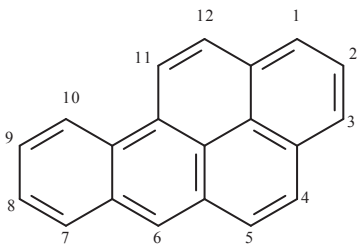
BENZO[*a*]PYRENE

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: B*a*P; 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 m^3/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/m^3; 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/m^3. Mid-range levels are observed in roofing and paving (e.g. 10–20 µg/m^3) and the lowest concentrations (i.e. at or below 1µg/m^3) 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 (Sparnins *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 haematopoeitic 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 *et al.* (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 *et al.* (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 *et al.* (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_4OH), 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_4OH (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 *et al.* (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 *et al.* (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: *Experiment 1*–0/16, 0/20, 15/18 (83%), 12/19 (63%) *Experiment 2*–0/20, 0/18, 14/18 (78%), 7/19 (37%) *Experiment 3*–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: 1.5, 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 ($CSP^{\pm}$ 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, B6C3F1; C3A/JF1 (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	B6C3F1 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%)	*$P < 0.005$ **$P < 0.05$	> 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%)	*$P < 0.005$ **[$P < 0.0005$]	> 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)	*$P < 0.05$ **$P < 0.001$	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/m3, 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 trioctanoin)
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 *et al.* (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 *et al.* (1977)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/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)	+	97% (0.9% saline solution or Tris buffer)
	Experiment 2: 0, 4, 8, 16 mg in Tris buffer/animal, 1 × 30/group	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)	+	

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 & Kruysse (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_2O_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)	+	NR (0.9% saline solution); ferric oxide
		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)	+	

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, 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 anaplasic 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, 1AdC, 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)	*[$P < 0.01$]	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] (5th 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 ± 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, C57B1 (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 µg]/animal, 1 × 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 suspendents 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 Intramammilary administration

In three studies in rats, benign and malignant mammary gland tumours developed after intrammilary 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 streochemical 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 diolepoxide 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^2-10*S*-(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)-*N7*-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 immunosupression 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 & Gengozian, 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 pleotropic 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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