenz[*a,h*]anthracene to 98.6 ng/m³ phenanthrene in the winter, and from 3.0 ng/m³ benz[*a*]anthracene to 35.1 ng/m³ phenanthrene in the summer. The most abundant PAH in both sampling periods was phenanthrene, followed by fluoranthene and pyrene. B[*a*]P toxic

equivalency concentrations were found to be 3-fold higher in the winter months ([Gaga *et al.*, 2012](#)). A similar outcome was observed in a study of children aged 5–6 years (n = 260) in New York City when measurements were conducted in the heating and non-heating seasons ([Jung *et al.*, 2010](#)). In the United Kingdom, the Toxic Organic Micropollutants programme measured temporal trends in PAH in the atmosphere from 1991 to 2005 at six different sampling sites. Most showed a reduction in PAH levels and had concentrations that were lower than the new air quality standard of 0.25 ng/m³. However, this value was exceeded in urban areas in the winter months ([Meijer *et al.*, 2008](#)).

Indoor PAH levels usually range from 1 ng/m³ to 50 ng/m³ due to tobacco smoke and residential heating with wood, coal, and other materials ([WHO, 1998](#)). Environmental tobacco smoke is a major contributor to air pollution and dust, and surfaces remain contaminated long after the smoking has ceased (called third-hand smoke). Measurement of PAHs in settled household dust in 132 homes showed that total PAHs were 990 ng/g in smoking households versus 756 ng/g in nonsmoking households, and when corrected

Table 7.3 Mean profiles of individual PAHs in ambient air (relative to benzo[*a*]pyrene = 1.0)

Compound	Point source	Near mobile source	Home heating	Transport	Geometric mean
Anthracene	5.5	7.6	1.0	1.8	2.9
Phenanthrene	38	200	39	43	60
Fluoranthene	14	48	12	13	18
Pyrene	9.3	28	11	7.1	12
Benz[<i>a</i>]anthracene	1.4	0.82	1.0	0.78	0.97
Perylene	0.33	0.25	0.22	0.24	0.26
Benzo[<i>e</i>]pyrene	1.5	1.3	1.6	1.4	1.4
Benzo[<i>g,h,i</i>]perylene	1.4	1.5	2.4	1.3	1.6
Indeno[1,2,3- <i>cd</i>]pyrene	1.5	1.3	1.5	1.4	1.4
Anthanthrene	0.19	0.15	0.13	0.20	0.17
Chrysene and triphenylene	3.0	2.7	3.5	2.9	3.0
Benzo[<i>k</i>]fluoranthene	3.6	2.9	3.6	4.4	3.6

Source: [WHO \(1998\)](#); reproduced with permission from the publisher.

for loading (dust/m³), the fold change was greater than 2-fold ([Hoh *et al.*, 2012](#)).

PAHs in the ambient air can react with nitrates, hydroxyl radicals, or ozone, leading to the production of more water-soluble compounds. These compounds are rarely included in routine PAH measurements. However, nitro-PAHs have been detected on soot, and the formation of B[*a*]P-nitroquinone has been identified ([Schauer *et al.*, 2004](#)). Exposure levels of nine different nitroarenes resulting from diesel and gasoline exhaust have recently been reviewed by the International Agency for Research on Cancer; diesel exhaust was ranked as a Group 1 known human carcinogen ([Benbrahim-Tallaa *et al.*, 2012](#)).

Generally the mobile sources differ in their PAH profile, with the heavy diesel vehicles being characterized by lower-molecular-weight components than gasoline vehicles. However, per driven kilometre, total emissions from a gasoline-fuelled car are much lower than emissions from a diesel car. The three-way converter does not change the PAH profile of a gasoline-fuelled car significantly but reduces the total levels considerably. PAH levels vary with season, with higher levels being observed in the winter than in the summer. Data from Stockholm, Sweden, indicate that during

the winter the levels of low-molecular-weight PAHs are increased compared with the summer ([Prevedouros *et al.*, 2004](#)).

Biomonitoring

Significant progress has been made in biomonitoring of human exposure to PAH. External dose can be measured using personalized air monitoring devices where PM is trapped on filters and then analysed for PAH content. Internal dose can be assessed by measuring blood and urinary biomarkers of exposure. Different analytes have been used as biomarkers of PAH exposure and effect. These include measuring PAH metabolites in the urine and intermediate biomarkers of effect (e.g. DNA and haemoglobin adducts). Analysis using urinary metabolites has given the most clear-cut results. Particulate pyrene is well correlated with total PAH in the breathing zone.

Urinary 1-hydroxypyrene may also reflect inter-individual variation in PAH metabolism. Occupational exposure has been found to lead to a 10–100 times greater urinary 1-hydroxypyrene content. Danish bus drivers excreted more 1-hydroxypyrene than mail carriers did, but outdoor working mail carriers had more

PAH metabolites in their urine than those working indoors, indicating the impact of outdoor air pollution ([Hansen et al., 2004](#)). The use of 1-hydroxypyrene as a biomarker of PAH exposure has been criticized on the grounds that pyrene is not a carcinogenic PAH. This has led to the substitution of 3-hydroxy-B[a]P, but sensitive methods of detection have been a challenge. The detection of 3-hydroxy-B[a]P has also been criticized as a biomarker since this metabolite is not derived from any of the known pathways of B[a]P activation.

Measurements of urinary 1-hydroxypyrene-glucuronide, 2-naphthol, and malondialdehyde by synchronous fluorescence spectroscopy or high-performance liquid chromatography were used to evaluate seasonal and regional variations in PAH exposure and oxidative stress in Korean adults and women. Higher levels were found in individuals from industrialized areas and in the winter. Further elevation of 1-hydroxypyrene-glucuronide was observed in children exposed to environmental tobacco smoke ([Yoon et al., 2012](#)). In a study in Chinese children from polluted and non-polluted areas, the levels of nine urinary monohydroxylated PAH metabolites and 8-oxo-2'-deoxyguanosine (8-oxo-dG) were compared. Children from the polluted area had a higher PAH burden than those from the non-polluted area, but no significant difference in 8-oxo-dG levels was noted ([Fan et al., 2012](#)). The effect of involuntary tobacco smoke exposure on urinary levels of 23 monohydroxylated metabolites of PAH in 5060 subjects aged > 6 years was studied in the National Health and Nutrition Examination Survey (NHANES). After correcting for other confounders, significant increases in urinary 1-hydroxypyrene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxypyrene, and 1-2-hydroxy-phenanthrene were observed. Increases of 1.1–1.4-fold for involuntary exposure were noted, which increased to 1.6–6.9-fold

increases when children were actively exposed ([Suwan-ampai et al., 2009](#)).

As there is compelling evidence for the conversion of PAH to diol-epoxides as an activation pathway (see below), there have been recent advances in measuring their corresponding tetraol hydrolysis products in humans. Progress has been made in developing stable isotope dilution liquid chromatographic mass spectrometric methods to detect phenanthrene tetraols ([Hecht et al., 2010](#); [Zhong et al., 2011](#)). Phenanthrene contains a bay region and undergoes similar metabolic transformation to B[a]P to form diol-epoxides, which hydrolyse to tetraols. The detection of phenanthrene tetraols has also been criticized, since it is not a carcinogenic PAH. Recently, methods have been developed to measure urinary B[a]P tetraols with femtomole sensitivity ([Hecht et al., 2010](#)), and these techniques can now be applied to biomonitoring studies.

Efforts have also been made to detect stable covalent diol-epoxide DNA and haemoglobin adducts in exposed humans. Repaired diol-epoxide DNA adducts in blood can be measured using ELISA and chemiluminescence-based methods, while unrepaired DNA adducts can be measured in lymphocytes by [³²P]-postlabelling methods. For example, (+)-7 β ,8 α -dihydroxy-9 α ,10 α -oxo-7,8,9,10-tetrahydro-B[a]P-N²-deoxyguanosine [(+)-anti-B[a]PDE-N²-dGuo] adducts have also been detected in human maternal and umbilical white blood cells after exposure to air pollution, using ELISA-based methods ([Whyatt et al., 1998](#); [Santella, 1999](#)). Total DNA and B[a]P-like DNA adducts were measured by [³²P]-postlabelling in lymphocytes of nonsmoking policemen in Prague (n = 109) working 8 hour shifts. While there was no significant change in total DNA adducts, there was a marked increase in B[a]P-like DNA adducts correlated to personal exposure to PAHs collected on respirable particles ([Topinka et al., 2007](#)). Diol-epoxide DNA adducts are

short-lived; therefore, attention has also focused on the development of methods to detect haemoglobin diol-epoxide adducts since the half-life of the red blood cell is 7–10 days ([Day et al., 1990](#)).

Toxicokinetics, including metabolic activation

Parent PAHs have low chemical reactivity and must be metabolically activated to electrophilic intermediates to exert their carcinogenic effects ([Sims and Grover, 1974](#); [Conney 1982](#); [Thakker et al., 1985](#)). Three pathways of PAH activation have been proposed in the literature and are best exemplified with B[a]P ([Figure 7.2](#)). In the first pathway, B[a]P is metabolically activated by either P450 peroxidase or another peroxidase by acting as a co-reductant of complex-1 (Fe^{V}). This leads to a radical cation on the most electron-deficient C6 atom, which is highly reactive and capable of forming unstable C8-guanine [8-(benzo[a]pyren-6-yl)guanine], N7-guanine [7-benzo[a]pyren-6-yl)guanine], and N7-adenine [7-benzo[a]pyren-6-yl)adenine] depurinating DNA adducts ([Cavalieri and Rogan, 1995](#)). Evidence for this pathway comes from *in vitro* reactions with B[a]P, microsomes, and a peroxide substrate, which has led to the trapping of DNA adducts, as well as from mouse skin studies ([Cavalieri et al., 1990, 1991](#)). Data exist that B[a]P and dibenzo[a,l]pyrene can exert their tumorigenicity through this mechanism in mouse skin and rat mammary gland ([Cavalieri et al., 1991, 2005](#)). In addition, trace amounts of B[a]P-depurinating DNA adducts have been detected in the urine of smokers and in women exposed to household smoke ([Casale et al., 2001](#)). However, apart from this single study, the evidence to support this mechanism due to inhalation exposure to PAH is not strong.

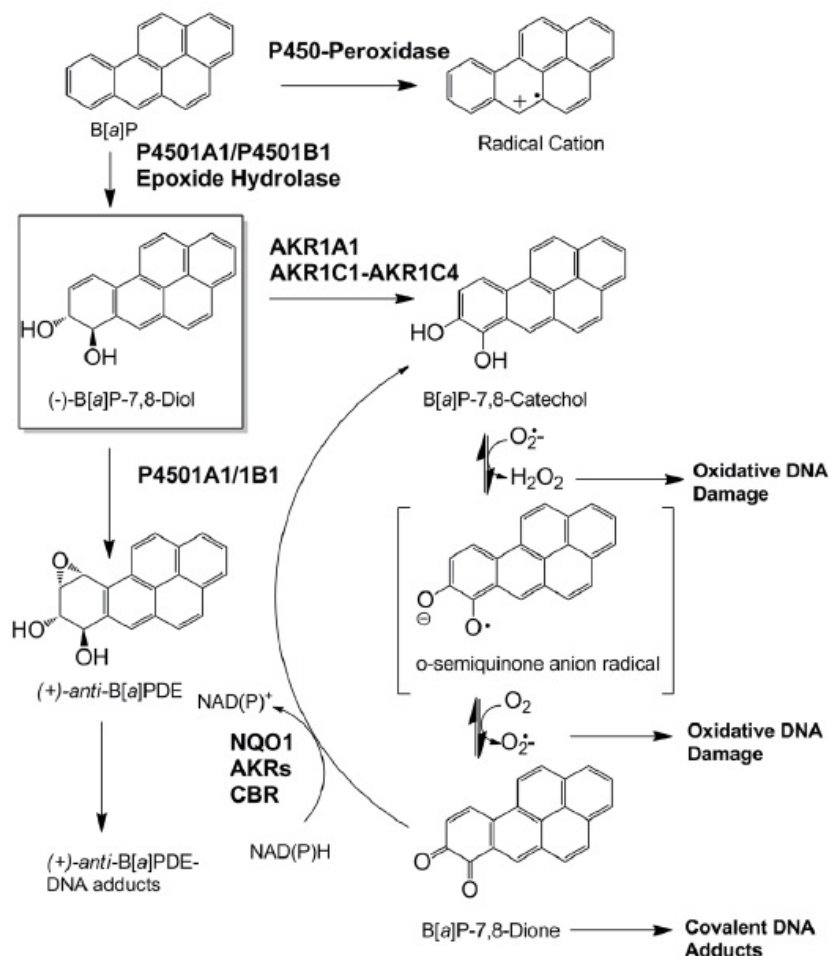
In the second pathway, B[a]P is metabolically activated to vicinal diol-epoxides ([Jerina et al., 1991](#)) formed through a three-step process

involving oxidation and hydrolysis reactions ([Figure 7.2](#)). In the first step, B[a]P is converted preferentially in the lung by the cytochrome P450 isozyme P4501B1 to the major (+)-7R,8S-epoxide and minor (–)-7S,8R-epoxide. In the second step, the 7R,8R-*trans*-dihydrodiol is predominately formed by the action of epoxide hydrolase. In the third step, diol-epoxide diastereomers are generated by another oxidation reaction via various P450 enzymes, including P4501B1 ([Thakker et al., 1985](#); [Petruska et al., 1992](#); [Guengerich, 1993](#); [Constantin et al., 1994](#); [Cavalieri and Rogan, 1995](#); [Shimada et al., 1999, 2001](#)).

Diol-epoxides have been studied in various animal carcinogenicity models. It has been revealed that the diol-epoxides with the highest carcinogenic activity are in general the *anti*-diastereomers and especially the enantiomers with *R*-absolute configuration at the benzylic arene carbon ([Thakker et al., 1985](#); [Glatt et al., 1991](#)). In studies of interactions of diol-epoxides with DNA, they demonstrate a high preference for the exocyclic amino group of deoxyguanosine and deoxyadenosine, where the major adduct derived from B[a]P is (+)-*anti*-B[a]PDE-N²-dGuo ([Jeffrey, 1985](#); [Gräslund and Jernström, 1989](#); [Jerina et al., 1991](#); [Geacintov et al., 1997](#)). This pathway of metabolic activation has been observed for many PAHs in ambient air, including 5-methylchrysene ([Melikian et al., 1983](#), [Koehl et al., 1996](#)), benz[a]anthracene ([Cooper et al., 1980](#)), benzo[b]fluoranthene ([Ross et al., 1992](#)), B[a]P (as outlined above), dibenz[a,h]anthracene ([Platt et al., 1990](#)), and dibenzo[a,l]pyrene ([Luch et al., 1997, 1999](#)), in *in vitro* systems (cell extracts, microsomes, and cell culture systems), and in some cases in *in vivo* studies in animals and humans. For example, PAHs within airborne PM_{2.5} produced DNA bulky stable adducts in human lung cell co-cultures ([Abbas et al., 2013](#)).

In the third pathway, PAHs are metabolically activated to *o*-quinones by the action of aldo-keto reductases (AKRs) ([Penning et al., 1999](#); [Penning, 2004](#)). For B[a]P, the sequence involves

Fig 7.2 Pathways of PAH activation using benzo[a]pyrene as an example.



Source: [Park and Penning \(2008\)](#); reproduced with permission from John Wiley & Sons.

the $NAD(P)^+$ -dependent oxidation of the 7R,8R-*trans*-dihydrodiol to a ketol catalysed by AKR1A1, AKR1C1–AKR1C4 ([Figure 7.2](#)). The ketol then spontaneously rearranges to a catechol, which undergoes air-oxidation to yield B[a]P-7,8-dione and reactive oxygen species (ROS) ([Palackal et al., 2001, 2002](#); [Penning et al., 1996](#)). B[a]P-7,8-dione is both electrophilic (will react with DNA) and redox-active. In the presence of reducing equivalents and NQO1, AKRs themselves, and carbonyl reductase, the quinones can be reduced back to the corresponding catechols, and if they are not intercepted a futile redox cycle will ensue in

which NADPH is depleted and ROS is amplified ([Shultz et al., 2011](#)). This pathway of metabolic activation has been observed for several PAHs in ambient air, including phenanthrene, chrysene, 5-methyl-chrysene, benz[a]anthracene, and B[a]P in *in vitro* systems (recombinant enzymes) and cultures of human lung cells ([Palackal et al., 2001, 2002](#); [Park et al., 2008b](#)).

Efforts have been made to assess the contribution of each of these pathways to the metabolic activation of B[a]P in human lung cells. Using a stable isotope dilution liquid chromatographic mass spectrometric method, signature

metabolites of each of the three pathways were measured: B[a]P-1,6-dione and B[a]-3,6-dione (radical cation metabolites), B[a]P-tetraol-1 (diol-epoxide metabolites), and B[a]P-7,8-dione (*o*-quinone metabolites) in human bronchoepithelial (H358) cells in the presence and absence of the aryl hydrocarbon receptor (AhR) agonist TCDD. It was found that each of the pathways contributed equally to B[a]P metabolism in the presence and absence of TCDD ([Lu et al., 2011](#)).

The rate of absorption of PAHs from the tracheobronchial epithelium after inhalation exposure is determined by their high lipophilicity ([Gerde et al., 1993](#)). For lipophilic carcinogens such as B[a]P, the delayed absorption in the airway mucosa is a result of slow passage through the airway epithelium, yielding a very high dose to these target cells. Because of the long retention time, the metabolic activation can be considerable even at low enzyme activities ([Bond et al., 1988](#)).

Modes of action

Carcinogenic PAHs are generally positive in short-term tests for mutagenicity ([Table 7.4](#)), for example the bacterial *Salmonella* mutagenicity (Ames) assay and the *HPRT*-mammalian cell mutagenicity assay, provided a metabolic activation system is present ([Malaveille et al., 1977](#); [MacLeod et al., 1988](#); [Chen et al., 1990](#); [Wei et al., 1993](#)). In the Ames assay, a rat liver S9 activation system is used; in the *HPRT* assay, recombinant P4501A1 and P4501B1 are co-expressed. The mutagenic species has been identified by comparing the mutagenic potency of different PAH metabolites, which demonstrates that of the known metabolites the diol-epoxides are the most potent mutagens ([Malaveille et al., 1977](#)). Treatment of a plasmid containing *K-Ras* with the (+)-*anti*-B[a]PDE followed by transfection into NIH3T3 cells led to cell transformation with increased foci in soft agar. Rescue of the plasmid showed that there were single point mutations of

the 12th and 61st codons, which could explain the transformation potential of the diol-epoxide. The dominant mutation observed was a G → T transversion, consistent with DNA-adduct formation on deoxyguanosine ([Marshall et al., 1984](#)). One of the most compelling pieces of data has shown that by using ligation-mediated polymerase chain reaction, the (+)-*anti*-B[a]PDE preferentially forms DNA adducts in *hot spots* on the *p53* tumour suppressor gene, which is one of the most mutated genes in human lung cancer. These hot spots correspond to the same codons that are mutated in tumours obtained from humans with lung cancer. The dominant mutation observed was again a G → T transversion, consistent with DNA adduct formation on deoxyguanosine ([Denissenko et al., 1996](#); [Hainaut and Pfeifer, 2001](#)).

In a separate *in vitro* study, the mutagenic potency of (±)-*anti*-B[a]PDE and B[a]P-7,8-dione (AKR product) were compared in a yeast-reporter gene assay for *p53* mutation. It was found that B[a]P-7,8-dione was 80-fold more mutagenic than the diol-epoxide provided it was permitted to redox cycle ([Yu et al., 2002](#)). In these experiments there was a linear correlation between (±)-*anti*-B[a]PDE mutagenicity and the formation of (+)-*anti*-B[a]PDE-N²-dGuo adducts, and a linear correlation between B[a]P-7,8-dione mutagenicity and the formation of 8-oxo-dGuo adducts ([Park et al., 2008a](#)). In addition, B[a]P-7,8-dione gave predominately G → T transversions, consistent with the base mispairing of 8-oxo-dGuo with adenine. The position of the point mutations within *p53* was quite random until there was biological selection for dominance, and then the spectrum of mutations was similar to that seen in lung cancer ([Park et al., 2008b](#)). These data suggest that B[a]P-7,8-dione formed by AKRs has the potential to contribute to the carcinogenic mode of action of B[a]P.

Planar PAHs can induce their own metabolism. Compounds such as B[a]P can bind to the AhR ([Nebert and Jensen, 1979](#); [Nebert et al.,](#)

Table 7.4 Genotoxicity of individual PAHs that are carcinogenic in experimental animals after inhalation or intratracheal instillation

Compound	Results
Anthanthrene	Positive, limited database
Benzo[<i>b</i>]fluoranthene	Positive
Benzo[<i>j</i>]fluoranthene	Positive
Benzo[<i>k</i>]fluoranthene	Positive
Benzo[<i>a</i>]pyrene	Positive
Chrysene	Positive
Dibenz[<i>a,h</i>]anthracene	Positive
Dibenzo[<i>a,i</i>]pyrene	Positive
Indeno[1,2,3- <i>cd</i>]pyrene	Positive
Naphthalene	Negative for gene mutations, positive for clastogenicity <i>in vitro</i>

Source: [WHO \(1998\)](#); reproduced with permission from the publisher.

1993, 2004). This leads to nuclear localization of the liganded AhR, where it can act as a transcription factor by binding to the xenobiotic response element to induce the *CYP1A1* and *CYP1B1* genes ([Denison et al., 1988a, 1988b, 1989](#)), which will result in enhanced monooxygenation of the parent PAH. PAH metabolism leads to the production of electrophiles (e.g. quinones), which can activate the Nrf2-Keap 1 system. Nrf2 acts as a transcription factor and binds to the antioxidant response element to induce γ GCS, *NQO1* and *AKR1C1*–*AKR1C3*, and *AKR1B10* ([Burczynski et al., 1999](#); [Jin and Penning 2007](#); [Penning and Drury, 2007](#)). Importantly, *AKR1C1*–*AKR1C3* are involved in the metabolic activation of PAH *trans*-dihydrodiols to the electrophilic and redox active PAH *o*-quinones, which could further exacerbate PAH activation via induction of AKRs. The PAH *o*-quinones produced by this pathway are also ligands for the AhR ([Burczynski and Penning, 2000](#)). Thus, both the parent PAH and their downstream metabolites can lead to the metabolic activation of PAHs in ambient air.

PAHs may, in addition to initiating carcinogenesis via a genotoxic mechanism, exert promotional effects through various modes of action. Certain PAHs induce inflammatory processes ([Casale et al., 1997](#)). The binding of PAHs to the AhR also leads to transcriptional upregulation of

genes involved in growth as well as biotransformation and differentiation ([Nebert et al., 1993](#)). Studies also indicate the ability of both PAHs and their metabolites to activate kinases involved in survival signalling, thus giving DNA-damaged cells a survival advantage ([Burdick et al., 2003](#)). At higher concentrations some PAHs induce apoptosis ([Solhaug et al., 2004](#)). In addition, PAHs show inhibitory effects on gap junctional intercellular communication ([Upham et al., 1996](#); [Weis et al., 1998](#)).

Carcinogenicity studies in animals

Most investigations of PAH carcinogenesis by the respiratory route are intratracheal instillation studies ([WHO, 1998](#)). In all, 10 PAHs have been found to be carcinogenic in experimental animals after inhalation or intratracheal instillation ([WHO, 1998](#); [NTP, 2000](#)) (Table 7.5). Only B[a]P and naphthalene have been studied by the inhalation route. In one inhalation study in hamsters, groups of 24 males were exposed to B[a]P condensed onto sodium chloride particles at concentrations of 2.2, 9.5, and 46.5 mg/m³ for 4.5 hours per day, 7 days per week for the first 10 weeks, then for 3 hours per day for 2 years. Exposure was by nose breathing only. There were no tumours in the controls or in the low-exposure

group. In the other two groups, exposure-related tumours were found in the nasal cavity, larynx, trachea, pharynx, oesophagus, and forestomach, but not in the lung ([Thyssen et al., 1981](#)). [RIVM \(1989\)](#) cites two other inhalation studies with B[a]P not found in the open literature: one in mice ([Knizhnikow et al., 1982](#); see [RIVM, 1989](#)) and one in rats with co-exposure with sulfur dioxide ([Laskin et al., 1970](#); see [RIVM, 1989](#)). In both studies malignant lung tumours were observed.

In recent bioassay inhalation studies with naphthalene, Fischer 344/N rats developed neuroblastomas of the nasal olfactory epithelium after being exposed in inhalation chambers to 0, 10, 30, or 60 ppm (80, 52, 157, or 314 mg/m³) for 6 hours per day, on 5 days per week, for 105 weeks ([NTP, 2000](#)). The observed rates in males were 0/49, 0/49, 4/48, and 3/48, respectively, and in females 0/49, 2/49, 3/49, and 12/49, respectively. In addition, adenomas of the nasal respiratory epithelium were observed in 0/49, 06/49, 8/48, and 15/48 males and in 0/49, 0/49, 4/49, and 2/49 females, respectively. In the study with B6C3F₁ mice subjected to whole-body exposure of 0, 10, or 30 ppm (0, 52, or 157 mg/m³) naphthalene in inhalation chambers for 6 hours per day, 5 days per week, for 104 weeks, a statistically significant increase in the incidence of bronchioloalveolar adenomas in high-dose female mice was observed ([NTP, 2000](#)). Increased incidences of bronchioloalveolar adenomas and carcinomas were observed in the male mice, but the increases were not statistically significant.

PAHs and their metabolites will also cause lung cancer in animals when administered by other routes. Classically, the newborn mouse model of lung cancer was used to rank the tumorigenicity of different B[a]P metabolites, given that the developing lung is more susceptible to carcinogen exposure. Studies such as these showed that the (+)-*anti*-B[a]PDE was the most potent lung tumorigen of the known B[a]P metabolites ([Buening et al., 1978](#);

[Kapitulnik et al., 1978](#)). Similarly, in the A/J mouse lung model of B[a]P-induced carcinogenesis, *anti*-B[a]PDE-DNA adducts were early lesions that could be detected in the initiation phase ([Nesnow et al., 1998](#)).

Carcinogenesis experiments with mixtures containing PAHs have also been reported. [Heinrich et al. \(1994\)](#) exposed groups of 72 female Wistar rats to a coal tar/pitch aerosol containing either 20 or 46 µg/m³ B[a]P for 17 hours per day, 5 days per week, for 10 or 20 months, followed by a clear air period of up to 20 or 10 months, respectively. The cumulative doses of inhaled B[a]P of the four exposure groups were 71, 143, 158, and 321 mg B[a]P/m³ hours, and the corresponding lung tumour rates were 4.2%, 33.3%, 38.9%, and 97.2%, respectively, whereas there were no tumours in the control group. In similar experiments in which rats were exposed to coal tar/pitch vapour condensed on the surface of fine carbon black particles, the resulting lung tumour rate was about twice as high.

[Pott and Heinrich \(1990\)](#) have also performed a lifelong inhalation study with rats exposed to diesel exhaust. In this study, tumour rates similar to those in the study with pitch pyrolysis vapours were induced, although the PAH content (measured as B[a]P) was 100–1000 times lower. This result indicates that diesel exhaust contains other potent carcinogenic or tumour-promoting compounds besides unsubstituted PAHs.

Numerous carcinogenicity studies have been performed using dermal application and subcutaneous and intramuscular injection (for overview, see [WHO, 1998](#)). An oral gavage study with B[a]P revealed tumour development in the liver, forestomach, auditory canal, oral cavity, skin, and intestines in both sexes of rats, and additionally the kidney in males and the mammary gland and oesophagus in females ([RIVM, 2001](#)). However, no lung tumours were observed after this route of administration. In a feeding study of B[a]P in mice, tumours in the tongue,

Table 7.5 Carcinogenicity of individual PAHs in experimental animals after inhalation or intratracheal instillation

Compound	Carcinogenicity (weight of evidence)	Species	No. of studies with positive, negative, and questionable results		
			+	–	±
Anthanthrene	Positive	Mouse	1		
Anthracene	Negative	Rat		1	
Benzo[<i>b</i>]fluoranthene	Positive	Rat Hamster	1	1	
Benzo[<i>j</i>]fluoranthene	Positive	Rat	1		
Benzo[<i>k</i>]fluoranthene	Positive	Rat	1		
Benzo[<i>g,h,i</i>]perylene	Negative	Rat		1	
Benzo[<i>a</i>]pyrene	Positive	Mouse Rat Hamster	1 9 11	1	1
Benzo[<i>e</i>]pyrene	Negative	Rat		1	
Chrysene	Positive	Rat	1		
Dibenz[<i>a,h</i>]anthracene	Positive	Rat Hamster	1 1	1	
Dibenzo[<i>a,i</i>]pyrene	Positive	Hamster	2		
Indeno[1,2,3- <i>cd</i>]pyrene	Positive	Rat	1		
Naphthalene	Positive	Mouse Rat	1		2
Phenanthrene	Negative	Rat		1	
Pyrene	Negative	Hamster		1	

Source: [WHO \(1998\)](#); reproduced with permission from the publisher; [IARC \(2002\)](#).

oesophagus, forestomach, and larynx, but not lung, were observed ([Culp *et al.*, 1998](#)).

Carcinogenicity studies in humans

Occupational exposures

A review and meta-analysis on the association between occupational exposure to PAHs and lung cancer development in 39 cohorts found an average relative risk of 1.20 per 100 µg/m³ years cumulative B[*a*]P ([Armstrong *et al.*, 2004](#)). For some occupations relative risks were considerably higher, but confidence intervals were very wide. For exposures in coke ovens, gas works, and aluminium industries, the risk is equivalent to a relative risk of 1.06 for a working lifetime of 40 years at 1 µg/m³.

Ambient air exposures

Few studies have addressed the impact of exposure to PAHs in ambient air on human cancer. Studies using other exposure indicators (PM or NO₂) have shown associations between air pollution and lung cancer; however, no PAH exposure information was available ([Pope *et al.*, 2002](#); [Hoek *et al.*, 2002](#); [Nafstad *et al.*, 2003](#)). An analysis of the United States data on lung cancer, PM exposure, and older PAH and metal air concentration data, supports the plausibility that known chemical carcinogens may be responsible for the lung cancer attributed to PM_{2.5} exposure in the American Cancer Society study ([Harrison *et al.*, 2004](#)). A study by [Cordier *et al.* \(2004\)](#) found an increased risk of childhood brain cancer associated with PAH exposure. Both paternal

preconception occupational PAH exposure and paternal smoking were associated with increased risks for childhood brain tumours.

Human susceptibility

PAHs are metabolically activated by phase I P450 isozymes (*CYP1A1*, *CYP1B1*) in combination with epoxide hydrolase (*EPHX*) and phase I AKR isozymes (*AKR1A1*, *AKR1C1*-*AKR1C4*) and are detoxified by phase II enzymes including *GSTs*, *UTGs*, *SULTs*, and *COMT*. In addition, bulky covalent diol-epoxide DNA adducts can be repaired by nucleotide excision repair proteins (*XPD* [helicase], *XPA*, and *XPC* [damage recognition]), and oxidative DNA lesions can be repaired by base excision repair enzymes (*hOGG1* and *APE*). Each of these genes is highly polymorphic in the human population. (A complete list of these variants is available at the NCBI database: <http://www.ncbi.nlm.nih.gov/>.) Many of these variants are non-synonymous single-nucleotide polymorphisms (nSNPs) that can affect enzyme activity. Combinations of these nSNPs rather than an individual SNP may affect human genetic susceptibility to PAH emissions in ambient air.

In a study of Prague policemen occupationally exposed to polluted air, B[a]P-like DNA adducts were detected and found to be positively associated with SNPs in *XPD* and *GSTM1* (Binková *et al.*, 2007). In another lung cancer case-control study, exposure to environmental tobacco smoke and polymorphisms in *CYP1B1* *Leu(432)Val* was significantly associated with lung cancer susceptibility, with an odds ratio for at least one allele of 2.87 (95% confidence interval [CI], 1.63–5.07) (Wenzlaff *et al.*, 2005a). Combinations of the polymorphism in this phase I enzyme gene along with those selected from either phase II enzyme genes (*GSTM1* null, *GSTP1* *Ile(105)Val*) or NADPH-quinone oxidoreductase (*NQO1* *C(609)T*) were also evaluated. Here the combination of the *CYP1B1* *Leu(432)Val* allele and the *NQO1* *C(609)T* allele was associated with

the highest risk of lung cancer (odds ratio [OR], 4.14; 95% CI, 1.60–10.74) (Wenzlaff *et al.*, 2005a). In the same study cohort, variants in *GSTM1*, *GSTT1*, and *GSTP1* were examined to determine whether there was an association of the genotype with lung cancer incidence in never-smokers. Individuals who had been exposed to household environmental tobacco smoke for > 20 years, and who were carriers of either the *GSTM1* null allele or the *GSTP1* Val allele, were at a 4-fold increased risk of developing lung cancer (OR, 4.56; 95% CI, 1.21–17.21) (Wenzlaff *et al.*, 2005b). In a lung cancer case-control study in China, women who were never-smokers were found to be at a significant increased risk of adenocarcinoma if they were carriers of the variants in the nucleotide excision repair variant *XRCC1* 399 Gln/Gln versus the Arg/Arg genotype (OR, 14.12; 95% CI, 2.14–92.95). The OR of lung adenocarcinoma for the *XRCC1* 399Gln allele with exposure to cooking oil smoke was 6.29 (95% CI, 1.99–19.85) (Li *et al.*, 2005). DNA integrity was investigated in 50 bus drivers, 20 garage men, and 50 controls in the Czech Republic and associated with variants in the base excision repair gene *hOGG1*. Carriers of at least one variant (Cys allele) had a higher degree of DNA damage (Bagryantseva *et al.*, 2010). To date, no molecular epidemiological study has been performed whereby combinations of polymorphic variants in phase I, phase II, and DNA repair genes have been pooled. However, based on the studies described, carriers of variants in all three classes of genes might be at higher risk of developing lung cancer from emissions of PAHs in ambient air.

Conclusions

PAHs generated from the incomplete combustion of organic material are ubiquitous contaminants in urban air. There are numerous unsubstituted PAHs (pyrogenic) and substituted PAHs (petrogenic). The pyrogenic PAHs may occur in the gas phase, particulate phase,

or mixtures of both phases. The major world-wide source is the combustion of biofuels, while other sources such as combustion plants, various industrial and production processes, road transport, and waste incineration can contribute. Total PAH levels in some urban areas are in the range of 100–200 ng/m³ but may be even higher in more polluted areas and can show distinct seasonal variation. However, measurements of total PAHs are relatively scarce. B[a]P is the traditional marker for PAHs, but various other individual PAHs have also been proposed, such as fluoranthene, B[a]P, and benzo[b]fluoranthene. Biomarkers of exposure include 1-hydroxypyrene, 3-hydroxy-B[a]P, and tetraols, but DNA and protein adducts can also be measured as intermediate cancer biomarkers. The major disease end-point of interest is lung cancer, and approximately 10–15% of all lung cancer cases are seen in never-smokers. Parent PAHs must be metabolically activated to electrophilic intermediates (radical cations, vicinal diol-epoxides, and *o*-quinones) to act as lung carcinogens. All three routes have been observed in human lung cells. Various promotional effects of PAHs may contribute to their carcinogenic action. In all, 10 PAHs have been found to be carcinogenic in experimental animals after inhalation or intratracheal instillation. Naphthalene seems to be an exception compared with other carcinogenic PAHs as it appears to not be genotoxic. A meta-analysis of occupational cohort studies found a 20% increase in relative risk per 100 µg/m³ years cumulative B[a]P exposure. Studies of ambient air pollution and cancer have demonstrated an association between carriers of polymorphic variants in phase I, phase II, and DNA repair enzyme genes.

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PART 1.

CONCORDANCE BETWEEN CANCER IN HUMANS AND IN EXPERIMENTAL ANIMALS

CHAPTER 7.

Polycyclic aromatic hydrocarbons and associated occupational exposures

Charles William Jameson

Properties of PAHs

Most polycyclic aromatic hydrocarbons (PAHs) with potential biological activity have been determined to have a molecular structure that ranges in size from two to six fused aromatic rings (IARC, 2010). The physicochemical properties of these PAHs that are critical to their biological activity vary greatly, because their molecular weights cover a vast range.

Aqueous solubility of PAHs decreases approximately logarithmically with increasing molecular mass (Johnsen et al., 2005). Two-ring PAHs, and, to a lesser extent, three-ring PAHs, dissolve in water;

this makes them more readily available for biological uptake and degradation (Mackay and Callcott, 1998; Choi et al., 2010). Furthermore, two- to four-ring PAHs volatilize sufficiently to appear in the atmosphere predominantly in gaseous form, although the physical state of four-ring PAHs can depend on temperature (Atkinson and Arey, 1994; Srogi, 2007).

In contrast, PAHs with five or more rings have low solubility in water and low volatility. They therefore occur predominantly in solid form, bound to particulates in polluted air, soil, or sediment (Choi et al., 2010). In the solid state, these compounds are less accessible for biological

uptake or degradation, which means that their persistence in the environment is increased (Johnsen et al., 2005; Haritash and Kaushik, 2009).

The properties that influence the biological activity of PAHs include their vapour pressure, their adsorption on surfaces of solid carrier particles, their absorption into liquid carriers, their lipid–water partition coefficient in tissues, and their limits of solubility in the lipid and aqueous phases of tissues. These properties are linked with the metabolic activation of PAHs, as well as their deposition and disposition.

PAHs share a similar mechanism of carcinogenic action in both humans and experimental animals.

This includes metabolic conversion to oxides and dihydrodiols, which in turn are oxidized to diol epoxides. These oxides and diol epoxides are the ultimate DNA-reactive metabolites of PAHs. The oxides form stable DNA adducts, and the diol epoxides form stable adducts but also unstable adducts (so-called depurinating adducts) with DNA through formation of electrophilic carbonium ions.

Occupational exposure to PAHs

Occupational exposure to PAHs occurs predominantly through inhalation and dermal contact. Industrial processes that involve the pyrolysis or combustion of coal and the production and use of coal-derived products, including coal tar and coal tar-derived products, are major sources of occupational exposure to PAHs. Workers at coal-tar production plants, coking plants, bitumen production plants, coal-gasification sites, smokehouses, aluminium production plants, coal-tarring facilities, and municipal waste incinerators are exposed to PAHs. Exposure may also result from inhalation of engine exhaust and from use of products that contain PAHs in a variety of other industries, such as mining, oil refining, metalworking, chemical production, transportation, and the electrical industry (Vanrooij et al., 1992).

Studies in Germany measured concentrations of PAHs in the breathing zone of chimney sweeps during “black work”; the PAHs in the air samples varied depending on the type of fuel burned (oil, oil/solid, or solid) (Knecht et al., 1989). Concentrations of PAHs in coal-tar products may range from less than 1% to 70% or more (ATSDR, 2002). Occupational exposure can lead to

PAH body burdens among exposed workers that are considerably higher than those in the general population.

There is growing awareness that uptake of PAHs through the skin is substantial (Jongeneelen, 2001). Dermal uptake has been shown to contribute to the internal exposure of workers to PAHs; a study in the creosote industry found that the total internal dose of PAHs did not necessarily correlate with levels of inhalation exposure alone, and that dermal exposure contributed significantly (Vanrooij et al., 1992).

Classification of PAHs

The *IARC Monographs Programme* has reviewed experimental data for 60 individual PAHs (IARC, 2010). Of these 60 PAHs, one, benzo[a]pyrene, is classified as *carcinogenic to humans* (Group 1). Other PAHs reviewed by IARC include cyclopenta[cd]pyrene, dibenz[a,h]anthracene, and dibenzo[a,l]pyrene, which are classified as *probably carcinogenic to humans* (Group 2A), and benz[j]aceanthrylene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[c]phenanthrene, chrysene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, indeno[1,2,3-cd]pyrene, and 5-methylchrysene, which are classified as *possibly carcinogenic to humans* (Group 2B). It should be noted that in the evaluations of benz[j]aceanthrylene, benzo[c]phenanthrene, benzo[a]pyrene, cyclopenta[cd]pyrene, dibenzo[a,h]anthracene, and dibenzo[a,l]pyrene, the mechanistic data available for these compounds were

critical for determining the overall evaluation for each one (IARC, 2010).

The remaining 45 PAHs reviewed by IARC were acenaphthene, acetyrene (3,4-dihydrocyclopenta[cd]pyrene), anthanthrene, anthracene, 11*H*-benz[bc]aceanthrylene, benz[*l*]aceanthrylene, benzo[*b*]chrysene, benzo[*g*]chrysene, benzo[*a*]fluoranthene, benzo[*ghi*]fluoranthene, benzo[*a*]fluorene, benzo[*b*]fluorene, benzo[*c*]fluorene, benzo[*ghi*]perylene, benzo[*e*]pyrene, coronene, 4*H*-cyclopenta[*def*]chrysene, 5,6-cyclopenteno-1,2-benzanthracene, dibenz[*a,c*]anthracene, dibenz[*a,j*]anthracene, dibenzo[*a,e*]fluoranthene, 13*H*-dibenzo[*a,g*]fluorene, dibenzo[*h,rsf*]pentaphene, dibenzo[*a,e*]pyrene, dibenzo[*e,l*]pyrene, 1,2-dihydroaceanthrylene, 1,4-dimethylphenanthrene, fluoranthene, fluorene, 1-methylchrysene, 2-methylchrysene, 3-methylchrysene, 4-methylchrysene, 6-methylchrysene, 2-methylfluoranthene, 3-methylfluoranthene, 1-methylphenanthrene, naphtho[1,2-*b*]fluoranthene, naphtho[2,1-*a*]fluoranthene, naphtho[2,3-*e*]pyrene, perylene, phenanthrene, picene, pyrene, and triphenylene. These compounds were determined to be *not classifiable as to their carcinogenicity to humans* (Group 3), because of limited or inadequate experimental evidence (IARC, 2010).

As noted above, benzo[a]pyrene is the only PAH classified by IARC in Group 1. A review of the data available for this PAH indicates that the complete sequence of steps in the metabolic activation pathway of benzo[a]pyrene to mutagenic and carcinogenic diol epoxides has been demonstrated in humans, in human tissues, and in experimental animals. After exposure, humans

Table 7.1. Group 1 agents associated with dermal exposures to polycyclic aromatic hydrocarbons (PAHs) that cause squamous cell carcinoma of the skin in humans and in rodents

Agent	Target organ		
	Humans	Mice	Rats
Benzo[a]pyrene	No data	Skin	Skin
Chimney sweep soots	Skin, including scrotum	Skin	No data
Coal tar	Skin, including scrotum	Skin	No data
Coal-tar pitch	Skin, including scrotum	Skin	No data
Mineral oils, untreated and mildly treated	Skin, including scrotum	Skin	No data
Shale oils	Skin, including scrotum	Skin	No data

metabolically activate benzo[a]pyrene to benzo[a]pyrene diol epoxides that form DNA adducts. The *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adduct has been measured in populations (e.g. coke-oven workers and chimney sweeps) exposed to PAH mixtures that contain benzo[a]pyrene. The reactive *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide induces mutations in rodent and human cells. Mutations (G → T transversions) in the *K-ras* proto-oncogene in lung tumours from mice treated with benzo[a]pyrene are associated with *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide–deoxyguanosine adducts. Similar mutations in the *KRAS* proto-oncogene and mutations in the tumour suppressor gene *TP53* were found in lung tumours from non-smokers exposed to PAH-rich products of coal combustion that are known to contain benzo[a]pyrene (as well as many other PAHs). In an in vitro study, the codons in *TP53* that are most frequently mutated in lung cancer in humans were shown to be targets for DNA adduct formation and mutation induced by benzo[a]pyrene. The strong and extensive experimental evidence for the carcinogenicity of benzo[a]pyrene in many animal species, supported by the consistent and coherent

mechanistic evidence from studies in exposed humans and in experimentally exposed animals, and from in vitro studies in human and animal tissues and cells, provides biological plausibility to support the overall classification of benzo[a]pyrene as *carcinogenic to humans* (Group 1) (IARC, 2010, 2012).

Studies of cancer in humans

There are no epidemiological studies on human exposure to individual PAHs, because these chemicals never occur in isolation in the environment but are present as components of complex chemical mixtures. PAHs are very widespread environmental contaminants, because they are formed during incomplete combustion of materials such as coal, oil, gas, wood, or waste, or during pyrolysis of other organic materials, such as tobacco. Data on the carcinogenicity of PAHs to humans are available primarily from studies in occupational settings where workers are exposed to mixtures containing PAHs. It is difficult to ascertain the carcinogenicity of the component PAHs in these mixtures, because of

potential chemical interactions and the presence of other carcinogenic substances.

Certain occupations associated with high exposure to PAHs have been classified by IARC as *carcinogenic to humans* (Group 1); these include coal gasification, coke production, coal-tar distillation, chimney sweeping, paving and roofing with coal-tar pitch, and work involving mineral oils, shale-oil production, and aluminium production. In most cases the classification is based on epidemiological studies of increased cancer incidence without reference to supporting evidence from bioassays in experimental animals. The roles of individual PAHs in the genesis of cancer observed in these occupations could not be defined (IARC, 2010).

Tumour site concordance

There are six IARC Group 1 agents that cause non-melanoma tumours of the skin (Rice, 2005). Five of these are related to occupations where PAH exposures are high and are believed to be the causative agents (Table 7.1). There is a precise correlation between carcinogenicity to human skin and carcinogenicity to mouse skin for these five

Table 7.2. Group 1 carcinogens associated with inhalation exposures to polycyclic aromatic hydrocarbons (PAHs) that cause lung cancer in humans and in rodents

Agent	Target organ		Route/target organ
	Humans	Mice	Rats
Benzo[a]pyrene	No data	Intraperitoneal injection of and oral exposure to soot extracts/lung	Intratracheal and intrapulmonary instillation of soot extracts/lung
Chimney sweep soots	Lung	No data	Intratracheal instillation of soot extracts/lung
Coal-tar vapours from coke ovens	Lung	Inhalation/lung	Inhalation/lung
Soots and vapours from aluminium production	Lung, bladder	No data	No data

PAH-associated exposures when the complex mixtures isolated from the occupational environment are applied topically.

In 1775, Pott made the pioneering observation that cancer of the scrotum in chimney sweeps was an occupational disease resulting from direct contact with soot (Pott, 1775). All five established PAH-based chemical carcinogens for human skin to which exposures occur by direct dermal contact are complex mixtures: coal tar, coal-tar pitch, untreated and mildly treated mineral oils, shale oils, and soots. Because these mixtures contain PAHs, all have a genotoxic component to their mode of action in rodents. Most of the individual PAHs classified by IARC as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B) (listed above) are genotoxic and have been shown to cause skin cancer and/or be initiators of skin cancer in rodents (IARC, 1983, 2010).

Soots and vapours from coke production, aluminium production, and related industries also cause lung cancer in humans, but only extracts of soot and vapours from coke production have been tested in rodents

by an appropriate route (Table 7.2). Both mice and rats developed lung tumours after inhalation of coal-tar vapours from coke ovens. Soot extracts caused lung tumours in rats after intratracheal instillation. There appears to be a good correlation between lung cancer in humans and in rodents for these two mixtures when studied by an appropriate route in mice and rats. All these complex mixtures have genotoxic activity, which is recognized to underlie their carcinogenic activity in the lung. In summary, many of the individual PAHs in these complex mixtures that have been classified by IARC as either *probably carcinogenic* or *possibly carcinogenic* to humans are also genotoxic and have been shown to cause lung tumours in rodents when administered by an appropriate route (IARC, 2010).

The various tissue compartments of the respiratory tract are metabolically active towards exogenous chemicals in both humans and experimental animals and are clearly capable of transforming many metabolism-dependent chemicals, including carcinogenic PAHs, to their chemically reactive metabolites (Rice, 2005). In the lung, met-

abolically active cell types include pulmonary macrophages as well as epithelial cells.

Benzo[a]pyrene is the only PAH that has been classified by IARC as *carcinogenic to humans* (Group 1). As indicated above, the basis for this classification is the extensive knowledge of the mechanism of carcinogenic action of benzo[a]pyrene in humans and experimental animals. None of the many remaining PAHs shown to be carcinogens in animals have been classified as an IARC Group 1 carcinogen, most likely because much less mechanistic information is available for these agents than for benzo[a]pyrene. These other PAHs are classified as either *probably carcinogenic to humans* (Group 2A) or *possibly carcinogenic to humans* (Group 2B). Most marked human occupational exposure to these compounds involves complex mixtures that contain more than one of these PAHs and that often contain other, non-PAH carcinogens. Therefore, the carcinogenic activity of these mixtures cannot confidently be ascribed to any one of their individual components.

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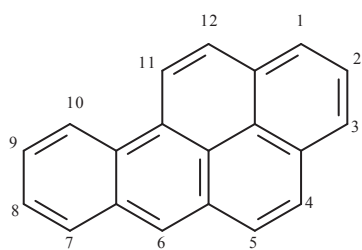
BENZO[a]PYRENE

Benzo[a]pyrene was considered by previous IARC Working Groups in 1972, 1983, and 2005 ([IARC, 1973, 1983, 2010](#)). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Services Reg. No.: 50-32-8
Chem. Abstr. Name: Benzo[a]pyrene
IUPAC Systematic Name: Benzo[a]pyrene
Synonyms: BaP; benzo[def]chrysene;
3,4-benzopyrene*; 6,7-benzopyrene*;
benz[a]pyrene; 3,4-benz[a]pyrene*;
3,4-benzpyrene*; 4,5-benzpyrene*
(*alternative numbering conventions)



$C_{20}H_{12}$

Relative molecular mass: 252.31

Description: Yellowish plates, needles from benzene/methanol; crystals may be monoclinic or orthorhombic

Boiling-point: 310–312 °C at 10 mm Hg

Melting-point: 179–179.3 °C; 178.1 °C

Spectroscopy data: Ultraviolet/visual, infrared, fluorescence, mass and nuclear

magnetic-resonance spectral data have been reported

Water solubility: 0.00162 mg/L at 25 °C;
0.0038 mg/L at 25 °C

log K_{ow} (octanol–water): 6.35

Henry's Law Constant: 0.034 Pa m³/mol at 20 °C

From [IARC \(2010\)](#)

1.2 Occurrence and exposure

Benzo[a]pyrene and other polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants formed during incomplete combustion or pyrolysis of organic material. These substances are found in air, water, soils and sediments, generally at trace levels except near their sources. PAHs are present in some foods and in a few pharmaceutical products based on coal tar that are applied to the skin. Tobacco smoke contains high concentrations of PAHs ([IARC, 2010](#)).

1.2.1 Exposure of the general population

The general population can be exposed to benzo[a]pyrene through tobacco smoke, ambient air, water, soils, food and pharmaceutical products. Concentrations of benzo[a]pyrene in

sidestream cigarette smoke have been reported to range from 52 to 95 ng/cigarette — more than three times the concentration in mainstream smoke. Major sources of PAHs in ambient air (both outdoors and indoors) include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires. Average concentrations of individual PAHs in the ambient air in urban areas typically range from 1 to 30 ng/m³; however, concentrations up to several tens of nanograms per cubic metre have been reported in road tunnels, or in large cities that make extensive use of coal or other biomass as residential heating fuel. Estimates of PAH intake from food vary widely, ranging from a few nanograms to a few micrograms per person per day. Sources of PAHs in the diet include barbecued/grilled/broiled and smoke-cured meats; roasted, baked and fried foods (high-temperature processing); bread, cereals and grains (at least in part from gas/flame-drying of grains); and vegetables grown in contaminated soils, or in areas with surface contamination from atmospheric PAH fall-out ([IARC, 2010](#)).

1.2.2 Occupational exposure

Occupational exposure to PAHs occurs primarily through inhalation and via skin contact. Monitoring by means of ambient air-sampling or personal air-sampling at the workplace, to determine individual PAHs, sets of PAHs or surrogates (e.g. coal-tar pitch volatiles) has been used to characterize exposure via inhalation; more recently, biological monitoring methods have been applied to characterize the uptake of certain specific PAHs (e.g. benzo[a]pyrene) to be used as biomarkers of total exposure ([IARC, 2010](#)).

Industries where occupational exposure to benzo[a]pyrene has been measured and reported include: coal liquefaction, coal gasification, coke production and coke ovens, coal-tar distillation, roofing and paving (involving coal-tar pitch), wood impregnation/preservation with creosote, aluminium production (including anode manufacture), carbon-electrode manufacture, chimney sweeping, and power plants. Highest levels of exposure to PAHs are observed in aluminium production (Söderberg process) with values up to 100 µg/m³. Mid-range levels are observed in roofing and paving (e.g. 10–20 µg/m³) and the lowest concentrations (i.e. at or below 1 µg/m³) are observed in coal liquefaction, coal-tar distillation, wood impregnation, chimney sweeping and power plants ([IARC, 2010](#)).

2. Cancer in Humans

No epidemiological data on benzo[a]pyrene alone were available to the Working Group.

3. Cancer in Experimental Animals

Benzo[a]pyrene was considered by three previous Working Groups ([IARC, 1973](#), [1983](#), [2010](#)).

In *IARC Monograph Volume 3* ([IARC, 1973](#)) it was concluded that benzo[a]pyrene produced tumours in all species tested (mouse, rat, hamster, guinea-pig, rabbit, duck, newt, monkey) for which data were reported following exposure by many different routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, intravenous). Benzo[a]pyrene had both a local and a systemic carcinogenic effect, was an initiator of skin carcinogenesis in mice, and was carcinogenic in single-dose studies and following prenatal and transplacental exposures.

In *IARC Monograph* Volume 32 ([IARC, 1983](#)) no evaluation was made of studies of carcinogenicity in experimental animals published since 1972, but it was concluded that there is *sufficient evidence* for the carcinogenicity of benzo[a]pyrene in experimental animals.

Carcinogenicity studies with administration of benzo[a]pyrene by multiple route of exposure, reported after the initial evaluations, were subsequently reviewed in *IARC Monograph* Volume 92 ([IARC, 2010](#)) and are summarized below ([Table 3.1](#)). See [Table 3.2](#) for an overview of malignant tumours induced in different animal species.

3.1 Skin application

In several studies in which benzo[a]pyrene was applied to the skin of different strains of mice, benign (squamous cell papillomas and keratoacanthomas) and malignant (mainly squamous-cell carcinomas) skin tumours were observed ([Van Duuren et al., 1973](#); [Cavalieri et al., 1977, 1988a](#); [Levin et al., 1977](#); [Habs et al., 1980, 1984](#); [Warshawsky & Barkley, 1987](#); [Albert et al., 1991](#); [Andrews et al., 1991](#); [Warshawsky et al., 1993](#)). No skin-tumour development was seen in *AhR*^{-/-} mice that lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice developed squamous-cell carcinomas of the skin ([Shimizu et al., 2000](#)).

In a large number of initiation–promotion studies in mice, benzo[a]pyrene was active as an initiator (mainly of squamous-cell papillomas) when applied to the skin ([IARC, 2010](#)).

3.2 Subcutaneous injection

In subcutaneous injection studies of benzo[a]pyrene, malignant tumours (mainly fibrosarcomas) were observed at the injection site in mice ([Kouri et al., 1980](#); [Rippe & Pott, 1989](#)) and rats ([Pott et al., 1973a, b](#); [Rippe & Pott, 1989](#)). No fibrosarcomas were observed in *AhR*^{-/-} mice that

lacked the aryl hydrocarbon receptor, whereas the heterozygous and wild-type mice did develop these tumours ([Shimizu et al., 2000](#)).

In another study, male and female newborn Swiss mice that were given benzo[a]pyrene subcutaneously showed a significant increase in lung-adenoma incidence and multiplicity ([Balansky et al., 2007](#)).

A single study in 12 strains of hamsters resulted in sarcomas at the site of injection in both sexes of all 12 strains ([Homburger et al., 1972](#)).

3.3 Oral administration

After administration of benzo[a]pyrene by gavage or in the diet to mice of different strains ([Spornins et al., 1986](#); [Estensen & Wattenberg, 1993](#); [Weyand et al., 1995](#); [Kroese et al., 1997](#); [Culp et al., 1998](#); [Hakura et al., 1998](#); [Badary et al., 1999](#); [Wijnhoven et al., 2000](#); [Estensen et al., 2004](#)), increased tumour responses were observed in lymphoid and haematopoietic tissues and in several organs, including the lung, forestomach, liver, oesophagus and tongue.

Oral administration of benzo[a]pyrene to *XPA*^{-/-} mice resulted in a significantly higher increase of lymphomas than that observed in similarly treated *XPA*^{+/-} and *XPA*^{+/+} mice ([de Vries et al., 1997](#)). Benzo[a]pyrene given by gavage to *XPA*^{-/-}/*p53*^{+/-} double-transgenic mice induced tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at higher incidences than in similarly treated single transgenic and wild-type counterparts. These cancer-prone *XPA*^{-/-} or *XPA*^{-/-}/*p53*^{+/-} mice also developed a high incidence of tumours (mainly of the forestomach) when fed benzo[a]pyrene in the diet ([van Oostrom et al., 1999](#); [Hoogervorst et al., 2003](#)).

Oral administration of benzo[a]pyrene by gavage to rats resulted in an increased incidence of mammary gland adenocarcinomas ([el-Bayoumy et al., 1995](#)).

Table 3.1 Carcinogenicity studies of benzo[*a*]pyrene in experimental animals

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Skin application				
Mouse, Swiss ICR/ Ha (F) 52 wk Van Duuren <i>et al.</i> (1973)	0 (untreated), 0 (vehicle control), 5 µg/animal, 3 × /wk, 52 wk 50/group	Skin T: 0/50, 0/50, 23/50 (46%; 13 P; 10 C)	+	NR (acetone)
Mouse, Swiss (F) 38–65 wk Cavalieri <i>et al.</i> (1977)	0 and 0.396 µmol [0.1 mg] per animal, twice/wk, 30 wk 40/group	Skin T: 0% [0/29], 78.9% [30/38] (7 P, 7 K, 36 C, 1 malignant Schwannoma)	+	99% (acetone)
Mouse, C57BL/6J (F) 60 wk Levin <i>et al.</i> (1977)	Experiment 1 and 2: 0 (DMSO/ acetone), 0.02 [5.28 µg], 0.1 [26.43 µg], 0.4 [105.75 µg] µmol/ animal, once/2 wk, 60 wk (high dose given in two paintings, 30 min apart) Experiment 3: 0 (acetone/NH ₄ OH), 0.025 [6.6 µg], 0.05 [13.21 µg], 0.1 [26.43 µg] µmol/animal, once/2 wk, 60 wk 30/group	Skin T (mainly SCC): Experiment 1–0%, 0%, 38% (13 T), 100% (44 T) Experiment 2–0%, 4% (1 T), 50% (15 T), 100% (40 T) Experiment 3–0%, 7% (2 T), 59% (20 T), 91% (24 T)	+	NR (DMSO/acetone (1:3) or acetone/NH ₄ OH (1 000:1)) Effective number of animals not clearly specified At most, seven animals/group died prematurely without a skin tumour.
Mouse, NMRI (F) 63–109 wk Habs <i>et al.</i> (1984)	0, 2, 4 µg/animal, twice/wk 20/group	Skin T: 0/20, 9/20 (45%; 2 P, 7 C), 17/20 (85%; 17 C)	+	> 96% (acetone)
Mouse C3H/HeJ (M) 99 wk Warshawsky & Barkley (1987)	0 (untreated), 0 (vehicle control) or 12.5 µg/animal, twice/wk 50/group	Skin T: 0/50, 0/50, 48/50 (96%; 47 C, 1 P)	+	99.5% (acetone)
Mouse, Swiss (F) 42 wk Cavalieri <i>et al.</i> (1988a)	0, 0.1 [26.4 µg], 0.4 [105.7 µg] µmol/ animal, twice/wk, 20 wk 30/group	Skin T incidence: 0/30, 26/29 (90%; SGA, 3 P, 23 SCC), 26/30 (90%; 2 P, 26 SCC)	+	Purified [NR] (acetone)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, C3H/fCum (M) Experiment 1: 15 mo Experiment 2: 18 mo Experiment 3: 18 mo Kouri et al. (1980)	Experiment 1: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or DMSO, Experiment 2: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or in DMSO, Experiment 3: 0 (trioctanoin/ DMSO, 100:1), 0.9 µmol [0.23 mg] in trioctanoin/DMSO (100:1), 1 × 20 or 40/group	FibroS at injection site: <i>Experiment 1</i> – 0/16, 0/20, 15/18 (83%), 12/19 (63%) <i>Experiment 2</i> – 0/20, 0/18, 14/18 (78%), 7/19 (37%) <i>Experiment 3</i> – 0/20, 36/40 (90%)	+	Pure (trioctanoin, DMSO)
Mouse, NR (F) 78 wk Rippe & Pott (1989)	0, 10, 100 µg/ animal, 1 × NR/group	S at injection site: 1/30 (3%), 13/30 (43%), 20/30 (67%)	+	NR (tricaprylin)
Mouse, Swiss (newborn) (M, F) 75–200 d Balansky et al. (2007)	0 and 1.0 mg/animal, 1 × 12–15 M/group, 12–15 F/group	Lung A: M – 0/15, 9/12; F – 0/15, 11/12	$P < 0.001$	Pure (olive oil)
Rat, Wistar (F) ~530 d Pott et al. (1973a)	0, 33, 100, 300, 900, 2 700 µg/ animal, 1 × 50/group	T (mainly fibroS) at injection site [incidence derived from dose–response curves]: 2/50 (~4%), 4/50 (~8%), 7/50 (~14%), 23/50 (~46%), 35/50 (~70%), 38/50 (~76%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 1 mg, 1 × NR/group	S at injection site: 0/24 (0%), 20/24 (83%)	+	NR (tricaprylin)
Rat, NR (F) 132 wk Rippe & Pott (1989)	0 and 15 mg, 1 × NR/group	S at injection site: 1/24 (4%), 19/24 (79%)	+	NR (DMSO)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian, RB (randomly bred), BIO inbred strains designated as: I.5, 4.22, 4.24, 7.88, 12.14, 15.16, 45.5, 54.7, 82.73, 86.93, 87.20 (M, F) 53 wk Homburger et al. (1972)	500 µg/animal, 1 × 25 M/group, 25 F/group	FibroS at injection site: RB–M, 4/25 (16%); F, 6/23 (26%) 1.5–M, 5/25 (20%); F, 4/23 (17%) 4.22–M, 3/25 (12%); F, 8/25 (32%) 4.24–M, not tested; F, 9/25 (36%) 7.88–M, 13/25 (52%); F, 5/23 (23%) 12.14–M, 3/25 (12%); F, 9/22 (41%) 15.16–M, 9/25 (36%); F, 16/25 (64%) 45.5–M, 12/25 (48%); F, 7/15 (47%) 54.7–M, 5/25 (20%); F, 5/25 (20%) 82.73–M, 4/21 (19%); F, 4/24 (17%) 86.93–M, 9/25 (36%); F, 8/25 (32%) 87.20–M, 16/25 (64%); F, 11/25 (42%)	+	NR (tricaprylin) No subcutaneous T observed in historical controls
Oral administration				
Mouse, A/J (F) 260 d Weyand et al. (1995)	0, 16, 98 ppm (total dose; 0, 11, 67 mg) in the diet 30/group	Lung T: 4/21 (19%; 4 A; 0.19 ± 0.09 A/animal), 9/25 (36%*; 7 A, 2 AdC; 0.48 ± 0.14 T/animal), 14/27 (52%*; 14 A; 0.59 ± 0.12 A/animal) Forestomach T: (0%) 0/21, (5/25) (20%; 3 P, 2 C; 0.24 ± 0.11** T/animal), 27/27 (100%*; 13 P, 14 C; 4.22 ± 0.41**)	*P < 0.05 **P < 0.001	NR (gel diet)
Mouse B6C3F1 (F) 2 yr Culp et al. (1998)	0 (acetone control diet), 5 ppm, 25 ppm, 100 ppm in the diet 48/group	Liver (A): 2/48 (4%), 7/48 (15%), 5/47 (11%), 0/45 (0%) Lung (A and/or C): 5/48 (10%), 0/48 (0%), 4/45 (9%), 0/48 (0%) Forestomach (P and/or C): 1/48 (2%), 3/47 (6%), 36/46 (78%****), 46/47 (98%****) Oesophagus (P and/or C): 0/48 (0%), 0/48 (0%), 2/45 (4%), 27/46 (59%**) Tongue (P and/or C): 0/48 (0%), 0/48 (0%), 2/46 (4%), 23/48 (48%****) Larynx (P and/or C): 0/35 (0%), 0/35 (0%), 3/34 (9%), 5/38 (13%*)	*P < 0.014 **P < 0.0014 ***P < 0.0003 ****P < 0.00001	98.5% (acetone)
Mouse, Swiss albino, inbred (F) 27 wk Badary et al. (1999)	0 and 1 mg/animal by gavage, twice/wk, 4 wk 10/group	0, 10/10 (100%) (forestomach P; multiplicity, 7.11 ± 1.05)	+	Highest purity grade (corn oil) Drinking-water contained 0.005% ethanol

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, CSB ^{-/-} or wild- type (CSP [±] or CSB ^{+/+}) (M, F) 52 wk Wijnhoven et al. (2000)	0 and 13 mg/kg bw by gavage, 3 × / wk, 13 wk 6–18 M/group, 6–13 F/group	Wild-type: 5/27 (14 M, 13 F; 19%; 4 bronchiolo- alveolar A, 2 lymphoma), 17/29* (18 M, 11 F; 59%; 6 bronchiolo-alveolar A, 10 forestomach P, 2 forestomach SCC, 2 histiocytic S, 2 hepatocellular A, 1 intestinal AdC, 1 skin P) CSB ^{-/-} : 0/13 (6 M, 7 F), 7/12** (6 M, 6 F; 58%; 2 bronchiolo-alveolar A, 2 uterine S, 1 forestomach SCC, 1 intestinal AdC, 1 skin histiocytic S)	*P = 0.0023 **P = 0.0017	NR (soya oil)
Rat, Crl:CD(SD)BR (F) 49 wk el-Bayoumy et al. (1995)	0 and 50 µmol/animal, once/wk, 8 wk by gavage 30/group	Mammary T incidence: 11/30 [37%] [incidence not clearly specified] (8 desmoplastic A, 2 A, 1 AdC), 29/30 (96.7%; 8 fibroA**, 17 desmoplastic A*, 7 A, 22 AdC**) Numbers of mammary T: controls, 14 desmoplastic A, 2 A, 1 AdC; treated animals, 14 fibroA*, 35 desmoplastic A, 11 A, 56 AdC**	*P < 0.05 **P < 0.01	99% (trioctanoin)
Intraperitoneal injection				
Mouse, B6C3F ₁ ; C3A/ JF ₁ (M, F) 90 wk or lifetime Vesselinovitch et al. (1975a, b)	0, 75, 150 µg in 10 µL/g bw, 1 × at 1, 15, 42 d of age 30–63/group, 96–100 controls/ group	B6C3F ₁ mice (all ages combined): Liver T (A and hepatocellular C)– M, 1/98 (1%), 69/162 (43%), 81/165 (49%); F, 0/96 (0%), 7/147 (5%), 10/126 (8%) Lung T (A and AdC)– M, 7/98 (7%), 57/162 (35%), 73/165 (44%); F, 2/90 (2%), 53/147 (36%), 50/126 (40%) Forestomach T (P and SCC)– M, 0/98 (0%), 39/162 (24%), 64/165 (39%); F, 0/96 (0%), 22/147 (15%), 40/126 (32%) Lymphoreticular T (mainly reticulum-cell S)– M, 2/98 (2%), 104/314 (33%) (high- and low-dose groups combined); F, 2/96 (2%), 148/281 (53%) (high- and low-dose groups combined)	+	NR (trioctanoin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Vesselinovitch et al. (1975a, b) Contd.			+	
		C3A/JF1 mice (all ages combined): Liver T (A and hepatocellular C)– M, 3/97 (3%), 30/148 (20%), 33/137 (24%); F, 0/100 (0%), 1.3% 2/126 (1.3%), 2/153 1.3%) Lung T (A and AdC)– M, 49/97 (49%), 1 438/148 (93%), 125/137 (91%); F, 26/100 (26%), 115/126 (91%), 141/153 (92%) Forestomach T (P and SCC)– M, 0/97 (0%), 18/148 (12%), 42/137 (31%); F, 0/100 (0%), 18/126 (14%), 31/153 (20%) Lymphoreticular T (mainly reticulum-cell S)– M, 0/97 (0%), 26/285 (9%); F, 2/100 (2%), 50/278 (18%) (high- and low-dose groups combined)		
Mouse, CD-1 (M, F) 1 yr Wislocki et al. (1986)	0 and 560 nmol [148 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 0, 8, 15) 37 M/group, 27 F/group	Liver T: M, 2/28 (7%; 2 A), 18/37* (49%; 11 A, 7 C*); F, no liver T found Lung T: M, 1/28 (4%; 1 A), 13/37** (35%; 13 A); F, 0/31, 13/27** (48%) (13 A) Malignant lymphoma: M, 1/28 (4%), 2/37 (5%); F, 1/31 (3%), 4/27 (15%)	* <i>P</i> < 0.005 ** <i>P</i> < 0.05	> 99% (DMSO)
Mouse, CD-1 (M, F) 52 wk Lavoie et al. (1987)	0 and 1.1 µmol [290 µg] (total dose; given as 1/6, 2/6, 4/6 on PND 1, 8, 15) 17 M/group, 14–18 F/group	Liver T: M, 1/17 (6%; 1 H), 13/17* (76%; 9 hepatic A, 4 H); F, 0/18, 0/14 Lung A: M, 0/17, 14/17* (82%); F, 0/18, 9/14** (64%)	* <i>P</i> < 0.005 **[<i>P</i> < 0.0005]	> 99% (DMSO)
Mouse, Swiss-Webster BLU: Ha(ICR) (M, F) 26 wk Busby et al. (1989)	0 and 59.5 µg (total dose; given as 8.5, 17, 34 µg on PND 1, 8, 15) NR/group	Lung T: M, 12/91 (13%; 12 A, 1 AdC; 0.15 ± 0.04 T/mouse), 13/28 (46%; 13 A; 0.71 ± 0.19 A/mouse); F, 7/101 (7%; 7 A; 0.08 ± 0.03 A/mouse), 18/27 (67%; 18 A, 1 AdC; 1.19 ± 0.21 T/mouse)	+	> 99% (DMSO) statistics NR
Mouse, NR, newborn (M, F) 30 wk Rippe & Pott (1989)	0, 10, 100 µg/animal, 1 × NR/group	Lung T: 13% [5/38] (0.13 T/animal), 16% [5/31] (0.23 T/animal), 64% [21/33] (2.52 T/animal)	+	NR (saline solution + 1% gelatine + 0.4% Tween 20) Type of lung tumour NR; statistics NR
Mouse, A/J (F) 260 d Weyand et al. (1995)	0 (untreated), 0 (vehicle control), 1.79 mg/animal, 1 × 29–30/group	Lung T: 7/30 (23%; 7 A; 0.27 ± 0.12 A/animal), 11/30 (37%; 11 A; 0.43 ± 0.11 A/animal), 29/29* (100%; 27 A, 2 AdC; 15.8 ± 1.28** T/animal); forestomach T: 0/30 (0%), 0/30 (0%), 24/29** (83%; 15 P, 9 C; 1.83 ± 0.25** T/animal)	* <i>P</i> < 0.05 ** <i>P</i> < 0.001	NR (tricaprylin)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Mouse, B6C3F1 infant (M, F) 26 wk, 39 wk, 52 wk Rodriguez et al. (1997)	0 (untreated), 0 (vehicle controls), 125, 250, 375 µg/7 g bw, 1 × > 30 M/group, > 30 F/group	Liver T (M): At wk 26: 0/41, 0/58, 0/29, 0/25, 3/34 (9%; multiplicity, 1.0); at wk 39: 0/34, 0/59, 6/26 (23%; multiplicity, 1.0), 13/34 (38%); multiplicity, 1.9), 15/23 (65%); multiplicity, 1.9); at wk 52: 4/64 (6%; multiplicity, 1.0), 3/63 (5%); multiplicity, 1.0), 13/29 (45%); multiplicity, 1.8), 14/27 (52%); multiplicity, 2.2), 19/24 (79%); multiplicity, 2.5) No liver T in F	+	NR (corn oil) No forestomach tumours
Mouse, CD-1 (M) 12 mo Von Tungeln et al. (1999)	0, 100, 400 nmol [26, 111 µg]/ animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15) 24/group	Liver T: 3/20 (15%); 1 A, 2 C; 1.7 T/liver section), 5/21 (24%); 4 A, 1 C; 1.5 T/liver section), 9/20 (45%; 7 A*, 2 C; > 2.3 T/liver section) Lung T: 4/20 (20%); 4 A; 1.0 T/lung section), 1/21 (5%); 1 A; 1.0 T/lung section), 9/20 (45%); 7 A, 2 C; 1.9 T/lung section)	*P = 0.0234	> 99% (DMSO)
Rat, Wistar (F) ~112 wk Roller et al. (1992)	0 and 5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 3/41 (7.3%), 33/37 (89.2%)	+	NR (3:1 mixture of tricaprylin/ beeswax) Limited reporting
Rat, Wistar (F) ~116 wk Roller et al. (1992)	5 mg/animal, 1 × NR/group	Abdominal mesothelioma and S: 19/38 (50%); historical controls, 11/369 (3%)	+	NR (saline solution) No control; limited reporting of tumour data
Inhalation				
Hamster, Syrian golden (M) Lifetime Thyssen et al. (1981)	0, 2.2, 9.5, 46.5 mg/m ³ , 4.5 h/d, 7 d/ wk, 10 wk; thereafter 3 h/d, 7 d/wk (total average doses: 0, 29, 127, 383 mg/animal) 24/group (+ animals added during the study)	Respiratory tract T: (polyps, P, SCC) – 0/27, 0/27, 34.6% [9/26; 3 nasal, 8 laryngeal, 1 tracheal], 52% [13/25; 1 nasal, 13 laryngeal, 3 tracheal; no bronchogenic T] Upper digestive tract T: (polyps, P, SCC) – 0/27, 0/27, 26.9% [6/26; 6 pharyngeal, 1 forestomach], 56% [14/25; 14 pharyngeal, 2 oesophageal, 1 forestomach]	+	NR (0.1% saline solution); particle size, > 99% diameter 0.2–0.5 µm, > 80% diameter 0.2–0.3 µm Survival decreased for high dose-exposed animals (59 wk) vs other groups (96 wk).

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Intrapulmonary injection				
Rat, OM (F) 64 (high-dose group)–133 wk (untreated controls) Deutsch-Wenzel et al. (1983)	0 (untreated), 0 (vehicle control), 0.1, 0.3, 1.0 mg/animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [10/35] (28.6%) (4 epidermoid C; 6 pleomorphic S), [23/35] (65.7%) (21 epidermoid C; 2 pleomorphic S), [33/35] (94.3%) (33 epidermoid C)	+	99.1% (1:1 mixture of beeswax and tricaprylin)
Rats, F344/NSlc (M) 104 wk Iwagawa et al. (1989)	0, 0.03, 0.1, 0.3, 1.0 mg/ animal, 1 × NR/group	Lung T: 0/40, 1/29 (3%; 1 undifferentiated T), 7/30 (23%; 6 SCC, 1 undifferentiated T), 22/29 (76%; 20 SCC, 2 undifferentiated T), 9/13 (69%; 9 SCC)	+	NR (1:1 mixture of beeswax/ tricaprylin)
Rat, Osborne-Mendel (F) 134 wk (low-dose group)–140 wk (vehicle controls) Wenzel-Hartung et al. (1990)	0 (untreated), 0 (vehicle control), 30, 100, 300 µg/ animal, 1 × 35/group	Lung T: [0/35] (0%), [0/35] (0%), [3/35] (8.6%; 3 SCC), [11/35] (31.4%; 11 SCC), [27/35] (77.1%; 27 SCC).	+	99.6% (beeswax/trioctanoin mixture of varying composition) SCC predominantly keratinized
Rat, F344/DuCrj (M) 100 wk Horikawa et al. (1991)	0, 50, 100, 200 µg/animal, 1 × 9–10/group	Lung T: 0/19, 0/10, 3/10 (30%; 2 SCC, 1 AdSC), 4/9 (44.4%; 3 SCC, 1 undifferentiated T)	+	NR (1:1 mixture of beeswax/ tricaprylin)
Intratracheal administration				
Rat, Wistar-WU/ Kisslegg (F) 124–126 wk Pott et al. (1987)	0 and 1 mg/animal, once/wk, 20 wk NR/group	Lung T: 0/40, 7/36 (19%; 1 A, 5 SCC, 1 mixed AdC/SCC)	+	NR (0.9% saline solution)
Rat, Sprague-Dawley (M, F) Controls, 131 wk; treated animals, 112 wk Steinhoff et al. (1991)	0 and 0 (physiological saline), 7 mg/kg bw/instillation (physiological saline with Tween 60), once/2 wk, 44 wk 20 or 50/group	M: 0/50, 0/50, 19/20 (95%; 19 malignant lung T) F: 0/50, 0/50, 19/20 (95%; 18 malignant, 1 benign lung T)	+	NR (physiological saline solution with or without Tween 60) Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 78 wk Feron (1972)	0 (only for M), 1 mg/animal, once/ wk, 36 wk 35/group	Respiratory tract T/adenomatoid lesions: M–6/27 (22%; 1 tracheal P, 5 pulmonary adenomatoid lesion), 19/29 (66%; 1 tracheal P, 17 SCC, 26 pulmonary adenomatoid lesion, 5 A, 1 AdC, 1 SCC) F–22/27 (81%; 1 laryngeal SCC, 16 tracheal SCC, 2 bronchial A, 1 AdC, 21 pulmonary adenomatoid lesion, 8 A, 1 AdC)	+	> 99% (0.9% saline solution) No female controls; Statistics NR
Hamster, Syrian golden (M) 78 wk Feron et al. (1973)	0, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/ animal, once/wk, 52 wk 30/group	Respiratory tract T: 0/29, 3/30 (10%; 3 tracheal P, 1 pulmonary A), 4/30 (13%; 1 tracheal P, 4 pulmonary A), 9/30 (30%; 5 tracheal P, 7 pulmonary A), 25/29 (86%; 2 tracheal polyp, 9 P, 5 SCC, 1 AdSC, 1 fibroS, 2 bronchial polyp, 1 P, 2 SCC, 1 AdSC), 26/28 (93%; 6 tracheal P, 11 SCC, 1 AdSC, 1 bronchial polyp, 2 P, 4 SCC, 2 AdSC, 4 AdC, 1 anaplastic C, 16 pulmonary A, 4 SCC, 3 AdSC, 1 AdC, 2 anaplastic C)	+	NR (0.9% saline solution)
Hamster, Syrian golden (M, F) M, 67–88 wk; F, 60–88 wk Henry et al. (1973)	0, 13.3–15.5 mg/animal, once/wk, 8 wk 50/group, 25 controls/group	Respiratory tract T: Controls– 1 tracheal polyp, 6 pulmonary bronchiolar adenomatoid lesions/47 animals Treated animals– 26/65 (40%; 1 nasal polyp; 6 laryngeal polyps, 1 P, 1 A, 1 AdC, 7 tracheal polyps, 1 AdC, 1 SCC, 1 fibroS, 2 bronchial AdC, 13 pulmonary bronchiolar adenomatoid lesion, 3 A, 5 AdC, 1 SCC, 2 anaplastic C, 1 mixed C, 1 myelogenous leukaemia, 1 neurofibroS) T at other sites: Controls– 1 renal A Treated animals– 3 blast-cell leukaemia, 2 adrenocortical A, 1 renal AdC, 1 oesophageal fibroS, 1 haemangioma	+	NR (0.5% gelatine in 0.9% saline solution) Tumour data for M and F combined; statistics NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 60 wk Kobayashi (1975)	0 and 1 mg/animal, once/wk, 30 wk 20–32 M/group, 20–28 F/group	Respiratory tract T: M–0/20, 11/26 (42.3%); 1 laryngeal polyp, 1 tracheal polyp, 1 P, 1 bronchial SCC, 9 lung A, 7 AdC, 3 SCC, 1 anaplastic C, 2 AdSC) F–0/20, 14/26 (53.8%); 1 laryngeal P, 2 tracheal polyps, 1 bronchial SCC, 10 lung A, 3 AdC, 1 SCC)	+	NR (0.9% saline)
Hamster, Syrian golden (M, F) 78 wk Kruysse & Feron (1976)	0 (untreated), 0 (vehicle controls), 1 mg/animal, once/2 wk, 52 wk 17 or 40/group	Respiratory tract T: M–0/40, 0/40, 13/14 (93%); 2 laryngeal P, 1 SCC, 4 tracheal P, 3 SCC, 1 anaplastic C, 1 S, 1 bronchial SCC, 1 AdC, 5 pulmonary A, 1 AdC) F–0/40, 0/40, 7/12 (58%); 2 tracheal P, 3 SCC, 1 bronchial P, 5 pulmonary A)	+	> 99% (saline solution)
Hamster, Syrian golden (M) 100 wk Sellakumar <i>et al.</i> (1976)	0 (untreated), 3 mg/animal, once/ wk, 10 wk 48/group	Respiratory tract T: 0/48, 7/48 (15%); 2 laryngeal P, 4 tracheal P, 1 lung A) T at other sites: 6/48 (13%); 3 forestomach P, 2 lymphoma, 1 anaplastic C), 26/48 (54%); 21 forestomach P, 1 skin melanoma, 1 liver haemangioma, 1 adrenocorticoA, 3 adrenocorticoC)	+	> 99% (0.9% saline solution)
Hamster, Syrian golden (M, F) Experiment 1: up to 89 wk for M and 70 wk for F Experiment 2: up to 83 wk for M and 68 wk for F Ketkar <i>et al.</i> (1977)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/animal, 1 × 30/group Experiment 2: 0, 4, 8, 16 mg in Tris buffer/ animal, 1 × 30/group	Respiratory tract T: Experiment 1– M 0/24, 3/30 (10%); 1 laryngeal P, 1 tracheal P, 1 lung S), 5/28 (18%); 1 laryngeal SCC, 1 tracheal P, 4 lung S), 4/27 (15%); 3 tracheal P, 1 lung A, 1 S) F 0/28, 3/29 (10%); 1 tracheal P, 2 lung A), 1/30 (3%); 1 lung A), 3/28 (13%); 1 laryngeal P, 2 lung A) Experiment 2– M 0/27, 5/24 (21%); 1 tracheal P, 5 lung A), 13/25 (52%); 1 laryngeal P, 7 tracheal P, 4 lung A, 3 AdC), 8/27 (30%); 2 laryngeal P, 1 SCC, 3 tracheal P, 3 lung A) F 0/27, 3/27 (11%); 2 tracheal P, 1 lung AdC), 2/29 (7%); 2 tracheal P), 8/29 (28%); 1 laryngeal P, 4 tracheal P, 5 lung A)	+	97% (0.9% saline solution or Tris buffer)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 81 wk Feron & Krusysse (1978)	0 (untreated), 0 (vehicle controls), 0.35, 0.7 mg/animal, once/wk, 52 wk 15 or 30/group	Respiratory tract T: M-0/30 (untreated and vehicle controls combined), 4/29 (14%; 2 tracheal P, 1 bronchial P, 2 pulmonary A), 19/30 (63%; 1 laryngeal P, 5 tracheal P, 1 SCC, 1 anaplastic C, 1 S, 2 bronchial P, 1 AdC, 11 pulmonary A, 2 AdC, 1 SCC, 1 anaplastic C) F-0/28 (untreated and vehicle controls combined), 3/27 (11%; 1 laryngeal P, 1 bronchial P, 1 pulmonary A), 7/24 (29%; 1 tracheal P, 2 SCC, 1 bronchial AdC, 5 pulmonary A)	+	> 99% (0.9% saline solution) Statistics NR
Hamster, Syrian golden (M, F) Average survival up to 41 wk for M and 35 wk for F Ketkar et al. (1978)	0, 0.1, 0.33, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: M-0/29, 5/26 (19%; 5 bronchiogenic A), 7/29 (24%; 5 tracheal P, 2 bronchiogenic A), 6/27 (22%; 5 tracheal P, 2 bronchiogenic A) F-0/30, 12/30 (40%; 1 tracheal P, 1 SCC, 10 bronchiogenic A), 10/28 (36%; 7 tracheal P, 5 bronchiogenic A, 1 SCC), 6/30 (20%; 3 tracheal P, 3 bronchiogenic A, 3 SCC)	+	97% (10% bovine serum albumin) Average survival time much lower in the high-dose group than in the other groups
Hamster, Syrian golden (M, F) Lifetime, up to 90 wk Stenbäck & Rowland (1978)	0, 3 mg large particles, 3 mg small particles/animal, once/wk, 18 wk 48 (M + F)/group	Respiratory tract T (M + F combined): 0/46, 31/47 (66%; 5 laryngeal P, 12 tracheal P, 20 SCC, 2 unspecified T, 2 bronchial P, 9 SCC, 3 A, 2 anaplastic C), 5/46 (11%; 1 laryngeal P, 1 SCC, 4 tracheal P)	+	99.4% (0.9% saline solution); particle size by weight: large-98% < 30 µm, 90% < 20 µm, 36% < 10 µm, 10% < 5 µm; small-98% < 10 µm, 79% < 5 µm, 5% < 1 µm
Hamster, Syrian golden (M) Average survival up to 88 wk Ketkar et al. (1979)	0 (untreated), 0 (vehicle controls), 0.125, 0.25, 0.5, 1.0 mg/animal, once/wk 30/group	Respiratory tract T: 0/29, 0/28, 9/29 (31%; 2 laryngeal polyps/P, 1 tracheal P, 1 SCC, 2 lung A, 2 SCC, 5 AdC), 24/29 (83%; 1 nasal SCC, 2 laryngeal polyps/P, 4 tracheal P, 9 SCC, 5 lung A, 5 SCC, 11 AdC), 19/29 (66%; 1 laryngeal P, 2 SCC, 5 tracheal P, 11 SCC, 7 lung SCC, 2 AdC), 9/29 (31%; 1 laryngeal P, 1 SCC, 1 tracheal P, 5 SCC, 1 lung A, 4 SCC)	$P < 0.001$, all treated groups	97% (Tris buffer + 0.9% saline solution); particle size: majority < 10 µm but particles up to 80 µm also present Average survival in two highest-dose groups much lower than that in the other groups due to many early deaths from pulmonary lesions other than tumours

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) 105 wk Feron et al. (1980)	0 (untreated), 0 (gelatine in 0.9% saline), 0.5, 1.0 mg fine particles, 0.5, 1.0 mg coarse particles, 1.0 mg wide-range particles/animal, once/wk, 52 wk 30–35/group	Respiratory tract T: M–0/29, 2/34 (6%); 2 laryngeal P, 7/34 (21%); 1 laryngeal P, 6 tracheal P, 1 lung A, 6/31 (19%); 2 laryngeal P, 1 tracheal P, 1 S, 1 pulmonary A, 13/31 (42%); 2 laryngeal P, 3 tracheal P, 9 pulmonary A, 25/34 (74%); 2 laryngeal P, 9 tracheal P, 4 SCC, 2 S, 1 pulmonary A, 1 AdC), 23/34 (68%); 2 laryngeal P, 1 SCC, 6 tracheal P, 2 SCC, 1 bronchial P, 1 SCC, 13 pulmonary A, 2 AdC, 2 anaplastic C) F–0/28, 2/33 (6%); 1 tracheal P, 1 pulmonary A), 2/34 (6%); 1 bronchial P, 1 A), 5/32 (16%); 1 laryngeal P, 2 tracheal P, 3 pulmonary A), 9/32 (28%); 2 laryngeal P, 5 tracheal P, 6 pulmonary A), 19/32 (31%); 4 tracheal P, 1 SCC, 1 S, 1 bronchial P, 7 pulmonary A, 1 AdC), 11/34 (34%); 1 laryngeal P, 3 tracheal P, 2 bronchial P, 7 pulmonary A, 1 AdC)	+	NR; particles size by weight: fine, 77% < 5.2 µm, 60% < 3.9 µm; coarse, 77% < 42 µm, 3% < 16 µm; wide-range, 72% < 30 µm, 19% < 10 µm (gelatine in 0.9% saline solution) Statistics NR
Hamster, Syrian golden (M) 129 wk Godleski et al. (1984)	0 and 5 mg/animal, once/wk, 15 wk 80/group	Malignant T: 4/80 (5%); 1 multicentric undifferentiated lung C, 3 lymphoma), 25/80* (31%); 9 SCC, 2 undifferentiated C of the respiratory tract, 5 lymphoma, 1 SCC, 2 AdC of the gastrointestinal tract, 2 soft-tissue T, 1 hepatoma, 2 mouth SCC, 1 skin C)	*P < 0.001	> 99% (0.5% gelatine in 0.9% saline solution)
Intratracheal administration of combinations of benzo[a]pyrene and 'particles/fibres'				
Rat, Sprague-Dawley (M, F) Up to 130 wk Steinhoff et al. (1991)	0, (untreated), 0 (physiological saline), 10–40 mg/kg bw Bayferrox 130 (96.2% cubic α-Fe ₂ O ₃), 10–40 mg/kg bw Bayferrox 920 (86.1% fibrous α-FeOOH), 7 mg/kg bw, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 130, 7 mg/kg bw + 10–40 mg/kg bw Bayferrox 920, ~once/2 wk, 44–~130 wk 20 or 50/group	Lung T: M–0/50, 0/50, 0/50, 0/50, 19 malignant T in 20 animals, 21 malignant and 1 benign T in 20 animals, 17 malignant and 1 benign T in 20 animals F–0/50, 0/50, 0/50, 1 malignant and 1 benign T in 50 animals, 18 malignant and 1 benign T in 20 animals, 16 malignant T in 20 animals, 17 malignant and 2 benign T in 20 animals	+	NR (physiological saline solution with or without Tween 60); Bayferrox 130, Bayferrox 920 Limited histology

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 140 wk) Saffiotti et al. (1972)	Experiment 1 0, 50 mg ferric oxide, 5 mg + 45 mg ferric oxide, 12.5 mg + 37.5 mg ferric oxide/ animal, 1 × Experiment 2 (2 groups/dose level) 5 mg + 5 mg ferric oxide, 10 mg + 10 mg ferric oxide, 15 mg + 15 mg ferric oxide, once/wk, 15 wk 23–110 M/group, 18–107 F/group	Experiment 1: Respiratory tract T– M 0/45, 0/101, 3/92 (3%); 1 tracheal polyp, 1 P, 1 bronchial A, 3/27 (11%); 1 bronchial A, 1 bronchogenic SCC, 1 anaplastic C) F 0/44, 0/89, 4/97 (4%); 1 tracheal polyp, 1 P, 1 bronchiolar A, 1 AdC, 6/33 (18%); 1 bronchial P, 1 A, 2 bronchogenic SCC, 1 anaplastic C, 2 bronchiolar A) Forestomach P– M 5/45 (11%; 6 T), 5/101 (5%; 5 T), 15/92 (16%; 35 T), 8/27 (30%; 16 T) F 2/44 (5%; 2 T), 2/89 (2%; 3 T), 5/97 (5%; 5 T), 4/33 (12%; 6 T) Experiment 2: Respiratory tract T (M + F combined)– 7/50 (14%; 2 tracheal P, 1 SCC, 1 bronchial P, 1 A, 2 SCC, 1 anaplastic C, 1 pulmonary SCC), 8/58 (14%; 2 tracheal polyps, 1 bronchial polyp, 2 SCC, 2 AdC, 2 pulmonary A, 2 AdC), 17/61 (28%; 2 tracheal polyps, 2 P, 5 SCC, 5 bronchial SCC, 1 pulmonary A, 1 SCC, 1 AdC, 1 anaplastic C), 25/60 (42%; 4 tracheal polyps, 3 P, 3 SCC, 4 anaplastic C, 1 bronchial P, 1 SCC, 2 anaplastic C, 4 AdC, 1 A, 2 pulmonary SCC, 2 anaplastic C, 6 A), 25/39 (64%; 1 tracheal P, 10 SCC, 1 anaplastic C, 3 bronchial P, 7 SCC, 11 anaplastic C, 2 AdC, 2 pulmonary SCC, 2 A), 35/55 (64%; 2 laryngeal SCC, 11 tracheal P, 1 polyp, 12 SCC, 1 carcinoS, 2 fibroS, 16 bronchial SCC, 10 anaplastic C, 6 AdC, 3 A, 2 pulmonary A) Forestomach T– M 8/22 (36%; 13 P, 1 SCC), 6/28 (21%; 9 P), 11/34 (32%; 28 P, 1 SCC), 11/30 (37%; 18 P), 5/22 (23%; 10 P), 1/28 (4%; 1 P) F 9/28 (32%; 14 P), 6/30 (20%; 9 P), 5/27 (19%; 20 P), 8/30 (27%; 10 P, 1 SCC), 5/17 (29%; 11 P), 3/27 (11%; 3 P)	+	NR (0.9% saline solution); ferric oxide

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (F) Presumably lifetime Pott et al. (1973b)	340 µg in tricaprylin, the larynx, trachea or bronchi): 340 µg in Tween 60/saline solution, 340 µg in Tween 60/saline solution + 850 µg atmospheric dust/animal, 45 × within a period of 6.5 mo (total dose, ~15 mg; dust, 38 mg) 48/group	Respiratory tract T (benign and malignant T of the larynx, trachea or bronchi): 2/48 (4%), 14/48 (29%), 16/48 (33%)	+	NR (tricaprylin, Tween 60/ saline solution); atmospheric dust from Bochum, Germany (particle size < 5 µm)
Hamster, Syrian (M, F) 100 wk Sellakumar et al. (1973)	0 (untreated), 3 mg + 3 mg ferric oxide, 3 mg + 6 mg ferric oxide, 3 mg + 9 mg ferric oxide, once/2 wk, 20 wk 36/group, 193 controls/group	Respiratory tract T (M + F combined): 0/193, 26/67 (39%); 3 laryngeal polyp, 3 P, 3 SCC, 7 tracheal polyp, 6 P, 2 SCC, 2 bronchial polyp, 5 SCC, 9 AdC, 1 anaplastic C, 7 lung A, 1 AdC), 28/64 (44%); 1 laryngeal polyp, 3 P, 6 SCC, 3 tracheal polyp, 9 P, 3 SCC, 3 bronchial polyp, 1 P, 4 SCC, 3 AdC, 1 anaplastic C, 7 lung A, 4 AdC), 26/66 (39%); 3 laryngeal polyp, 6 SCC, 6 tracheal polyp, 11 P, 1 SCC, 1 bronchial polyp, 1 P, 4 SCC, 4 AdC, 2 anaplastic C, 6 lung A, 6 AdC) Forestomach T: M–0/193 (M + F), 17/32 (53%; 37 P), 10/31 (32%; 16 P, 1 SCC), 6/35 (17%; 15 P) F–0/193 (M + F), 10/35 (29%; 30 P), 12/33 (36%; 25 P), 15/31 (48%; 33 P)	+	NR (0.9% saline solution); ferric oxide
Hamster, Syrian golden (M, F) Lifetime (up to 120 wk) Stenbäck et al. (1975)	0 (untreated), 2 mg + 1 mg magnesium oxide/ animal, once/wk, 20 wk, 3 mg + 3 mg ferric oxide/animal, once/wk, 15 wk 48 or 90/group	Respiratory tract tumours (M + F combined): 0/89, 32/45 [71%] (11 laryngeal P, 3 SCC, 1 tracheal polyp, 20 P, 5 SCC, 1 AdC, 1 bronchial P, 3 A, 8 AdC, 9 SCC, 1 AdSC), 31/44 (70%; 10 laryngeal P, 4 SCC, 8 tracheal P, 12 SCC, 2 anaplastic C, 2 bronchial P, 4 A, 2 AdC, 17 SCC, 3 anaplastic C)	+	NR (0.2% saline solution); ferric oxide, magnesium oxide

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Hamster, Syrian golden (M, F) Lifetime (up to 100 wk) Stenbäck & Rowland (1979)	0 (untreated), 0 (saline), 0 (gelatine in saline), 3 mg silicon dioxide in saline, 1.5 mg manganese dioxide in saline, 3 mg in saline, 3 mg in gelatine/saline, 3 mg + 3 mg silicon dioxide in saline, 1.5 mg + 1.5 mg manganese dioxide in saline/ animal, once/wk, 20 wk 50/group	All T (M + F combined): 2/100 (2%); 2 lymphoma), 1/48 (2%); 2 forestomach P), 2/45 (4%); 2 lymphoma), 0/48 (0%); 2/48 (4%); 1 forestomach P, 1 lymphoma), 18/46 (39%); 1 laryngeal P, 1 SCC, 4 tracheal P, 15 forestomach P), 11/47 (23%); 2 tracheal P, 1 SCC, 3 bronchial SCC, 1 splenic haemangioma, 1 adrenal cortical A, 1 lymphoma, 2 forestomach SCC), 25/48 (52%); 1 laryngeal SCC, 8 tracheal P, 2 SCC, 3 bronchial SCC, 6 lung A, 3 AdC, 10 forestomach P, 1 thyroid A, 1 uterine fibroma, 1 A, 1 lymphoma), 20/48 (42%); 1 laryngeal P, 3 tracheal P, 1 SCC, 1 bronchial SCC, 24 forestomach P, 1 ovarian fibroma, 1 thyroid A, 2 forestomach SCC, 1 squamous-cell fibroma)	+	> 99% (saline, 0.5% gelatine in saline); manganese dioxide, silicon dioxide
Hamster, Syrian golden (M, F) 82 wk Reynders et al. (1985)	0 and 8 mg + 6 mg ferric oxide/ animal, once/wk, 6 wk 35/group	Respiratory tract T: M-0/32, 12/24 (50%); 15 T: 3 laryngeal P, 1 tracheal P, 1 SCC, 2 bronchial polyp, 2 SCC, 1 AdC, 3 pulmonary SCC, 1 AdSC, 1 AdC) F-0/35, 9/26 (35%); 12 T: 1 laryngeal P, 5 tracheal P, 2 bronchial polyp, 2 pulmonary SCC, 1 AdSC, 1 AdC)	+	NR (0.9% saline solution); ferric oxide
Buccal pouch				
Hamster, Syrian golden (M) Up to 40–44 wk with interim kills after 5, 20, and 24–32 wk Solt et al. (1987)	Painting of both buccal pouch with 0, 20 mM solution/animal, twice/ wk, 20 wk 28/group, 20 controls/group	Forestomach P: 0/6, 8/10* (after 40–44 wk) Buccal pouch SCC: 0/6, 1/10 (after 40–44 wk)	*[<i>P</i> < 0.01]	NR (paraffin oil)
Intramammary or intramamillary administration				
Rat, Sprague-Dawley (F) 20 wk Cavalieri et al. (1988a)	0 and 0 (untreated contralateral mammary gland), 4 [1 mg], 16 µmol [4.2 mg] (5 th right mammary gland), 1 × 20/group	Mammary gland T: [0/20] (0%), [0/20] (0%), [10/20] (50%); 6 AdC, 4 fibroS), [16/20] (80%); 8 AdC, 2 fibroA, 10 fibroS)	+	> 99% (no vehicle)

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Rat, Sprague-Dawley (F) 45 wk Cavalieri et al. (1988a, b)	0 and 4 µmol [1 mg]/mammary gland (2nd, 3rd, 4th and 5th mammary gland on both sides injected), 1 × 20/group	Epithelial mammary T: [3/20] (15%; 3 fibroA), [14/20] (70%; 13 AdC, 3 fibroA); multiplicity: controls, 3/3 [1]; treated rats: AdC, 18/13 [1.4]; fibroA, 4/3 [1.3] Mesenchymal (mammary) T: [0/20] (0%), [11/20] (55%; 11 fibroS; multiplicity, 20/11 [1.8]) Skin T: [0/20] (0%), [9/20] (45%; 9 SCC; multiplicity, 11/9 [1.2])	+	> 99% (trioctanoin)
Rat, Sprague-Dawley (F) 24 wk Cavalieri et al. (1991)	0, 0.25 [66 µg], 1 µmol [264 µg]/mammary gland (the 2nd, 3rd, 4th and 5th on both sides), 1 × 20/group	Epithelial mammary gland T: 1/18 (6%; 1 fibroA), 1/20 (5%; 1 AdC), 0/20 (0%) Mesenchymal (mammary) T: 0/18 (0%), 6/20 (30%; 6 fibroS; multiplicity, 7/6), 8/20 (40%; 8 fibroS; multiplicity, 10/8) Skin T: 0/18 (0%), 0/20 (0%), 1/20 (5%; 1 SCC)	+	> 99% (trioctanoin) Statistics NR
Intracolonic instillation				
Mouse, Swiss albino (M, F) 120 wk Toth (1980)	0, 200, 2000 µg/g bw (total doses); control and high-dose group, 10 × /wk instillations of 0 and 200 µg, respectively; low-dose group, 1 instillation 50/group/sex	Malignant lymphoma: M–0/50, 6/50* (12%; 1 histiocytic, 4 lymphocytic, 1 mixed), 7/50** (14%; 2 histiocytic, 3 lymphocytic, 2 mixed) F–11/49 (22%; 5 histiocytic, 6 lymphocytic), 21/50*** (42%; 5 histiocytic, 16 lymphocytic), 18/49 (36%; 6 histiocytic, 8 lymphocytic, 4 mixed) Oesophagus T: M–no tumour F–0/49, 0/50, 5/49 (10%) Forestomach T: M–0/50, 2/50 (4%; 2 P), 10/50**** (20%; 9 P, 1 SCC) F–1/49 (2%; 1 SCC), 5/10 (20%; 3 P, 2 SCC), 11/49**** (22%; 9 P, 2 SCC)	*P < 0.04 **P < 0.02 ***P < 0.053 ****P < 0.006 *****P < 0.0001	98% (olive oil) Anal and skin tumours probably due to release of benzo[a]pyrene through the anal orifice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Result or significance	Purity (vehicle) Comments
Toth (1980) Contd.		Anal T : M-0/50, 0/50, 7/50** (14%; 4 P, 3 SCC) F-0/49, 1/50 (2%; 1 P), 6/49* (12%; 1 P, 4 SCC, 1 K) Skin T : M-1/50 (2%; 1 K), 0/50, 13/50***** (26%; 5 P, 7 SCC, 1 K) F-0/49, 2/50 (4%; 2 SCC), 11/49***** (22%; 4 P, 5 SCC, 2 K)		
Mouse, C57Bl/6 (F) 18 mo Anderson et al. (1983)	0 (untreated, olive oil or β -naphthoflavone in olive oil), 1 mg/animal (in olive oil), once/ wk, 14 wk 45–60/group	Forestomach P: 7/34 (21%; multiplicity, 1.1 ± 0.4), 17/18* (94%; multiplicity, $3.2 \pm 2.3^*$) Peritoneal S: 0/40, 5/32* Lymphoma: 1/40 (2.5%), 9/32* (28%)	* $P < 0.05$	99% (olive oil, enzyme inducer β -naphthoflavone) No colon tumours found
Intravaginal application				
Mouse, C57Bl (F) 5 mo Näslund et al. (1987)	Cotton swab soaked in acetone (controls) or 1% solution of benzo[a]pyrene in acetone, twice/ wk 10 or 76/group	0/10, 17/76 (22%; invasive cervical C)	+	NR (acetone)
Intrafetal injection				
Mouse, Swiss (M, F) 12 wk Rossi et al. (1983)	0, 0.4, 4.0, 9.9, 19.8 nmol [0, 0.1, 1, 2.6, 5.2 μ g]/animal, 1 \times 43–56/group	Lung A (M + F combined): 0/37, 1/39 (3%), 10/42 (25%), 10/38 (26%), 12/31 (39%)	+	> 99% (trioctanoin-acetone mixture (1:1))

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; bw, body weight; C, carcinoma; d, day or days; DMSO, dimethyl sulfoxide; F, female; H, hepatoma; K, keratocanthoma; M, male; min, minute or minutes; mo, month or months; NH_4OH , ammonium hydroxide; NR, not reported; P, papilloma; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; SGA, sebaceous gland adenoma; T, tumour; vs, versus; wk, week or weeks; yr, year or years

Table 3.2 Summary of reports of malignant tumours clearly induced in experimental animals by benzo[a]pyrene

Organ site/ species	Lung	Trachea	Larynx	Forestomach	Liver	Lymphoid tissue (lymphoma)	Sarcoma (injection site)	Skin	Mammary gland
Mouse	x			x	x	x	x	x	
Rat	x						x		x
Hamster	x	x	x	x			x		

3.4 Intraperitoneal injection

In a series of studies in newborn and adult mice, intraperitoneal injection of benzo[a]pyrene increased the incidence of liver (adenomas and carcinomas) and lung (adenomas and adenocarcinomas) tumours and, occasionally, forestomach (squamous cell papillomas and carcinomas) and lymphoreticular tumours ([Vesselinovitch et al., 1975a, b](#); [Wislocki et al., 1986](#); [Lavoie et al., 1987](#); [Busby et al., 1989](#); [Rippe & Pott, 1989](#); [Mass et al., 1993](#); [Nesnow et al., 1995](#); [Ross et al., 1995](#); [Weyand et al., 1995](#); [Rodriguez et al., 1997](#); [Von Tungeln et al., 1999](#)).

In one study in rats with a single intraperitoneal injection of benzo[a]pyrene, a high incidence of abdominal mesotheliomas and sarcomas was observed ([Roller et al., 1992](#)).

3.5 Inhalation

In a lifetime inhalation study ([Thyssen et al., 1981](#)) in male hamsters, benzo[a]pyrene induced dose-related increases in the incidence of papillomas and squamous-cell carcinomas in both the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach).

3.6 Intrapulmonary injection

Dose-related increases in the incidence of malignant lung tumours (mainly epidermoid and squamous-cell carcinomas and a few pleomorphic sarcomas) were found after injection of benzo[a]pyrene into the lung of rats ([Deutsch-Wenzel et al., 1983](#); [Iwagawa et al., 1989](#); [Wenzel-Hartung et al., 1990](#); [Horikawa et al., 1991](#)).

3.7 Intratracheal administration

Intratracheal administration of benzo[a]pyrene alone or mixed with particulates and suspended in saline with or without suspensions resulted in benign and malignant respiratory tumours in mice ([Heinrich et al., 1986a](#)), rats ([Pott et al., 1987](#); [Steinhoff et al., 1991](#)) and in numerous studies in hamsters ([IARC, 2010](#)). This treatment also induced forestomach tumours in hamsters ([Saffiotti et al., 1972](#); [Sellakumar et al., 1973](#); [Smith et al., 1975a, b](#); [Stenbäck & Rowland, 1979](#)). Larger benzo[a]pyrene particles were generally more effective than smaller ones.

Mice that lack the nucleotide excision-repair gene *XPA* (*XPA*^{-/-} mice) showed a stronger lung-tumour response after intratracheal instillation of benzo[a]pyrene than did their similarly treated *XPA*^{+/+} and *XPA*^{+/-} counterparts ([Ide et al., 2000](#)).

3.8 Buccal pouch application

Repeated application of benzo[a]pyrene to the buccal pouch mucosa of male hamsters resulted in a high incidence of forestomach papillomas ([Solt *et al.*, 1987](#)).

3.9 Subcutaneous tracheal grafts transplantation

In one study conducted in rats transplanted with subcutaneous rat tracheal grafts exposed to beeswax pellets containing various amounts of benzo[a]pyrene, a high incidence of squamous-cell carcinomas was reported ([Nettesheim *et al.*, 1977](#)).

3.10 Intramammary administration

In three studies in rats, benign and malignant mammary gland tumours developed after intramammary injection of benzo[a]pyrene ([Cavalieri *et al.*, 1988a](#), [b](#), [1991](#)).

3.11 Intracolonic instillation

In three experiments in mice, intracolonic instillation of benzo[a]pyrene induced lymphomas and a variety of benign and malignant tumours in various organs including the forestomach ([Toth, 1980](#); [Anderson *et al.*, 1983](#)).

3.12 Intravaginal application

Intravaginal application of benzo[a]pyrene in mice produced invasive cervical carcinoma; no such tumours were seen in controls ([Näslund *et al.*, 1987](#)).

3.13 Intrafetal injection

In one study in male and female Swiss mice, intrafetal injection of benzo[a]pyrene produced lung adenomas ([Rossi *et al.*, 1983](#)).

4. Other Relevant Data

Benzo[a]pyrene is a carcinogen that induces tumours in many animal species. Some of the examples relevant for this review are: lung tumours in mice, rats, and hamsters; skin tumours in mice; liver tumours in mice; forestomach tumours in mice and hamsters; and mammary gland tumours in rats ([Osborne & Crosby, 1987](#); [IARC, 2010](#)). In humans, occupational exposures to benzo[a]pyrene-containing mixtures have been associated with a series of cancers: coke production: lung; coal gasification: lung, bladder; paving and roofing: lung; coal tar distillation: skin; soots: lung, oesophagus, haematolymphatic system, skin; aluminum smelting: lung, bladder; tobacco smoking: lung, lip, oral cavity, pharynx, oesophagus, larynx, bladder ([IARC, 1984, 1985, 1986, 2010](#)).

Studies on the mechanisms of action of benzo[a]pyrene have been reviewed ([Xue & Warshawsky, 2005](#); [IARC, 2010](#)).

4.1 Metabolism

Benzo[a]pyrene is metabolized by both phase-I and phase-II enzymes to form a series of arene oxides, dihydrodiols, phenols, and quinones and their polar conjugates with glutathione, sulfate, and glucuronide ([Osborne & Crosby, 1987](#)). Benzo[a]pyrene-7,8-diol is a key metabolite that is formed by the action of epoxide hydrolase on benzo[a]pyrene-7,8-epoxide. This dihydrodiol can be further metabolized by cytochrome P450s (CYPs) to a series of benzo[a]pyrene-7,8-diol-9,10-epoxides, which form one class of ultimate carcinogenic metabolites of benzo[a]pyrene.

Both CYPs and peroxidases (e.g. prostaglandin-H synthase) can oxidize benzo[*a*]pyrene. The major cytochrome P450s involved in the formation of diols and diolepoxides are CYP1A1, CYP1A2 and CYP1B1 ([Eling *et al.*, 1986](#); [Shimada, 2006](#)). Cytochrome P450s are inducible by benzo[*a*]pyrene and other PAHs through binding to the aryl hydrocarbon-receptor (AhR) nuclear complex, leading to changes in gene transcription of CYPs and phase-II enzymes. Mice lacking the AhR receptor are refractory to benzo[*a*]pyrene-induced tumorigenesis ([Shimizu *et al.*, 2000](#)). Both CYPs and peroxidases can form radical cations by one-electron oxidation. These cations comprise another class of ultimate carcinogenic metabolites ([Cavalieri & Rogan, 1995](#)). Some polymorphisms in human CYPs and phase-II enzymes (glutathione S-transferases, uridine 5'-diphosphate glucuronosyltransferases and sulfotransferases modulate susceptibility to cancer ([Shimada, 2006](#)). In another metabolic pathway, benzo[*a*]pyrene-7,8-dihydrodiol is oxidized to benzo[*a*]pyrene-7,8-quinone by enzymes of the aldo-keto reductase (AKR1) family. Among these, gene polymorphisms that influence susceptibility have been identified. NAD(P)H: quinone oxidoreductase-1 (NQO1) catalyses the reduction of benzo[*a*]pyrene quinones to hydroquinones, which may be re-oxidized and generate reactive oxygen species. Polymorphisms in this gene have also been described ([Penning & Drury, 2007](#); [IARC, 2010](#)).

The current understanding of mechanisms underlying benzo[*a*]pyrene-induced carcinogenesis in experimental animals is almost solely based on two complementary pathways: those of the diolepoxides and the radical cations. Each provides a different explanation for the effects observed in experimental animals in specific tissues.

4.2 Diolepoxide mechanism

The diolepoxide mechanism for benzo[*a*]pyrene features a sequence of metabolic transformations: benzo[*a*]pyrene → benzo[*a*]pyrene-7,8-oxide (by CYP1A1 and CYP1B1) → benzo[*a*]pyrene-7,8-diol (by epoxide hydrolase) → benzo[*a*]pyrene-7,8-diol-9,10-epoxides (by CYP1A1 and CYP1B1) ([Xue & Warshawsky, 2005](#)). Each class of metabolic intermediate has been shown to be genotoxic and carcinogenic ([Osborne & Crosby, 1987](#)). The stereochemistry of the metabolic transformation of benzo[*a*]pyrene to diols and diolepoxides is an important component of this mechanism of action. Due to the creation of chiral carbons during the metabolic conversions, many of the metabolic intermediates of benzo[*a*]pyrene have multiple stereochemical forms (enantiomeric and diastereomeric). As the metabolism proceeds the complexity of the stereo-chemical forms increases, eventually leading to four benzo[*a*]pyrene-7,8-diol-9,10-epoxides [(+)- and (-)-*anti*, (+)- and (-)-*syn*]. Diolepoxides react with DNA, mainly with the purines, deoxyguanosine and deoxyadenosine, and each diol-epoxide can form both *cis* and *trans* adducts thus giving a total of 16 possible benzo[*a*]pyrene-7,8-diol-9,10-epoxide DNA adducts. However, in most cases far fewer DNA adducts are actually observed. The most ubiquitous benzo[*a*]pyrene adduct detected in isolated mammalian DNA after metabolic conversion in metabolically competent mammalian cells in culture, or in mammals, is the *N*²-deoxyguanosine adduct, (+)-*N*²-10S-(7*R*,8*S*,9*R*-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-yl)-2'-deoxyguanosine (BPDE-deoxyguanosine), derived from 7*R*,8*S*-dihydroxy-9*R*,10*R*-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (*anti*-benzo[*a*]pyrene-7,8-diol-9,10-epoxide, or BPDE). This adduct was first fully identified after isolation from benzo[*a*]pyrene-treated human and bovine bronchial explants ([Jeffrey *et al.*, 1977](#)). This diolepoxide is considered to be an ultimate, DNA-reactive,

metabolite of benzo[a]pyrene ([Osborne & Crosby, 1987](#)). The *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide can form both stable and unstable (so-called 'depurinating') adducts with DNA, mediated by electrophilic carbonium ions ([Chakravarti et al., 2008](#)). *In vivo*, *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide produces stable adducts that were formed primarily with guanines in many species and organs ([IARC, 2010](#)).

Mice treated with benzo[a]pyrene had *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide-*N*²-deoxyguanosine adducts in their lung tissue, while the lung tumours induced by benzo[a]pyrene had G→T and G→A mutations in the *K_i-Ras* gene at codon 12 ([Mass et al., 1993](#)). In mice treated with benzo[a]pyrene the major stable DNA adduct in the epidermis was the *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-deoxyguanosine adduct ([Melendez-Colon et al., 1999](#)). Skin tumours from benzo[a]pyrene-treated mice or in preneoplastic skin from benzo[a]pyrene-treated mice had G→T mutations in codon 13 and A→T mutations in codon 61 of the *Ha-Ras* gene ([Chakravarti et al., 2008](#)).

Benzo[a]pyrene-induced skin tumours harboured G→T transversion mutations in the *Tp53* tumour-suppressor gene ([Ruggeri et al., 1993](#)). The *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts occurred at guanine positions in codons 157, 248, and 273 of the *TP53* gene in *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide-treated human HeLa cells. The same positions are the major mutational hotspots found in human lung cancers ([Denissenko et al., 1996](#)).

4.3 Radical-cation mechanism

The radical-cation mechanism for benzo[a]pyrene has been studied exclusively in connection with mouse-skin tumorigenesis ([Cavalieri & Rogan, 1995](#)). One-electron oxidation of benzo[a]pyrene by CYPs or peroxidases creates a radical cation localized on carbon 6, as a consequence of

the ionization potential and geometric configuration. In mouse skin, this radical cation gives rise to the formation of covalent adducts with guanine (at the C8 carbon and N7 nitrogen) and adenine (at the N7 nitrogen). These adducts are unstable and are thought to generate apurinic sites in mouse skin. However, only low levels of apurinic sites were measured in the epidermis of mice treated with benzo[a]pyrene ([Melendez-Colon et al., 1999](#)) and no studies to date have shown an increase in the number of apurinic sites in lung tissues treated with benzo[a]pyrene. In two *in vivo* studies, rats treated intraperitoneally with benzo[a]pyrene were shown to excrete 7-(benzo[a]pyrene-6-yl)-*N*7-guanine in faeces and urine, while the same adduct was detected in lung tissue of mice treated intraperitoneally with benzo[a]pyrene ([Rogan et al., 1990](#); [Banasiewicz et al., 2004](#)). Skin papillomas obtained from mice treated topically with benzo[a]pyrene showed mutations (at guanine and/or adenine) at codons 12, 13 and 61 in the *Ha-Ras* oncogene ([Wei et al., 1999](#)). Similar studies in preneoplastic skin from benzo[a]pyrene-treated mice showed *Ha-Ras* mutations at codons 13 and 61 ([Chakravarti et al., 2008](#)). The *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide can also form depurinating DNA adducts at guanine and adenine (at the N7 nitrogen). The distribution and chemical nature of the depurinating adducts (from both radical-cation and diolepoxide intermediates) in mouse skin and the distribution and chemical nature of the specific benzo[a]pyrene-induced mutations in mouse-skin papillomas have been reported ([Chakravarti et al., 2008](#)).

4.4 Other activation mechanisms of benzo[a]pyrene

4.4.1 Meso-region mechanism

The mechanism of meso-region biomethylation and benzylic oxidation features biomethylation of benzo[a]pyrene to 6-methylbenzo[a]

pyrene, with S-adenosylmethione as the carbon donor (Flesher *et al.*, 1982). This process has been shown to occur *in vitro*, and *in vivo* in rat liver (Stansbury *et al.*, 1994). 6-Methylbenzo[a]pyrene is further metabolized by CYPs to 6-hydroxymethylbenzo[a]pyrene (Flesher *et al.*, 1997) and then conjugated to sulfate by 3'-phosphoadenosine-5'-phosphosulfate sulfotransferase to 6-[(sulfooxy)methyl]-benzo[a]pyrene. This reactive sulfate ester forms DNA adducts *in vivo* (Stansbury *et al.*, 1994). These benzo[a]pyrene-DNA adducts have only been measured in rat liver (Surh *et al.*, 1989), which is not a target for benzo[a]pyrene-induced carcinogenesis. There is no evidence to date that this mechanism operates in lung.

4.4.2 Mechanism via formation of ortho-quinone/ reactive oxygen species

This mechanism features enzymatic oxidation of benzo[a]pyrene-7,8-diol to the *ortho*-quinone, benzo[a]pyrene-7,8-quinone, by aldo-keto reductases (Mangal *et al.*, 2009). Benzo[a]pyrene-7,8-quinone can react with DNA to yield both stable and depurinating DNA adducts *in vitro* (McCoull *et al.*, 1999; Balu *et al.*, 2006) and can also undergo repetitive redox cycling which generates reactive oxygen species that damage DNA (Penning *et al.*, 1999). In human A549 lung-tumour cells benzo[a]pyrene-7,8-quinone increased the formation of 8-oxo-deoxyguanosine and DNA strand-breaks (Park *et al.*, 2008; Mangal *et al.*, 2009). In a yeast reporter-assay, benzo[a]pyrene-7,8-quinone (in the presence of redox cycling) induced 8-oxo-deoxyguanosine formation and G→T transversions in the *Tp53* tumour-suppressor gene. The mutational spectra induced in the yeast reporter-assay closely matched those seen in DNA from human lung tumours (Shen *et al.*, 2006). Benzo[a]pyrene-7,8-quinone inhibited the activity of protein kinase C in MCF-7 cell lysates suggesting an ability to alter cell signalling (Yu *et al.*, 2002). Rats treated

with benzo[a]pyrene showed increased urinary concentrations of 8-oxo-deoxyguanosine, but lower levels in liver and lung tissues. This suggested that reactive oxygen species are generated during the CYP-dependent metabolism of benzo[a]pyrene, but induction of DNA-repair mechanisms may reduce these levels in target tissues (Briedé *et al.*, 2004). To date this mechanism has been studied only in *in-vitro* systems.

It is noted that formation of reactive oxygen species is not limited to the redox cycling of the *ortho*-quinone of benzo[a]pyrene (benzo[a]pyrene-7,8-quinone). There are several other sources of benzo[a]pyrene-induced reactive oxygen species. *In vivo*, both mice and rats metabolize benzo[a]pyrene to benzo[a]pyrene-1,6-quinone, benzo[a]pyrene-3,6-quinone and benzo[a]pyrene-6,12-quinone and these quinones enter into redox cycling and induce mutations (Osborne & Crosby, 1987; Joseph & Jaiswal, 1998). Many of the reactive intermediates of benzo[a]pyrene (oxides, diol-epoxides, radical cations) and quinone-generated reactive oxygen species can disrupt the balance of cellular oxidants and anti-oxidants by reducing the anti-oxidant levels thus leading to an imbalance and an excess of reactive oxygen species.

4.4.3 Aryl hydrocarbon-receptor mechanism

The AhR regulates the transcription of a series of genes including *Cyp1A1*, *Cyp1A2*, *Nqo1*, *Aldh3a1* (encoding aldehyde dehydrogenase 3A1), *UGT1a6* (uridine 5'-diphosphate-glucuronosyl transferase), and *Gsta1* (glutathione S-transferase A1). All these genes are activated by AhR-ligands, including benzo[a]pyrene, via the AhR-mediated aromatic hydrocarbon response element. The AhR plays a role in the response to oxidative stress in cell-cycle regulation and apoptosis. In addition, the CYP1A1/1A2-mediated metabolism generates oxidative stress (Nebert *et al.*, 2000). Mitochondrial hydrogen-peroxide production was induced by an AhR-ligand in

wild-type mice but not in *AhR*^{-/-} knockout mice (Senft *et al.*, 2002). These mice were shown to be refractory to benzo[a]pyrene-induced carcinogenicity (Shimizu *et al.*, 2000). Benzo[a]pyrene induced oxidative stress in mouse lung (Rajendran *et al.*, 2008).

4.4.4 Immunosuppression mechanism

Benzo[a]pyrene induces immunosuppression in adult mice by altering the cell-mediated responses (Wojdani & Alfred, 1984). Immune development in offspring is also altered following *in utero* exposure to benzo[a]pyrene (Urso & Gengoian, 1984). It is postulated that PAHs, including benzo[a]pyrene, act principally through their AhR-mediated CYP-derived metabolites (diolepoxides, quinones) to activate oxidative and electrophilic signalling pathways in lymphoid and nonlymphoid cells, including myeloid cells, epithelial cells, and other cell types. Furthermore, there is evidence that PAHs suppress immunity by p53-dependent pathways, by modulating signalling pathways in lymphocytes via non-genotoxic mechanisms, and by oxidative stress (Burchiel & Luster, 2001).

4.4.5 Epigenetic mechanisms

Benzo[a]pyrene and/or its metabolites have been shown to increase cell proliferation in several human cell lines, including terminally differentiated human bronchial squamous epithelial cells and in lung-cancer cells where increased expression of the *Cdc25B* gene (cell-division cycle 25B) was observed, along with reduced phosphorylation of Cdk1 (cyclin-dependent kinase 1) (Oguri *et al.*, 2003). Treatment with benzo[a]pyrene increased the number of human embryo lung-fibroblasts in the G1-S transition via the activation of c-Jun, through the p53-dependent PI-3K/Akt/ERK (phosphatidylinositol-3-kinase/protein kinase β /extracellular signal-regulated kinase) pathway (Jiao *et al.*, 2008).

Benzo[a]pyrene and/or its metabolites also affect apoptosis. Benzo[a]pyrene induced apoptosis in human MRC-5 lung fibroblasts via the JNK1/FasL (c-Jun N-terminal kinase 1/Fas Ligand) and JNK1/p53 signalling pathways (Chen *et al.*, 2005). Apoptosis induced by *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide in H460 human lung-cancer cells was associated with induction of Bak (BCL2-antagonist/killer) and with activation of caspase, but it was independent of Bcl-2 (Xiao *et al.*, 2007).

Altered DNA methylation has been reported to be associated with exposure to benzo[a]pyrene and/or its metabolites. After treatment of immortalized bronchial epithelial cells with *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide, the concentration of cytosine-DNA methyltransferase-1 was increased and was associated with hypermethylation of the promoters of 5–10 genes, including members of the cadherin gene-family (Damiani *et al.*, 2008).

4.5 Human exposure to PAH-rich mixtures

4.5.1 Biomarkers of exposure and effect

Molecular-epidemiological studies of cancer associated with occupational and environmental exposures to PAH have provided biomarkers that may be used to estimate internal exposure as well as to inform about molecular mechanisms that may be relevant to cancer causation, particularly in defining the early events in the carcinogenesis process. Biomarkers can be detected in the target organ, in surrogate tissues, or in tumours. These can be categorized into *biomarkers of exposure*, which are generally specific to the PAH of concern (e.g. DNA or protein adducts), *biomarkers of effect* (e.g. genotoxic and cytogenetic effects, 8-oxo-deoxyguanosine, sister chromatid exchange (SCE), micronuclei, chromosomal aberrations, mutations in oncogenes, tumour-suppressor genes, or indicator genes),

and *biomarkers of susceptibility* (DNA-repair enzymes, e.g. XPA, XPC – *xeroderma pigmentosum* complementation groups A and C), bioactivation enzymes (e.g. CYPs), detoxification enzymes (e.g. GSTs), and mutagenic metabolites in urine ([Kalina et al., 1998](#); [Pilger et al., 2000](#); [Simioli et al., 2004](#); [Raimondi et al., 2005](#); [Vineis & Husgafvel-Pursiainen, 2005](#); [Matullo et al., 2006](#); [Farmer & Singh, 2008](#); [Gyorffy et al., 2008](#)). Although biomarkers of effect and susceptibility are generally not unique to any specific PAH exposure, several these biomarkers may provide insight into the mechanism of carcinogenesis induced in humans by PAHs or PAH-rich exposures.

4.5.2 Exposure to benzo[a]pyrene and relationship with specific biomarkers

Biomarkers of exposure to complex mixtures that contain benzo[a]pyrene have been studied in populations exposed in industrial settings: coke production, coal-tar distillation, the aluminium industry, roofing and paving with coal-tar pitch, coal gasification, chimney sweeping, and iron and steel founding. Most if not all of these biomarkers are genotoxic markers. Populations of patients who undergo coal-tar therapy and groups exposed to combustion emissions, and tobacco smokers have also been evaluated. Studies on biomarkers of exposure are dominated by those focusing on the *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct, the most commonly studied PAH-DNA adduct because of the availability of specific analytical methods and standards ([Gyorffy et al., 2008](#)). In one study the depurinating adducts resulting from radical-cation formation, *viz.* 7-(benzo[a]pyrene-6-yl)guanine and 7-(benzo[a]pyrene-6-yl)adenine were found in the urine of women exposed to coal smoke ([Casale et al., 2001](#)). Concomitantly, several biomarkers of effect have also been evaluated in these studies: chromosomal aberrations, sister chromatid exchange ([Kalina et al., 1998](#)),

DNA damage (measured by the comet assay) and 8-oxo-deoxyguanosine formation ([Marczynski et al., 2002](#)). It is important to note that these genotoxic effects observed in humans in relation to exposure to benzo[a]pyrene-containing mixtures have also been observed in experimental studies where benzo[a]pyrene or *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide has been shown to induce sister chromatid exchange ([Pal et al., 1980](#); [Brauze et al., 1997](#)), chromosomal aberrations, micronuclei ([Kliesch et al., 1982](#)), DNA damage ([Nesnow et al., 2002](#)), and 8-oxo-deoxyguanosine ([Thaiparambil et al., 2007](#)). Tobacco smoke, dietary habits and indoor ambient air are also important sources of exposure to benzo[a]pyrene, which has been implicated as one of the components of tobacco smoke related to the induction of lung cancer in smokers ([Watanabe et al., 2009](#)). In a large study of 585 smokers and nonsmokers, smoking and diet were highly correlated with *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct levels ([Pavanello et al., 2006](#)). Several studies have demonstrated moderately increased levels of 8-oxo-deoxyguanosine from lungs, sperm, and leukocytes of smokers. Increased urinary excretion of 8-oxo-deoxyguanosine has also been reported ([Hecht, 1999](#)). In rats exposed to benzo[a]pyrene via oral, intratracheal and dermal routes, *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts were formed in white blood cells independently of the exposure route and their numbers correlated with those found in lung DNA, suggesting that *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct levels in white blood cells may be used as a surrogate for pulmonary *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts ([Godschalk et al., 2000](#)).

4.5.3 Relationship of biomarkers to human cancer

Mutations in *TP53* are common in lung cancers from smokers and less common in nonsmokers. These mutations are G→T transversions with hotspots in codons 157, 248 and 273 ([Hainaut & Pfeifer, 2001](#); [Pfeifer et al., 2002](#)) and they are associated with *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts. The active metabolite *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide causes a unique spectrum of *TP53* mutations distinct from those found in cancers that are not associated with smoking ([Campling & el-Deiry, 2003](#)). Similar G→T mutations have been reported in lung tumours from nonsmoking Chinese women whose tumours were associated with exposure to PAHs from smoke generated by burning smoky coal in unventilated homes. The mutations were clustered at the CpG rich codons 153–158 of the *TP53* gene, and at codons 249 and 273. The mutation spectrum was fully consistent with exposure to PAHs ([DeMarini et al., 2001](#)).

4.6 Synthesis

Benzo[a]pyrene is metabolically activated to a series of reactive intermediates by CYP450 and related enzymes under control of the aryl-hydrocarbon receptor. There is strong evidence that the benzo[a]pyrene diolepoxide mechanism operates in mouse-lung tumorigenesis, while there is also strong evidence that both the radical-cation and the diolepoxide mechanisms are involved in mouse-skin carcinogenesis. The meso-region mechanism has been studied only in rat liver, while the mechanism that involves the formation of *ortho*-quinone/reactive oxygen species has only been studied *in vitro*, although reactive oxygen species can be formed *in vivo* by other benzo[a]pyrene-mediated mechanisms. All these pathways reflect genotoxic mechanisms, as they involve alterations to DNA. Benzo[a]pyrene is pleiotropic and has the ability to affect many

cell- and organ-based systems. Therefore, there are probably many modes of carcinogenic action operating to different extents *in vivo*. These include mechanisms that involve AhR, oxidative stress, immunotoxicity and epigenetic events.

Based on the best available, consistent and strong experimental and human mechanistic evidence it is concluded that benzo[a]pyrene contributes to the genotoxic and carcinogenic effects resulting from occupational exposure to complex PAH mixtures that contain benzo[a]pyrene. The most commonly encountered – and most widely studied – mechanistically relevant DNA lesion is the *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adduct. The formation of this adduct is consistent with *anti*-benzo[a]pyrene-7,8-diol-9,10-epoxide-associated genotoxic effects in surrogate tissues and with the mutation pattern in the *TP53* gene in lung tumours from humans exposed to PAH mixtures that contain benzo[a]pyrene. The fact that those PAH mixtures and benzo[a]pyrene itself induce genotoxic effects like sister chromatid exchange, chromosomal aberrations, micronuclei, DNA damage (comet assay) and 8-oxo-deoxyguanosine, supports the notion that benzo[a]pyrene contributes to human cancer.

5. Evaluation

There is *sufficient evidence* for the carcinogenicity of benzo[a]pyrene in experimental animals.

[No epidemiological data on benzo[a]pyrene alone were available to the Working Group.]

The genotoxic mechanism of action of benzo[a]pyrene involves metabolism to highly reactive species that form covalent adducts to DNA. These *anti*-benzo[a]pyrene-7,8-diol-9,10-oxide-DNA adducts induce mutations in the *K-RAS* oncogene and the *TP53* tumour-suppressor gene in human lung tumours, and

in corresponding genes in mouse-lung tumours. Exposure to benzo[a]pyrene and benzo[a]pyrene-containing complex mixtures also induce other genotoxic effects, including sister chromatid exchange, micronuclei, DNA damage and 8-oxo-deoxyguanosine, all of which can contribute to the carcinogenic effects of benzo[a]pyrene and benzo[a]pyrene-containing complex mixtures in exposed humans.

Benzo[a]pyrene is *carcinogenic to humans* (Group 1).

In making the overall evaluation, the Working Group took the following into consideration:

The strong and extensive experimental evidence for the carcinogenicity of benzo[a]pyrene in many animal species, supported by the consistent and coherent mechanistic evidence from experimental and human studies provide biological plausibility to support the overall classification of benzo[a]pyrene as a human carcinogen (Group 1).

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