3. Studies of Cancer in Experimental Animals

Acenaphthene

Dermal application (see also Table 3.1)

Mouse

A solution of acenaphthene in 90% benzene was tested in a group of 100 mice [strain, sex and age unspecified] by dermal application for a period of 9 months. No tumours were observed (Kennaway, 1924a,b). [The Working Group noted that no controls were available.]

Dermal initiation-promotion (see also Table 3.2)

Mouse

A group of 85 male white mice was treated weekly for 1 year with three drops of a 5% croton oil solution that contained approximately 3% 'pure' acenaphthene. A group of 160 control mice received croton oil solution only. After 12 months, 5/85 acenaphthene-treated mice were still alive, two of which had skin tumours, and 13/160 croton oil control mice were still alive, one of which had a skin tumour (Graffi *et al*., 1953). [The Working Group noted the poor survival.]

Acepyrene

Dermal application (see also Table 3.1)

Mouse

Groups of 30 female Swiss mice, 9 weeks of age, received twice weekly applications of the same doses of acepyrene for 30 weeks. Repeated applications resulted in tumour incidences of 0/30 (0%), 0/28 (0%), 1/30 (3%) and 1/30 in the control, low-, mid- and high-dose groups, respectively (Cavalieri *et al.*, 1981).

Dermal initiation–promotion (see also Table 3.2)

Mouse

In a mouse skin initiation–promotion study, groups of 30 female CD-1 mice, 9 weeks of age, received dermal applications of a total dose of 0.02, 0.06 or 0.18 μmol acepyrene in

16.7 μL acetone as 10 single subdoses administered on alternate days. The control group received acetone alone. One week later, all mice received dermal applications of 0.017 μmol 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 33.3 μL acetone twice a week for 40 weeks. After 40 weeks of promotion, the incidences of skin papillomas were 3/29 (10%), 0/30 (0%), 1/30 (3%) and 4/30 (13%) in the acetone controls, low-, mid- and high-dose groups, respectively, with an average number of skin tumours/mouse of 0.14, 0.0, 0.03 and 0.13, respectively (Cavalieri *et al.*, 1981).

Anthanthrene

Previous evaluation

Anthanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated four bioassays in which anthanthrene was administered dermally to mice, one of which produce skin tumours. Three initiation–promotion studies in mice were also evaluated, one of which gave positive results. A study in mice by subcutaneous administration was judged to be inadequate, whereas intrapulmonary administration to rats was considered to give positive results. These studies are summarized in Tables 3.1–3.4. On the basis of the available data, the Working Group concluded that there was *limited evidence* that anthanthrene was carcinogenic to experimental animals. Additional studies that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 27 female SENCAR mice [weight unspecified], 8 weeks of age, received a single dermal application of 800 nmol [221 μg] anthanthrene (purity >99% by high-performance liquid chromatography (HPLC)) in 100 μL dioxane:dimethylsulfoxide (DMSO) (75:25). A vehicle-control group of 23 mice was treated with 100 μL dioxane:DMSO alone. Starting 1 week later, all mice were treated topically with 4.26 nmol (2.6 μg) TPA in 100 μL acetone twice weekly for 25 weeks. The mice were killed after the 25th week of promotion and complete necropsies were performed. At the end of the experiment, 3/27 mice in the anthanthrene-treated group and 2/23 mice in the control group had developed skin papillomas. The first skin papillomas appeared after 15 weeks in the anthanthrene-treated group compared with 20 weeks in the control group (Cavalieri *et al*., 1989).

Table 3.1. Carcinogenicity studies of dermal application of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Acenaphthene									
Mouse, NS	NS	100	NS (benzene)	NS	9 mo	0/100	–	No control or histopathology	Kennaway (1924a,b)
Acepyrene									
Mouse, Swiss	F	30	>99% (acetone)	0, 0.2, 0.6, 1.8 μmol 2×/wk, 30 wk	30 wk	Skin: 0/30, 0/30, 1 SGA/30 (3%) and 1 SCC/30 (3%)	–		Cavalieri *et al.* (1981)
Anthanthrene									
Mouse, NS	NS	30	NS (benzene)	0.3% solution; 1×/wk every 3–5 wk	Life; last mouse died on day 712	1/30 (3%) (lung A); no statistics	–	No control	Badger *et al.* (1940)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05 or 0.1% solution, 3×/wk, 12 mo	15 mo	0/20 (0.05%), 0.20 (0.1%) vs 0/20 solvent controls	–		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss	F	30	Recrystallized (toluene)	43 μg in 20 μL, 2×/wk, 75 wk (72 wk in the vehicle-control groups)	Up to 100 wk	1/30 (3%) skin C vs 2/30 skin P and C toluene controls, 0/30 acetone controls	–	No statistics	Lijinsky & Garcia (1972)
Mouse, Swiss	F	40	98.65% (acetone)	119 μg in 16.7 μL, 2×/wk, 30 wk	70 wk	Skin: 18/38 (47%) (7 P, 2 K, 14 C, 1 SGA) vs 0/29 solvent controls	+	Purity questionable; no statistics	Cavalieri *et al.* (1977)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Mouse, MS	NS	100	NS (lanolin or ether solution)	40% suspension or solution, NS	5 mo	Skin P: lanolin suspension, 0/100; ether solution, 1/100	–	No control; limited reporting	Kennaway (1924a,b)
Mouse, albino	NS	41	NS (water or sesame oil)	5 mg in 5 ml, 1×/wk	10 mo	0/41	–	No control	Pollia (1939)
Mouse, Swiss	F	5	NS (acetone)	10% solution, 3×/wk, 20 mo	20 mo	0/5	–	Small numbers; no control	Wynder & Hoffmann (1959)
Mouse, C3H/Hej	M	20; 50 controls	99.5% (toluene)	50 µg in 50 µL, 2×/wk, 104 wk	104 wk	0/14 vs 0/39 solvent controls	–		Warshawsky *et al.* (1993)
Benz[*a*]anthracene									
Mouse, NS	NS	50	NS (benzene)	NS	NS	1/50 (2%) (skin P, regressed)	–	No control	Kennaway (1930)
Mouse, NS	NS	30	Purified (benzene)	200 µg in 100 µL, NS	>1 year	1/30 (3%) (skin E)	–	No control	Barry *et al.* (1935)
Mouse, CAF1	M, F	20	NS (mineral oil)	0.4%, 1 drop 2×/wk, 68 wk	68 wk	1/20 (5%) (skin P)	–	No control	Hill *et al.* (1951)
Mouse, C3H	NS	20	NS (acetone)	0.5%, 2×/wk	638 days	0% vs 0% in solvent-treated controls	–		Stevenson & von Haam (1965)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/He	NS	30–40	Pure (toluene or *n*-dodecane)	0.002%, 0.02%, 0.2%, 1% in toluene or 0.0002%, 0.002%, 0.02%, 0.2%, 1% in *n*-dodecane, 3×/wk; total dose, 150 mg	Up to 88 wk	Toluene: 0/32, 1/18 (6%), 3/32 (9%), 5/29 (17%) (malignant skin T); 0/32, 0/18, 0/32, 3/29 (10%) (benign skin T) with increasing dose level *n*-Dodecane: 2/31 (6%), 4/21 (19%), 4/20 (20%), 7/21 (33%), 16/22 (73%) (malignant skin T); 2/31 (6%), 4/21 (19%), 0/20, 4/21 (19%), 1/22 (5%) (benign skin T)	+	No control	Bingham & Falk (1969)
Mouse, Swiss	F	40	Recrystallized (acetone)	0.396 µmol [90.4 µg] in 16. µL, 2×/wk, 30 wk	70 wk	1/39 (3%) (skin P) vs 0/29 solvent-treated controls; no statistics	–		Cavalieri *et al.* (1977)
Rat, Donryu	M	25	NS (acetone)	Saturated solution, 1×daily, 5 mo	18 mo	0/9	–	No control	Tawfic (1965)
Hamster, Syrian golden	M, F	10	NS (mineral oil)	0.5%, 2×/wk, 10 wk	85 wk	0/10	–	No control	Shubik *et al.* (1960)
Benzo[*a*]pyrene									
Mouse, Swiss ICR/Ha	F	50	NS (acetone)	0 (untreated), 0 (vehicle control), 5 µg/animal, 3×/wk, 52 wk	52 wk	Skin T: 0/50, 0/50, 23/50 (46%; 13 P; 10 C)	+		Van Duuren *et al.* (1973)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss	F	40	99% (acetone)	0, 0.396 μmol [0.1 mg]/animal, 2×/wk, 30 wk	38–65 wk	Skin T: 0% [0/29], 78.9% [30/38] (7 P, 7 K, 36 C, 1 malignant Schwannoma)	+		Cavalieri *et al.* (1977)
Mouse, C57BL/6J	F	30	NS (DMSO/ acetone (1:3) or acetone/ NH_4OH (1000:1))	Experiment 1 and 2: 0 (DMSO/ acetone), 0.02 [5.28 μg], 0.1 [26.43 μg], 0.4 [105.75 μg] μmol/ animal, 1×/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, 1×/ 2 wk, 60 wk	60 wk	Skin T (mainly SCC based on past experience)*: 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)	+	Effective no. of animals not clearly specified *At most, 7 animals/group died prema- turely without a skin tumour.	Levin *et al.* (1977)
Mouse, NMRI	F	40	>96% (acetone)	0, 1.7, 2.8, 4.6 μg/animal, 2×/wk for life	~88–~130 wk	Local T: 0/35, 8/34 (23.5%; age-standardized, 24.8%), 24/35 (68.6%; age-standardized, 89.3%), 22/36 (61.1%; age-standardized, 91.7%)	+	Type of local tumours NS	Habs *et al.* (1980)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, NMRI	F	20	>96% (acetone)	0, 2, 4 µg/animal, 2×/wk for life	63–109 wk	Skin T: 0/20, 9/20 (45%; 2 P, 7 C); 17/20 (85%; 17 C)	+		Habs *et al.* (1984)
Mouse C3H/HeJ	M	50	99.5% (acetone)	0 (untreated), 0 (vehicle control) or 12.5 µg/animal, 2×/wk, 99 wk	99 wk	Skin T: 0/50, 0/50, 48/50 (96%; 47 C, 1 P)	+		Warshawsky & Barkley (1987)
Mouse, Swiss	F	30	Purified [NS] (acetone)	0, 0.1 [26.4 µg], 0.4 [105.7 µg] µmol/animal, 2×/wk, 20 wk	42 wk	Skin T incidence: 0/30, 26/29 (90%; SGA, 3 P, 23 SCC), 26/30 (90%; 2 P, 26 SCC) Multiplicity: low dose, SGA 1/1 [1], P 9/3 [3], SCC 111/23 [4.8]; high dose, P 3/2 [1.5], SCC 153/26 [5.9]	+		Cavalieri *et al.* (1988b)
Mouse, ICR/Harlan	F	43–50	NS (acetone)	0 (untreated), 0 (vehicle control), 16, 32, 64 µg/ animal, 1×/wk, 29 wk	34 wk	No skin T reported in 86 untreated and vehicle controls combined; 127 skin T in the high-dose group (mainly P) [type of skin T and incidences in the mid- and low-dose groups NS]. Skin tumours/animal: 0, ~1.1, ~1.4, ~8.0 [derived from dose–response curves]	+	Limited reporting of tumour data	Albert *et al.* (1991)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M	6	NS (acetone)	0 (untreated), 0 (vehicle control), 100 µg/ animal, 2×/wk for life (3 wk–6 mo)	3 wk–6 mo	No skin T reported in the control groups; mean time (wk) to skin T onset, 19.8 (range 15–27); mean skin T weight, 0.57 ± 0.17 g; type of skin T, mainly SCC, 1 pleiomorphic SCC, 1 K	+	Small no. of animals; limited data on tumours	Andrews *et al.* (1991)
Mouse, SENCAR	F	24	>99% (acetone)	0, 100 nmol [26.4 µg]/animal, 1×	27 wk	0/24, 1/24 (4%) (1 skin P; multiplicity, 1/1)	–		Cavalieri *et al.* (1991)
Mouse, Swiss	F	23–27	>99% (acetone)	0, 1, 4, 8 nmol [0, 0.264,1.057, 2.11 µg]/animal, 2×/wk, 40 wk	48 wk	No skin T found (effective no. of animals, 23–27); tumours at other sites: 1/27 (1 lung A), 3/24 (4%; 2 lung A, 1 splenic malignant lymphoma), 3/23 (13%; 1 lung A, 1 splenic malignant lymphoma, 1 liver haemangioma), 3/23 (13%; 1 lung A, 1 splenic malignant lymphoma, 1 malignant lymphoma in multiple organs)	–	Low doses	Higginbotham *et al.* (1993)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/HeJ	M	Experiment 1, 20; experiment 2, 20 or 50; experiment 3, 24–100	99.5% (toluene)	Experiment 1: 0, 0.2% [presumably 100 µg/animal], 2×/wk, 6 mo	Experiment 1: 6 mo	Experiment 1: 0/20, 20/20 (100%) (1 benign and 19 malignant T)	Experiment 1: +	Site and type of T NS	Warshawsky *et al*. (1993)
				Experiment 2: 0 (untreated), 0 (toluene control), 0.001% [0.5 µg/animal], 2×/wk, 104 wk [not clearly specified]	Experiment 2: 104 wk	Experiment 2: 0/32, 0/39, 0/14	Experiment 2 and 3: –		
				Experiment 3: 0, 0.0006% [0.3 µg]/animal, 2×/wk, 66 wk	Experiment 3: 66 wk	Experiment 3: 0/23, 0/66			
Mouse, C3H/Hej	M	20, 50 controls	95% (toluene)	50 µg/in 50 µl 2×/wk, 104 wk	104 wk	1/15 vs 0/39 solvent controls	–		Warshawsky *et al* 1993
Mouse, $AhR^{-/-}$, $AhR^{+/-}$, $AhR^{+/+}$	F	NS	NS (acetone)	200 µg/animal, 1×/wk, 25 wk	28 wk	Skin T: $AhR^{-/-}$, 0/10 (0%); $AhR^{+/-}$, 13/14 (92.4%; 4.6 ± 2.4 T/animal; 12 SCC, 1 P; p <0.01); $AhR^{+/+}$, 15/16 (93.8%; 4.2 ± 1.9 T/animal; 13 SCC, 1 P, 1 K; p <0.01)	+		Shimizu *et al.* (2000)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Cyclopenta[*cd*]pyrene									
Mouse, Swiss	F	30	>99% (acetone)	0, 22.2, 66.6, 200 nmol, 2×/wk, 48 wk	48 wk	Skin SCC, SGA, P: (0/29; 0%), (2/29; 7%), (2/29; 7%), (24/29; 83%)	+		Cavalieri *et al.* (1983)
5,6-Cyclopenteno-1,2-benzanthracene									
Mouse, NS	NS	10	Pure (melting-point [mp] or picrate) (benzene)	0.1%, 0.3% [volume, no. and frequency of treatment NS]	Up to 339 days	0.1% (mp): 0/10 (skin P), 4/10 (40%; skin E); 0.3% (mp): 2/10 (20%), 1/10 (10%) (group 1), 1/10 (10%), 4/10 (40%) (group 2); 0.3% (picrate): 0/10, 8/10 (80%)	+	No control; limited reporting	Cook (1932)
Mouse, NS	NS	10–40	NS (benzene)	0.03, 0.1, 0.3% [volume, no. and frequency of treatments NS]	Up to 795 days (low-dose); 358 days (high-dose)	0.03%: 1/20 (5%; skin P), 1/20 (5%; skin E); 0.1%: 0/10, 5/10 (50%); 0.3%: 5/40 (12%), 14/40 (35%)	+	No control; limited reporting	Barry *et al.* (1935)
Dibenz[*a*,*c*]anthracene									
Mouse, Swiss	F	30, 20 controls	>99% (acetone)	85 µg in 16–20 µL, 2×/wk, 65 wk	Lifetime (>100 wk)	9/16 (56%; 1/16 skin P, 8/16 skin C) vs 0/14 acetone controls.	+	No statistics	Lijinsky *et al.* (1970)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, Swiss (Millerton)	F	20	NS (acetone)	0.001, 0.01, 0.1% [volume NS], 3×/wk, life	≤6, 13 or 21 mo	0.001%: 6/20 (30%) skin P, 6/20 (30%) skin C; 0.01%: 19/20 (95%) skin P, 18/20 (90%) skin C; 0.1%: 18/20 (90%) skin P, 15/20 (75%) skin C	+	No control; no statistics	Wynder & Hoffmann (1959)
Mouse, C3H (Jax), DBA	F	9–11	NS (benzene)	0.25% solution [volume NS], 2×/wk	NS	C3H: 10/11 (91%) mammary T vs 5/10 (50%) controls; DBA: 9/10 (90%) mammary T vs 0/9 controls	+ for DBA	Small numbers; no statistics	Ranadive & Karande (1963)
Mouse, Swiss	F	20, 50 controls	Purified (acetone: benzene (9:1))	38 µg, 2×/wk, 44 wk	≤60 wk	Skin P: 16/20 (80%) vs 2/50 (4%) solvent controls	+	No statistics	Lijinsky *et al.* (1965)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss (ICR/Ha)	F	30–50	NS (acetone or DMSO)	1, 10, 100 µg in 100 µL, 3×/wk	374–663 days	1 µg (in acetone): 1/30 (3%) skin P, 1/30 (3%) skin C vs 0/30 acetone controls; 1 µg (in DMSO): 1/30 (3%) skin P vs 0/30 DMSO controls; 10 µg (in acetone): 43/50 (86%) skin P, 39/50 (78%) skin C vs 0/50 acetone controls; 100 µg (in acetone): 39/40 (97%) skin P, 32/40 (80%) skin C vs 0/40 acetone controls	+	No statistics	Van Duuren *et al.* (1967)
Mouse, IF/Bcr	M	48, 30	NS (acetone)	1.5 mg, 1×/wk, 18 wk	≤29 wk	Thymectomized, 10/10 (100%) skin P; intact, 27/27 (100%) skin P	+	No control	Johnson (1968)
Mouse, NMRI	F	50	>99% (acetone)	0.1, 0.4, 1.1 µg in 17 µL, 3×/wk, 112 wk; total doses, 37.8, 125, 378 µg	112 wk	0.1 µg, 3/50 (6%); 0.4 µg, 4/50 (8%); 1.1 µg, 16/50 (32%) skin P vs 2/48 (4%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)
Hamster, Syrian golden	M, F	5	NS (mineral oil)	~320 µg in ~160 µL, 2×/wk, 10 wk	≤75 wk	0/5 M and 0/5 F	–	No control; small numbers; only 1 M and 1 F survived 75 wk; no statistics	Shubik *et al.* (1960)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*j*]anthracene									
Mouse, Swiss	F	30, 20 controls	>99% (acetone)	39, 78 µg in 16–20 µL, 2×/wk, 81 wk (39 µg) or 60 wk (78 µg)	Lifetime (>100 wk)	Skin T: 39 µg, 2/9 (22%; P), 2/9 (22%; C); 78 µg, 2/20 (10%; P), 6/20 (30%; C) vs 0/14 acetone controls	+	No statistics	Lijinsky *et al*. (1970)
Dibenzo[*a*,*g*]fluorene									
Mouse, CF1	M, F	20	NS (acetone)	One drop (0.02 mL) of 0.3% in acetone (~60 µg)/animal, 2×/wk, 31 wk	48 wk	Skin T: experiment 1, 6/20 (30%; 6 SCC, 1 S); experiment 2, 9/20 (45%; 9 SCC, 2 S)	+	No control, limited histology	Riegel *et al.* (1951)
Dibenzo[*a*,*e*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	40 or 20, 20 controls	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS), 3×/wk, 12 mo	15 mo	Skin T: 0.05%, 16/40 (40%; P), 9/40 (22%; E); 0.1%, 9/20 (45%; P), 6/20 (30%; E) vs 0/20 solvent controls	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*h*]pyrene									
Mouse, NS	M, F	74	NS (benzene)	50 M: 1 drop of a 0.15–0.18% solution, every other day, 86×; 14 M, 10 F: 1 drop of a 0.15–0.18% solution, every 3rd day, 55×	4.5 mo	86×, 32/49 (65%; skin E); 55×, 11/23 (48%; skin E)	+	No control; no statistics	Kleinenberg (1939)
Mouse, NS	NS	30	NS (benzene)	0.3% solution (volume NS), 2×/wk	350 days	10/30 (33%; skin E)	+	No control; no statistics	Badger *et al.* (1940)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS), 3×/wk, 12 mo	11 (0.05%), 15 (0.1%) mo	Skin: 0.05%, 16/20 (80%; P), 13/20 (65%; E); 0.1%, 15/20 (75%; P), 15/20 (75%; E) vs 0/20 solvent controls (p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss	F	40	99.6% (acetone)	119 µg in 16.7 µL, 2×/wk, 30 wk	45 wk; control, 70 wk	35/39 (88%; skin P and C) vs 0/29 solvent controls	+	No statistics	Cavalieri *et al.* (1977)
Dibenzo[*a*,*i*]pyrene									
Mouse, XVII	M	23, NS	NS (*ortho*-dichloro-benzene)	1 drop of saturated solution (concentration, volume NS), 2×/wk	14 mo	21/23 (91%; skin P, 8 skin E) vs 0 solvent control	+	Control group not treated simultaneously; no statistics	Lacassagne *et al.* (1958)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, albino	M	12	Recrystallized (benzene or DMF)	Saturated solution (~0.04% in benzene; concentration in DMF, volume NS), 2×/wk, 3.5 mo (no. of applications NS), followed by a single application as a solid (dose NS) covered with resin	>8 mo	0/12 after solution, 3/12 (25%; skin P) after application as a solid	±	No control; limited duration; no statistics	Schoental (1959)
Mouse, Swiss (Millerton)	F	10	NS (acetone)	0.01, 0.1% solution (volume NS), 3×/wk	Life (up to 17 wk)	0.01%, 1/10 (10%; skin P), 0/10 (skin C); 0.1%, 5/10 (50%; skin P), 1/10 (10%; skin C)	+	No control; no statistics	Wynder & Hoffmann (1959)
Mouse, Swiss	F	12	NS (benzene)	25 µg in 25 µL, 3×/wk, 18–22 mo	Up to 22 mo	4/12 epidermoid C	±	No control; no statistics	Pai & Ranadive (1965)
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.1% solution (volume NS); 3×/wk, 12 mo	15 mo	0.05%, 16/20 (80%; skin P), 13/20 (65%; skin E); 0.1%, 16/20 (80%; skin P), 15/20 (75%; skin E) vs 0/20 solvent controls	+	No statistics	Hoffmann & Wynder (1966); LaVoie *et al*. (1979)
Rabbit	NS	3	Recrystallized (benzene)	~0.04% (volume NS), 2×/wk, 6 mo	6 mo	0/3	–	No control; small number	Schoental (1959)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, ICR Swiss albino	F	19–21	Pure (NS)	1 µg [55×], 5 µg [40×], 10 µg [24×], 50 µg [7×], 100 µg [7×] in 100 µL, 3×/wk	7 mo	Skin T: 1 µg, 20/20 (100%); 5 µg, 19/19 (100%); 10 µg, 21/21 (100%); 50 µg, 19/20 (95%); 100 µg, 16/20 (80%)	+	No control; no histology; limited reporting; no statistics	Masuda & Kagawa (1972)
Mouse, SENCAR	F	22–24, 27 controls	>99% (acetone)	0.3, 1.2, 2.4 µg in 100 µL, 2×/wk, 40 wk	48 wk	0.3 µg, 1/24 (4%) skin squamous P (3 T/TBA), 5/24 (21%) lung A; 1.2 µg, 16/23 (70%) skin C (1.8 T/TBA), 9/23 (39%) skin squamous P (1.9 T/TBA), 2/23 (9%) SGA (1.5 T/TBA), 9/23 (39%) T at other sites; 2.4 µg, 20/22 (91%) skin C (2.6 T/TBA), 16/22 (73%) skin squamous P (1.9 T/TBA), 3/22 (14%) SGA (1.7 T/TBA), 14/22 (64%) T at other sites vs 0/27 skin T, 1/27 (4%) lung A solvent controls	+		Higginbotham *et al*. (1993)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57BL/6J, *AhR*$^{+/+}$, *AhR*$^{-/-}$	NS	*AhR*$^{+/+}$, 17; *AhR*$^{-/-}$, 15	NS (NS)	30 µg (volume NS), followed (elapsed time NS) by 6 µg (volume NS), 1×/wk, 20 wk	Up to 2 years	*AhR*$^{+/+}$, 17/17 (76% skin P, 24% skin C; 2.7 ± 1.4 T/mouse); *AhR*$^{-/-}$, 5/15 (100% skin P; 0.46 ± 0.83 T/mouse)	+	No control; limited reporting	Nakatsuru *et al*. (2004)
Dibenzo[*e*,*l*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	Recrystallized (dioxane)	0.05, 0.07% solution (volume NS), 3×/wk, 12 mo	15 mo	Skin T: 0.05%, 0/20; 0.07%, 0/20 vs 0/20 solvent controls	–		Hoffmann & Wynder (1966)
Fluoranthene									
Mouse C3H/HeJ	M	20	NS (toluene)	0.1% fluoranthene, 0.001% benzo[*a*]pyrene 2×/wk	104 wk	1/12 skin (8%) vs 0% fluoranthene alone, benzo[*a*]pyrene alone or solvent controls	±		Warshawsky *et al*. (1993)
1-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	No skin T observed; 7 animals survived	–		Hecht *et al.* (1974); LaVoie *et al.* (1979)
2-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 11/11 (100%; 7 C; 1.0 T/mouse); 10/20 (50%) treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
3-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 5/8 (63%; 4 C in 4 animals; 0.8 T/mouse); 8/20 treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
4-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 3/10 (30%; 2 C in 2 animals; 0.5 T/mouse); 10/20 treated animals survived to study termination	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
5-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 20/20 (100%) (37 C; in 12 animals; 5 T/mouse); no animals alive when the study was terminated at 35 wk	+		Hecht *et al.* (1974); LaVoie *et al*. (1979)
6-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1 mg, 3×/wk, 17 mo	17 mo	Skin T: 0/20 (0%), 3/12 (25%; 1 C in 1 animal; 1 T/mouse)	–		Hecht *et al.* (1974); LaVoie *et al*. (1979)
2-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0.2% solution, 3×/wk, 12 mo	15 mo	Skin C: 4/10 (40%; 2 T/mouse)	±	No control	Hoffman *et al.* (1972)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Naphtho[2,3-*e*]pyrene									
Mouse, Swiss albino Ha/ICR	F	20	NS (dioxane)	100 µg of 0.1% solution, 3×/wk	15 mo	Skin P: 0/20 (0.05%); 1/20 (0.1%) vs 0/20 solvent controls	–		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Perylene									
Mouse, Swiss	M	20	NS (benzene)	2 drops of a 0.3% solution every 4th day, 22 wk	22 wk	0%	–	No control	Finzi *et al.* (1968)
Mouse, Swiss albino Ha/ICR	F	20	'Rigorously purified' (benzene)	0, 800 µg in 200 µL, 1×, alone or followed by (2.5 µg TPA in 100 µL), 3×/wk, 58–60 wk	58–60 wk	0/20 skin P vs 0/20 solvent controls; 3/20 (15%) skin P vs 1/20 TPA controls	–	No statistics	Van Duuren *et al.* (1970)
Mouse, C3H	M	20	Pure (decalin or decalin + *n*-dodecane (1:1))	0, 50 mg in 60 µL, 2×/wk, 82 wk	82 wk	50 mg (decalin), 0/16 skin P, 0/16 skin C; 50 mg (decalin + *n*-dodecane), 1/15 (7%) skin P, regressed, 0/15 skin C vs 2/13 (15%) skin P, 0/13 skin C decalin + *n*-dodecane controls	–	Small number; no control in decalin experiment; no statistics	Horton & Christian (1974)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Phenanthrene									
Mouse, NS	NS	100	NS (90% benzene)	NS	9 mo	0/100 skin	–	Limited reporting; no control	Kennaway (1924a,b)
Mouse, white	NS	100	'Pure' (5% croton oil)	3 drops of a 3% solution, 1×wk	1 year	1/6 (17%)	±	Low survival; no statistics	Graffi *et al.* (1953)
Mouse, C3H/HeJ	M	20, 50 controls	>99% (toluene)	0, 50 μg in 50 μL, 2×/wk, 104 wk	104 wk	1/12 (8%) benign skin T vs 0/39 benign or malignant skin T solvent controls	–	No statistics	Warshawsky *et al.* (1993)
Picene									
Mouse, NMRI	F	50	>99% (acetone)	0, 0.1, 0.4, 1.1 μg in 17 μL, 3×/wk, 112 wk (total doses, 38, 125, 378 μg)	112 wk	Skin T: 0.1 μg, 3/49 (6%); 0.4 μg, 11/48 (23%); 1.1 μg, 11/50 (22%) vs 2/48 (4%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)

Table 3.1 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Triphenylene									
Mouse	NS	10	NS (benzene)	0.3% solution in 100 µL, 2×/wk, 548 days	548 days	0/10 skin T	–	Small number; no control	Barry *et al*. (1935)
Mouse, C3H	M	15 or 20	Pure (decalin or decalin + *n*-dodecane (1:1))	0, 300 µg in 60 µL, 2×/wk, 82 wk	82 wk	300 µg (decalin), 0/14 skin P, 0/14 skin C; 300 µg (decalin + *n*-dodecane): 4/11 (36%) skin P, 1/11 (9%) skin C vs 2/13 (15%) skin P, 0/13 skin C decalin-*n*-dodecane controls	±	Small number; no control for decalin alone; no statistics	Horton & Christian (1974)

A, adenoma; C, carcinoma; DMF, *N,N*- dimethylformamide; DMSO, dimethylsulfoxide; E, epithelioma; F, female; K, keratocanthoma; M, male; mo, month; NH_4OH, ammonium hydroxide; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; SGA, sebaceous gland adenoma; T, tumour; TBA, tumour-bearing animal; TPA, 12-*O*-tetradecanoyl-13-acetate; vs, versus; wk, week

[a] –, negative; +, positive; ±, equivocal

Table 3.2. Dermal initiation–promotion studies of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Acenaphthene									
Mouse, NS	M	85; 160 controls	'Pure' (acetone)	3 drops of a ~3% solution, 1×/wk, co-administered with 1 drop of 5% croton oil; 1 year	1 year	Skin: 2/5 (40%) vs 1/13 (8%) croton oil controls	±	Low survival	Graffi *et al.* (1953)
Acepyrene									
Mouse, CD-1	F	30	>99% (acetone)	0, 0.02, 0.06, 0.18 μmol, ×10, followed by 2×/wk 0.017 μmol TPA, 40 wk	44 wk	Skin P: 3/29 (10%), 0/30 (0%), 1/30 (3%), 4/30 (13%); 0.14, 0.03, 0.31, 0.31 T/mouse	–		Cavalieri *et al.* (1981)
Anthanthrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	25 μg in 25 μL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone	6 mo	Skin P: 2/25 (8%) vs 2/26 (8%) promoter controls	–	Short duration of study	Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, Swiss ICR/Ha	F	13	Pure (benzene)	4 × 250 μg in 0.1 mL, followed 2 wk later by 25 μg croton oil in 0.1 mL acetone (3×/wk)	54–66 wk	Skin P: 2/13 (15%) vs 0/13 non-promoted group, 0/20 untreated controls, 0/20 vehicle controls, 6/40 (15%) promoter controls Skin C: 1/40 (2%) promoter controls	–	No statistics	Van Duuren *et al.* (1968)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	Pure (benzene)	690 µg 1×, followed 1 wk later by 5 µmol (3.1 mg) TPA 2×/wk (10 µmol TPA in promoter controls), 34 wk	35 wk	Skin P: 5/28 (18%) vs 0/30 promoter controls	+	No statistics	Scribner (1973)
Mouse, SENCAR	F	27, 23 controls	>99% (dioxane: DMSO (75:25))	221 µg in 100 µL, 1×, followed 1 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 25 wk	26 wk	Skin P: 3/27 (11%) vs 2/23 (9%) promoter controls	–		Cavalieri *et al.* (1989)
Anthracene									
Mouse, NS	NS	44; 44; 100	NS (petroleum jelly–olive oil)	5% solution on ears, 3×/wk; 5% solution, 3×/wk, then 40 or 60 min UV; 5% solution, 3×/wk, then 90 min UV	11 mo; 9 mo; 9 mo	0/44 (only 1 alive); 0/44 (none alive after 9 mo); 0/100 (none alive after 9 mo)	–	No controls	Miescher (1942)
Mouse, 'S'	NS	20; 20 controls	NS (acetone)	1.5 mg in 300 µL, 2×/day, 3 days/wk, 20×; followed by 300 µL 0.17% croton oil 1×/wk, 16 wk; 300 µL 0.085% 1×/wk, 2 wk	21 wk	Skin P: 3/17 (18%) vs 4/19 (21%) acetone, croton oil control	–	No statistics	Salaman & Roe (1956)
Mouse, CD-1	F	30; 30 controls	Purified (acetone)	1.78 mg 1×; followed by 5 µg TPA, 2×/wk, 34 wk	35 wk	Skin P: 4/28 (14%) vs 1/30 (3%) acetone, TPA control	–	No histo-pathology; no statistics	Scribner (1973)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino Crl:CD/1 (ICR)BR	F	20; 20 controls	>99% (acetone)	100 μg in 100 μL every other day, 10×; followed by 2.5 μg TPA in 100 μL, 3×/wk, 20 wk	24 wk	Skin P: 3/20 (15%) vs 2/20 (10%) acetone, TPA control	–		LaVoie *et al.* (1983, 1985)
11*H*-Benz[*b*,*c*]aceanthrylene									
Mouse, CD-1	F	20	>99% (acetone)	0.05, 0.2, 0.4 μmol [12, 48, 96 μg] in 100 μL; 10× on alternate days; followed 10 days later by 2.5 μg TPA in 100 μL acetone, 3×/wk, 20 wk	24 wk	0.05 μmol, 15/20 (75%); [type NS]; 0.2 μmol, 18/20 (90%); 0.4 μmol, (90%) 18/20 vs 1/20 (5%) solvent-treated controls (p <0.005 at all doses); 1.60, 4.90, 7.60 T/ mouse vs 0.05 solvent controls	+		Rice *et al.* (1988)
Benz[*j*]aceanthrylene									
Mouse, SENCAR	F	20	NS (acetone)	40, 200, 400 μg in 200 μL, 1×; followed by 2 μg TPA, 2×/wk, 21 wk	22 wk	Skin P: 40 μg, 20/20 (100%) (8.7 P/mouse); 200 μg, 20/20 (100%) (10.8 P/mouse); 400 μg, 20/20 (100%) (7.7 P/mouse) vs ~1/20 (5%) acetone, TPA controls	+	Limited histo-pathology; no statistics	Nesnow *et al.* (1993)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*l*]aceanthrylene									
Mouse, SENCAR	M, F	20–22	NS (acetone)	12.6, 25.2, 63.1, 126, 252 µg in 200 µL, 1×; followed by 2 µg TPA in 200 µL acetone, 2×/wk, 30 wk	31 wk	Skin P: males – 12.6 µg, 12/20 (60%; 1.4 P/mouse); 25.2 µg, 16/17 (94%; 2.3 P/mouse); 63.1 µg, 21/21 (100%; 8.4 P/mouse); 126 µg, 16/16 (100%; 10.8 P/mouse); 252 µg, 19/20 (95%; 8.7 P/mouse) vs 0/20 acetone, TPA-treated controls; females – 12.6 µg, 13/20 (65%; 1.1 P/mouse); 25.2 µg, 18/19 (95%; 3.1 P/mouse); 63.1 µg, 19/21 (90%; 4.7 P/mouse); 126 µg, 20/21 (95%; 6.6 P/mouse); 252 µg, 20/20 (100%; 10.8 P/mouse) vs 1/19 (5%; 0.05 P/mouse) acetone, TPA-treated controls	+	No histopathology; no statistics	Nesnow *et al.* (1984a)
Benz[*a*]anthracene									
Mouse, NS	NS	75	NS (acetone)	0.05%, alternating applications (1×/wk) with 5% croton oil in mineral oil; NS	12 mo	Skin P: 9/18 (50%) vs 1/13 (8%) croton oil controls	+		Graffi *et al.* (1953)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, albino S	M	20	NS (acetone)	1% solution (300 µL), 2×/wk (total dose, 6 mg), followed 3 wk later by 300 µL of 0.5% solution of croton oil in acetone, 1×/wk	21 wk	Skin: 7/18 (39%; 1.17 T/mouse) vs 0/8 without promotion, 1/20 (5%) croton oil controls	+		Roe & Salaman (1955)
Mouse, Swiss	F	20	'Purified' (acetone)	0.9 µg in 50 µL, 1×; followed by 0.5% croton oil in 200 µL acetone, 2×/wk, 60 wk	61 wk	8/20 (40%; mostly skin P) vs 0/20 without promotion	+	No control	Hadler *et al.* (1959)
Mouse, Swiss (ICR/Ha)	F	20	'Rigorously purified' (benzene)	1.0 mg in 100 µL, 1×; 1.0 mg in 100 µL, 1×; followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 56–58 wk	58–60 wk	Skin P: 1.0 mg, 0/20 vs 0/20 solvent controls; 1.0 mg + TPA, 10/20 (50%) vs 1/20 (5%) TPA controls	+	No statistics	Van Duuren *et al.* (1970)
Mouse, CD-1	F	30	Pure (acetone)	2.2 µmol [502 µg], 1×, followed 1 wk later by 10 µmol (6.2 mg) TPA, 2×/wk, 34 wk	35 wk	18/29 (62%; skin P) vs 0/30 TPA controls	+	TPA dose probably 10 µg; no statistics	Scribner (1973)
Mouse, CD-1	F	30	>99% (acetone)	2 µmol [457 µg], 1×, followed 1 wk later by 10 µg TPA, 2×/wk, 26 wk	27 wk	17/30 (57%; skin P; 1.2 P/mouse) vs 2/29 (6%) in TPA controls	+	No statistics	Slaga *et al.* (1978)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	NS (acetone)	1.0, 2.5 μmol [2.28, 571 μg] in 200 μL, 1×, followed 1 wk later by 16 nmol (9.9 μg) TPA in 200 μL acetone, 2×/wk, 26 wk	27 wk	Skin: 1.0 μmol, 5/30 (17%; 0.17 ± 0.07 T/mouse); 2.5 μmol, 11/29 (38%; 0.67 ± 0.17) vs 1/25 (4%; 0.04 ± 0.04) (p <0.05 at 2.5 μmol)	+	No statistics	Wood *et al.* (1980)
Mouse, CD-1	F	30	>99% (acetone)	0.4, 2.5 μmol [91, 571 μg] in 200 μL, 1×, followed 2 wk later by 16 nmol [9.9 μg] TPA in 200 μL acetone, 2×/wk, 25 wk	27 wk	Skin: 0.4 μmol, 4/28 (14%;0.14 ± 0.07 T/mouse); 2.5 μmol, 10/27 (36%; 0.64 ± 0.20) vs 2/29 (7%; 0.07 ± 0.05) in solvent controls (p <0.05 for T incidence and T/mouse at 2.5 μmol)	+		Levin *et al.* (1984)
Benzo[*b*]chrysene									
Mouse, CD-1	F	30	Pure (benzene)	2.5 mmol [695 μg], 1×, followed 1 wk later by 5 μmol (3.1 mg) TPA 2×/wk (10 μmol TPA in promoter controls), 34 wk	35 wk	14/29 (48%) (skin P) vs 0/30 in promoter controls	+	No statistics	Scribner (1973)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	Skin P: 2/20 (10%), 19/20 (95%; p <0.001), 18/20 (90%; p <0.001); 0, 1, 3.3, 4.3 T/animal in TPA control, low- and high-dose groups	+		Weyand *et al.* (1990)
Benzo[*b*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 10, 30, 100 µg; 10 subdoses, 1× every other day followed by TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 45%, 60%, 80%; 0, 0.9, 2.3, 7.1 T/animal	+		LaVoie *et al.* (1982a)
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	20	>99% (acetone)	0, 40, 100 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk	34 wk	Skin P: 10%, 45%, 95%; 0.2, 0.9, 3.3 T/animal	+		Amin *et al.* (1985a)
	F	20	>99% (acetone)	0, 40, 100 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA in 100 µL acetone, 3×/wk	34 wk	Skin P: 5%, 42%, 53%; 0.1, 0.5, 0.9 T/animal	+		
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	25	>99% (acetone)	400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 92%; 6.0 T/animal	+		Geddie *et al.* (1987)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 75%; 2.8 T/animal	+		Geddie *et al.* (1987) (contd)
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 70%; 1.8 T/animal	+		
	F	20	>99% (acetone)	100 nmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	34 wk	Skin P: 53%; 0.9 T/animal	+		
Mouse, CD-1	F	20	NS (acetone)	0, 30, 100 μg; 10 subdoses, 1× every other day followed by 2.5 μg TPA in 100 μL acetone, 3×/wk, 20 wk	24 wk	Skin P: 15%, 65%, 100%; 0.1, 1.4, 5.4 T/animal	+		Weyand *et al.* (1989)
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 μmol; 10 subdoses, 1× every other day followed by 2.5 μg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10%, 100% (p <0.001), 100% (p <0.001), 0.1, 8.5, 11.0 T/animal	+		Weyand *et al.* (1990)
Mouse, Crl:CD-1 (ICR) BR outbred albino	F	20	NS (acetone)	0, 30, 100 μg; 10 subdoses, 1× every other day followed by 2.5 μg TPA in 100 μL acetone, 3×/wk, 30 wk	34 wk	Skin P: 15%, 65%, 100%; 0.1, 1.4, 5.4 T/animal	+		Amin *et al.* (1991)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 outbred albino	F	20	>99% (acetone)	0, 120, 400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 15%, 70% (p <0.001), 95%; 0.15, 1.40, 7.15 T/animal	+		LaVoie *et al.* (1993)
	F	20	>99% (acetone)	0, 40, 120, 400 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 5%, 35% (p <0.05), 90% (p <0.001), 95% (p <0.001); 0.05, 0.45, 3.70, 8.65 T/animal	+		
Benzo[*j*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 30, 100, 1000 µg; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 30%, 55%, 95%; 0, 0.6, 1.9, 7.2 T/animal	+		LaVoie *et al.* (1982a)
Mouse, CD-1	F	20	NS (acetone)	0, 1, 3 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10% (p <0.005), 70% (p <0.005), 90% (p <0.005); 0.1, 3.4, 7.8 T/animal	+		Rice *et al.* (1987)
Mouse, Crl:CD-1 outbred albino	F	20–24	>99% (acetone)	0, 0.3, 1.0, 2.0 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 5% (p <0.01), 55% (p <0.01), 88% (p <0.01), 100% (p <0.01); 0.05, 1.75, 4.08, 7.17 T/animal	+		Weyand *et al.* (1992)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	NS (acetone)	0, 25, 50, 100, 1000 nmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA. 3×/wk, 20 wk	24 wk	Skin P: 0%, 5% (p <0.05), 10% (p <0.05), 45% (p <0.001), 95% (p <0.001); 0, 0.05, 0.40, 0.65, 8.75 T/animal	+		LaVoie *et al.* (1993)
Benzo[*k*]fluoranthene									
Mouse, Crl:CD-1 (ICR)BR	F	20	>99% (acetone)	0, 30, 100, 1000 µg; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 5%, 25%, 75%; 0, 0.1, 0.4, 2.8 T/animal	+		LaVoie *et al.* (1982a)
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	20	>99% (acetone)	0, 4 µmol; 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 30 wk	30 wk	Skin P: 0%, 37%; 0.7 T/animal	+		Amin *et al.* (1985b)
Benzo[*a*]pyrene									
Mouse, SENCAR	F	Experiment 1, 30; experiment 2, 60	98.5% (acetone or acetone + DMSO)	Experiment 1: 50, 100, 200 nmol [13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol TPA, 2×/wk, 19–28 wk	28/50 wk	Experiment 1: Skin P (after 28 wk): 19/30 (63%; 1.7 P/animal), 27/30 (89%; 3.8 P/animal), 29/30 (97%; 7.8 P/animal) Skin C (after 50 wk): 5/30 (18%), 9/30 (30%), 17/30 (55%)	+	No control in experiment 1	DiGiovanni *et al.* (1980)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
				Experiment 2: 0, 10, 50, 100, 200 nmol [0, 2.64, 13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol TPA, 2×/wk, 25 wk	25 wk	Experiment 2: Skin P: 6/60 (10 %; 0.1 P/animal), 25/60 (42%; 0.9 P/animal), 36/60 (60%; 1.6 P/animal), 48/60 (80%; 3.8 P/animal), 60/60 (100%; 8.2 P/animal)			DiGiovanni *et al.* (1980) (contd)
Mouse, CD-1	F	30	98.5% (acetone or acetone + DMSO)	0, 10, 50, 100, 200 nmol [0, 2.64, 13.21, 26.43, 52.87 µg]/animal, 1× followed after 1 wk by 8.5 nmol croton oil, 2×/wk, 25 wk	25 wk	Skin P: 6/60 (10%; 0.1 P/animal), 6/60 (10%; 0.1 P/animal), 24/60 (40%; 0.7 P/animal), 35/60 (58%; 1.8 P/animal), 43/60 (72%; 3.8 P/animal)	+		DiGiovanni *et al.* (1980)
Mouse, Crl/CD-1(ICR)BR	F	20	>99% (acetone)	0, 5 µg animal, 1×/2 days, 20 days (total dose, 50 µg/animal) followed after 10 days by 2.5 µg TPA/animal, 3×/wk, 25 wk	25 wk	Skin T (predominantly P): 1/20 (5%; 0.1 T/animal), 18/20 (90%; 7.1 T/animal) (p <0.01)	+		El-Bayoumy *et al.* (1982)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	30	NS (acetone)	0, 10, 25, 50, 100, 200 µg/animal, 1× followed 1 wk later by 2 µg TPA/animal, 2×/wk, 25 wk	25 wk	Skin P: 3/29 (10%; 0.2 T/animal), 17/29 (58%; 1.3 T/animal), 21/28 (76%; 3.8 T/animal), 24/28 (87%; 6.2 T/animal), 27/27 (100%; 8.8 T/animal), 26/26 (100%; 9.0 T/animal)	+		Raveh *et al.* (1982)
Mouse, SENCAR	M, F	22–40	NS (acetone or acetone/DMSO mixture (1:1))	Experiment 1: 0, 51 µg/animal in acetone, 1× followed after 1 wk by 2.0 µg TPA/animal, 2×/wk, 30 wk	30 wk	Skin P Experiment 1: M: 37/37 (100%; 7.2 P/animal), 39/40 (97%; 5.3 P/animal) F: 35/35 (100%; 7.7 P/animal), 36/40 (90%; 4.5 P/animal)	+		Nesnow *et al.* (1984b)
				Experiment 2: 0 (acetone/DMSO mixture), 51 µg/animal (in acetone) followed after 1 wk by 2.0 µg TPA/animal, 2×/wk, 30 wk		Experiment 2:M: (0/21; 0%), 11/18 (61%; 1.5 P/animal); F: (0/20; 0%), 9/19 (47%; 0.8 P/animal)			
Mouse, SENCAR	F	20	NS (acetone)	0, 396 nmol [0.1mg]/animal, 1× followed after 7 days by 3.24 nmol TPA, 2×/wk, 11 wk	11 wk	Skin P: 0/20, 20/20 (100%; 6.6 P/animal)	+		Mukhtar *et al.* (1986)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24, 29 controls	NS (acetone)	0, 0.8 μmol [0, 0.2 mg]/ animal, 1× followed after 1 wk by 4.26 nmol TPA, 2×/wk, 23 wk	23 wk	Skin P: 1/29 (3%; multiplicity, 2), 22/24 (92%; multiplicity, 6.6; p <0.001)	+		Cavalieri *et al.* (1988b)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.01 μmol [2.64 μg]/animal, 1×/2 days, 20 days, followed 10 days after the final dose by 2.5 μg TPA, 3×/wk, 20 wk	20 wk	Skin T: 1/20 (5%; 0.05 T/animal), 17/19 (89%; 5.53 T/animal; (p <0.005)	+	Type of skin T NS	Rice *et al.* (1988)
Mouse, CD-1	F	30	>99% (acetone)	0, 10 nmol [2.64 μg]/animal, 1×/2 days, 20 days, followed 10 days after the last dose by 2.5 μg TPA, 3×/wk, 20 wk	20 wk	Skin P: 24/25 (0, 96%; 3.4 T/animal; p <0.005)	+		Rice *et al.* (1990)
Mouse, SENCAR	F	24	>99% (acetone)	0, 33.3, 100, 300 nmol [0, 8.8, 26.4, 79 μg]/ animal, 1× followed by 3.24 nmol TPA, 2×/wk, 1 wk, then stopped for 2 wk then resumed and continued with 2 ×/wk for 11 wk	15 or 16 wk	Skin T (predominantly P): 0/24, 10/23 (43%; 15 T, 0.65 T/animal), 17/24 (71%; 66 T, 2.75 T/animal), 21/23 (91%; 120 T, 5.22 T/animal)	+		Cavalieri *et al.* (1991)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	>99% (acetone)	0, 4, 20, 100 nmol [0, 1, 5.3, 26.4 μg]/animal, 1×, followed after 1 wk with 3.24 nmol TPA, 2×/wk, 24 wk	27 wk	Skin T (predominantly P): 0/24, 1/24, (4%; 1 T, 0.04 T/animal), 10/24 (42%; 18 T, 0.75 T/animal), 22/24 (92%; 82 T, 3, 42 T/ animal)	+		Cavalieri *et al.* (1991)
Mouse, SENCAR	F	24	>99% (acetone)	0, 1 nmol [0.26 μg]/animal, 1× followed after 1 wk by 2.16 nmol TPA, 2×/wk, 27 wk	27 wk	No skin T found	–	Very low dose	Higginbotham *et al.* (1993)
Mouse, CD-1	F	20	NS (acetone)	0, 4, 10, 25 nmol [1, 2.5, 6.6 μg]/animal, 10]×, 1×/2 days, 20 days followed after 10 days by 2.5 μg TPA/animal, 3×/wk, 20 wk	25 wk	Skin T: 3/20 (15%; 0.15 T/animal), 3/20 (15%; 0.15 T/animal), 2/20 (10%; 0.10 T/ animal), 5/20 (25%; 0.4 T/animal; p >0.05)	–	Type of skin T NS	LaVoie *et al.* (1993)
Benzo[*e*]pyrene									
Mouse, CD-1	F	30	>99% (acetone/DMSO)	0, 1.0, 2.5, 6.0 μmol, 1×; followed by TPA (16 nmol/200 μL acetone), 2×/wk, 25 wk	36 wk	Skin P: 7% 15%, 11%, 14%; 0.07, 0.15, 0.11, 0.14 T/animal	±		Buening *et al.* (1980)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Chrysene									
Mouse CD-1	F	30	NS (acetone/DMSO)	0, 0.4, 1.2 µmol, 1×, followed by 16 nmol TPA, 2×/wk, 25 wk	26 wk	Skin P: 10%, 43% (p <0.05), 43% (p <0.05); 0.10 ± 0.06, 0.77 ± 0.25, 1.07 ± 0.34 P/animal	+		Chang *et al.* (1983)
				0, 1.2 µmol, 1×, followed by 16nmol TPA, 2×/wk, 25 wk	26 wk	Skin P: 0%, 30% (p <0.05);0, 0.93 ± 0.27 P/animal	+		
Mouse, CD-1	F	25	>99% (acetone)	0,1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 2/25 (8%), 23/25 (92%)	+		Rice *et al.* (1985)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	20 wk	Skin P: 20%, 25% (p <0.05), 90% (p <0.005), 95% (p <0.005); 0.05, 0.45, 2.95, 4.45 P/animal	+		Rice *et al.* (1988)
Mouse, CD-1	F	20	>99% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 10%, 10%; 0.6, 0.1 T/animal	–		Amin *et al.* (1990)
Mouse, SENCAR	M, F	16	NS (toluene)	0, 1600 nmol, 1×, followed by croton oil/toluene (1:99, v/v), 2×/wk, 100 wk	101 wk	No skin T in treated or control mice	–		Bhatt & Coombs (1990)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
4*H*-Cyclopenta[*def*]chrysene									
Mouse, CD-1	F	25	>99% (acetone)	0, 0.1 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 2/25 (8%), 25/25 (100%)	+		Rice *et al*. (1985)
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 1/20 (5%; 0.05), 13/20 (65%; 1.0), 19/19 (100%; 6.85), 19/19 (100%; 8.47) (p <0.05)	+		Rice *et al*. (1988)
Dibenz[*a*,*c*]anthracene									
Mouse, Swiss (ICR/Ha)	F	20, 40 controls	Purified (benzene)	1 mg in 100 µL, 1×; 1 mg in 100 µL, 1×, followed 2 wk later by 25 µg croton oil in 100 µL acetone, 3×/wk	54–66 wk	1.0 mg, 0/20 vs 0/20 solvent controls; 1.0 mg + croton oil, 5/20 (25%) skin P, 2/20 (10%) skin C vs 6/40 (15%) skin P, 1/40 (2%) skin C in croton oil controls	±	No statistics	Van Duuren *et al*. (1968)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss (ICR/Ha)	F	20 or 50, 20 controls	'Rigorously purified' (benzene)	1.0 mg in 100 µL, 1×; 1.0 mg in 100 µL, 1×, followed 2 wk later by 2.5 µg TPA in 100 µL acetone, 3×/wk	58–60 wk	1.0 mg, 0/50 vs 0/20 solvent controls; 1.0 mg + TPA, 19/20 (95%; skin P), 4/20 (20%; skin C) vs 1/20 (5%; skin P) TPA controls	+	No statistics	Van Duuren *et al.* (1970)
Mouse, CD-1	F	30	Pure (benzene)	696 µg, 1×, followed 1 wk later by TPA [5 µmol (3.1 mg); solvent and volume NS], 2×/wk (10 µmol TPA in promoter controls), 34 wk	35 wk	63% skin P vs 0% in TPA controls	+	No statistics; incidence NS	Scribner (1973)
Mouse, SENCAR	F	30	>5% (acetone)	2 µmol [557 µg], 1×; followed 1 wk later by TPA [2 µg, solvent and volume NS], 2×/wk [duration NS]	At least 15 wk	27% skin P vs 10% in TPA controls	±	Limited reporting and histo-pathology; no statis-tics	Slaga *et al.* (1980)
Mouse, CD-1	F	39–40	Pure (acetone)	25, 50 µg in 100 µL, followed 1 wk later by 0.64 µg TPA in 100 µL acetone, 2×/wk; 29 wk, then 1 µg, 38 wk	68 wk	25 µg, 5/39 (13%; skin P); 50 µg, 8/40 (20%) vs 3/40 (8%) TPA controls	±		Chourou-linkov *et al.* (1983)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, Swiss albino	M	97	NS (acetone)	20, 160 ng in 200 µL, 1×, followed by croton oil [1%, volume NS], 1×/wk, 25 wk	210–218 days	20 ng, 10/30 (33%) skin T (0.5 T/mouse; p <0.05); 160 ng, 14/37 (38%; 0.9 T/mouse; p <0.05) vs 4/32 (13%; 0.1 T/mouse) acetone controls	+		Klein (1960)
Mouse, Swiss (ICR/Ha)	F	20, 50 controls	NS (benzene)	0, 150 µg, 1×, followed by croton oil [100 µL, 0.025%], 3×/wk, 45 wk	331 days	18/20 (90%; skin P), 10/20 (50%; skin C) vs 8/50 (16%; skin P), 0/50 (skin C) croton oil controls	+	No statistics	Van Duuren *et al.* (1967)
Mouse, NMRI	F	16, 30 controls	>99% (acetone or THF)	0, 83.5 µg in 100 µL acetone, 167 µg in 100 µL tetrahydrofuran, 100 µL acetone, 1×, followed by 10 nmol TPA, 2×/wk, 23 wk	24 wk	83.5 µg, 6/16 (38%; skin P); 167 µg, 15/16 (93%; skin P) vs 0/30 acetone controls	+	No histology; no statistics	Platt *et al.* (1990)
Dibenz[*a*,*j*]anthracene									
Mouse, SENCAR	F	30	NS (acetone)	0, 111, 223 µg [400, 800 nmol] in 200 µL, 1×, followed by 2.1 µg TPA, 2×/wk, 20 wk	22 wk	Skin P: 111 µg, 70% (1.3 P/mouse); 223 µg, 97% (3.0 P/mouse) vs 19% (0.19 P/mouse) acetone controls	+	No histology	Sawyer *et al*. (1987, 1988)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	NS (peroxide-free THF)	0, 111 μg (400 nmol) in 200 μL, 1×, followed by 2.1 μg TPA, 2×/wk, 14 wk	16 wk	Skin P: 111 μg, 29% (0.58 P/mouse) vs 5% (0.05 P/mouse) THF controls (p <0.05)	+	Limited histology	Harvey *et al.* (1988)
Mouse, SENCAR	F	24	NS (peroxide-free THF)	0, 111, 223 μg [400, 800 nmol] in 200 μL, 1×; followed by 2.1 μg TPA, 2×/wk, 20 wk	22 wk	Skin P: 111 μg, 39% (0.86 P/mouse); 223 μg, 65% (1.83 P/mouse) vs 5% (0.05 P/mouse) THF controls	+	No histology	Sawyer *et al*. (1988)
Dibenzo[*a*,*e*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Recrystallized (dioxane)	0, 25 μg in 25 μL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	10/28 (36%; skin P) vs 2/30 (7%) croton oil controls	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)
Mouse, SENCAR	F	21, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 μg [800 nmol] in 100 μL, 1×, followed 1 wk later by 2.6 μg TPA/100 μL acetone, 2×/wk, 25 wk	26 wk	5/21 (24%; skin P) vs 2/23 (9%) TPA controls	–		Cavalieri *et al*. (1989)
Dibenzo[*a*,*h*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Recrystallized (dioxane)	0, 25 μg, 10×/20 days, followed by 2.5% [2.3 mg] croton oil (volume NS), 3×/wk)	6 mo	21/29 (74%; skin P) vs 2/30 (7%; skin P) croton oil control (p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al*. (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	31, 32 controls	NS (acetone)	0, 200 µg, 1×, followed by 10 µg TPA, 2×/wk, 26 wk	27 wk	26/28 (93%; skin T) vs 2/32 (6%) TPA controls	+	No statistics	Sardella *et al.* (1981)
Mouse, CD-1	F	30	'Essentially pure' (10% DMSO in THF)	0, 15, 60, 180 µg in 200 µL, 1×, followed by 16 nmol TPA, 2×/wk, 16 or 24 wk	15 µg, 25 wk; 60 and 180 µg, 17 wk	Skin P: 15 µg, 72% (3.97 P/mouse) vs 0% in TPA control; 60 µg, 79% (4.72 P/mouse); 180 µg, 72% (5.52 P/mouse) vs 10% (0.10 P/mouse) in TPA control	+	No histology	Chang *et al.* (1982)
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 240 µg [800 nmol] in 100 µL, 1×, followed by 4.26 nmol TPA, 2×/wk, 25 wk	26 wk	18/24 (75%) skin P vs 2/23 (9%) TPA control	+		Cavalieri *et al.* (1989)
Dibenzo[*a*,*i*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	0, 25 µg in 25 µL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	12/30 (40%; skin P) vs 2/30 (7%; skin P) croton oil controls	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Mouse, 20Ha/ICR Swiss	F	20	95% (acetone)	0, 10, 50 µg in 100 µL (total dose, 100, 500 µg), 10×/20 days, followed 10 days later by 2.5 µg TPA in 100 µL acetone 3×/wk, 20 wk	NS	Skin T: 100 µg, 40% (0.5 T/mouse, type NS); 500 µg, 85% (5.8 T/mouse, type NS) vs 0% solvent controls	+		Hecht *et al.* (1981)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	30	'Essentially pure' (10% DMSO in THF)	0, 15, 60, 180 µg in 200 µL, 1×, followed 1 wk later by 10 µg TPA in 200 µL, 2×/wk, 16 wk (all dose groups) and 24 wk (15 µg group)	NS	Skin P: 15 µg (16 wk), 28% (0.52 P/mouse) vs 0% in TPA controls; 60 µg, 67% (5.33 P/mouse); 180 µg, 79% (5.25 P/mouse) vs 10% (0.10 P/mouse) in TPA controls; 15 µg (24 wk), 69% (2.07 ± 0.44 (mean ± SE) P/mouse) vs 0% in TPA controls	+	No histology	Chang *et al*. (1982)
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 µg (800 nmol) in 100 µL, 1×, followed 1 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 25 wk	26 wk	15/24 (62%; skin P) vs 2/23 (9%) TPA controls	+		Cavalieri *et al*. (1989)
Dibenzo[*a*,*l*]pyrene									
Mouse, SENCAR	F	24, 23 controls	>99% (dioxane: DMSO (75:25))	0, 242 µg (800 nmol) in 100 µL, 1×, followed 3 wk later by 2.6 µg TPA in 100 µL acetone, 2×/wk, 22 wk	26 wk	22/24 (92%; skin P) vs 2/23 (9%) TPA controls	+		Cavalieri *et al*. (1989)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, SENCAR	F	24	>99% (acetone)	0, 10.1, 30.2, 90.7 μg in 100 μL, 1×, followed 1 wk later by 2.0 μg TPA in 100 μL acetone; promotion suspended after first treatment and resumed by 4 wk, 2×/wk, 12 wk	15 wk	Skin P: 10.1 μg, 23/24 (96%; 6.75 T/mouse); 30.2 μg, 22/24 (92%; 7.92 T/mouse); 90.7 μg, 24/24 (100%; 8.50 T/mouse) vs 0/24 TPA controls	+		Cavalieri *et al*. (1991)
Mouse, SENCAR	F	24	>99% (acetone)	1.2, 6.0, 30.2 μg in 100 μL, 1×, followed 2 wk later by 2.0 μg TPA in 100 μL acetone, 2×/wk, 24 wk	27 wk	Skin P: 1.2 μg, 22/24 (92%; 6.96 T/mouse); 6.0 μg, 20/24 (83%; 5.29 T/mouse); 30.2 μg, 20/24 (83%; 3.29 T/mouse) vs 0/24 TPA controls	+		Cavalieri *et al*. (1991)
Mouse, SENCAR	F	24	>99% (acetone)	75.5, 302 ng in 100 μL, 1×, followed 1 wk later by 1.3 μg TPA in 100 μL acetone, 2×/wk, 27 wk	27 wk	Skin P: 75.5 ng, ~30%; 302 ng, ~80% vs 0% in TPA controls	+		Higginbotham *et al*. (1993)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	NS (acetone)	0.3, 1.2, 3.0, 7.6 μg (total doses); 10 subdoses in 100 μL, every other day, followed 10 days later by 2.5 μg TPA in 100 μL, 3×/wk, 20 wk	>22 wk	Unspecified skin T: 0.3 μg, 18/19 (95%; 5.0 T/mouse); 1.2 μg, 20/20 (100%; 17.8 T/mouse); 3.0 μg, 18/20 (90%; 11.3 T/mouse); 7.6 μg, 20/20 (100%; 15.0 T/mouse) vs 3/20 (15%; 0.15 T/mouse) solvent controls (p <0.001 for all dose groups)	+	No histology	LaVoie *et al*. (1993)
Mouse, SENCAR	F	23–25	Pure (acetone)	0.4, 1.2, 3.6 μg in 100 μL, 1×, followed 1 wk later by 1 μg TPA in 100 μL, 2×/wk, 28 wk	29–30 wk	Skin T: 0.4 μg, 16/23 (70%; 5.22 T/mouse; 2 SCC in 2 mice); 1.2 μg, 19/23 (83%; 7.09 T/mouse); 3.6 μg, 23/25 (92%; 9.28 T/mouse; 7 SCC in 5 mice)	+	No control	Gill *et al*. (1994)
Mouse, NMRI	F	16	>99.7% (acetone)	0, 12 μg in 100 μL, 1×, followed 1 wk later by TPA (6.2 μg in 100 μL acetone, 2 ×/wk, 30 wk)	31 wk	15/16 (94%; skin T; 6.5 T/mouse) vs 0/16 acetone controls	+	No histology	Luch *et al*. (1999)
Mouse, SENCAR	F	35, 10 controls	NS (toluene)	0, 0.6 μg in 200 μL, 1×, followed 2 wk later by 1 μg TPA in 200 μL acetone, 2×/wk, 25 wk	26 wk	30/30 (100%; skin P; 7.97 T/mouse) vs 1/9 (11%; 0.25 T/mouse) TPA controls	+		Marston *et al*. (2001)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*e*,*l*]pyrene									
Mouse, Swiss albino Ha/ICR/Mil	NS	30	Recrystallized (dioxane)	0, 25 µg in 25 µL, 10×/20 days, followed 1 wk later by 2.5% (2.3 mg) croton oil in acetone (frequency, duration NS)	6 mo	0/30 skin P vs 2/30 (7%) promoter controls	–		Hoffmann & Wynder (1966)
1,4-Dimethylphenanthrene									
Mouse, Swiss albino (Ha/ICR)	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10×, every other day, followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	95% skin P (8.2 T/ mouse) vs 0% in TPA controls	+	No histology; no statistics	LaVoie *et al*. (1981)
Mouse, outbred albino Crl:CD-1 (ICR) BR	F	20	>99.5% (acetone)	0, 30, 100 µg in 100 µL, 10×, every other day, followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 20 wk	24 wk	30 µg, 80% skin T (3.25 T/mouse); 100 µg, 100% (5.30 T/mouse) vs 0% in TPA control; (p <0.01)	+		LaVoie *et al*. (1982b)
Indeno[1,2,3-*cd*]pyrene									
Mouse, Crl:CD-1 (ICR) BR (outbred albino)	F	25	NS (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 90% (2.83 T/mouse) vs 5% in controls	+		Rice *et al.* (1986)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	25	>99% (acetone)	0, 4.0 µmol [1105 µg], 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P: 0%, 72% (2.1 T/mouse) (p <0.005)	+		Rice *et al.* (1990)
1-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 6/19 (32%; 0.32 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
2-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 8/19 (42%; 0.68 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
3-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 0.1, 0.3 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T (T/mouse): 0/20, 3/17 (18%; 0.18), 4/16 (25%; 0.50)	+		Hecht *et al.* (1974)
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 14/20 (70%; 1.3 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
4-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 7/20 (35%; 0.45 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
5-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0.1, 0.3 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 20/20 (100%; 5.5 T/mouse), 20/20 (100%; 8.0 T/mouse)	+		Hecht *et al.* (1974)
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0 mg, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 17/18 (94%; 5.3 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al.* (1979)
Mouse, CD-1	F	20	>99.9% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 5% (0.05), 65% (1.7; p <0.01)	+		Amin *et al.* (1985c)
Mouse, CD-1	F	20	100% (NS)	0, 33, 100 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	26 wk	Skin P (T/mouse): 10% (0.1), 80% (3.9), 90% (5.2)	+		Hecht *et al.* (1985)
Mouse, CD-1	F	20	>99.9% (acetone)	0, 100 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	25 wk	Skin P (T/mouse): 10% (0.1), 90% (5.2)	+		El-Bayoumy *et al.* (1986)
Mouse, CD-1	F	20	>99% (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P: 0%, 84% (4.8 T/mouse)	+		Hecht *et al.* (1987)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	F	20	>99% (acetone)	0, 0.15, 0.5, 1.5 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 20% (0.3), 100% (9.2; p <0.005), 100% (10.68; p <0.005), 100% (9.42; p <0.005)	+		Rice *et al.* (1988)
Mouse, CD-1	F	22	NS (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 20 wk	22 wk	Skin P (T/mouse): 10% (0.6), 85% (4.3; p <0.05)	+		Amin *et al.* (1990)
Mouse, CD-1	F	20	NS (acetone)	0, 33 nmol, 1×, followed by 2.5 µg TPA, 3×/wk, 25 wk	25 wk	Skin P (T/mouse): 10% (0.01), 65% (2.80; p <0.01)	+		Amin *et al.* (1992)
6-Methylchrysene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.9% (acetone)	0, 1.0, 10 subdoses, 1× every other day followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin T: 0/20 (0%), 7/19 (37%; 1.5 T/mouse)	+		Hecht *et al.* (1974); LaVoie *et al*. (1979)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
2-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0, 1.0 mg (2 studies), 10 subdoses in 50 µL, 1× every other day, followed by 2.5% croton oil, 3×/wk, 20 wk	4 mo	**Skin T (T/mouse)**: Study 1: 1/30 (3%; 0.03), 9/30 (30%; 0.47) Study 2: 1/30 (3%; 0.03), 10/29 (34%; 0.55; p <0.01)	+		Hoffman *et al.* (1972); LaVoie *et al.* (1979)
3-Methylfluoranthene									
Mouse, Swiss albino Ha/ICR/Mil	F	30	Pure (acetone)	0, 1.0 mg (2 studies), 10 subdoses of 50 µL, 1× every other day, followed by 2.5% croton oil, 3×/wk, 20 wk	4 mo	Skin T (T/mouse): 1/30 (3%; 0.03), 9/28 (32%; 0.5; p <0.01)	+		Hoffman *et al.* (1972); LaVoie *et al.* (1979)
1-Methylphenanthrene									
Mouse, Swiss albino Ha/ICR/Mil	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10× every other day, followed by 2.5 µg TPA in 100 µL, 3×/wk, 20 wk)	24 wk	0% skin P vs 0% in TPA control	–	No histology	LaVoie *et al.* (1981)
Naphtho[1,2-*b*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 10% (0.1), 65% (2.5), 100% (6.6; p <0.001)	+		Weyand *et al.* (1990)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Naphtho[2,1-*a*]fluoranthene									
Mouse, CD-1	F	20	NS (acetone)	0, 1.0, 4.0 µmol, 10 subdoses, 1× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 10% (0.1), 90% (5.9), 100% (7.3; p <0.001)	+		Weyand *et al.* (1990)
Naphtho[2,3-*e*]pyrene									
Mouse, Swiss albino Ha/ICR	F	30	NS (dioxane)	0, 250 µg, 10 subdoses, 1× every other day followed by 2.5% croton oil, 3×/wk, 20 wk	24 wk	Skin P (T/mouse): 7.6% (0.1), 33% (0.5; p <0.01)	+		Hoffmann & Wynder (1966); LaVoie *et al.* (1979)
Perylene									
Mouse, Swiss albino Ha/ICR	F	20	'Rigorously purified' (benzene)	0, 800 µg in 200 µL, 1×, alone or followed by 2.5 µg TPA in 100 µL acetone, 3×/wk, 56–58 wk	58–60 wk	0/20 skin P vs 0/20 solvent controls; 3/20 (15%) skin P vs 1/20 TPA controls	±	No statistics	Van Duuren *et al.* (1970)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 (ICR)Br	F	20	>99% (acetone)	0, 100 µg in 100 µL, 10× every other day followed by 2.5 µg TPA in 100 µL, 3×/wk, 25 wk	29 wk	5% skin T vs 5% in TPA controls	–		El-Bayoumy *et al.* (1982)
Phenanthrene									
Mouse, 'S'	NS	20	NS (acetone)	0, 54 mg, 10×, 3×/wk, followed by (0.17%) croton oil, 300 µL, 1×/wk, 16 wk; 300 µL (0.085%) 1×/wk, 2 wk	24 wk	5/20 (25%) skin P (12 T) vs 4/19 (21%) skin P (4 T) croton oil controls	±		Salaman & Roe (1956)
Mouse, 'stock albino'	M, F	10	'High purity' (acetone)	0, 300 µg on days 0, 2, 6, 8, followed by 250 µl (0.1%) croton oil, 1×/wk, 20 wk	24 wk	4/19 (21%) skin P vs 2/20 (10%) skin P croton oil controls	±		Roe (1962)
Mouse, CD-1	F	30	Purified (acetone)	0, 1.78 mg, 1×, followed by 5 µmol TPA, 2×/wk, 34 wk	35 wk	12/30 (40%) skin P vs 1/30 (3%) skin P TPA controls	+	No histology; no statistics	Scribner (1973)
Mouse, CD-1	F	30	>98% (acetone: ammonium hydroxide (1000:1))	0, 1.78 mg, 1×, followed by 16 nmol TPA, 2×/wk, 35 wk	36 wk	4/30 (13%) skin P vs 2/30 (7%) skin P TPA controls	±	No histology	Wood *et al.* (1979)
Mouse, Swiss albino Ha/ICR	F	20	>99.5% (acetone)	0, 100 µg in 100 µL, 10× every other day, followed by 2.5 µg TPA, 3×/wk, 20 wk	24 wk	0/20 skin P vs 0/20 skin P TPA controls	–	No histology	LaVoie *et al.* (1981)

Table 3.2 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Picene									
Mouse, CD-1	F	30	Purified (benzene)	0, 2.8 mg (volume NS), 1×, followed 1 wk later by 10 μmol TPA, 2×/wk, 34 wk	35 wk	8/30 (27%) skin P vs 0/30 TPA controls	+	No histology; the TPA dose was probably 10 μg; no statistics	Scribner (1973)
Mouse, NMRI	F	16 or 30	>99% (acetone, THF, or benzene)	83.5 μg in 100 μL acetone, 167 μg in 100 μL tetrahydrofuran, 2800 μg in 100 μL benzene, 1×, followed by 6.2 μg TPA in 100 μL, 2×/wk, 23 wk	24 wk	83.5 μg in acetone, 0% skin T; 167 μg in THF, 0%; 2800 μg in benzene, 19% vs 0% in acetone controls	+	No histology; no statistics	Platt *et al*. (1990)

C, carcinoma; DMSO, dimethylsulfoxide; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; SCC, squamous-cell carcinoma; SE, standard error; SGA, sebaceous gland adenoma; T, tumour; THF, tetrahydrofuran, TPA, 12-*O*-tetradecanoylphorbol-19-acetate; UV, ultraviolet; vs, versus; wk, week

[a]–, negative; +, positive; ±, equivocal

Table 3.3. Carcinogenicity studies of subcutaneous administration of various PAHs in experimental animals

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene									
Mouse, XVII	M, F	7 M, 7 F	NS (olive oil)	600 μg in 200 μL, 1×/mo, 3 mo	NS	M: 0/7; F: 0/7	–	No control; small no. of animals; short duration of treatment; no statistics	Lacassagne *et al.* (1958)
Anthracene									
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	5 mg 1×	22–28 mo	0/26 mice surviving >4 mo	–	No control	Steiner (1955)
Mouse, NMRI	M, F	40; 40 controls	99.9% (aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20))	71.3 μg in 50 μL (400 nmol), 1× on PND 2	40 wk	Pulmonary T: F, 1/12 (8%) vs 1/19 (5%) solvent control; M, 2/17 (12%) vs 1/14 (7%) solvent control	–	No statistics	Platt *et al.* (1990)
Rat, NS	NS	10	NS; aqueous suspension	1 mg in 2 mL, 1×/wk, 103 wk	103 wk	0/10 (only 2 survived 18 mo)	–	Small no. of animals; limited reporting; no control	Boyland & Burrows (1935)
Rat, Wistar	NS	5	NS (sesame oil)	5 mg, 1×/wk, 6–7 wk	10 mo	0/5 (only 4 survived 10 mo)	–	Small no. of animals; short duration; no control; no statistics	Pollia (1941)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, BDI and BDIII	NS	10	Pure (oil)	20 mg, 1×/wk, 33 wk	Lifetime	5/9 (55%) (1 myosarcoma, 4 fibroma, some of which had sarcomatous proliferations)		No control	Druckrey & Schmähl (1955); Schmähl (1955)
11*H*-Benz[*b*,*c*]aceanthrylene									
Mouse, C3H	M	15	NS (tricaprylin)	1 mg in 500 µL, 1×	>539 days	1/15 (7%) (3 T/TBA, pulmonary A, hepatic A, benign haemangioma of rump)	–		Dunlap & Warren (1946)
Benz[*a*]anthracene									
Mouse, A	M, F	10; 20 controls	NS (tricaprylin)	500 µg in 100 µL, 1×	14 wk	0/10 vs 1/20 control	–		Andervont & Shimkin (1940)
Mouse, C57BL	M, F	50	NS (tricaprylin)	5.0 mg in 500 µL, 1×	22 mo	S: 8/50 (16%; S) vs 3/304 (1%) tricaprylin controls	+		Steiner & Falk (1951)
Mouse, C57BL	M, F	16 (10 mg); 36–45 (≤5 mg)	Purified (tricaprylin)	0.05, 0.2, 1, 5, 10 mg in 500 µL, 1×	22–28 mo	S: 0.05 mg, 5/44 (2%); 0.2 mg, 11/45 (24%); 1 mg, 15/44 (34%); 5 mg, 20/36 (56%); 10 mg, 5/16 (31%)	+	No control	Steiner & Edgcomb (1952); Steiner (1955)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M, F	60	NS (1% aqueous gelatine)	50 µg in 20 µL, 1× on PND1, 2, 4 or 8	36–43 wk	Pulmonary A + AdC: PND1, 24/52 (46%); PND2, 11/39 (28%); PND4, 10/33 (30%); PND8, 10/41 (24%) vs 2/21 (10%) solvent controls	+		Roe *et al.* (1963)
Mouse, C3H	NS	20	NS (tricaprylin)	5 mg, 1×	638 days	0% vs 0% in solvent controls	–		Stevenson & von Haam (1965)
Mouse, C57BL	M, F	10	NS (arachis oil)	1 mg in 100 µL, 1×/wk, 10 wk	80 wk	S: M, 8/10 (80%); F, 6/10 (60%) vs 0/10 solvent controls	+		Boyland & Sims (1967)
Mouse, C57BL/6J	M	40–50	NS (tricaprylin)	500 µg in 100 µL, 1×; 500 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 8–24 wk later	Up to 52 wk	FibroS: no transplant, 4.1%; transplant, 67% (8 wk), 80% (12 wk), 20% (16 wk), 44% (24 wk) vs 0% in transplanted vehicle controls	+		Homburger & Treger (1970)
Rat, albino	M	20	NS (tricapry lin)	2 mg in 250 µL, 1×	14.5 mo	0/20 vs 0/19 solvent controls	–		Miller & Miller (1963)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Mouse, NMRI	F	60–90	NS (tricaprylin, 0.9% saline solution or lutrol 9 (polyethylene-oxide))	Experiment 1: 0, 25, 50, 100, 200, 400 µg/animal (in tricaprylin), 1×	420–500 days	T (mainly fibroS but also myofibroS, carcinoS, SCC, AdC and reticuloS) at injection site [incidence derived from dose–response curves] Experiment 1: [0/90] (0%), [24/90] (~ 27%), [49/90] (~54%), [51/90] (~57%), [66/90] (~73%), [56/90] (~62%)	+		Pott *et al*. (1973a)
				Experiment 2: 0, 25, 50, 100, 200 µg/animal (in tricaprylin), 1×	~420 days	Experiment 2: [1/80] (~1%), [40/80] (~50%), [50/80] (~62%), [60/80] (~74%), [61/80] (~76%)			
				Experiment 3: 0, 200, 400, 800 µg/animal (in 0.9% saline solution), 1×	~270 days	Experiment 3: [0/ 60] (0%), [2/60] (~3%), [18/60] (~30%), [26/60] (~44%)			
				Experiment 4: 100 µg/animal in lutrol or tricaprylin, 1×	~410 days	Experiment 4: [2/60] (~3%), [30/60] (~50%)			
				Experiment 5: 50 µg in 1 mL or 0.1 mL tricaprylin, 1×	~310 days	Experiment 5: [11/60] (~18%), [37/60] (~62%)			

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, NMRI	F	80	NS (tricaprylin, 0.9% saline solution, Tween 60 or Lutrol 9 (polyethylene oxide))	Experiment 1: 0, 25, 50, 100, 200 µg/animal in tricaprylin, 1×	19 mo	**Subcutaneous T at injection site** Experiment 1: 1/80 (1%), 44/80 (~55%), 52/80 (~65%), 60/80 (~75%), 66/80 (~83%)	+		Pott *et al.* (1973b)
		60		Experiment 2: 0, 100, 200, 400, 800 µg /animal in 0.9% saline/Tween 60 solution, 1×	17 mo	Experiment 2: 1/60 (2%), 1/60 (2%), 5/60 (~8%), 22/60 (~37%), 32/60 (~54%)			
		120		Experiment 3: 100 µg/animal in Lutrol 9, 1×	14 mo	Experiment 3: 2/97 (2%)			

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C3H/fCum	M	20 or 40	Pure (trioctanoin, DMSO)	Experiment 1: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or DMSO, 1×	15 mo	**FibroS at injection site** Experiment 1: 0/16, 0/20, 15/18 (83%), 12/19 (63%)	+		Kouri *et al.* (1980)
				Experiment 2: 0 (trioctanoin control), 0 (DMSO control), 0.9 µmol [0.23 mg] in trioctanoin or in DMSO, 1×	18 mo	Experiment 2: 0/20, 0/18, 14/18 (78%), 7/19 (37%)			
				Experiment 3: 0 (trioctanoin/ DMSO; 100:1), 0.9 µmol [0.23 mg] in trioctanoin/ DMSO (100:1), 1×	18 mo	Experiment 3: 0/20, 36/40 (90%)			
Mouse, NS, newborn	M, F	NS	NS saline (solution + 1% gelatine + 0.4% Tween 20)	0, 10, 100 µg/ animal, 1×	30 wk	Lung T: 5/38 (13%; 0.13 T/ animal), 5/31 (16%; 0.23 T/animal), 21/33 (64%; 2.52 T/animal)	+	Type of lung tumours NS; statistics NS	Rippe & Pott (1989)
Mouse, NS	F	NS	NS (tricaprylin)	0, 10,100 µg/ animal, 1×	78 wk	S at injection site: 1/30 (3%), 13/30 (43%), 20/30 (67%)	+		Rippe & Pott (1989)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, $AhR^{-/-}$, $AhR^{+/-}$, $AhR^{+/+}$	M	17, 16 controls	NS (olive oil)	2 mg/animal, on day 1, day 8	18 wk	$AhR^{-/-}$: 0/16 (0%); $AhR^{+/-}$: 17/17 (100%; 15 fibroS, 1 rhabdomyoS, 1 SCC; p <0.01); $AhR^{+/+}$: 17/17 (100%; 13 fibroS, 2 rhabdomyoS, 2 SCC; p <0.01)	+		Shimizu *et al.* (2000)
Rat, Wistar	F	50	NS (tricaprylin)	0, 33, 100, 300, 900, 2700 µg/ animal, 1×	~530 days	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%)	+		Pott *et al.* (1973a)
Rat, NS	F	NS	NS (tricaprylin)	0, 1 mg, 1×	132 wk	S at injection site: 0/24 (0%), 20/24 (83%)	+		Rippe & Pott (1989)
Rat, NS	F	NS	NS (DMSO)	0, 15 mg, 1×	132 wk	S at injection site: 1/24 (4%), 19/24 (79%)	+		Rippe & Pott (1989)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
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	25 M, 25 F	NS (tricaprylin)	500 µg/animal, 1×	53 wk	**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%)	+	No sub-cutaneous T observed in historical controls	Homburger *et al*. (1972)
Monkey, Old World	NS	17	NS (NS)	30–90 mg/kg; multiple doses [no. of injections and duration of treatment NS]	Under observation up to 18 years	No T found	–		Adamson & Sieber (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*c*]anthracene									
Mouse, C57BL6/J	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 1/30 (3%) (fibroS)	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)
Mouse, DBA2/J	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 0/30	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)
Mouse, B6D2F$_1$	M, F	30, 10 controls	NS (trioctanoin)	150, 300 µg in 50 µL, 1×	12 mo	150 µg, 0/30; 300 µg, 1/30 (3%) (fibroS)	–	No incidence reported for the solvent control group; no statistics	Kouri *et al*. (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Mouse, CH3	M	19–79	Melting-point (tricaprylin)	1.95, 7.8, 15.6, 31, 62, 125, 250, 500 µg, 1, 2, 4 mg in 250 µL, 8 mg in 500 µL, 1×	NS	S: 1.95 µg, 2/79 (3%); 7.8 µg, 6/40 (15%) 0; 15.6 µg, 6/19 (31%); 31 µg, 16/21 (76%); 62 µg, 20/20 (100%); 125 µg, 21/23 (91%); 250 µg, 19/21 (90%); 500 µg, 20/21 (96%); 1 mg, 22/22 (100%); 2 mg, 19/19 (100%); 4 mg, 17/20 (85%); 8 mg, 16/21 (76%)	+	No control	Bryan & Shimkin (1943)
Mouse, C57BL	M, F	50, 304 controls	NS (tricaprylin)	20 µg, 1×	≤22 mo	S: 28/48 (58%) vs 3/280 (1%) solvent-treated controls	+	No statistics	Steiner & Falk (1951)
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	20, 40 µg in 500 µL, 1×	22–28 mo	S: 20 µg, 7/21 (33%); 40 µg, 6/18 (33%)	+	No control; no statistics	Steiner (1955)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, General Purpose/NTH	M, F	592, 79 controls	NS (olive oil)	0, 0.003, 0.01, 0.03, 0.08, 0.2, 0.7, 2.2, 6.7 µg in 50 µL, 1× as newborn	M, 54–55 wk; F, 78–79 wk	0.003 µg, 1/61 (2%; fibroS), 23/60 (38%; pulmonary T); 0.01 µg, 1/45 (2%; fibroS), 10/45 (22%; pulmonary T); 0.03 µg, 0/54 fibroS, 15/53 (28%; pulmonary T); 0.08 µg, 5/42 (12%; fibroS), 10/42 (24%; pulmonary T); 0.2 µg, 5/48 (10%; fibroS), 18/48 (37%; pulmonary T); 0.7 µg, 11/45 (24%; fibroS), 15/45 (33%; pulmonary T); 2.2 µg, 13/38 (34%; fibroS), 14/38 (37%; pulmonary T); 6.7 µg, 29/50 (58%; fibroS), 22/50 (44%; pulmonary T) vs 0/79 fibroS, 26/79 (33%; pulmonary T) solvent controls	+	No dose–response for pulmonary T; no statistics	O'Gara *et al.* (1965)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57BL	M, F	20	NS (arachis oil)	0, 1 mg, 1×/wk, 10 wk	60–80 wk	S: M, 20/20 (100%); F, 17/19 (90%) vs 0/40 solvent controls	+	No statistics	Boyland & Sims (1967)
Mouse, NMRI	F	60	NS (tricaprylin)	10, 30, 90, 270, 810 µg, 1×	≤16 mo	Mainly fibroS: 10 µg, 24/60 (40%); 30 µg, 21/60 (35%); 90 µg, 39/60 (65%); 270 µg, 45/60 (75%); 810 µg, 54/60 (90%)	+	No control; no statistics	Pott *et al.* (1973a)
Mouse, NMRI	F	100	NS (tricaprylin)	0, 2.35, 4.7, 9.3, 18.7, 37.5, 75.0 µg in 500 µL, 1×	114 wk	S: 2.35 µg, 37/100; 4.7 µg, 39/100; 9.3 µg, 44/100; 18.7 µg, 56/100; 37.5 µg, 65/100; 75.0 µg, 69/100	+	Control data not reported; no statistics	Pfeiffer (1977)
Mouse, B6, D2, $B6D2F_1$	M	30 B6, 30 D2, 57 or 60 $B6D2F_1$; 10 controls of each	NS (tricaprylin)	0, 150, 300 µg in 50 µL, 1×	12 mo	FibroS: B6: 150 µg, 16/30 (53%); 300 µg, 14/30 (46%); D2: 150 µg, 1/30 (3%); 300 µg, 0/30; $B6D2F_1$: 150 µg, 8/57 (14%); 300 µg, 33/60 (55%)	+	No histology; control data not reported; no statistics	Kouri *et al.* (1983)
Mouse, C3H/HeJ, C57BL/6J, AKR/J, DBA/J2	M	30, 10 controls	NS (trioctanoin)	0, 150 µg in 50 µL, 1×	9 mo	FibroS: C3H/HeJ, 24/30 (80%) vs 0/10 controls; C57BL/6J, 16/30 (53%) vs 0/10 controls; AKR/J, 0/30 vs 0/10 controls; DBA/J2, 1/30 (3%) vs 0/10 controls	+	No statistics	Lubet *et al.* (1983)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mice, NMRI	F	50	>99% (tricaprylin)	0, 10, 11, 30, 86, 112 µg in 500 µL, 1×	112 wk	FibroS: 10 µg, 25/48 (52%); 11 µg, 16/47 (34%); 30 µg, 25/50 (50%); 86 µg, 31/49 (63%); 112 µg, 38/48 (79%) vs 3/49 (6%) and 1/50 (2%) solvent controls	+	No histology; no statistics	Platt *et al.* (1990)
Mouse, NMRI		40, 44 and 49 controls	>99% (aqueous solution [1% gelatine, 0.9% saline, 0.4% Tween 20])	0, 11, 111 µg in 50 µL, 1× on PND 2	40 wk	Pulmonary T: F: 11 µg, 6/13 (46%; 3.3 T/mouse); 111 µg, 16/17 (94%; 30.6 T/mouse) vs 1/19 (5%; 1.0 T/mouse) solvent controls; M: 11 µg, 6/22 (27%; 3.8 T/mouse); 111 µg, 19/21 (90%; 27.3 T/mouse) vs 1/14 (7%; 2.0 T/mouse) solvent control	+	No statistics	Platt *et al.* (1990)
Rat, NS	NS	2 series of 10, 20 controls	NS (lard)	2 mg in 1 mL, 1×/wk, then 6 mg in 3 mL at unspecified intervals	161–217 days	S: 1/10 (10%), 7/10 (70%) vs 0/20 solvent controls		Limited survival and reporting	Barry & Cook (1934)
Rat, albino	NS	40	Melting-point (lard)	8 mg, 1×/mo; 4×	1 year	11/18 (61%) (T)	+	No control or histology	Shear (1936)
Rat, Wistar	NS	5	NS (sesame oil)	5 mg in 500 µL, 1×/wk, 4, 5 or 8×	10 mo	2/5		Small numbers; no histology	Pollia (1941)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, NS	M, F	10	NS (olive oil)	0.1, 1 mg in 1 mL, 1×	0.1 mg, 21 mo; 1 mg, 15 mo	S: 0.1 mg, 3/9 (33%); 1 mg, 6/10 (60%)	+	No control	Roussy & Guérin (1942)
Rat, Sprague-Dawley	F	12	Pure (sesame oil:DMSO (9:1))	300 µg in 100 µL, 3×/wk, 20×	37 wk	S: 12/12 (100%) vs 0/12 solvent controls	+	No statistics	Flesher *et al.* (2002)
Dibenz[*a,j*]anthracene									
Mouse, Swiss	F	25	>99% (olive oil)	400 µg in 200 µL, 1×	Life	S: 3/21 (14%); vs 0/16	±	No statistics	Lijinsky *et al*. (1970)
Dibenzo[*h,rst*]pentaphene									
Mouse XVII/nc/Z	M, F	8 M, 19 F	NS (olive oil)	600 µg in 200 µL, 1×/mo, 3 mo	320 days	S: M, 3/8 (37%; S); F, 6/19 (32%)	+	No control; no statistics	Lacassagne *et al*. (1964)
Dibenzo[*a,e*]pyrene									
Mouse, XVII	M, F	12 or 21 M, 15 or 14 F	NS (olive oil)	600 µg in 200 µL, 1×; or 1×/mo, 3 mo	NS	600 µg: M, 10/12 (83%; S); F, 10/15 (67%; S); 1.8 mg: M, 18/21 (86%; S); F, 14/14 (100%)	+	No control; no statistics	Lacassagne *et al*. (1963)
Dibenzo[*a,h*]pyrene									
Mouse, white	NS	20	NS (olive oil)	6 mg, 1×	9.5 mo	17/20 (85%) papillomas at 3.5 mo; none survived 9.5 mo		No control; no statistics	Kleinenberg (1938)
Mouse, XVII	M, F	35 M, 10 F	NS (olive oil)	600 µg in 200 µL; 1×/mo, 3 mo	NS	M, 34/35 (97%; S); F, 1/10 (10%; S)		No control; no statistics	Lacassagne *et al.* (1958)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*i*]pyrene									
Mouse, XVII	NS	11	NS (peanut oil)	600 µg (volume NS), 1×/mo, 3 mo	Up to 135 days	11/11 (100%; S); none survived 135 days	+	No control; low survival; no statistics	Lacassagne *et al*. (1957)
Mouse, XVII	M, F	17 M, 18 F	NS (olive oil)	600 µg in 200 µL, 1×/mo, 3 mo	NS	M, 17/17 (100%; S); F, 16/18 (89%; S)	+	No control; limited duration; no statistics	Lacassagne *et al*. (1958)
Mouse, hybrid XVII × C57BL	M, F	8 M, 8 F	NS (propylene glycol)	2 mg in 200 µL, 1×	NS	M, 8/8 (100%; S); F, 8/8 (100%; S)	+	No control; limited duration; no statistics	Waravdekar & Ranadive (1958)
Mouse, albino	M	12	Recrystallized (paraffin oil)	2 mg in 100 µL, 4 mg in 200 µL, 1×	Up to 4.5 mo	2 mg, 6/6 (100%; S); 4 mg, 6/6 (100%; S)	+	No control; limited duration; no statistics	Schoental (1959)
Mouse, C57Br/cd	M	10–12	NS (peanut oil, tricaprylin or tricaprylin/ cholesterol (% NS)	0.01–600 µg (volume NS), 1×	66 wk	<1 µg, 0%; 1 µg, 9%; 2 µg, 33%; 6.25 µg, 50%; 12.5 µg, 64%; 25 µg, 92%; ≥50 µg, 100%	+	No control; T were fibroS; leiyomioS; no statistics	Homburger & Tregier (1960)
Mouse, C57Br/cd	M	8850	NS (peanut oil)	500 µg (volume NS), 1×	>22 wk	'Nearly' 100% (fibroS or leiyomioS)	+	No control; no statistics	Homburger & Tregier (1960)
Mouse, C57BL/6	M	438	NS (peanut oil)	500 µg (volume NS), 1×	>22 wk	100% fibroS	+	No control; no statistics	Homburger *et al*. (1962)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BALB/c	M, F	8 M, 22 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 8/8 (100%; fibroS); F, 22/22 (100%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, C3H/An	M, F	9 M, 22 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 9/9 (100%; fibroS); F, 20/22 (91%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, I strain	M, F	10 M, 20 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 5/10 (50%; fibroS); F, 17/20 (85%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, (BALB/c × C3H)F_1	M, F	10 M, 25 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 10/10 (100%; fibroS); F, 25/25 (100%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, (C3H × I)F_1	M, F	9 M, 18 F	NS (sesame oil)	50, 100 µg, 1×	8 mo	M, 6/9 (67%; fibroS); F, 15/18 (83%; fibroS) (both doses combined)	+	No control; limited reporting; no statistics	Old *et al*. (1962)
Mouse, Swiss	M, F	8 M, 5 F	NS (tricaprylin)	2 mg in 200 µL, 1×	8 mo	M, 8/8 (100%; fibroS); F, 5/5 (100%; fibroS)	+	No control; no statistics	Pai & Ranadive (1965)
Mouse, XVII × C57BL	M, F	8 M, 8 F,	NS (tricaprylin)	2 mg in 200 µL, 1×	10 mo	M, 8/8 (100%; fibroS); F, 8/8 (100%; fibroS)	+	No control; no statistics	Pai & Ranadive (1965)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss ICR/Ha	F	138	NS (tricaprylin)	100 mg in 200 µL, 1×	38 wk	125/138 (91%; fibroS); 2/138 (1%; local C); 1/138 (1%; lymphoma); 6/138 (4%; solitary pulmonary A); 2/138 (1%; multiple pulmonary A)	+	No control; the dose was probably 100 µg; no statistics	Epstein *et al*. (1967)
Mouse, C57BL/6	M	20–80	NS (peanut oil)	500 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 1–8 wk later	26 wk	No transplant: M, 95% (fibroS); F, 90% (fibroS); with transplant: decreased latency times for the same incidences in both M and F	+	No control; no statistics	Homburger & Treger (1967)
Mouse, C57BL/6J	M	40	NS (tricaprylin)	25 µg (volume NS), 1×, followed by transplantation of injection sites to secondary hosts 7 wk later	30 wk	No transplant, 90% fibroS; with transplant, 90% fibroS at 18 wk	+	No control; no statistics	Homburger & Baker (1969)
Mouse, C57BL/6J	M	40–50	NS (tricaprylin)	25 µg in 100 µL, 1×; 25 µg in 100 µL, 1×, followed by transplantation of injection sites to secondary hosts 6 wk later	34 wk	No transplant: 50% fibroS at 19.5 wk; 92% fibroS at 34 wk; with transplant: 50% fibroS at 14.5 wk; 100% fibroS at 31 wk vs 0% in transplanted vehicle controls	+	No statistics	Homburger & Treger (1970)
Mouse, NS	NS	50, 25 controls	NS (tricaprylin)	100 µg (volume NS), 1×	75 wk	40/50 (80%; local S) vs 0/25 solvent controls	+	No histology; no statistics	Sardella *et al*. (1981)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian	M	6–10	NS (tricaprylin)	250–2000 µg in 400 µL, 1×	>17 wk	250 µg, 5/9 (56%; fibroS); 500 µg, 9/10 (90%; fibroS); 1000 µg, 10/10 (100%; fibro S); 2000 µg, 6/6 (100%; fibroS) vs 0 solvent controls	+	Limited reporting; no statistics	Wodinsky *et al.* (1964)
Hamster, Syrian	F	8–20	NS (tricaprylin)	250–2000 µg in 200 µL, 2500 µg in 400 µL, 1×	40 wk	250 µg, 85% fibroS; 500 µg, 87% fibroS; 1000 µg; 100% fibroS; 1500 µg, 100% fibroS; 2000 µg, 100% fibroS; 2500 µg, 95% fibroS	+	No control; no statistics	Wodinsky *et al.* (1965)
Rabbit, NS	NS	3	Recrystallized [arachis or paraffin oil]	100–300 µg in 100–300 µL, 5× [periodicity NS], 3 mo	7 mo	1/3 (33%) C (developed after biopsy)	±	No control; limited reporting; small number; no statistics	Schoental (1959)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, XVII nc/ZE	M, F	19 M, 17 F, 500 controls	NS (olive oil)	600 µg in 200 µL, 1×/mo, 2×; 1 additional 600 µg injection 2 mo later [only to mice without strong fibrous reaction at injection site; sex, no. NS]	M, 195 days; F, 217 days	M, 12/12 (100%; S; av. latency, 130 days); F, 12/12 (100%; S av. latency, 113 days) vs 'inactive' solvent	+	No histology; no statistics	Lacassagne *et al*. (1968)
1,2-Dihydroacenanthrylene									
Mouse, NS	F	9	Crystalline (NS)	5 mg (volume NS), 1×	20 mo	0/9	–	No control; limited duration; limited reporting; no statistics	Shear (1938)
Phenanthrene									
Mouse, C57BL	M, F	40–50	NS (tricaprylin)	5 mg, 1×	22–28 mo	0/27 surviving >4 mo	–	No control	Steiner (1955)
Mouse, 'stock albino'	M, F	10	NS (3% aqueous gelatine)	0, 300 µg on days 0, 2, 6, 8, followed by 250 µL croton oil, 1×/wk, 20 wk	24 wk	3/17 (18%) skin P vs 2/20 (10%) skin P croton oil control	–		Roe (1962)

Table 3.3 (contd)

Chemical species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, 'stock albino'	M, F	57, 45 controls	'Highest purity' (1% aqueous gelatine)	0, 40 µg in 20 µL, 1× neonatal treatment	62 wk	3/49 (6%) lung A vs 8/34 (24%) lung A solvent control	–	No histology	Grant & Roe (1963); Roe & Waters (1967)
Picene									
Mouse, NMRI	F	50	>99% (tricaprylin)	10, 11, 30, 86, 111 µg in 500 µL, 1×	112 wk	10 µg, 9/50 (18%) fibroS; 11 µg, 12/46 (26%); 30 µg, 17/49 (35%); 86 µg, 31/49 (63%); 111 µg, 29/50 (58%) vs 3/49 (6%), 1/50 (2%) solvent control	+	No histology; no statistics	Platt *et al*. (1990)
Mouse, NMRI	M, F	45 M, 50 F, 49 controls	>99% (aqueous solution (1% gelatine, 0.9% saline, 0.4% Tween 20))	0, 11, 111 µg [40, 400 nmol] in 50 µL, 1× on PND 2	40 wk	**Pulmonary T (T/mouse)**: F: 11 µg, 4/16 (25%; 2.8); 111 µg, 8/23 (35%; 2.1) vs 1/19 (5%; 1.0) solvent controls; M: 11 µg, 1/22 (5%; 1.0); 111 µg, 2/13 (15%; 1.5) vs 2/14 (14%; 2.0) solvent controls	+	No statistics	Platt *et al*. (1990)
Rat, Sprague-Dawley	F	12	Pure (sesame oil:DMSO (9:1))	0, 300 µg in 100 µL, 3×/wk, 20 ×	37 wk	No skin T found	–		Flesher *et al*. (2002)

A, adenoma; AdC, adenocarcinoma; av., average; C, carcinoma; DMSO, dimethylsulfoxide; F, female; M, male; mo, months; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TBA, tumour-bearing animal; vs, versus; wk, week

[a]–, negative; +, positive; ±, equivocal

Table 3.4. Carcinogenicity studies of intrapulmonary administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene									
Rat, Osborne-Mendel	F	35	99.4% (beeswax:tricaprylin (1:1))	160 of 830 µg in 50 µL, 1×	Life; av. 88 wk for high-dose group, 118 wk for untreated group	SCC: 1/35 (3%) low-dose, 19/35 (54%) high-dose vs 0/35 untreated, 0/35 vehicle controls	+		Deutsch-Wenzel *et al.* (1983)
Anthracene									
Rat, Osborne-Mendel	F	60, 108 controls	'Recrystallized' (beeswax:tricaprylin (1:1))	500 µg in 50 µL, 1×	>2 years	0/60 (approx. 50% killed after 1 year) vs 0/108 solvent control	–	No statistics	Stanton *et al.* (1972)
Benzo[*b*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.5% (beeswax/trioctanoin)	0, 0.1, 0.3, 1.0 mg, 1×	Lifetime	Lung SCC/S: 0%, 2.9%, 8.6%, 37.1%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*j*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.9% (beeswax/trioctanoin)	0, 0.2, 1.0, 5.0 mg, 1×	Lifetime	Lung SCC: 0%, 2.9%, 8.6%, 51.4%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*k*]fluoranthene									
Rat, Osborne-Mendel	F	35	>99.5% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC: 0%, 0%, 9.7%, 44.4%	+		Deutsch-Wenzel *et al.* (1983)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*ghi*]perylene									
Rat, Osborne-Mendel	F	35	>98.5% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC/S: 0%, 0%, 2.9%, 11.8%	+		Deutsch-Wenzel *et al.* (1983)
Benzo[*a*]pyrene									
Rat, OM	F	35	99.1% (1:1 mixture of beeswax and trioctanoin)	0 (untreated), 0 (vehicle control), 0.1, 0.3, 1.0 mg/animal, 1×	64 (high-dose group)–133 wk (untreated controls)	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)	+		Deutsch-Wenzel *et al.* (1983)
Rats, F344/NSlc	M	NS	NS (beeswax/tricaprylin mixture (1:1))	0, 0.03, 0.1, 0.3, 1.0 mg/animal, 1×	104 wk	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)	+		Iwagawa *et al.* (1989)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Osborne-Mendel	F	35	99.6% (beeswax/tri-octanoin mixture of varying composition)	0 (untreated), 0 (vehicle control), 30, 100, 300 µg/animal, 1×	134 wk (low-dose group)–140 wk (vehicle controls)	Lung T: [0/35] (0%), [0/35] (0%), [3/35] (8.6%; 3 SCC), [11/35] (31.4%; 11 SCC), [77.1%; 27/35] (27 SCC).	+	SCC predominantly keratinized	Wenzel-Hartung *et al.* (1990)
Rat, F344/DuCrj	M	9–10	NS (beeswax/trica-prylin mixture (1:1))	0, 50, 100, 200 µg/animal, 1×	100 wk	Lung T: 0/19, 0/10, 3/10 (30%; 2 SCC, 1 AdSC), 4/9 (44.4%; 3 SCC, 1 undifferen-tiated T)	+		Horikawa *et al.* (1991)
Benzo[*e*]pyrene									
Rat, Osborne-Mendel	F	35	>99.7% (beeswax/trioctanoin)	0, 0.2, 1.0, 5.0 mg	Lifetime	Lung SCCS: 0%, 0%, 3.3%, 2.9%	±		Deutsch-Wenzel *et al.* (1983)
Chrysene									
Rat, Osborne-Mendel	F	35	>99.6% (beeswax/trioctanoin)	0, 1, 3 mg	Lifetime	Lung C: 0/35 (0%), 5/35 (14.3%), 10/35 (28.6%)	+		Wenzel-Hartung *et al.* (1990)
Dibenz[*a,h*]anthracene									
Mice, Street	M, F	80	NS (paraffin)	20 µg in 10 µL, 1×	<27 mo	4/16 (25%) pulmonary T vs 1/41 (2%) controls	+	Controls not solvent-treated; no statistics	Rask-Nielsen (1950)

Table 3.4 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Osborne/Mendel	F	35	99.3% (beeswax, tricaprylin)	100 μg, 1×	123 wk	20/35 (57%) vs 0/35 solvent control	+	No statistics	Wenzel-Hartung *et al.* (1990)
Indeno[1,2,3-*cd*]pyrene									
Rat, Osborne-Mendel	F	35	>99.4% (beeswax/trioctanoin)	0, 0.16, 0.83, 4.15 mg	Lifetime	Lung SCC S: 0%, 11.4% (4/35), 22.9% (8/35), 60.0% (21/35)	+		Deutsch-Wenzel *et al.* (1983)
Phenanthrene									
Rat, Osborne/Mendel	F	35	99.9% (beeswax:tricaprylin)	0, 1, 3, 10 mg, 1×	135 wk	1 mg, 0/35 lung C; 3 mg, 0/35; 10 mg, 1/35(3%) vs 0/35 solvent control	–	No statistics	Wenzel-Hartung *et al.* (1990)

AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycylic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus; wk, week

[a] –, negative; +, positive; ±, equivocal

Intramamillary administration (see also Table 3.5)

Rat

A group of 20 female Sprague-Dawley rats [weight unspecified], 8 weeks of age, received a single intramamillary injection of 4 μmol [1.1 mg]/gland anthanthrene (purity >99% by HPLC; total dose, 32 μmol [8.8 mg]) dissolved in 100 μL trioctanoin. One control group of 21 rats was treated with 100 μL solvent and another control group of 20 rats remained untreated. The animals were monitored weekly by palpation for tumour development and were killed when tumours were ≥2 cm in diameter; all the remaining animals were killed and necropsied at 40 weeks. Survival rates (mean ± standard deviation (SD)) were 38 ± 0, 37 ± 4 and 40 ± 0 weeks in the anthanthrene-treated, untreated and vehicle control groups, respectively. Tumour latencies were 36 ± 0 and 25 ± 13 weeks in the anthanthrene-treated and untreated groups, respectively. At the end of the study, 1/20 rats in the anthanthrene-treated group and 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma in the anthanthrene-treated rat; one adenofibroma and one adenocarcinoma in the untreated rats). No tumours were observed in the vehicle control group (Cavalieri *et al.*, 1989).

Anthracene

Previous evaluation

Anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which anthracene was fed to rats, administered dermally to mice, administered by subcutaneous, intraperitoneal or pulmonary injection to rats and implanted into the brain or eyes of rabbits. These studies are summarized in Tables 3.1–3.4 and 3.6–3.8. On the basis of the available data, the Working Group concluded that there was *inadequate evidence* that anthracene was carcinogenic to experimental animals (IARC, 1987). Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 20 male C3H/HeJ mice, 6–8 weeks of age, was treated twice weekly with dermal applications of 50 μL of a 0.1% toluene solution of anthracene (99.5% pure by HPLC; 50 μg per treatment) for 104 weeks. A control group of 50 male mice was treated with toluene alone. Lesions (≥1 mm^3) that persisted for at least 1 week were diagnosed as papillomas. After 104 weeks, no benign or malignant skin tumours had developed in the surviving 14 experimental or 39 control mice. Gross examination of internal organs indicated no tumours in either the experimental or control groups (Warshawsky *et al.*, 1993).

Table 3.5. Carcinogenicity studies of intramammary or intramamillary administration of various PAHs in female experimental animals

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthanthrene								
Rat, Sprague-Dawley	20	>99% (trioctanoin)	1.1 mg/gland in 100 µL, 1×; 8 glands total	40 wk	1/20 (5%) mammary epithelial T (AdF) vs 0/21 vehicle controls, 2/20 (10%) (1 AdC, 1 AdF) untreated controls	–		Cavalieri *et al.* (1989)
Benz[*a*]anthracene								
Rat, Sprague-Dawley	20	>99% (none)	4 [913 µg] or 16 µmol [3.65 mg] applied as powder to 1 gland, 1×; untreated contralateral gland used as negative control	20 wk	0/20 at both doses	–		Cavalieri *et al.* (1988a)
Benzo[*a*]pyrene								
Rat, Sprague-Dawley	20	>99% (no vehicle)	0 (untreated contralateral mammary glands), 4 [1 mg], 16 µmol [4.2 mg], 1×	20 wk	Mammary gland T: [0/20] (0%), [10/20] (50%; 6 AdC, 4 fibroS; multiplicity: AdC, 6/6; fibroS 4/4), [16/20] (80%; 8 AdC, 2 fibroA, 10 fibroS; multiplicity: AdC, 8/8; fibroA, 2/2; fibroS, 10/10)	+		Cavalieri *et al.* (1988a)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	20	>99% (trioctanoin)	0, 4 μmol [1 mg]/mammary gland (2nd, 3rd, 4th and 5th mammary gland on both sides injected), 1×	45 wk	Epithelial mammary T: [3/20] (15%; 3 fibroA), [14/20] (70%; 13 AdC, 3 fibroA) Multiplicity: controls, 3/3 [1]; dosed 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])	+		Cavalieri *et al.* (1988a,b)
Rat, Sprague-Dawley	20	>99% (trioctanoin)	0, 0.25, 1 μmol [66, 264 μg]/mammary gland (the 2nd, 3rd, 4th and 5th on both sides), 1×	24 wk	Epithelial mammary gland T: 1/18 (6%; 1 fibroA; multiplicity, 1/1), 1/20 (5%; 1 AdC; multiplicity 1/1), 0/20 (0%) Mesenchymal (mammary) T: 0/18 (0%), 6/20 (30%; 6 fibroS; multiplicity, 7/6 (1.2)), 8/20 (40%; 8 fibroS; multiplicity, 10/8 (1.3)) Skin T: [0/18] (0%), (0/20, 0%), (1/20, 5%); 1 SCC; multiplicity 1/1	+	Statistics not specified	Cavalieri *et al.* (1991)
Dibenz[*a*,*h*]anthracene								
Rat, Sprague-Dawley	20	>99% (fine powder)	1.1, 4.5 mg at 50 day of age, 1×	20 wk	0/20 vs 0/20 control	–	Control was untreated contralateral breast; no statistics	Cavalieri *et al.* (1988a)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*e*]pyrene								
Rat, Sprague-Dawley	19, 21 controls	>99% (tricaprylin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	40 wk	1/19 (5%; mammary epithelial T (AdF) vs 0/21 solvent controls	–		Cavalieri *et al.* (1989)
Dibenzo[*a*,*h*]pyrene								
Rat, Sprague-Dawley	20, 21 controls	>99% (tricaprylin)	1.2 µg/gland in 100 µL, 1×; 8 glands total	40 wk	19/20 (95%; fibroS) vs 0/20 solvent controls; 4/20 (20%; mammary gland AdF, AdC) vs 0/21 solvent controls	+		Cavalieri *et al.* (1989)
Dibenzo[*a*,*i*]pyrene								
Rat, Sprague-Dawley	20, 21 controls	>99% (tricaprylin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	40 wk	18/19 (95%; fibroS) vs 0/21 solvent controls; 11/19 (58%; mammary AdC), 1/19 (5%; mammary AdF) vs 0/21 solvent controls	+		Cavalieri *et al*. (1989)

Table 3.5 (contd)

Chemical, species and strain	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene								
Rat, Sprague-Dawley	9 [from 19; 10 died within 9 wk], 21 controls	>99% (trioctanoin)	1.2 mg/gland in 100 µL, 1×; 8 glands total	15 wk; controls, 40 wk	100% [8/9 mammary AdC, 0/9 mammary AdF vs 2/20 solvent controls; 7/9 fibroS vs 0/20 solvent controls; 8/9 skin SCC vs 0/20 solvent controls]	+		Cavalieri *et al.* (1989)
Rat, Sprague-Dawley	20	>99% (trioctanoin)	75.6, 302 µg/gland in 50 µL, 1×; 8 glands total	24 wk	75.6 µg, 20/20 (100%) mammary AdC, 0/20 mammary AdF, 4/20 (20%) fibroS, 1/20 (5%) SCC; 302 µg, 19/19 (100%), 0/19, 14/19 (74%), 1/19 (5%) vs 0/18, 1/18 (6%), 0/18, 0/18 solvent controls	+		Cavalieri *et al.* (1991)

A, adenoma; AdC, adenocarcinoma; AdF, adenofibroma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus, wk, week

[a] –, negative; +, positive

Table 3.6. Carcinogenicity studies of oral administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Rat, BDI and BDIII	NS	28	Pure (oil)	5 mg/day, then 15 mg/day, 6 days/wk, 91 wk; total dose, 4.5 g/animal	Lifespan; mean survival time, 700 days	2/28 (7%) (1 liver S, 1 uterine AdC)	–	No controls; both tumours considered to be unrelated to treatment	Druckrey & Schmähl (1955); Schmähl (1955)
Benz[*a*]anthracene									
Mouse, C57BL	NS	8–19; 6–16 controls	NS (heavy mineral oil)	500 µg in 10 µL, 1, 8 or 16× at 3–7-day intervals	16 months	Forestomach P: 1×, 0/13; 8×, 1/19 (5%); 16×, 1/8 (12%) vs 0/12, 0/16, 0/6 solvent controls	±		Bock & King (1959)
Mouse, B6AF1/J	M	20 or 40	99% (methocel-aerosol)	3% suspension in 50 µL, 3×/wk, 15×	340–444 or 547–600 days	340–444 days: 18/39 (46%) H, 37/39 (95%) pulmonary A, 1/39 (3%) reticulum-cell neoplasm, 2/39 (5%) forestomach P vs 0/39, 4/39 (10%), 0/39, 0/39 solvent controls 547–500 days: 20/20 (100%), 19/20 (95%), 0/20, 0/20 vs 2/20 (10%), 7/20 (35%), 0/0, 0/0, 2/20 (10%) lymphocytic neoplasms in solvent controls	+		Klein (1963)
Rat, Sprague-Dawley	F	18	NS (sesame oil)	200 mg	NS	0/18	–		Huggins & Yang (1962)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*c*]fluorene									
Mouse, A/J	F	30	≥98% (mixed with diet)	27, 397 µmol/kg diet; 260 days	260 days	Lung A: 27 µmol/kg, [13/28] (46%; 0.57 ± 0.13 T/mouse; mean ± SE); 397 µmol/kg, [29/29] (100%; 46.0 ± 2.8) (p <0.001) vs [7/29] (24%; 0.31 ± 0.11) in basal diet controls	+		Weyand *et al.* (2004)
Benzo[*a*]pyrene									
Mouse, A/J	F	15	NS (cottonseed oil or corn oil [not clearly specified])	2 mg/animal or 2 mg/animal preceded by oral intubation of 0.2 mL cottonseed oil (96 and 48 h before) 3× with 18-day intervals	26 wk	Forestomach T: 15/15 (100%; 12 P, 3 C; 2.9 ± 0.5 T/animal), 14/14 (100%; 14 P; 3.7 ± 0.4 P/animal) Pulmonary A: 15/15 (100%; 24 ± 1.8 A/animal), 14/14 (100%; 18 ± 2.0 A/animal)	+	No control	Sparnins *et al.* (1986)
Mouse, A/J	F	15–16	>98% (cottonseed oil)	0, 2, 3 mg/animal on day 1, 4, 7 of 1-wk period at age 9 wk	22 wk	Forestomach P: 4.1 ± 0.8, 8.7 ± 1.1 and 9.9 ± 0.9 P/animal Pulmonary A: 28.3 ± 3.0, 64.1 ± 5.8 and 52.3 ± 4.3 A/animal	+	No control; limited data on tumour incidence	Estensen & Wattenberg (1993)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, A/J	F	30	NS (gel diet)	0, 16, 98 ppm (total dose; 0, 11, 67 mg/animal)	260 days	Lung T: 4/21 (19%; 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)	+		Weyand *et al.* (1995)
Mouse B6C3F1	F	48	98.5% (acetone)	0 (untreated), 0 (acetone control diet), 0.0005 % (5 ppm), 0.0025% (25 ppm), 0.01% (100 ppm) in the diet	2 years	Liver (A): 4/21 (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)	+		Culp *et al.* (1998)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino, inbred	F	10	Highest purity grade (corn oil) (drinking-water contained 0.005% ethanol)	0, 1 mg/animal, 2×/wk, 4 wk	27 wk	0, 10/10 (100%) (forestomach P; multiplicity, 7.11 ± 1.05)	+		Badary *et al.* (1999)
Mouse, A/J	F	11 or 18	>99% (cottonseed oil)	100, 125, 150 [dose not clearly specified] mg/kg bw on day 1, 3, 7 of a 1-wk period at age 8–14 wk	34 or 36 wk	Lung T/cm^2 lung tissue: 100 mg/kg bw, 4.2 ± 2.2 A, 2.9 ± 2.7 A with progression* combined with C; 100 [or 125 or 150] mg/kg bw, 6.3 ± 5.6 A, 2.2 ± 1.6 A with progression*, 2.1 ± 1.9 C *A with clones of 10 cells or more with hyperchromatic nuclei and nuclear size greater than that of nuclei in A cells	+	No control; lack of data on dose and lung tumour incidence	Estensen *et al.* (2004)
Mouse, Eμ-*pim*-1 transgenic, wild-type counterpart	M	20–31	NS (soya oil)	0, 4.3, 13, 39 mg/kg bw, by gavage 3×/wk, 13 wk (0, 4.3 mg/kg bw only administered to Eμ-*pim*-1 transgenic mice)	287 days	**Eμ-*pim*-1 transgenic mice** Lymphoma: 2/20 (10%), 4/30 (13%), 15/31* (48%), 24/29** (83%) *(p <0.005), **(p <0.0001) Forestomach T (P + C combined): 0/20, 4/30 (13%), 8/31 (26%), 6/29 (21%) **Wild-type mice** Lymphoma: 2/30 (7%), 6/20 (30%) Forestomach T (P + C combined): 5/30 (17%), 12/20 (60%)	+	No control for wild-type mice; statistics NS	Kroese *et al.* (1997)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, *XPA*$^{+/+}$, *XPA*$^{+/-}$, *XPA*$^{-/-}$	NS	17–34	NS (soya oil)	0, 4.3, 13 mg/kg bw/animal by gavage, 3×/wk, 13 wk	273 days	*XPA*$^{+/+}$: 1/26 (4%; 1 lymphoma), 1/14 ([7%] 1 lymphoma), 5/17 (29%; 2 lymphoma, 3 other types of T); *XPA*$^{+/-}$: 0/34, 0/28, 6/28 (21%; 3 lymphoma, 3 other T); *XPA*$^{-/-}$: 2/13 (15%; 2 hepatocellular A), 2/11 (18%; 2 lymphoma), 5/9 (56%; 4 lymphoma, 1 other T)	+	Limited reporting of tumours other than lymphomas; tumour response in high-dose *XPA*$^{-/-}$ mice significantly stronger than that in high-dose *XPA*$^{+/-}$ or *XPA*$^{+/+}$ mice (p <0.005)	de Vries *et al.* (1997)
Mouse, Muta^{TM} (λ*lacZ* transgenic)	M	8–12	NS (corn oil)	0, 75, 125 mg/kg bw/day, 5 consecutive days	41 wk	**Forestomach** SCC: 0/8, 2/11 (18%), 2/11 (18%) P: 0/8, 4/11 (36%), 5/11 (45%) (p ≤0.05) Splenic malignant lymphoma: 0/8, 0/11, 2/11 (18%)	+		Hakura *et al.* (1998)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, wild-type (C57BL/6), *XPA*$^{-/-}$, *p53*$^{+/-}$, *XPA*$^{-/-}$/*p53*$^{+/-}$	M, F	5–31	NS (soya oil)	0, 13 mg/kg bw/animal, by gavage, 3×/wk, 13 wk	52 wk	Wild type: 0/11, 6/17 (35%; total no. of T, 7; 3 lymphomas, 1 bronchiolo-alveolar A, 1 histiocytic S, 1 hepatocellular C, 1 intestinal A) *XPA*$^{-/-}$: 0/5, 5/7 (71%; total no. of T, 9; 3 lymphoma, 2 fore-stomach P, 1 bronchiolo-alveolar A, 1 histiocytic S, 1 ovary T, 1 intestinal A) *p53*$^{+/-}$: 3/18 (17%; 1 lymphoma, 1 osteosarcoma, 1 mammary AdC), 22/31 (71%; total no. of T, 33; 6 lymphoma, 9 forestomach P, 4 bronchiolo-alveolar A, 4 fibroS, 3 histiocytic S, 2 osteoS, 2 mammary AdC, 1 skin SCC, 1 hepatocellular C, 1 intestinal A) *XPA*$^{-/-}$/*p53*$^{+/-}$: 0/8, 15/15 (100%; total no. of T, 25; 8 lymphoma, 8 forestomach tumours, 6 histiocytic S, 1 rhabdomyoS, 1 haemangioma, 1 leukaemia)	+	Treated double transgenic *XPA*$^{-/-}$/*p53*$^{+/-}$ mice developed tumours much earlier and in higher incidences than their similarly treated single transgenic *XPA*$^{-/-}$ or *p53*$^{+/-}$ counterparts; statistics NS	van Oostrom *et al.* (1999)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, $CSB^{-/-}$ or wild-type ($CSP^{+/-}$ /$CSB^{+/+}$)	M, F	6–18 M; 6–13 F	NS (soya oil)	0, 13 mg/kg bw, 3×/wk, 13 wk	52 wk	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) *($p = 0.0023$) **($p = 0.0017$)	+		Wijnhoven *et al.* (2000)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, wild-type (C57BL/6), *Xpa*$^{-/-}$, *Xpa*$^{-/-}$/*p53*$^{+/-}$	M, F	5 or 10 M, 5 or 10 F of each genotype	NS	0, 75 ppm in the diet, 13 wk	9 months	Wild-type (M + F combined): 1/10 (10%; 1 intestinal A), 2/20 (10%; 2 forestomach P) *Xpa*$^{-/-}$ (M + F combined): 1/10 (10%; 1 bronchiolo-alveolar A), 16/20 (80%; 15 forestomach P, 1 SCC) *Xpa*$^{-/-}$/*p53*$^{+/-}$ (M + F combined): 2/10 (20%; 1 forestomach P, 1 mammary AdC), 13/15 (87%; total no. of T, 21; 3 oesophageal P, 11 forestomach P, 1 SCC, 2 haemangioS, 1 intestinal A, 1 abdominal osteoS, 1 widespread C, 1 abdominal S)	+	Treated cancer-prone nucleotide excision repair-deficient mice (*Xpa*$^{-/-}$ or *Xpa*$^{-/-}$/*p53*$^{+/-}$ mice) showed a much stronger tumour response than their treated wild-type counterparts (C57Bl/6 mice); no statistics	Hoogervorst *et al.* (2003)
Rat, Sprague-Dawley	M, F	32 M, 32 F	Highly pure (caffeine solution)	0, 0.15 mg/kg bw, 1×/9 days (6 mg/kg bw/year), 0.15 mg/kg bw, 5×/wk (39 mg/kg bw/year) for life in diet	Lifespan median survival times, 128–131 wk	T incidence (M + F combined): 3/64 (47%; 2 forestomach P, 1 oesophageal P), 3/64 (47%; 1 forestomach P, 1 oesophageal P, 1 laryngeal P), 10/64 (16%; 9 forestomach P (p <0.1), 1 oesophageal P)	+		Brune *et al.* (1981)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Crl:CD(SD)BR	F	30	99% (trioctanoin)	0, 50 µmol/ animal, 1×/wk, 8 wk by gavage	49 wk	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**) No. 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)	+		El-Bayoumy *et al.* (1995)
Dibenz[*a*,*h*]anthracene									
Mouse, C57 strain A BL, C3H, DBA,	M, F	10 or 20	Melting-point (olive oil emulsion in drinking-water)	500–800 µg/day, 156–303 days	156–303 days	C57 BL, 2/20 (10%; small intestine AdC); C3H, 6/20 (30%; small intestine AdC), 15/20 (75%; pulmonary T); DBA, 3/20 (15%; small intestine AdC), 15/20 (75%; pulmonary T); A: 7/30 (23%; small intestine AdC), 22/30 (73%; pulmonary T)	+	No. of controls NS; only observation in controls: pulmonary T in strain A mice; no statistics	Lorenz & Stewart (1947)
Mouse, A back-cross	M	10	Melting-point (mineral oil/aerosol emulsion)	400 µg/day, 406 days	406 days	2 forestomach SCC, 11 forestomach P		Small no. of animals; controls not treated with identical vehicle; no statistics	Lorenz & Stewart (1948)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss	M	42	NS (polyethylene glycol-400)	1.5 mg, 1× by gavage	30 wk	2/42 (5%) forestomach P	±	No control	Berenblum & Haran (1955)
Mouse, BALB/c	F	44, 30 controls	NS (almond oil)	500 µg, 2×/wk, 15 wk	60 wk	Virgin, 1/20 (5%; mammary gland T); pseudo-pregnant, 13/24 (54%; mammary gland T) vs untreated control pseudo-pregnant, 2/30 (7%; mammary gland T)	+	No solvent-treated control; no statistics	Biancifiori & Caschera (1962)
Mouse, DBA/2	M, F	21; 25 M, 10 F controls	Melting-point (olive oil emulsion in drinking-water)	800 µg/day, 8–9 months	8–9 months	M: 14/14 (100%; lung A), 14/14 (100%; alveologenic C), 10/14 (71%; haemangio-endotheliomas) versus 1/25 (4%; lung A), 0/25 (alveologenic C), 0/25 (haemangio-endothelioma) solvent controls; F: 13/13 (100%; lung A), 10/13 (77%; alveologenic C), 6/13 (46%; haemangio-endotheliomas), 12/13 (92%; mammary C) 0/10 (lung A), 0/10 (alveologenic C), 0/10 (haemangio-endotheliomas), 0/10 (mammary C) solvent controls	+	No statistics	Snell & Stewart (1962)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Fish, Japanese medaka (*Oryzias latipes*)	M, F	65, 75 controls	>97% (menhaden oil)	100 ppm in diet, 5×/wk, 28 days	10 months	17/65 (26%) vs 6/75 (8%) diet controls (p <0.05); hepatic neoplasia, 12/65 (18%) vs 0/75 (p <0.001); hepatocellular C, 7/65 vs 0/75 predominated	+		Reddy *et al*. (1999a)
Fish, Shasta rainbow trout (*Oncorhynchus mykiss*)	M, F	260	NS	200 ppm in diet, 4 wk, 500 ppm, 2 wk	200 ppm, 9 months; 500 ppm, 11 months	200 ppm, 36% liver T (hepatocellular C, 43% relative incidence; hepatocellular A, 44% relative incidence; 2.00 T/TBA), 48% stomach papillary A (3.80 T/TBA), 30% swimbladder papillary A (2.40 T/TBA); 500 ppm, 61% liver T (hepatocellular C, 64% relative incidence; hepatocellular A, 14% relative incidence; 2.58 T/TBA), 91% stomach papillary A (5.67 T/TBA), 53% swimbladder papillary A (2.25 T/TBA) vs 0% liver, stomach and swimbladder T in the diet control group	+		Reddy *et al*. (1999b)

Table 3.6 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Phenanthrene									
Rat, Sprague-Dawley	F	10	NS (sesame oil)	200 mg, 1×	60 days	0/10 vs 8/164 (5%) mammary gland T non-concurrent controls after 310 days	–	Small numbers; no statistics	Huggins & Yang (1962)

A, adenoma; AdC, adenocarcinoma; C, carcinoma; F, female; H, hepatoma; M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; SE, standard error; T, tumour, TBA, tumour-bearing animal; vs, versus; wk, week

[a]–, negative; +, positive; ±, equivocal

Table 3.7. Carcinogenicity studies of intraperitoneal administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Mouse, Swiss	M	5	NS (olive oil)	25 mg in ~750 µL, 1×	5 months	0/4 remaining mice vs 0/4 remaining solvent controls	–	Small no. of animals; limited reporting; no statistics	Shubik & Della Porta (1957)
Rat, BDI and BDIII	NS	10	Pure (oil)	20 mg, 1×/wk, 33 wk	Lifetime (>2 years)	1/10 (10%; spindle-cell S)	±	No control; no statistics	Schmähl (1955)
Benz[*j*]aceanthrylene									
Mouse, A/J	M	27	NS (tricaprylin)	20, 50, 100 mg/kg bw, 1×	8 months	Lung A: 20 mg/kg, 12/12 (100%; 60.3 ± 14.6 A/mouse); 50 mg/kg, 13/13 (100%; 140.6 ± 21.5 A/mouse); 100 mg/kg, 14/14 (100%; 97.6 ± 28.2 A/mouse) vs 19/34 (56%; 0.85 ± 0.9 A/mouse) solvent controls	+	Limited histopathology; no statistics	Mass *et al.* (1993); Nesnow *et al.* (1998a)
Benz[*a*]anthracene									
Mouse, BLU:Ha (ICR)	M, F	140, 100 controls	NS (DMSO)	2800 nmol [63.9 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	Pulmonary T: M, 10/47 (21%; 0.26 T/mouse) vs 7/43 (16%; 0.19 T/mouse) solvent controls; F, 4/38 (11%; 0.08 T/mouse) vs 2/24 (8%; 0.08 T/mouse) solvent controls	±		Wislocki *et al.* (1979)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BLU:Ha(ICR)	M, F	90, 80 controls	≥99% (DMSO)	2.8 μmol [639 μg] in 35 μL (total dose, given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26–32 wk	M: 17/27(63%; pulmonary A; 1.44 T/mouse), 2/27 (8%; hepatic T, mostly A or neoplastic nodules; 0.08 T/mouse) vs 1/28 (4%; 0.04 T/mouse), 4% (1/28; 0.04 T/mouse) in solvent controls; F: 14/22 (64%; pulmonary A; 2.00 T/ mouse), 0/22 (0%; hepatic T) vs 4/37 (11%; 0.11 T/ mouse 0/37 (0%) in solvent controls (p <0.05 for lung T incidence and number of T/mouse (M + F combined))	+		Levin *et al.* (1984)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	39 M; 32 F; 28 M controls, 31 F controls	>99% (DMSO)	2.8 μmol [639 μg] in 70 μL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	1 year	M: 31/39 (79%; liver T; 6/39 A, 25/39 C; 3.0 nodules/TBA) vs 2/28 (7%; 2/28 A, 0/28 C; 1.0/TBA) solvent controls (p <0.05 for C and combined liver T); 6/39 (15%; lung T; 5/39 A, 1/39 C) vs 1/28 (4%; 1/28 A, 0/28 C) in solvent controls; 3% (1/39, malignant lymphoma) vs 4% (1/28) solvent controls F: 0/32 (0%; liver T) vs 0/31 (0%) solvent controls; 6/32 (19%; lung T; 6/32 A, 0/32 C) vs 0/32(0%) solvent controls (p <0.025); 3/32 (9%; malignant lymphoma) vs 1/31 (3%) solvent controls	+		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*b*]fluoranthene									
Mouse, CD-1	M	15	>99% DMSO	0, 0.5 µmol (total dose; given as on 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/17 (0%), 2/15 (13.3%); liver A + H: 1/17 (5.6%), 8/15 (53.3%)	+		LaVoie *et al.* (1987)
	F	17		0, 0.5 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/18 (0%), 3/17 (17.6%); liver A: 0%, 0%	+		
Mouse, strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg, 1×, tricaprylin, negative control; 1000 mg/kg urethane, positive control	8 months	Lung A: 55%, 50%, 80% (p <0.05), 100% (p <0.05), 100% (p <0.05); 0.60 ± 0.58, 0.67 ± 0.75, 2.00 ± 1.82, 5.30 ± 3.21, 6.95 ± 03.52 T/animal	+		Nesnow *et al.* (1995); Ross *et al.* (1995); Mass *et al.* (1996); Nesnow *et al.* (1998a)
Benzo[*j*]fluoranthene									
Mouse, CD-1	M	40	>99% (DMSO)	0, 1.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 0/17 (0%), 11/21 (52.3%) (p <0.005); liver H: 1/17 (5.6%), 11/21 (52.3%) (p <0.005)	+		LaVoie *et al.* (1987)
	F	40				Lung A: 0/17 (0%), 4/18 (22.2%) (p <0.05) [p = 0.058, Fisher's exact test, one-tailed]; liver H: 0%, 0%	±		

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M	80	>99% (DMSO)	0, 1.10, 0.275, 1.10 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	Lung A: 18.2% (p >0.05), 21.6% (p >0.05), 50.0% (p <0.01), 100% (p <0.001); liver A: 9.1%, 27.0% (p >0.05), 38.2% (p <0.005), 56.0% (p <0.001); 0.18, 0.30, 0.65, 2.96 lung T/animal; 0.18, 0.35, 0.47, 2.20 liver T/animal	+		LaVoie *et al.* (1994a)
	F	80				Lung A: 21.2% (p >0.05), 20.7% (p >0.05), 43.8% (p >0.05), 92.1% (p <0.001); liver A: 0%, 6.8%, 0%, 2.6%; 0.24, 0.28, 0.53, 2.63 lung T/animal; 0, 0.07, 0, 0.03 liver T/animal	+		
Benzo[*k*]fluoranthene									
Mouse, CD-1	M, F	16 M, 18 F	>99% (DMSO)	0, 2.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M : liver T, 3/16 (18.8%; 2 A, 1 H; p <0.05); lung T, 1/16 (6.3%; 1 A; p <0.05) vs 1/17 (6%) solvent controls F : liver T, 0/18; lung T, 3/18 (16.7%; 3 A; p <0.05) vs 0/18 solvent controls	±		LaVoie *et al.* (1987)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*c*]fluorene									
Mouse, A/J	F	30	≥98% (tricaprylin)	1.75 mg (100 mg/kg bw) in 250 µL, 1×	260 days	Lung A: [26/28] (92%; 4.0 ± 0.53 T/mouse; p <0.01) vs 14/29 (48%; 0.6 ± 0.14) solvent controls	+		Weyand *et al.* (2004)
Benzo[*c*]phenanthrene									
Mouse, CD-1	M, F	75	>99% (DMSO)	0, 50, 150 nmol, 1×, (total dose; given as as 1/7, 2/7, 4/7 on PND 1, 8, 15)	33–39 wk	Lung T (T/mouse): M: 3% (0.06), 6% (0.06), 57% (1.6) F: 5% (0.07), 9% (0.09), 65% (2.6)	+		Levin *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Mouse, B6C3F1; C3A/JF1	M, F	30–63, 96–100 controls	NS (trioctanoin)	0, 75, 150 µg in 10 µL/g bw, 1× at 1, 15, 42 days of age	90 wk or lifespan	*B6C3F1 mice (all ages combined)* Liver T (A and hepatocelluar 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%) Stomach 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): 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)	+		Vesselinovitch *et al.* (1975a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						C3A/JF1 mice (all ages combined) Liver T (A and hepatocelluar 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%), 1438/148 (93%), 125/137 (91%); F, 26/100 (26%), 115/126 (91%), 141/153 (92%) Stomach 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): M, 0/97 (0%), 26/285 (9%); F, 2/100 (2%), 50/278 (18%) (high- and low-dose groups combined)			Vesselinovitch *et al.* (1975a,b) (contd)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss-Webster BLU:Ha (ICR)	NS	120–184	NS (DMSO)	0, 7, 14 nmol [0, 1.8, 3.7 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	34–37 wk	Lung T (mainly A + few AdC): 11%, [11/98] (0.12 [should be 0.11] T/animal), 22% [18/82] (0.22 T/animal), 15% [14/92] (0.15 T/animal)	–	Limited reporting of the type of pulmonary tumours; statistics NS; no significant differences in tumour incidence between M and F	Buening *et al.* (1978)
Mouse, CD-1	M, F	37 M, 27 F	>99% (DMSO)	0, 560 nmol [148 µg] (total dose; given as 1/7, 2/7, 4/7 on PND 0, 8, 15)	1 year	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)	+		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1,	M, F	17 M, 14 F	>99% (DMSO)	0, 1.1 μmol [290 μg]/animal (total dose; given as 1/6, 2/6, 4/6 on PND 1, 8, 15)	52 wk	Liver T: M, 1/17 (6%; 1 H), 13/17 (76%; 9 hepatic A, 4 H; p <0.005); F, 0 Lung A: M, 0/17, 14/17 (82%; p <0.005); F, 0/18, 9/14 (64%)	+	Statistics NS	LaVoie *et al.* (1987)
Mouse, Swiss-Webster BLU: Ha(ICR)	M, F	NS	>99% (DMSO)	0, 59.5 μg/animal (total dose; given as 8.5, 17, 34 μg on PND 1, 8, 15)	26 wk	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)	+	Statistics NS	Busby *et al.* (1989)
Mouse, NS, newborn	M, F	NS	NS (saline solution + 1% gelatine + 0.4% Tween 20)	0, 10, 100 μg/ animal, 1×	30 wk	Lung T: 13% [5/38] (0.13 T/animal), 16% [5/31] (0.23 T/animal), 64% [21/33] (2.52 T/animal)	+	Type of lung tumour NS; no statistics	Rippe & Pott (1989)
Mouse, A/J	NS	27	NS (tricaprylin)	0, 20, 50, 100 mg/kg bw/animal, 1×	8 months	Lung T: 19/34 [56%] (0.85 ± 0.9 T/animal), 10/16 [63%] (1.0 ± 1.0 T/animal), 15/16 [94%] (3.9 ± 2.9 T/animal), 14/14 [100%] (5.9 ± 3.3 T/animal)	+	Type of lung tumours NS; statistics NS	Mass *et al.* (1993); Ross *et al.* (1995)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, A/J	M	20	NS (tricaprylin, urethane)	0, 10, 50, 100 or 200 mg/kg bw, 1×; positive control, 1000 mg/kg bw urethane	8 months	Lung A/animal (partly derived from dose–response curve): 0.6, ~0.5, ~6, 12.8, ~35: at the 3 highest doses, T incidence was 100% (20/20); positive controls, 27.3 ± 4.7	+		Nesnow *et al.* (1995)
Mouse, A/J	M	55	NS (tricaprylin)	0, 5, 10, 50, 100, 200 mg/kg bw, 1×; positive control, 1000 mg/kg bw urethane, 1×	240 days	Lung A/animal: 0.6 ± 0.6, (~ 0), (~ 0), (~ 3), (~14), (~ 33); positive controls, 25.6 ± 5.7 [figures derived from dose–response curve except for those for both control groups; incidence of lung A NS]	+		Ross *et al.* (1995)
Mouse, A/J	F	29	NS (tricaprylin)	0 (untreated), 0 (vehicle control), 1.79 mg/animal, 1×	260 days	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; $p < 0.05$); forestomach T: 0/30 (0%), 0/30 (0%), 24/29 (83%; 15 P, 9 C; 1.83 ± 0.25 T/animal; $p < 0.001$)	+		Weyand *et al.* (1995)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, B6C3F1, infant	M, F	>30 M, >30 F	NS (corn oil)	0 (untreated), 0 (vehicle controls), 125, 250, 375 µg/7 g bw, 1×	26 wk, 39 wk, 52 wk	**Liver T in 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 found in F	+		Rodriguez *et al.* (1997)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M	24	>99% (DMSO)	0, 100, 26, 400 nmol [111 µg]/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15); positive controls, 100, 400 nmol 6-nitro-chrysene/animal (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	12 months	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) *(p = 0.0234) 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); positive controls: over 90% liver T (A and/or C) and 100% lung T (A and/or C) in both groups	+		von Tungeln *et al*. (1999a)
Rat, Wistar	F	NS	NS (tricaprylin/ beeswax mixture (3:1))	0, 5 mg/animal, 1×	~112 wk	Abdominal mesothelioma and S: 3/41 (7.3%), 33/37 (89.2%)	+	Limited reporting	Roller *et al.* (1992)
Rat, Wistar	F	NS	NS (saline solution)	5 mg/animal, 1×	~116 wk	19/38 (50%; abdominal mesothelioma and S); historical controls, 11/369 (3%)	+	No control; limited reporting of tumour data	Roller *et al.* (1992)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*e*]pyrene									
Mouse, Swiss-Webster BLU/Ha (ICR)	M, F	80	>99% (DMSO)	0, 2.8 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	62–66 wk	M: lung T, 42%, 41%; liver T, 11%, 21%; 0.53, 0.55 lung T/animal; 0.11, 0.21 liver T/animal F: lung T, 57%, 40%; liver T, 0%, 0%; 0.95, 0.57 lung T/animal	– –		Buening *et al.* (1980)
Mouse, Swiss-Webster BLU/Ha (ICR)	M, F	80	>99% (DMSO)	0, 5.6 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	62–66 wk	M: lung T, 31%, 48%; liver T, 0%, 12%; 0.48, 0.56 lung T/animal; 0, 0.12 liver T/animal F: Lung T, 40%, 26%; liver T, 0%, 0%; 0.43, 0.26 lung T/animal	+ –		Buening *et al.* (1980)
Chrysene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	80	NS (DMSO)	0, 1.4 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	37–41 wk	M: lung T, 8% (4/52), 15% (4/27); liver T, 0% (0/52), 22% (6/27); 0.08, 0.19 lung T/animal; 0.41 liver T/animal F: lung T, 15% (6/41), 9% (1/11); liver T, 0% (0/41), 0% (0/11); 0.09 lung T/animal	+ –		Chang *et al.* (1983)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 700 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M: lung A or AdC, 9%, 17%; liver A and C, 11% (5/45), 29% (10/35) [p <0.015]; lymphoma, 4%, 9% F: lung A or AdC, 6%, 6%; liver A or C, 0%, 0%; lymphoma, 0%, 3%	+ –		Wislocki *et al.* (1986)
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 2800 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	M: lung, A or AdC, 4% (1/28), 21% (7/34) [p <0.05]; liver A and C, 7% (2/28), 41% (14/44) [p <0.05]; lymphoma, 4%, 0% F: lung A and AdC, 0%, 4%; liver A and C, 0%, 0%; lymphoma, 3%, 0%	+ –		Wislocki *et al.* (1986)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	~297	>98% (DMSO)	0, 0.03, 0.92 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	M: lung A and AdC, 13/91 (14%), 2/27 (7%), 3/20 (15%); 0.15 ± 0.08, 0.07 ± 0.05, 0.15 ± 0.08 T/animal F: lung, A and AdC, 7/101 (7%), 3/29 (10%), 0/29 (0%); 0.08 ± 0.03, 0.10 ± 0.06, 0.0.06 ± 0.03 T/animal	– –		Busby *et al.* (1989)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Cyclopenta[*cd*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	~ 150	>99% (DMSO)	0, 1.55, 3.09, 4.64, 6.19, 7.73 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	M: lung A or AdC, 2/25 (8%), 5/8 (62%), 5/9 (56%), 6/7 (86%), 10/13 (77%), 8/9 (89%); 0.08 ± 0.06, 1.12 ± 0.48, 2.78 ± 1.10, 9.29 ± 4.78, 4.08 ± 0.98, 7.33 ± 1.80 T/animal F: lung A or AdC, 2/24 (8%), 6/10 (60%), 7/10 (70%), 13/14 (93%), 7/7 (100%), 9/9 (100%); 0.08 ± 0.06, 2.20 ± 0.83, 3.20 ± 1.04, 6.71 ± 2.01, 13.57 ± 5.50, 5.33 ± 1.62 T/animal	+ +		Busby *et al.* (1988)
Mouse, Strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg, 1×	8 months	Lung A: 55%, 40%, 100% (p <0.05), 100% (p <0.05), 100% (p <0.05); 0.60 ± 0.58, 0.58 ± 0.82, 4.63 ± 2.11 (p <0.05, one-way ANOVA), 32.8 ± 15.4 (p <0.05, one-way ANOVA), 97.7 ± 28.7 (p <0.05, one-way ANOVA) T/animal	+		Nesnow *et al.* (1994, 1995); Ross *et al.* (1995); Nesnow *et al.* (1998a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,c]anthracene									
Mouse, B6C3F$_1$	M	24	>99% (DMSO)	400 nmol [111 µg] in 35 µL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	12 months	9/24 (38%; liver A), 0/24 (liver C), 0/24 (lung T) vs 2/24 (8%), 1/24 (4%), 0/24 solvent controls	±		von Tungeln *et al.* (1999b)
Dibenz[*a*,*h*]anthracene									
Mouse, NS	NS	20	NS (aqueous suspension)	200 µg in 400 µL, 2×/wk, ~48 wk	~48 wk	5/20 (25%) peritoneal T	+	No control	Boyland & Burrows (1935)
Mouse, A/J	M	20	97% (tricaprylin)	0, 1.25, 2.5, 5, 10 mg/kg bw in 100 or 200 µL, 1×	8 months	Lung A: >2.5 mg/kg, 100%; 1.25 mg/kg, 1.44 A/mouse; 2.5 mg/kg, 3.05 A/mouse; 5 mg/kg, 13.1 A/mouse; 10 mg/kg, 32.1 A/mouse vs 0.6 A/mouse in solvent control significantly different (p <0.05) at doses >1.25 mg/kg	+	No histology	Nesnow *et al.* (1995); Ross *et al.* (1995); Nesnow *et al.* 1998a,b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*h*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	25 M, 14 F, 32 M controls, 39 F controls	'Essentially pure' (DMSO)	3.8, 7.6, 15.1 µg in 5, 10, 20 µL on PND 1, 8, 15	49–54 wk	Pulmonary T: F, 13/14 (93%) vs 11/39 (28%) solvent controls; M, 25/25 (100%) vs 7/32 (22%) solvent controls Hepatic T: F, 1/14 (7%) vs 0/39 solvent controls; M 11/25 (44%) vs 0/32 solvent controls	+		Chang *et al.* (1982)
Dibenzo[*a*,*i*]pyrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	39 M, 21 F, 32 M controls, 39 F controls	'Essentially pure' (DMSO)	3.8, 7.6, 15.1 µg in 5, 10, 20 µL on PND 1, 8, 15	49–54 wk	M, 38/39 (97%; pulmonary T; 3.64 T/mouse), 21/39 (54%; hepatic T; 0.82 T/mouse) vs 7/32 (22%; pulmonary T; 0.80 T/mouse), 0/32 (hepatic T) solvent controls; F, 21/21 (100%; pulmonary T; 5.80 T/mouse) vs 11/39 (28%; pulmonary T, 0.44 T/mouse) solvent controls	+	Limited histo-pathology	Chang *et al.* (1982)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenzo[*a*,*l*]pyrene									
Mouse, A/J	M	30–35 or 5	Pure analytical grade (tricaprylin)	0.3, 1.5, 3.0, 6.0 mg/kg bw or 12, 18, 24 mg/kg bw, 1× (volume NS)	8 months	Lung A: 0.3 mg/kg, 14/33 (42%; 0.42 ± 0.56 T/mouse); 1.5 mg/kg, 33/34 (97%; 4.30 ± 2.86); 3.0 mg/kg, 35/35 (100%; 7.50 ± 3.79); 6.0 mg/kg, 30/30 (100%; 16.1 ± 7.26); 12, 18 mg/kg, NS; 24 mg/kg, 5/5 (100%; 36.67 ± 10.64) vs 15/30 (50%; (0.67 ± 0.80) solvent controls	+		Prahalad *et al.* (1997)
Mouse, A/J	M	20	NS (tricaprylin)	0–6 mg/kg, 1× (volume NS)	8 months	Incidence of T, NS; lung A/mouse increased in a dose-dependent manner (p <0.005 for doses ≥1.5 mg/kg compared with solvent controls)	+		Nesnow *et al.* (1998a)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Crl:CD-1 (ICR)BR	M, F	25–40	NS (DMSO)	12.1, 121 µg in 80 µL, 3× (total dose; given as 1/8, 1/4, 5/8 on PND 1, 8, 15)	12.1 µg, 55 ± 1 wk; 121 µg, 17 wk	**Pulmonary T (T/mouse)** M: 12.1 µg, 84.8% (2.85 ± 0.44 [mean ± SEM]); 121 µg, 41.2% (0.65 ± 0.21) vs 25.0% (0.33 ± 0.14) in solvent controls F: 12.1 µg, 89.5% (2.95 ± 0.67); 121 µg, 35.7% (0.57 ± 0.29) vs 10.0% (0.10 ± 0.07) in solvent controls **Hepatic T (T/mouse)** M: 12.1 µg, 84.8% (5.67 ± 0.86); 121 µg, 35.3% (1.00 ± 0.38) vs 0% in solvent controls F: 12.1 µg, 10.5% (0.11 ± 0.07); 121 µg, 14.3% (0.21 ± 0.15) vs 0% in solvent controls **Other T (T/mouse)** M: 12.1 µg, 30.3% (0.58 ± 0.17); 121 µg, 23.5% (0.35 ± 0.19) vs 0% in solvent controls F: 12.1 µg, 47.4% (0.53 ± 0.14); 121 µg, 42.9% (0.50 ± 0.17) vs 0% in solvent controls	+		Platt *et al.* (2004)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
1,2-Dihydroacenanthrylene									
Mouse, CD-1	M, F	17–31 M, 13–23 F, 24 M controls, 34 F controls	Pure (DMSO)	175, 437.5, 875 µg in 35 µL (total dose; given as on 1/7, 2/7, 4/7 on PND 1, 8, 15)	9 months	**Liver T** M: 175 µg, 1/17 (6%); 437.5 µg, 1/25 (4%); 875 µg, 0/31 vs 0/24 solvent controls F: 175 µg, 0/23; 437.5 µg, 0/21; 875 µg, 0/13 vs 0/34 solvent controls **Lung A** M: 175 µg, 2/17 (12%); 437.5 µg, 2/25 (8%); 875 µg, 5/31(16%) vs 0/24 solvent controls F: 175 µg, 0/23; 437.5 µg, 1/21 (5%); 875 µg, 1/13 (8%) vs 1/34 (3%) solvent controls **Lung AdC** M: 175 µg, 2/17 (12%); 437.5 µg, 0/25; 875 µg, 1/31 (3%) vs 1/24 (4%) solvent controls F; 175 µg, 1/23 (4%); 437.5 µg, 0/21; 875 µg, 1/13 (8%) vs 0/34 solvent controls	±		Wang *et al.* (1999)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, BLU:Ha	M, F	10 M, 23 F, 22 M controls, 25 F controls	Pure (DMSO)	175 µg in 35 µL (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	6 months	M: 0/10 lung A, 0/10 lung AdC vs 1/22 (5%), 0/22 solvent controls F: 1/23 (4%) lung A, 0/23 lung AdC vs 1/25 (4%), 0/25 solvent controls	–		Wang *et al.* (1999)
Fluoranthene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	58 M, 41 F, 27 M controls, 27 F controls	98% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	24 wk	**Lung A/AdC (T/mouse)** M: 1/27 (4%; 0.04 ± 0.04), 7/31 (23%; 0.29 ± 0.15), 20/27 (74%; 1.52 ± 0.32) (p <0.014) F: 4/28 (14%; 0.14 ± 0.07), 3/20 (15%; 0.15 ± 0.11), 8/21 (38%; 0.52 ± 0.18)	+		Busby *et al.* (1984)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	23 M, 29 F, 91 M controls, 101 F controls,	>99% (DMSO)	0, 1.27 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	**Lung A/AdC (T/mouse)** M: 13/91 (14%; 0.15 ± 0.04), 5/23 (22%; 0.22 ± 0.09) (0.002 <p < 0.004) F: 7/101 (7%; 0.08 ± 0.03), 9/29 (31%; 0.41 ± 0.13) (0.002 <p <0.004)	+		Busby *et al.* (1989)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	57 M, 64 F, 23 M controls, 22 F controls	>99% (DMSO)	0, 3.46, 8.65, 17.30 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	6 months	**Lung A/AdC (T/mouse)** M: 0/23 (0%), 1/19 (5%; 0.05 ± 0.05), 2/18 (11%; 0.17 ± 0.12), 10/20 (50%; 0.70 ± 0.19); F: 0/22 (0%), 0/20 (0%), 2/21 (10%; 0.10 ± 0.07), 9/23 (39%; 0.43 ± 0.12) **Liver A (T/mouse)** M: 0/23 (0%), 1/19 (5%), 0/18 (0%), 2/20 (10%)	+		Wang & Busby (1993)
					9 months	**Lung A AdC (T/mouse)** M: 1/20 (5%; 0.05 ± 0.05), 3/18 (17%; 0.17 ± 0.09), 9/21 (43%; 0.52 ± 0.15), 9/14 (64%; 1.00 ± 0.26) F: 1/18 (6%; 0.06 ± 0.06), 6/19 (32%; 0.37 ± 0.14), 2/23 (9%; 0.09 ± 0.06), 7/24 (29%; 0.50 ± 0.21) **Liver A (T/mouse)** M: 0%, 4/18 (22%; p <0.03), 12/21 (57%; p <0.001), 5/14 (36%; p <0.01)	+		

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 17% (0.17), 43% (0.64; p <0.05), 65% (1.12; p <0.005) F: 12% (0.15), 35% (0.35; p <0.05), 86% (2.45; p <0.001) **Liver A/C (T/mouse)** M: 3/29 (10%; 0.17), 14/28 (50%; 0.64; p <0.012 χ^2), 14/17 (82%; 1.12; p <0.001 χ^2)	+		LaVoie *et al.* (1994b)
Indeno[1,2,3-*cd*]pyrene									
Mouse, CD-1	M, F	30	>99% (DMSO)	0, 2.1 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung T** M: 0/17 (0%), 1/11 (9.1%); F: 0/18 (0%), 0/9 (0%) **Liver T** M: 0/11 (0%), 0/11 (0%); F: 0/18 (0%), 0/9 (0%)	M, + F, –		LaVoie *et al.* (1987)
5-Methylchrysene									
Mouse, ICR/HA	M, F	100	Pure (DMSO)	0, 56 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	35 wk	**Lung A (T/mouse)** M: 4% (0.04), 20% (0.26) F: 7% (0.07), 21% (0.25) **Liver A** M: 2% (0.02), 23% (0.43) F: 2% (0.02), 12% (0.29)	+		Hecht *et al.* (1985)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, strain A/J	M	20	99% (tricaprylin)	0, 10, 50, 100, 200 mg/kg bw, 1×	8 months	Lung, A (T/mouse): 55% (0.6 ± 0.06), 65% (1.8 ± 1.6), 100% (39.0 ± 13.7; p <0.05), 100% (93.1 ± 19.9; p <0.05), 100% (too numerous to count; p <0.05)	+		You *et al.* (1994); Nesnow *et al.* (1995); Ross *et al.* (1995); Nesnow *et al.* (1998a)
2-Methylfluoranthene									
Mouse, CD-1, weanling	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol, (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 5/29 (17%; 0.17), 5/31 (16%; 0.25), 23/24 (96%; 3.04; p <0.001) F: 4/34 (12%; 0.15), 6/34 (18%; 0.35; p <0.001), 11/16 (69%; 2.45) **Liver A/C** M: 3/29 (10%), 8/31 (26%), 22/24 (92%; p <0.001) F: 0/34, 0/34, 5/16 (31%; p <0.001)	+		LaVoie *et al.* (1994b)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
3-Methylfluoranthene									
Mouse, CD-1, weanling	M, F	64–79	>99.5% (DMSO)	0, 3.46, 17.3 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC (T/mouse)** M: 5/29 (17%; 0.17), 6/24 (25%; 0.25), 5/26 (19%; 0.23) F: 4/34 (12%; 0.15), 5/33 (15%; 0.18), 6/28 (21%; 0.39) **Liver A/C** M: 3/29 (10%), 7/24 (30%), 15/26 (55%) F: 0/34, 0/33, 2.28 (7%)	+		LaVoie *et al.* (1994b)
Phenanthrene									
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	100	>98% (DMSO)	0, 35, 70, 140 µg on PND 1, 8, 15	38–42 wk	17/35 lung A vs 15/59 (25%) solvent controls; 0/35 liver T vs 0/59 solvent controls; 6/35 (17%) malignant lymphoma vs 0/59 solvent controls	–		Beuning *et al.* (1979)
Pyrene									
Mouse, CD-1	M, F	90 or 100	>99% (DMSO)	0, 700 nmol (total dose; given as 1/7, 2/7, 4/7 of total dose on PND 1, 8, 15)	52 wk	**Lung A/AdC** M: 9%, 8%; F: 6%, 10% **Liver A/C** M: 11%, 12%; F: 0%, 0% **Lymphoma** F: 0%, 10%	–		Wislocki *et al.* (1986)

Table 3.7 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, CD-1	M, F	90 or 100	>99%, (DMSO)	0, 200, 2800 nmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	52 wk	**Lung A/AdC** M: 4%, 3%, 7%; F: 0%, 3%, 6% **Liver A/C** M: 7%, 0%, 21%; F: 0%, 0%, 0% **Lymphoma** M: 4%, 0%, 0%; F: 3%, 3%, 6%	–		Wislocki *et al.* (1986)
Mouse, Swiss-Webster BLU:Ha (ICR)	M, F	NS	>98% (DMSO)	0, 0.43, 9.65 µmol (total dose; given as 1/7, 2/7, 4/7 on PND 1, 8, 15)	26 wk	**Lung A/AdC (T/mouse)** M: 13/91 (14%; 0.15 ± 0.04), 4/23 (17%; 0.17 ± 0.08), 2/27 (7%; 0.07 ± 0.05) F: 7/101 (7%; 0.08 ± 0.03), 1/28 (4%; 0.04 ± 0.04), 3/26 (12%; 0.12 ± 0.06)	M, –; F, ±		Busby *et al.* (1989)
Mouse, strain A/J	M	20	99.7% (tricaprylin)	0, 10, 50, 100, 200 mg/kg bw, 1 ×	8 months	No induction of lung T at any of the doses administered	–		Ross *et al.* (1995)

A, adenoma; AdC, adenocarcinoma; C, carcinoma; DMSO, dimethylsulfoxide; F, female; H, hepatoma; M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; PND, postnatal day; S, sarcoma; SCC, squamous-cell carcinoma; SEM, standard error of the mean; T, tumour; TBA, tumour-bearing animal; vs versus wk, week

[a]–, negative; +, positive; ±, equivocal

Table 3.8. Carcinogenicity studies of implantations of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Anthracene									
Rabbit, NS	NS	9	'Pure' (solid pellet)	4, 5, 10, 12, 20 mg, 1×	20–54 months	0/9	–	Cerebral implant; small no. of animals; no control; no statistics	Russell (1947)
Benz[*a*]anthracene									
Mouse, C57×IF F_1	NS	77	NS (paraffin wax)	~2 mg in a 12.5% suspension	40 wk	17/52 (33%) C (p <0.001), 1/52 (6%) P (p <0.001) vs 4/89 (4%), 1/89 (1%) controls	+	Bladder implantation	Clayson *et al.* (1968)

C, carcinoma; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; vs, versus; wk, week
[a] –, negative; +, positive

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 20 female Swiss albino mice (ICR/BR) [age and weight not specified] received dermal applications of 100 μL of a 0.1% solution of anthracene (purity >99%) 10 times on alternate days (total dose, 1 mg). Ten days after the last application, the mice received 2.5 μg TPA in 100 μL acetone thrice weekly for 20 weeks. Three treated mice (15%) developed skin tumours compared with two mice (10%) treated with acetone only and then TPA (LaVoie *et al*., 1983, 1985).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 40 male and female NMRI mice, 2 days of age, received a single subcutaneous injection of 50 μL of an aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20) that contained 71.3 μg anthracene (400 nmol; 99.9% pure). A control group of 49 male and female mice was treated with the solvent alone. After 40 weeks, 1/12 treated female and 2/17 treated male mice had developed pulmonary tumours compared with 1/19 control females and 1/14 control males (Platt *et al*., 1990)

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of five male Swiss albino mice received a single intraperitoneal injection of 25 mg anthracene in 750 μL olive oil. A control group of six males received olive oil only. After 5 months, no tumours had developed in the surviving four experimental mice or four control mice (Shubik & Della Porta. 1957).

11*H*-Benz[*b*,*c*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female CD-1 mice [age and body weight unspecified] were treated on alternate days with 10 subdoses of 0.05, 0.2 and 0.4 μmol [12, 48 and 96 μg] (total doses, 0.5, 2.0 and 4.0 μmol] 11*H*-benz[*b*,*c*]aceanthrylene (purity >99% by HPLC) in 100 μL acetone. A control group of 20 mice was treated with acetone alone. Ten days after the last dose, promotion began with applications of 2.5 μg TPA in 100 μL acetone thrice weekly for 20 weeks. At the end of the study, tumour incidences [type not specified] were 75% (15/20), 90% (18/20) and 90% (18/20) in the low-, mid- and high-dose treatment

groups, respectively, compared with 5% (1/20) in the control group (p <0.005 at all dose levels). The corresponding numbers of tumours/mouse were 1.60, 4.90 and 7.90 compared with 0.05 in the control group (Rice *et al*., 1988).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 15 male C3H mice, 3–5 months of age [body weight unspecified], received a single subcutaneous injection of 1 mg 11*H*-benz[*b*,*c*]aceanthrylene [purity unspecified] in 500 μL tricaprylin. The mice were monitored weekly and kept alive as long as possible; upon death, autopsies were performed and all tissues suggestive of neoplasia were examined histologically. No control group was used in the study. At 6 months, the survival rate was 14/15. At 539 days, one mouse had developed three tumours (a pulmonary adenoma, a hepatic adenoma and a benign haemangioma of the rump) (Dunlap & Warren, 1946).

Benz[*j*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female SENCAR mice, 7 weeks of age, received a single dermal application of 40, 200 or 400 μg benz[*j*]aceanthrylene [purity not specified] in 200 μL acetone. One week later, the mice were treated twice weekly for 21 weeks with 2 μg TPA in 200 μL acetone. A control group of 20 mice received TPA only. At the end of the study (22 weeks), the incidence of skin papillomas was 100% in each group treated with benz[*j*]aceanthrylene compared with ~5% in the TPA-treated control group (Nesnow *et al*., 1993).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 27 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 20, 50 or 100 mg/kg bw benz[*j*]aceanthrylene [purity not specified] in tricaprylin. A control group received tricaprylin alone. At the end of the study (after 8 months), the incidence of lung tumours (primarily adenomas) was 100% in each group treated with benz[*j*]aceanthrylene compared with 56% in the solvent-treated control group. The number of tumours/mouse was 0.85, 60.3, 140.6 and 97.6 in the 0-, 20-, 50- and 100-mg/kg bw benz[*j*]aceanthrylene-treated groups, respectively (Mass *et al*., 1993; Nesnow *et al*., 1998a).

Benz[*l*]aceanthrylene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20–22 male and 20–22 female SENCAR mice, 7 weeks of age, received a single dermal application of 50, 100, 250, 500 or 1000 nmol [12.6, 25.2, 63.1, 126 or 252 μg] benz[*l*]aceanthrylene [purity not specified] in 200 μL acetone or the solvent alone. One week later, all mice were treated twice weekly for 30 weeks with 2 μg TPA in 200 μL acetone. The experiment lasted 31 weeks. At the end of the study, the incidence of skin papillomas [histology not specified] was 12/20 (1.4 papillomas/mouse), 16/17 (2.3 papillomas/mouse), 21/21 (8.4 papillomas/mouse),16/16 (10.8 papillomas/mouse) and 19/20 (8.7 papillomas/mouse) male mice treated with 12.6, 25.2, 63.1, 126 or 252 μg benz[*l*]aceanthrylene compared with 0/20 control male mice. In females, the incidence of skin papillomas was 13/20 (1.1 papillomas/mouse), 18/19 (3.1 papillomas/mouse), 19/21 (4.7 papillomas/mouse), 20/21 (6.6 papillomas/mouse) and 20/20 (10.8 papillomas/mouse) after treatment with 12.6, 25.2, 63.1, 126 or 252 μg benz[*l*]aceanthrylene compared with 1/19 (0.05 papillomas/mouse) controls (Nesnow *et al*., 1984a).

Benz[*a*]anthracene

Previous evaluations

Benz[*a*]anthracene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated multiple bioassays in which benz[*a*]anthracene was administered orally to mice and dermally to mice, rats and hamsters, injected subcutaneously into adult and newborn mice or intramuscularly and intravenously into rats and implanted into the bladders of mice. The transfer of injection sites following subcutaneous injections to mice was also analysed. Among these studies (which are summarized in Tables 3.1–3.3, 3.6, 3.9 and 3.10), some gave negative results, while others were positive. Benz[*a*]anthracene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that benz[*a*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

Two groups of 40 female Swiss mice, 7 weeks of age [body weight unspecified], received twice-weekly dermal applications of 0.396 μmol [90.4 μg] benz[*a*]anthracene (recrystallized; purity verified by melting-point) in 16.7 μL acetone or solvent alone for

Table 3.9. Carcinogenicity studies of intravenous administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Mouse, strain A	M, F	10–11	NS (water)	250 µg suspended in 250 µL, 1×	8, 14 or 20 wk	8 wk, 0/10; 14 wk, 2/10 (20%); 20 wk, 2/11 (18%) (lung T, type NS)	±	No control	Andervont & Shimkin (1940)
Rat, Sprague-Dawley	F	28	NS (lipid NS)	0.25% (w/v), 2 mg (~13 mg/kg), 3× (on days 50, 53, 56 of age)	98 days	0/28	–	No control	Pataki & Huggins (1969)
Dibenz[*a*,*h*]anthracene									
Mouse, strain A	M, F	10, 20 controls	Melting-point (aqueous suspension)	250 µg in 250 µL, 1×	20 wk	10/10 (100%) pulmonary T vs 4/19 (21%) solvent controls	+	No statistics	Andervont & Shimkin (1940); Shimkin & Stoner (1975)
Mouse, strain A	M, F	44–55	NS (aqueous colloidal suspension)	100, 200, 300, 400, 500 µg, 1×	6 months	Lung T/mouse: 100 µg, 8.08 ± 0.542; 200 µg, 18.25 ± 1.225; 300 µg, 30.02 ± 1.663; 400 µg, 38.64 ± 1.923; 500 µg, 53.37 ± 2.166 vs 0.29 ± 0.033 in water controls	+	No histology	Heston & Scheidermann (1953)

F, female; M; male; NS, not specified; PAH, polycyclic aromatic hydrocarbon; T, tumour; vs, versus; wk, week; w/v, weight/volume

[a] –, negative; + positive; ±, equivocal

Table 3.10. Carcinogenicity studies of intramuscular administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Rat, Long-Evans	M	16	NS (sesame oil)	2.5 mg in 500 µL, 1×	270 days	0/16	–	No control	Pataki & Huggins (1969)
Dibenz[*a*,*h*]anthracene									
Fowl, NS	NS	31	NS (lard)	4 mg (volume NS), 1 ×	45 months	15/31 (48%; S)	+	No control	Peacock (1935)
Pigeon, NS	M, F	121, 32 controls	NS (benzene)	3 mg in 100 µL, 1×	13 months	14/109 (13%; fibroS) vs 0/32 untreated controls	+	No solvent-treated controls; no statistics	Prichard *et al.* (1964)
Dibenzo[*a*,*h*]pyrene									
Rat, NS	NS	4	NS (sunflower seed oil)	0.5–1.0 mg, 1×	7–8 months	2/4 (50%)		No control; small number; no statistics	Voronjansky *et al.* (1939)
Rat, NS	F	6	NS (rabbit fat)	0.5 mg, 1×	8 months	2/6 (33%)		No control; small number; no statistics	Pisareva *et al.* (1940)

F, female; M, male; NS, not specified; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; vs, versus
[a] –, negative; +, positive

30 weeks. The animals were observed until death or were killed when moribund. Mean survival rates were 65 ± 15 weeks (mean ± SD) in the benz[*a*]anthracene-treated group and 65 ± 11 weeks in the vehicle-control group. At the end of the experiment, 1/39 benz[*a*]anthracene-treated mice had developed a skin papilloma compared with 0/29 acetone-treated controls (Cavalieri *et al*., 1977).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single dermal application of 2.2 μmol [502 μg] chromatographically purified benz[*a*]anthracene in benzene [concentration unspecified], followed 1 week later by twice-weekly applications of 10 μmol TPA for 34 weeks. A control group of 30 female mice of the same strain was treated with 10 μmol TPA only [the dose of TPA was probably 10 μg] twice weekly for 34 weeks. At week 35, 29/30 benz[*a*]anthracene-treated animals and all controls were still alive. At that time, 62% [18/29] of benz[*a*]anthracene-treated mice had developed skin papillomas; one skin tumour had occurred in 1/30 (3%) TPA controls by week 25, but this had regressed by the end of the study, at which time no tumours were found in the control group (Scribner, 1973).

A group of 30 female CD-1 mice, 7–9 weeks of age [body weight unspecified], received a single dermal application of 2 μmol benz[*a*]anthracene [457 μg; volume unspecified]. One week later, mice received twice-weekly applications of 10 μg TPA in acetone [volume unspecified] for 26 weeks. A control group was treated with TPA alone. At the end of the study, 57% of the mice in the benz[*a*]anthracene-treated group had developed skin papillomas (1.2 papillomas/mouse) compared with 6% (0.1 papillomas/mouse) of the TPA-treated controls (Slaga *et al*., 1978).

Groups of 30 female CD-1 mice, 8 weeks of age [body weight unspecified], received a single dermal application of 0, 1.0 or 2.5 μmol [228 or 571 μg] benz[*a*]anthracene in 200 μL acetone. Beginning 1 week later, animals received twice-weekly applications of 16 nmol [9.9 μg] TPA in 200 μL acetone for 27 weeks. At the end of the study, 17% of the mice in the low-dose group and 38% of the mice in the high-dose group had developed skin tumours [type not specified] compared with 4% of the TPA-treated controls. The corresponding numbers of tumours/mouse in the benz[*a*]anthracene-treated groups were 0.17 ± 0.07 and 0.67 ± 0.17 (mean ± standard error (SE)) compared with 0.04 ± 0.04 in the TPA-treated controls (Wood *et al*., 1980).

In a similarly designed initiation-promotion study in CD-1 mice that used initiating doses of 0.4 or 2.5 μmol [91 or 571 μg] benz[*a*]anthracene and promotion with TPA for 25 weeks, skin tumours [type not specified] were found in 14% of the mice treated with 0.4 μmol (0.14 ± 0.07 tumours/mouse) and 36% of the mice treated with 2.5 μmol (0.64 ± 0.20 tumours/mouse) compared with 7% (0.07 ± 0.05 tumours/mouse) in the TPA-treated control group. At the highest dose, both the tumour incidence and the number of

tumours/mouse were significantly higher than those in the control group ($p < 0.05$) (Levin *et al*., 1984).

Buccal pouch application (see also Table 3.11)

Hamster

Two groups of 26 male Syrian golden hamsters [age unspecified] received twice-weekly applications of a 20 mM solution of benz[*a*]anthracene in paraffin oil [volume unspecified] to the buccal pouch for either 5 or 20 weeks. A group of 20 control animals was available. The animals were monitored for up to 44 weeks, with no evidence of tumours in any of the groups (Solt *et al.*, 1987).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 140 newborn male and female Swiss Webster BLU:Ha (ICR) mice received a total intraperitoneal dose of 0 (controls) or 2800 nmol [63.9 μg] benz[*a*]anthracene in DMSO [volume not specified] (1/7 of the dose was administered on postnatal day 1, 2/7 on postnatal day 8 and 4/7 on postnatal day 15). A control group of 100 mice was treated with DMSO alone. The incidence of pulmonary tumours was 10/47 treated males (0.26 tumours/mouse), 7/43 control males (0.19 tumours/mouse), 4/38 treated females (0.18 tumours/mouse) and 2/24 control females (0.08 tumours/mouse) (Wislocki *et al.*, 1979).

Groups of 90 newborn male and female BLU:Ha(ICR) mice received intraperitoneal injections of a total dose of 0 (controls) or 2.8 μmol [639 μg] benz[*a*]anthracene [purity >99%] in 35 μL DMSO [1/7, 2/7 and 4/7 of the dose on days 1, 8 and 15 of life, respectively] and were monitored for 26–32 weeks. A control group of 80 mice was treated with DMSO alone. The incidence of pulmonary adenomas was 63% [17/27] in treated males (1.44 tumours/mouse), 4% [1/28] in control males (0.04 tumours/mouse), 64% [14/22] in treated females (2.00 tumours/mouse) and 11% [4/37] in control females (0.11 tumours/mouse). Hepatic tumours (mostly type A and neoplastic nodules) were observed in 8% [2/27] of treated males (0.08 tumours/mouse) and 4% [1/28] of control males (0.04 tumours/mouse) but not in treated or control females. A significant increase in the incidence and number of pulmonary tumours per mouse compared with the controls was found when the results from male and female mice were combined (Levin *et al*., 1984).

A group of newborn male and female CD-1 mice received a total intraperitoneal dose of 2.8 mmol benz[*a*]anthracene in DMSO [1/7 on postnatal day 1, 2/7 on postnatal day 8 and 4/7 on postnatal day 15]. The incidence of liver tumour was 79% (6/39 adenomas and 25/39 carcinomas; 3.0 nodules/tumour-bearing animal) in treated male mice compared

Table 3.11. Carcinogenicity studies of application to the buccal pouch of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Hamster, Syrian golden	M	26, 20 controls	NS (paraffin oil)	20 mM, 2×/wk, 5 or 20 wk	Up to 44 wk	0% vs 0% in solvent-treated controls	–		Solt *et al.* (1987)
Benzo[*a*]pyrene									
Hamster, Syrian golden	M	28, 20 controls	NS (paraffin oil)	0, 20 mM [5046 mg]/animal, 2×/wk, 20 wk	Up to 40–44 wk with interim kills after 5, 20 and 24–32 wk	Forestomach P: only in treated animals killed after 40–44 wk: 80% [8/10] Buccal pouch tumours: only 1 (a SCC) in a treated animal killed in wk 41 Positive (DMBA) controls: 100% [19/19] forestomach (100% P, 68% SCC), 100% [19/19] buccal pouch (a total of 199 well-differentiated SCC) in animals surviving for 20 wk	+		Solt *et al.* (1987)

DMBA, 7,12-dimethylbenz[*a*]antracene; M, male; NS, not specified; P, papilloma; SCC, squamous-cell carcinoma; vs, versus; wk, week

[a] –, negative; +, positive

with 7% (2/28 adenomas and 0/28 carcinomas; 1.0 nodule/tumour-bearing animal) in control males (p <0.05 for liver carcinomas and for combined liver tumours); that of pulmonary tumours in treated males was 15% (5/39 adenomas and 1/39 carcinoma) compared with 4% (1/28 adenoma and no carcinomas) in control males. No liver tumours were observed in treated females which had an incidence of pulmonary tumours of 19% (6/32 adenomas and no carcinomas) compared with 0% in the control group (p <0.025) (Wislocki *et al*, 1986).

Intratratracheal administration (see also Table 3.12)

Hamster

A group of 48 male Syrian golden hamsters, 9–10 weeks of age (average weight, 98 g), was treated intratracheally with 30 weekly doses of 0.5 mg benz[a]anthracene [purity > 99% by thin layer chromagraphy; total dose, 15 mg] that was ground to a finely aggregated dust [1:1, w:w] with haematite [particle size < 1 μm] and then suspended in 200 μL saline [0.9% aqueous]. A second group of 36 male hamsters of the same strain and age was treated in a similar manner with 15 weekly doses of 3.0 mg benz[a]-anthracene [total dose, 45 mg] and an additional group of 90 hamsters remained untreated. No control group received intratracheal instillation of the vehicle alone. The animals were monitored daily, weighed once a week, died spontaneously or were killed when moribund. At 120 weeks, only one animal remained in the 3.0-mg treatment group and all animals in the remaining groups had died. Tumours of the respiratory tract were not observed in any of the groups (Sellakumar & Shubik, 1974).

Intramammary administration (see also Table 3.5)

Rat

A group of 20 female pathogen-free Sprague-Dawley rats, 50 days of age [body weight unspecified], was subjected to a small incision over the right fifth inguinal mammary gland, which was exposed, had finely powdered benz[*a*]anthracene (purity >99% by HPLC; 4 or 16 μmol) [913 μg or 3.65 mg, respectively] dispersed over it and was subsequently closed. The untreated contralateral left gland served as a negative control. All animals were killed at 20 weeks. No mammary tumours were present in the benz[*a*]anthracene-treated animals (Cavalieri *et al.,* 1988a).

Table 3.12. Carcinogenicity studies of intratracheal administration of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benz[*a*]anthracene									
Hamster, Syrian golden	M	36 or 48; 90 controls	>99% (0.9% saline)	Ground with haematite [1:1, <1 μm particles] in 200 μL; 0.5 mg 1×/wk, 30 wk [total dose, 15 mg], 3.0 mg, 1×/wk, 15 wk [total dose, 45 mg]	120 wk	15 mg: 0/47 (respiratory tract T), 6/47 (13%; other T); 45 mg, 0/33, 6/33 (18%) vs 0/82, 11/82 (13%) untreated controls	–	Control group not submitted to tracheal instillation of the vehicle	Sellakumar & Shubik (1974)
Benzo[*a*]pyrene									
Mouse, Iva; NMRI	F	NS	NS (0.9% saline solution)	0 (untreated), 0 (0.9% saline solution), 50 μg/animal, 1×/wk, 20 wk	2 years	Lung T: [9/28] (32%; 0.7 ± 1.7 T/animal), [30/55] (54%; 0.8 ± 1.0 T/animal), [28/50] (56%; 2.2 ± 3.7 T/animal)	+	Numbers not given; no histopathology; statistics NS	Heinrich *et al.* (1986a)
Mouse, *XPA*$^{-/-}$, *XPA*$^{+/-}$, *XPA*$^{+/+}$	F	5 or 30	NS (gelatine/ physiological saline)	0, 0.1 mg/ animal, 1×/wk, 4 wk	16 mo	**Lung T** *XPA*$^{-/-}$: 0/5, 15/21 (71%; 13 A, 2 C; 1.4 ± 0.3 T/animal) *XPA*$^{+/-}$: 0/5, 11/27 (41%; 11 A; 0.8 ± 0.2 A/animal) *XPA*$^{+/+}$: 0/5, 7/20 (35%; 7 A; 0.4 ± 0.1 A/animal)	+		Ide *et al* (2000)
Rat, Wistar-WU/Kisslegg	F	NS	NS (0.9% saline solution)	0, 1 mg/animal, 1×/wk, 20 wk	124–126 wk	Lung T: 0/40, 7/36 (19%; 1 A, 5 SCC, 1 mixed AdC/SCC)	+		Pott *et al.* (1987)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	M, F	20 or 50	NS (physiological saline solution with or without Tween 60)	0, 0 (physiological saline), 7 mg/kg bw/ instillation (physiological saline with Tween 60), 1×/2 wk, 44 wk	Controls, 131 wk; treated animals, 112 wk	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)	+	Limited histology; type of lung T NS	Steinhoff *et al.* (1991)
Hamster, Syrian golden	M, F	35	>99% (0.9% saline solution)	0 (only for M), 1 mg/animal, 1×/wk, 36 wk	78 wk	**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)	+	No female controls; no statistics	Feron (1972)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	NS (0.9% saline solution)	0, 0.0625, 0.125, 0.25, 0.5, 1.0 mg/animal, 1×/wk, 52 wk	78 wk	**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)	+		Feron *et al.* (1973)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	50, 25 controls	NS (0.5% gelatine in 0.9% saline solution)	0, 13.3–15.5 mg/animal, 1×/wk, 8 wk	M, 67–88 wk; F, 60–88 wk	**Respiratory tract T** Controls [effective no. of animals NS]: 1 tracheal polyp, 6 pulmonary bronchiolar adenomatoid lesions 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	+	Tumour data for M and F combined; statistics NS	Henry *et al.* (1973)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	20–32 M, 20–28 F	NS (0.9% saline)	0, 1 mg/animal, 1×/wk, 30 wk	60 wk	**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)	+		Kobayashi (1975)
Hamster, Syrian golden	M, F	17 or 40	>99% (saline solution)	0 (untreated), 0 (vehicle controls), 1 mg/animal, 1×/2 wk, 52 wk	78 wk	**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)	+		Kruysse & Feron (1976)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	48	>99% (0.9% saline solution)	0 (untreated) or 3 mg/animal, 1×/wk, 10 wk	100 wk	**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)	+		Sellakumar *et al.* (1976)
Hamster, Syrian golden	M, F	30	97% (0.9% saline solution or Tris buffer)	Experiment 1: 0, 4, 8, 16 mg in 0.9% saline solution/animal, 1×	Up to 89 wk for M and 70 wk for F	**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)	+		Kektar *et al.* (1977)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
				Experiment 2: 0, 4, 8, 16 mg in Tris buffer/ animal, 1×	Up to 83 wk for M and 68 wk for F	*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)			Ketkar *et al.* (1977) (contd)
Hamsters, Syrian golden	M, F	15 or 30	>99% (0.9% saline solution)	0 (untreated), 0 (vehicle controls), 0.35, 0.7 mg/animal, 1×/wk, 52 wk	81 wk	**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, 2 pulmonary A) 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)	+	Statistics NS	Feron & Kruysse (1978)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	30	97% (10% bovine serum albumin)	0, 0.1, 0.33, 1.0 mg/animal, 1×/wk for life	Average survival up to 41 wk for M and 35 wk for F	**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)	+	Average survival time drastically lower in the high-dose group than in the other groups	Ketkar *et al.* (1978)
Hamster, Syrian golden	NS	NS	NS (0.4% Tween 80 in saline solution)	0, 0.3, 0.9 mg/animal, 1×/wk, 20 wk	91 wk	Benign and malignant respiratory tract T: 3, 17, 68%	+	Type of T not further specified	Pott *et al.* (1978)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	48 (M + F)	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	0, 3 mg large particles, 3 mg small particles/ animal, 1×/wk, 18 wk	Lifespan, up to 90 wk	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)	+		Stenbäck & Rowland (1978)
Hamster, Syrian golden	M, F	70 (M + F)	NS (0.9% saline solution)	0, 0.5 mg/ animal, 1×/2 wk, 52 wk	104 wk	**Respiratory tract T** M: no T in 24 controls and 20 T (1 tracheal polyp, 6 P, 3 SCC, 1 anaplastic C, 1 bronchial polyp, 1 SCC, 7 pulmonary A) in treated animals F: no T in 25 controls and 18 T (8 tracheal P, 3 SCC, 6 pulmonary A, 1 AdC) in treated animals	+	No. of TBA NS	Feron (1979)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	97% (Tris buffer + 0.9% saline solution); particle size: majority <10 μm but particles up to 80 μm also present	0 (untreated), 0 (vehicle controls), 0.125, 0.25, 0.5, 1.0 mg/animal, 1×/wk, for life	Average survival up to 88 wk	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)	+	Average survival in two highest-dose groups very much lower than that in the other groups due to many early deaths from pulmonary lesions other than tumours	Ketkar *et al.* (1979)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	30–35	NS; 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)	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, 1×/wk, 52 wk	105 wk	**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)	+	Statistics NS	Feron *et al.* (1980)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	30	NS (Tris-buffer/saline)	0 (untreated), 0 (vehicle control), 5, 20, 40 µg/animal, 1×/2 wk for life	Mean survival time, 67 (low-dose) to 84 wk (high-dose)	0/29, 0/30, 0/28, 0/27, 2/28 (7%) (1 lung A, 1 respiratory tract mucoepidermoid C)	±	Statistics NS	Künstler (1983)
Hamster, Syrian golden	M	80	>99% (0.5% gelatine in 0.9% saline solution)	0, 5 mg/animal, 1×/wk, 15 wk	129 wk	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; $p < 0.001$)	+	No. of TBA NS	Godleski *et al*. (1984)
Hamster, Syrian golden	M, F	35	NS (gelatine in saline solution)	0, 1 mg/animal, 1×/2 wk, 52 wk	85 wk	**Respiratory tract T** M: 0/31, 6/ 34 [18%] (3 tracheal P, 1 SCC, 1 S, 1 bronchial P); F: 0/28, 1/29 [3%] (1 laryngeal C *in situ*)	+	Statistics NS	Feron *et al.* (1985)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a*,*h*]anthracene									
Hamster, Syrian golden	M	48, 90 controls	>99% (saline)	50, 250 µg (combined with an equal amount of ferric oxide) in 200 µL, 1×/wk, 30 wk	≤120 wk	Respiratory tract T: 50 µg, 0/46; 250 µg, 2/46 (4%) vs 0/82 untreated controls	–		Sellakumar & Shubik (1974)
Hamster, Syrian golden	NS	NS	NS (saline solution containing 0.4% Tween 80)	300, 900 µg in 150 µL, 1×/wk, 20 wk	≤2 years	Respiratory tract T: 300 µg, 55%; 900 µg, 65% vs 3% solvent controls	+	No statistics	Pott *et al.* (1978)
Dibenzo[*a*,*i*]pyrene									
Hamster, Syrian golden	M	36 or 48, 90 controls	>99% (0.9% saline)	Ground with haematite [1:1, <1 µm particles] in 200 µL, 2 mg 1×/wk, 4 wk (total dose, 8 mg); 500 µg, 1×/wk, 24 wk (total dose, 12 mg)	100 wk; controls, 120 wk	8 mg, 16/34 (47%; respiratory tract T, predominantly SCC; 1/34 (3%) larynx, 2/34 (6%) trachea, 13/34 (38%) bronchi, 1/34 (3%) lung); 12 mg, 39/44 (89%; respiratory tract T, predominantly SCC; 6/44 (14%) trachea, 37/44 (84%) bronchi, 1/44 (2%) lung, 2/44 (4%) malignant lymphoma) vs 0/82 (respiratory tract T), 11/82 (13%; T at other sites) untreated controls	+	Control group not submitted to tracheal instillation of the vehicle; no statistics	Sellakumar & Shubik (1974)

Table 3.12 (contd)

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	48	>99% (water)	1 mg, 1×/wk, 12 wk (total dose, 12 mg); 500 µg, 1×/wk, 17 wk (total dose, 8.5 mg) [volume NS]	NS	12 mg, 36/48 (respiratory tract T, predominantly SCC; bronchi, 62%; trachea, 19%); 8.5 mg, 39/48 (respiratory tract T, predominantly SCC; bronchi, 82%; trachea, 13%; larynx, lung and pleura T also observed)	+	No control; no statistics	Stenbäck & Sellakumar (1974)

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; mo, month; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TBA, tumour-bearing animal; vs, versus; wk, week

[a]–, negative; +, positive; ±, equivocal

Benzo[*b*]chrysene

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single dermal application of 2.5 μmol [695 μg] benzo[*b*]chrysene (chromatographically purified) in benzene [concentration unspecified], followed 1 week later by twice-weekly applications of 5 μmol TPA (total dose, 10 μmol) for 34 weeks. A control group of 30 female mice of the same strain was treated twice weekly with 10 μmol TPA only for 34 weeks. At week 35, 29/30 benzo[*b*]chrysene-treated mice and all controls were still alive. At that time, 48% (14/29) of benzo[*b*]chrysene-treated mice had developed skin papillomas; only one skin tumour had occurred in 3% (1/30) of TPA controls by week 25, but this had regressed by the end of the study, at which time no tumours were found in the control group (Scribner, 1973).

Benzo[*g*]chrysene

No data were available to the Working Group.

Benzo[*a*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

In a mouse skin initiation–promotion study, groups of 20 female CD-1 mice, 50–55 days of age, received 10 subdoses on alternate days of benzo[*a*]fluoranthene in 100 μL acetone (total initiating doses, 0, 1.0 or 4.0 μmol). Ten days later, 2.5 μg TPA in 100 μL acetone were applied thrice weekly for 20 weeks. The incidence of tumour-bearing animals was 95% (19/20) and 90% (18/20) with averages of 3.3 and 4.3 skin tumours/mouse in the low- and high-dose groups, respectively. Papillomas occurred in 10% (2/20) of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Benzo[*b*]fluoranthene

Previous evaluation

Benzo[*b*]fluoranthene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated three bioassays in which the compound was administered dermally or subcutaneously to mice. On the basis of the available data, the Working Group concluded that benzo[*b*]fluoranthene induced skin tumours and local sarcomas. Benzo[*b*]fluoranthene was also considered in February 1983 (IARC, 1983) by a Working

Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that benzo[*b*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since the previous evaluation are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In separate skin initiation–promotion studies with female outbred albino Crl:CD-1 (ICR) BR mice, total initiating doses of 0–100 nmol (Amin *et al.*, 1985a, 1991), 0–100 μg (LaVoie *et al.*, 1982a) and 0–400 nmol (LaVoie *et al.*, 1993) benzo[*b*]fluoranthene resulted in dose-related increases in the numbers of mice with skin papillomas. Similar results were obtained in female outbred albino Crl:CD-1 (ICR) BR mice with total initiating doses of 100 or 400 nmol benzo[*b*]fluoranthene (Geddie *et al.*, 1987), and in other studies in female CD-1 mice with total initiating doses of 0, 30 or 100 μg (Weyand *et al.*, 1989) and 0, 1.0 or 4.0 μmol benzo[*b*]fluoranthene (Weyand *et al.*, 1990).

Intraperitoneal administration (see also Table 3.7)

Mouse

Intraperitoneal injection of 0 or 0.5 μmol benzo[*b*]fluoranthene into newborn CD-1 mice induced lung and liver adenomas in treated males and lung adenomas in treated females (LaVoie *et al.*, 1987). A single injection of 0, 10, 50, 100 or 200 mg/kg bw benzo[*b*]fluoranthene into male strain A/J mice resulted in dose-related increases in the incidence and multiplicity of lung adenomas (Nesnow *et al.*, 1995; Ross *et al.*, 1995; Mass *et al.*, 1996; Nesnow e*t al.*, 1998a).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.1, 0.3 or 1.0 mg benzo[*b*]fluoranthene into Osborne-Mendel rats induced dose-related increases in the incidence of pulmonary squamous-cell carcinomas and sarcomas (Deutsch-Wenzel *et al.*, 1983).

Benzo[*ghi*]fluoranthene

Benzo[*ghi*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one study in which female mice were treated by dermal application; no skin tumours were observed. The Working Group concluded that the

available data were inadequate to permit an evaluation of the carcinogenicity of benzo[*ghi*]fluoranthene in experimental animals. No new studies were available.

Benzo[*j*]fluoranthene

Previous evaluation

Benzo[*j*]fluoranthene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated a bioassay in which the compound was administered dermally to mice and concluded that benzo[*j*]fluoranthene induced a high incidence of skin tumours. It was also considered by a Working Group in February 1983 (IARC, 1983) that, in addition to the previous assay, evaluated studies in which benzo[*j*]fluoranthene was administered dermally to mice (repeated administration and initiation–promotion protocols) and by intrapulmonary injection to rats. The Working Group concluded that there was *sufficient evidence* that benzo[*j*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In initiation–promotion studies in female CD-1 mice, total initiating doses of 0–3.0 μmol (Rice *et al.*, 1987), 0–2.0 μmol (Weyand *et al.*, 1992) and 0–1000 nmol (LaVoie *et al.*, 1993) benzo[*j*]fluoranthene resulted in dose-related increases in the incidence and multiplicity of skin papillomas. In female Crl:CD-1 (ICR)BR mice, total initiating doses of 0–1000 μg also resulted in dose-related increases in the incidence and multiplicity of skin papillomas (LaVoie *et al.*, 1982a).

Intraperitoneal administration (see also Table 3.7)

Mouse

In two studies in male and female newborn CD-1 mice, intraperitoneal injection of benzo[*j*]fluoranthene at doses of 0–1.1 μmol increased the incidence and multiplicity of lung (adenomas) and liver (hepatomas) tumours (LaVoie *et al*., 1987, 1994a).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.2, 1.0 or 5.0 mg benzo[*j*]fluoranthene into 35 Osborne-Mendel rats induced a dose-related increase in the incidence of pulmonary

squamous-cell carcinomas (0, 2.9, 8.6 and 51.4%, respectively) (Deutsch-Wenzel *et al.*, 1983).

Benzo[*k*]fluoranthene

Previous evaluation

Benzo[*k*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which the compound was administered dermally to mice (repeatedly and initiation–promotion protocols), by subcutaneous injection to mice and by intrapulmonary injection to rats. On the basis of the available data, the Working Group concluded that there was *sufficient evidence* that benzo[*k*]fluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In an initiation–promotion study, total initiating doses of 4 μmol benzo[*k*]-fluoranthene induced squamous-cell papillomas in 37% [effective number not specified] of female CD-1 mice (Amin *et al.*, 1985b).

Intraperitoneal administration (see also Table 3.7)

Mouse

Intraperitoneal injection of 2.1 μmol benzo[*k*]fluoranthene into newborn CD-1 mice induced lung adenomas in 6.3% and 16.7% of males and females, respectively, and liver hepatomas and adenomas in 18.8% of males (LaVoie *et al.*, 1987).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 0.16, 0.83 or 4.15 mg benzo[*k*]fluoranthene into 35 Osborne-Mendel rats induced a dose-related increase in the incidence of pulmonary squamous-cell carcinomas (0, 0, 9.7 and 44.4%, respectively) (Deutsch-Wenzel *et al.*, 1983).

Benzo[*a*]fluorene

Previous evaluation

Benzo[*a*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*a*]fluorene was administered dermally and subcutaneously to mice; each of the studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*a*]fluorene to experimental animals. No new studies were available.

Benzo[*b*]fluorene

Previous evaluation

Benzo[*b*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated an initiation–promotion bioassay in mice, which gave positive results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*b*]fluorene to experimental animals. No new studies were available.

Benzo[*c*]fluorene

Previous evaluation

Benzo[*c*]fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*c*]fluorene was administered dermally to mice (repeated administration and initiation–promotion protocols). Both studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*c*]fluorene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Oral administration (see also Table 3.6)

Mouse

Groups of 30 female A/J mice, 7 weeks of age, were fed benzo[*c*]fluorene (purity ≥98% by HPLC) at doses of 27 or 397 μmol/kg of diet for up to 260 days, at which time all surviving mice were killed. A group of 30 control mice was fed basal diet. Gross necropsies were performed on the lungs, liver, stomach, small intestine, kidney, ovaries, uterus and urinary bladder, and all stomachs were examined histologically. Lung adenomas were observed in 46% [13/28] of the mice fed 27 μmol/kg (0.57 ± 0.13 tumours/mouse) and 100% [29/29] of the mice fed 397 μmol/kg (46.0 ± 2.8 tumours/mouse) compared with 24% [7/29] of the control mice (0.31 ± 0.11 tumours/mouse). At

the highest dose, the number of tumours per mouse was significantly greater than that observed in the control group (p <0.001). There was a 15% incidence of squamous hyperplasia in the forestomach in both groups treated with benzo[*c*]fluorene compared with 3% in the control group. No carcinomas were found in the forestomach in any of the groups (Weyand *et al*., 2004).

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of 30 female A/J mice, 7 weeks of age, was administered a single intraperitoneal injection of 1.75 mg (100 mg/kg bw; purity ≥98% by HPLC) in 250 μL tricaprylin. A control group of 30 mice received tricaprylin alone. All surviving mice were killed at 260 days. Gross necropsies were performed on the lungs, liver, stomach, small intestine, kidney, ovaries, uterus and urinary bladder, and all stomachs were examined histologically. Lung adenomas were observed in 92% [26/28] of the mice administered benzo[*c*]fluorene (4.0 ± 0.53 tumours/mouse) compared with 48% [12/29] of the controls (0.6 ± 0.14 tumours/mouse). The number of tumours per mouse in the treated group was significantly greater than that observed in the control group (p <0.01). There was an 8% incidence of squamous hyperplasia in the forestomach in the group treated with benzo[*c*]fluorene compared with 0% in the control group. No carcinomas were found in the forestomach in any of the groups (Weyand *et al*., 2004).

Benzo[*ghi*]perylene

Previous evaluation

Benzo[*ghi*]perylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*ghi*]perylene was administered dermally to mice (repeated administration and initiation–promotion protocols), subcutaneously to mice and by intrapulmonary injection into rats. The study on intrapulmonary injection was considered to be inadequate for evaluation; the other studies gave negative results. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*ghi*]perylene to experimental animals. No new studies were available.

Benzo[*c*]phenanthrene

Previous evaluation

Benzo[*c*]phenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which benzo[*c*]phenanthrene was administered dermally to mice (repeated administration and initiation–promotion protocols) and by

subcutaneous injection into mice and rats. Benzo[*c*]phenanthrene was considered to be active as a tumour initiator in the initiation–promotion assay. The other bioassays (repeated dermal application to mice and subcutaneous injection into mice and rats) were considered to be inadequate for evaluation. The Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*c*]phenanthrene to experimental animals. An additional bioassay that has been published since that time is summarized below.

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of male and female newborn CD-1 mice received three intraperitoneal injections of benzo[*c*]phenanthene in DMSO on days 1, 8 and 15 of life (total doses, 0, 50 or 150 nmol). The incidence of lung tumours (number of tumours/tumour-bearing animal) was increased in the high-dose groups: male control, 3% [1/31] (0.06); male low-dose, 6% [2/36] (0.06); male high-dose, 57% [16/28] (1.6); female control, 5% [2/44] (0.07); female low-dose, 9% [3/32] (0.09); and female high-dose, 65% [23/35]. The carcinogenicity of seven suspected activated metabolites of benzo[*c*]phenanthene was also examined. Some of these metabolites substantially increased the incidence of lung tumours in both sexes of mice and those of liver tumours in male mice. The activated metabolites also showed a significant activity in an initiation–promotion experiment in the same study (Levin *et al.*, 1986) and in a previous experiment (Levin *et al.*, 1980).

Benzo[*a*]pyrene

Previous evaluation

This compound was considered by earlier Working Groups in December 1972 (IARC, 1973), October 1982 (IARC, 1982) and February 1983 (IARC, 1983). The Working Group of December 1972 (IARC, 1973) concluded that benzo[*a*]pyrene produced tumours in all species (mouse, rat, hamster, guinea-pig, rabbit, duck, newt, monkey) for which data were reported following exposure by different routes (oral, dermal, inhalation, intratracheal, intrabronchial, subcutaneous, intraperitoneal, intravenous). It 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 tranplacental exposure. The 1983 Working Group (IARC, 1983) did not evaluate the studies of carcinogenicity in animals published since 1972 but concluded that there is *sufficient evidence* that benzo[*a*]pyrene is carcinogenic to experimental animals. This conclusion was based on the data evaluated by the Working Group of December 1972 (IARC, 1973) and on a number of reviews, such as those of Dipple (1976), Freudenthal and Jones (1976), Bingham *et al.* (1980) and Conney (1982).

Studies described below are illustrative of the various routes of administration used in experimental animals or of various recently developed methods in carcinogenicity testing.

Inhalation exposure (see also Table 3.13)

Hamster

Groups of 24 male Syrian golden hamsters, 8–14 weeks of age, were exposed by inhalation to 0, 2.2, 9.5 or 46.5 mg/m^3 benzo[*a*]pyrene (mixed with an aerosol of 0.1% saline solution) for 4.5 h per day on 7 days per week for 10 weeks and then for 3 h per day for the rest of their life. Tumours (papillomas, polyps and squamous-cell carcinomas) were found in the upper respiratory tract (nose, larynx and trachea) and the upper digestive tract (pharynx, oesophagus and forestomach) of mid- and high-dose animals but not in controls or low-dose animals. The tumour response was dose-related. No bronchogenic tumours were detected (Thyssen *et al*., 1981). In a similar liftetime study with small numbers of animals (three groups of 10 males exposed to 0, 9.8 or 44.8 mg/m^3 benzo[*a*]pyrene) and short exposure periods (10 and 16 weeks for the high- and low-dose groups, respectively), only one tumour was found (a tracheal polyp in a low-dose animal) (Thyssen *et al*., 1980).

Oral administration (see also Table 3.6)

Mouse

Following oral administration of benzo[*a*]pyrene by gavage or in the diet to different strains of mice, increased tumour responses were found in several organs including the lung, forestomach, liver and lymphoreticular system (see Table 3.6). Compared with controls, significant dose-related increases in the incidence of tumours and in the number of tumours per animal were observed in the lung and the forestomach of female strain A/J mice fed a gel diet containing 16 or 98 ppm benzo[*a*]pyrene for 260 days (Weyand *et al*., 1995). In female B6C3F1 mice fed diets containing 0, 5, 25 or 100 ppm benzo[*a*]pyrene for 2 years, significantly increased incidences of squamous-cell papillomas and/or carcinomas were observed in the oesophagus and tongue of high-dose animals and in the forestomach of mid- and high-dose animals (Table 3.6) (Culp *et al*., 1998).

Table 3.13. Carcinogenicity studies of inhalation exposure of various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene									
Hamster, Syrian golden	M	10	NS (0.01% saline solution); particle size, generally 0.2–1.5 μm	0, 9.8, 44.8 mg/m^3, 4.5 h/day, 5 days/wk, 16 wk for the low-dose group and 10 wk for the high-dose group	Lifespan (average survival/group, 71–82 wk)	The only respiratory tract tumour found was a papillary polyp in the trachea of a low-dose animal	–	Short exposure period; small number of animals	Thyssen *et al.* (1980)
Hamster, Syrian golden	M	24 (+ animals added during the study)	NS (0.1% saline solution); particle size, >99% diameter 0.2–0.5 μm, >80% diameter 0.2–0.3 μm	0, 2.2, 9.5, 46.5 mg, 4.5 h/day, 7 days/wk, 10 wk; thereafter 3 h/day, 7 days/wk for life (total average doses: 0, 29, 127, 383 mg/animal)	Lifespan (average survival/group, 59–96 wk)	Respiratory tract T (P, polyps, 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 (P, polyps, SCC): 0/27, 0/27, 26.9% [6/26; 6 pharyngeal, 1 forestomach], 56% [14/25; 14 pharyngeal, 2 oesophageal, 1 forestomach]	+	Types of tumour/site NS	Thyssen *et al.* (1981)

M, male; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; SCC, squamous-cell carcinoma; T, tumour; wk, week

[a] –, negative; +, positive

Transgenic mouse

In a study of male Eμ-*pim*-1 transgenic mice administered benzo[*a*]pyrene by gavage at doses of 0, 4.3, 13 or 39 mg/kg bw thrice weekly for 13 weeks and observed for a period of up to 287 days, a significantly increased incidence of lymphomas and forestomach tumours was found in the two highest-dose groups (Kroese *et al*., 1997). Administration of benzo[*a*]pyrene by gavage to mice that lack the nucleotide excision repair gene ($XPA^{-/-}$) resulted in a high incidence of lymphomas; the tumour response was significantly stronger than that in similarly treated $XPA^{+/-}$ and $XPA^{+/+}$ mice (de Vries *et al*., 1997). When treated with benzo[*a*]pyrene by gavage, $XPA^{-/-}/p53^{+/-}$ double transgenic mice developed tumours (mainly splenic lymphomas and forestomach tumours) much earlier and at a higher incidence than similarly treated single transgenic ($XPA^{-/-}$ or $p53^{+/-}$) and wild-type counterparts (C57/B1/6) (van Oostrom *et al*., 1999). These cancer-prone $XPA^{-/-}$ or $XPA^{-/-}/p53^{+/-}$-deficient mice also developed a high incidence of tumours (80–87%; mainly forestomach tumours) after being fed a diet that contained 75 ppm benzo[*a*]pyrene for 13 weeks followed by a 6-month observation period, whereas similarly treated wild-type counterparts showed only a 10% incidence of forestomach papillomas (Hoogervorst *et al*., 2003).

Rat

In a lifespan study of Sprague-Dawley rats fed diets that contained benzo[*a*]pyrene at levels that resulted in total doses of 0, 6 or 39 mg/kg bw per year, a slightly higher incidence of forestomach papillomas was observed in high-dose animals (10/64 versus 3/64 controls; $p < 0.1$) (Brune *et al*., 1981). The incidence and total number of mammary gland tumours (fibroadenomas, adenomas and adenocarcinomas) were markedly increased in female CD rats treated with benzo[*a*]pyrene (50 μmol [13.21 mg]) by gavage once a week for 8 weeks followed by an observation period of 41 weeks (El-Bayoumy *et al*., 1995).

Dermal application (see also Table 3.1)

Mouse

Benzo[*a*]pyrene was found to induce skin tumours (mainly papilloma and squamous cell carcinomas) in different strains of mice following repeated skin painting for prolonged periods of time.

Mice that lack the aryl hydrocarbon receptor ($AhR^{-/-}$) did not develop tumours after weekly dermal application of 200 μg/animal benzo[*a*]pyrene for 25 weeks, whereas over 90% of their similarly treated AhR-positive counterparts ($AhR^{+/+}$ or $AhR^{+/-}$) developed skin tumours, mainly squamous-cell carcinomas (Shimizu *et al*., 2000).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Benzo[*a*]pyrene has been found to possess tumour-initiating activity in the skin of a range of strains of mice subsequently treated with TPA in acetone or in acetone/DMSO or, in once case, with croton oil (DiGiovanni *et al*., 1980) as the tumour-promoting agent. The predominant type of skin tumour was papilloma.

Buccal pouch application (see also Table 3.11)

Hamster

A high incidence of forestomach papillomas (8/10) and one buccal pouch tumour (a squamous-cell carcinoma) were observed in male Syrian golden hamsters that received applications to the surface of both buccal pouches with 20 mM 5.2g/L benzo[*a*]pyrene dissolved in paraffin oil twice a week for up to 20 weeks. No tumours were observed in these organs in controls (Solt *et al*., 1987).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn mice of various strains, intraperitoneal injection of benzo[*a*]pyrene resulted in an increased incidence of (mainly benign) liver and lung tumours. In some of these studies, an increased incidence of forestomach tumours (papillomas and squamous-cell carcinomas) and lymphoreticular tumours (mainly reticulum-cell sarcomas) was also found (Vesselinovitch *et al.*, 1975a; Weyand *et al.*, 1995).

Rat

In rats, a single intraperitoneal injection of benzo[*a*]pyrene resulted in a high incidence of abdominal mesotheliomas and sarcomas (Roller *et al*., 1992).

Subcutaneous administration (see also Table 3.3)

Mouse

In a series of experiments in female NMRI mice that received single subcutaneous injections of a range of doses of benzo[*a*]pyrene, malignant tumours (mainly fibrosarcomas) were found at the injection site. The tumour incidences were invariably dose-related but varied from one study to another and appeared to be affected by the

nature of the vehicle (tricaprylin, 0.9% saline solution or lutrol 9 (polyethylene-oxide)) and the concentration of benzo[*a*]pyrene in this vehicle (Pott *et al*., 1973a,b).

Transgenic mouse

Mice that lack the aryl hydrocarbon receptor ($AhR^{-/-}$) did not develop tumours after two subcutaneous injections of 2 mg benzo[*a*]pyrene/animal whereas 100% of their similarly treated AhR-positive counterparts ($AhR^{+/+}$ or $AhR^{+/-}$) developed malignant tumours at the injection site that were mainly fibrosarcomas (Shimizu *et al*., 2000).

Rat

Dose-related increases in the incidence of malignant tumours (mainly fibrosarcomas) were found at the site of injection in groups of female Wistar rats given a single subcutaneous injection of tricaprylin containing various concentrations of benzo[*a*]pyrene (Pott *et al*., 1973a,b). Rippe and Pott (1989) reported a high incidence of sarcomas at the injection site in female rats following a single subcutaneous injection of a suspension of benzo[*a*]pyrene in tricaprylin or DMSO.

Hamster

Groups of 25 male and 25 female Syrian hamsters of one randomly bred and 11 different inbred strains (one inbred strain comprised females only) received a single subcutaneous injection of 0.5 mg benzo[*a*]pyrene suspended in tricaprylin and were observed for life. Sarcomas at the site of injection were found in both sexes of all 12 strains; the incidence in males ranged from 12 to 64% and that in females from 17 to 64% (Homburger *et al*., 1972).

Monkey

No tumours were found in 17 Old World monkeys that received [an unspecified number of] subcutaneous injections of benzo[*a*]pyrene and were observed for up to 18 years (Adamson & Sieber, 1983).

Intratracheal instillation (see also Table 3.12)

After the landmark study on an effective method for the induction of tracheobronchial carcinomas in Syrian golden hamsters (Saffiotti *et al*., 1968) that received repeated intratracheal instillations of benzo[*a*]pyrene particles (attached to ferric oxide particles as a carrier) suspended in saline, this model has been used extensively in experimental studies of lung cancer. Studies of benzo[*a*]pyrene mixed with ferric oxide or other particles (or fibres) are summarized in Section 3.15. Studies on benzo[*a*]pyrene not

attached to ferric oxide particles (or other particulates) are summarized in Table 3.12 and are discussed below.

Mouse

An increased incidence and a larger number of lung tumours per animal were found in mice that received 20 weekly intratracheal instillations of a relatively low dose of benzo[*a*]pyrene (50 μg/instillation; total dose, 1 mg) and were observed for a maximum period of 2 years (Heinrich *et al*., 1986a).

Transgenic mouse

In *XPA*$^{-/-}$ mice, a stronger lung tumour response was found after four weekly intratracheal instillations of 100 μg benzo[*a*]pyrene (total dose, 0.4 mg) than in similarly treated *XPA*$^{+/-}$ and *XPA*$^{+/+}$ mice (Ide *et al*., 2000).

Rat

Following 20 weekly intratracheal instillations of 1 mg benzo[*a*]pyrene suspended in 0.9% saline solution, 7/36 (19%) female Wistar rats developed benign and malignant lung tumours (one adenoma, five squamous-cell carcinomas and one adenosquamous carcinoma) whereas no lung tumours were seen in 40 vehicle-treated controls (Pott *et al*., 1987). A very high incidence of malignant lung tumours (38/40) was also found in male and female Sprague-Dawley rats after 22 twice-weekly intratracheal instillations of 7 mg/kg bw benzo[*a*]pyrene, every other week (Steinhoff *et al*., 1991).

Hamster

Benzo[*a*]pyrene suspended in saline (with or without suspendents such as gelatine, lutrol or Tween 60) was found to induce a variety of benign and malignant respiratory tract tumours in male and female Syrian golden hamsters. Although the tumour response varied widely and ranged from only a few benign tumours (Sellakumar *et al*., 1976; Künstler, 1983; Feron *et al*., 1985) to a high incidence of malignant tracheobronchial and pulmonary carcinomas (Feron, 1972; Kobayashi, 1975; Kruysse & Feron, 1976), positive dose–response relationships were established (Feron *et al*., 1973; Feron & Kruysse, 1978; Pott *et al.*, 1978), and larger particles were found to be more effective than smaller ones (Stenbäck & Rowland, 1978; Feron *et al*., 1980).

Intrapulmonary administration (see also Table 3.4)

Rat

Malignant lung tumours (mainly squamous-cell carcinomas) were found in different strains of rat that received direct injections into the lung tissue of a fixed volume (generally 50 μL) of a mixture of beeswax/tricaprylin or beeswax/trioctanoin that contained different amounts of benzo[*a*]pyrene (30–1000 μg benzo[*a*]pyrene/animal) and were observed for life. The tumour incidence was invariably dose-related; low incidences (<10%) occurred at the lowest dose tested (30 μg) (Deutsch-Wenzel *et al*., 1983; Iwagawa *et al*., 1989; Wenzel-Hartung *et al*., 1990; Horikawa *et al*., 1991).

Tracheal graft (see also Table 3.14)

Rat

Malignant tumours (mainly squamous-cell carcinomas) were found in subcutaneously grafted rat tracheas exposed to beeswax pellets that contained 10–2490 μg benzo[*a*]-pyrene; tumour incidence ranged from 77% (40/52) to 100% (12/12) for the three-highest dose levels (1740, 2160 and 2490 μg, respectively); no tumours were observed in 52 controls (Nettesheim *et al*., 1977).

Intramammary administration (see also Table 3.5)

Rat

Intramammary injection or dispersion of benzo[*a*]pyrene over the exposed mammary glands led to a high incidence of malignant mammary gland tumours (mainly adeno-carcinomas and fibrosarcomas) in female Sprague-Dawley rats (Cavalieri *et al*., 1988a,b, 1991).

Intracolonic instillation (see also Table 3.14)

Mouse

No intestinal tumours were found in male or female mice of three different strains that were treated intracolonically with benzo[*a*]pyrene. However, treatment with benzo[*a*]-pyrene did cause significant increases in tumour incidence in various other organs including the lungs, forestomach, mammary glands, peritoneum, lymphoreticular tissue, oesophagus, anus and/or skin (Toth, 1980; Anderson *et al*., 1983).

Table 3.14. Carcinogenicity studies by miscellaneous routes of exposure to various PAHs in experimental animals

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Benzo[*a*]pyrene										
Rat, Fischer 344	NS	10–52	Subcutaneous. tracheal graft exposed to pellet	NS (beeswax)	0 (untreated), 0 (beeswax controls), 10, 45, 300, 480, 900, 1250, 1740, 2160, 2490 μg/graft, 1×	4–22 mo	Tracheal (graft) T: 0/52 (untreated and beeswax controls combined), 0/24, 0/10, 1/14 (7%; 1 SCC), 0/12, 2/12 (17%; 1 SCC, 1 S), 7/13 (54%; 6 SCC, 1 S), 7/13 (54%; 6 SCC, 1 P), 8/10 (80%; 7 SCC, 3 undifferentiated C, 3 non-invasive C, 2 P), 12/12 (100%; 7 SCC, 1 AdC, 5 non-invasive C, 5 P), 40/52 (77%; 32 SCC, 2 AdC, 3 non-invasive C, 1 P, 2 S)	+		Nettesheim *et al.* (1977)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Human; nude mouse, BALB/c-A	M	6 or 17	Human bronchial grafts (from 4 patients) transplanted subcutaneously in mice and treated by local application (intraluminal injection)	NS (distilled water)	0 (untreated), 10 mg/graft (animal), 3× (once in wk 4, once in wk 12 and once in wk 20 after transplantation)	16–21 wk after transplantation	One bronchial T (SCC) in 13 treated grafts examined histopathologically; 4/13 treated grafts showed preneoplastic epithelial changes (polypoid structures with squamous metaplasia, cellular atypia, unclear irregularity and mitotic changes). In addition, high incidence of basal-cell and goblet/mucus hyperplasia, and squamous metaplasia occurred in the treated grafts. No such changes were seen in the 6 control grafts which all showed well-preserved bronchial structure. Spindle-cell sarcoma of mouse origin developed in 7/13 animals bearing treated bronchial transplants	+		Ito *et al.* (1982)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, Swiss albino	M, F	50 M, 50 F	Intracolonic instillation	98% (olive oil)	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	120 wk	**Malignant lymphomas** 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) *(p <0.04), **(p <0.02), ***(p <0.053) **Oesophagus** M, no tumours; F, 0/49, 0/50 or 5/49 (10%) **Forestomach** 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 <0.005), **(p <0.006)	+	Anal and skin tumours probably due to release of benzo[*a*]pyrene through the anal orifice	Toth (1980)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
							Anus 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) *(p <0.02), ** (p <0.04) **Skin** 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) *(p <0.0001), ** (p <0.005)			Toth 1980 (contd)
Mouse, Swiss ICR/Ha	F	45–60	Intracolonic instillation	99% (olive oil, enzyme inducer β-naphtho-flavone)	0 (no treatment, olive oil or β-naphthoflavone in olive oil), 1 mg/animal, 1×/wk, 14 wk	18 mo	Lung T: 13/52 (25%; multiplicity, 1.4 ± 0.8), 27/37 (73%; multiplicity, 5.1 ± 3.9) (p <0.05 or less) Forestomach P: 4/20 (20%; multiplicity, 1.2 ± 0.5); 16/17 (94%; multiplicity, 1.9 ± 1.1; p <0.05 or less) Mammary gland C: 5/53 (9%), 10/43 (23%; p <0.05 or less)	+	Type of lung tumours NS; no colon tumours found	Anderson *et al.* (1983)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, C57/Bl/6	F	45–60	Intracolonic instillation	99% (olive oil, enzyme inducer β-naphtho-flavone)	0 (no treatment, olive oil or β-naphthoflavone in olive oil), 1 mg/animal (in olive oil), 1×/wk, 14 wk	18 mo	Forestomach P: 7/34 (21%; multiplicity, 1.1 ± 0.4), 17/18 (94%; multiplicity, 3.2 ± 2.3; p <0.05 or less) Peritoneal S: 0/40, 5/32 (16%; p <0.05 or less) Lymphoma: 1/40 (2.5%), 9/32 (28%; p <0.05 or less)	+	No colon tumours found	Anderson *et al.* (1983)
Mouse, C57B1	F	10 or 76	Intravaginal application	NS (acetone)	Cotton swab soaked in acetone (controls) or 1% solution of benzo[*a*]pyrene in acetone, 2×/wk, 5 mo	5 mo	0/10, 17/76 (22%; invasive cervical C)	+		Näslund *et al.* (1987)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mongrel dogs, Beagle	NS	3 or 6	Submucosal injection or sustained-release implant	NS (NS) Vehicle (NS)	Experiment 1: 3 dogs, 15–45 mg/wk (total dose, 1.02 g) plus topical application of 380×10^6 mg TPA administered over 4 mo Experiment 2: 6 dogs, 1 or more sustained-release bronchial implants containing 31–50 mg followed after 6 mo by sustained-release implants containing 0.1% TPA for 6 mo	5.5 years 2.6 (2.3–2.9) years	No lung cancer found	–		Benfield *et al.* (1986)
Mouse, Swiss	M, F	43–56	Intrafetal injection	>99% (trioctanoin-acetone mixture (1:1))	0, 0.4, 4.0, 9.9, 19.8 nmol [0, 0.1, 1, 2.6, 5.2 µg]/animal, 1×	12 wk	Lung A (M + F combined): 0/37, 1/39 (3%), 10/42 (25%), 10/38 (26%), 12/31 (39%)	+		Rossi *et al.* (1983)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Dibenz[*a,h*]anthracene										
Rat, NS	NS	68	Subcutaenous/ intraperitoneal (alternating)	NS (aqueous suspension)	1 mg in 2 mL, 2×/wk, ~44 wk	~44 wk	31/68 (46%; S)		No control	Boyland & Burrows (1935)
Frog, *Rana pipiens*	M, F	23, 10 and 192 controls	Intra-renal	NS (olive oil)	0.3–0.5 mg in 100 µL, 1×	<7 mo	6/23 (26%; kidney AdC) vs 0/10 solvent controls and 6/192 (3%) untreated controls	+	Small no. of solvent-treated controls; no statistics	Strauss & Mateyko (1964)
Dibenzo[*a,i*]pyrene										
Mouse, NS	F	30	Intrauterine	NS (NS)	500 µg (volume NS), 1×	32 wk	0/30	–	No control; limited design	Homburger & Tregier (1960)
Dibenzo[*a,l*]pyrene										
Hamster, Syrian golden	F	7, 4 controls	Tongue	NS (acetone)	0.25%, 0.01 µmol (3µg), 5×/wk, 30 wk	30 wk	6/7 (86%) oral cavity C (2.6 T/ animal) vs 0/4 solvent controls	+	No statistics	Schwartz *et al.* (2004)

Table 3.14 (contd)

Chemical, species and strain	Sex	No./group at start	Route of administration	Purity (vehicle)	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Mouse, mixed C57Bl/6 and 129/Sv [wild-type and P450 1B1-null]	F	18 wild-type, 13 P450 1B1 null, 27 controls	Intragastric	99.8% (corn oil)	1.07 mg/kg (~32 µg) in 100 µL, 5×/wk, 3 wk	12 mo	Wild-type: 18/18 (12/17 ovary T (83% granulosa-cell T), 5/17 lymphoma (60% lymphoblastic, 40% follicular), 1/17 liver A, 8/17 skin hyperplasia, 5/17 uterine T (40% haemangioS, 60% endometrial cystic hyperplasia), 0/17 lung T); P450 1B1-null: 8/13 (0/13 ovary T, 1/13 follicular lymphoma, 1/13 liver haemangioma, 5/13 endometrial cystic hyperplasia, 5/13 lung A) vs 4/27 [1/27 follicular lymphoma, 1/27 liver A, 1/27 endometrial cystic hyperplasia, 1/27 lung A) solvent controls (both genotypes combined)	+	No histology for one wild-type mouse	Buters *et al*. (2002)

A, adenoma; AdC, adenocarcinoma; bw, body weight; C, carcinoma; F, female; K, keratoacanthoma; M, male; mo, months; NS, not specified; P, papilloma; PAH, polycyclic aromatic hydrocarbon; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; vs, versus; wk, week

[a] –, negative; +, positive

Intravaginal application (see also Table 3.14)

Mouse

Intravaginal application of a 1% solution of benzo[*a*]pyrene in acetone twice a week for 5 months to C57BL mice produced invasive cervical carcinoma in 17/76 (22%) animals whereas no such tumours were seen in acetone-treated controls (0/10) (Näslund *et al*., 1987)

Intrafetal administration (see also Table 3.14)

Mouse

Groups of 43–56 male and female Swiss mice were injected intrafetally with 1 μL of a trioctanoin/acetone mixture (1:1) that contained benzo[*a*]pyrene at concentrations that produced doses of 0 (controls), 0.4, 4.0, 9.9 and 19.8 nmol [0, 0.1, 1, 2.61 and 5.23 μg]/animal respectively. Survivors were killed at 12 weeks of age. Lung adenomas were found in all treated groups at an incidence of 1/39 (3%), 10/42 (25%), 10/38 (26%) and 12/31 (39%) in the low-, low-mid-, high-mid- and high-dose groups, respectively. No lung tumours were found in 37 controls (Rossi *et al*., 1983)

Administration with particles and/or fibres (dusts) (see also Table 3.15)

Rat

Large numbers of malignant lung tumours (range, 17–21 tumours in 20 males and 16–18 tumours in 20 females) developed in Sprague-Dawley rats following 22 intratracheal instillations administered once a week every other week of 7 mg benzo[*a*]pyrene alone or mixed with 10–40 mg Bayferrox 130 (96.2% cubic α-Fe_2O_3) or 10–40 mg Bayferrox 920 (86.1% fibrous α-FeOOH) whereas no lung tumours were seen in controls and rats treated with Bayferrox 130 or Bayferrox 920 alone except for one benign and one malignant lung tumour in 50 females treated with Bayferrox 920 (Steinhoff *et al*., 1991).

Hamster

In a large number of studies of Syrian golden hamsters, repeated intratracheal instillation of benzo[*a*]pyrene mixed with ferric oxide (used as carrier particles) and suspended in saline or saline/gelatine was found to induce benign and malignant tumours in various segments of the respiratory tract (larynx, trachea, bronchi and lungs) (see Table 3.15). Although the tumour response was generally dose-related, wide variations in tumour incidence occurred between studies (see also review by Wolterbeek *et al*., 1995).

Table 3.15. Carcinogenicity studies in rats and hamsters exposed by intratracheal instillation to combinations of benzo[*a*]pyrene and 'particles/fibres'

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Rat, Sprague-Dawley	M, F	20 or 50	NS (physiological saline solution with or without Tween 60); Bayferrox 130, Bayferrox 920	0 (untreated), 0, 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, ~1×/2 wk, 44–~130 wk	Up to 920 days	Lung T (mainly malignant) 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	+	Type of lung tumour NS	Steinhoff *et al.* (1991)
Hamster, Syrian golden	M, F	23–110 M; 18–107 F	NS ferric oxide (0.9% saline solution)	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, 1×/wk, 15 wk	Lifespan (up to 140 wk)	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)	+		Saffiotti *et al.* (1972)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						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)			Saffiotti *et al.* (1972) (contd)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						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)			Saffiotti *et al.* (1972) (contd)
Hamster, Syrian golden	F	48	NS (tricaprylin, Tween 60/saline solution); atmospheric dust from Bochum, Germany (particle size <5 µm)	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 months (total dose, ~15 mg; dust, 38 mg)	Presum-ably lifespan	Respiratory tract T (mainly P or SCC of the larynx, trachea or bronchi): 2/48 (4%), 14/48 (29%), 16/48 (33%)	+		Pott *et al.* (1973b)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian	M, F	36; 193 controls	NS (0.9 % saline saline solution); ferric oxide	0, 3 mg + 3 mg ferric oxide, 3 mg + 6 mg ferric oxide, 3 mg + 9 mg ferric oxide, 1×/2wk, 20 wk	100 wk	**Respiratory tract T (M + F combined)** 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: 17/32 (53%; 37 P), 10/31 (32%; 16 P, 1 SCC), 6/35 (17%; 15 P) F: 10/35 (29%; 30 P), 12/33 (36%; 25 P), 15/31 (48%; 33 P) vs 0/193 untreated controls	+		Sellakumar *et al.* (1973)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	73–83	NS (0.9% saline solution); ferric oxide or carotene-free cottonseed oil	3 mg + 3 mg ferric oxide, 1×/wk, 12 wk followed after 1 wk by 100, 1600 or 3300/µg vitamin A (retinyl acetate), intragastric 1×/wk [signs of vitamin A toxicity appeared in animals given 3300 µg after 24 wk; dose reduced to 2400 µg/wk]	~60 wk	**Respiratory tract T** *Low-vitamin A group* 48/83 (58%; 37 laryngeal/tracheal T: 10 P, 4 polyp, 20 SCC, 2 AdC, 1 undifferentiated C; 35 bronchial/ bronchiolar/pulmonary T: 2 P, 9 A, 8 SCC, 7 AdC, 4 AdSC, 3 undifferentiated C, 2 fibroS) *Mid-vitamin A group* 52/74 (70%; 32 laryngeal/tracheal T: 3 P, 4 polyp, 18 SCC, 1 AdC, 1 carcinoS, 6 fibroS; 38 bronchial/bronchiolar/pulmonary T: 1 P, 11 A, 7 SCC, 6 AdC, 3 AdSC, 3 undifferentiated C, 1 carcinoS, 6 fibroS) *High-vitamin A group* 59/73 (81%; 32 laryngeal/tracheal T: 7 P, 1 polyp, 13 SCC, 4 AdC, 1 AsSC, 1 undifferentiated C, 3 carcinoS, 2 fibroS; 52 bronchial/ bronchiolar/pulmonary T: 11 A, 13 SCC, 16 AdC, 2 undifferentiated C, 1 carcinoS, 9 fibroS)	+		Smith *et al.* (1975a)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M	49–58	NS (0.15 M saline solution); ferric oxide or carotene-free cottonseed oil	3 mg + 3mg ferric oxide/animal, 1×/wk, 12 wk followed after 1 wk by 100, 1600 or 3300 μg vitamin A (retinyl acetate) intragastric 1×; half of the animals in each group were conventially housed (CH) while the other half was housed in laminar flow units (LFU), [signs of vitamin A toxicity appeared in animals given 3300 μg after 24 wk; doses reduced to 2400 μg/wk]	~100 wk	**Respiratory tract T** *Low-vitamin A group* CH: 34/57 (60%; 23 P, 11 polyps, 5 A, 26 SCC, 18 AdC, 3 ASC, 5 oat-cell C, 4 undifferentiated C, 2 carcinoS, 3 fibroS) LFU: 37/52 (71%; 25 P, 13 polyp, 14 A, 25 SCC, 11 AdC, 8 ASC, 2 undifferentiated C, 2 lymphoma) *Mid-vitamin A group*: CH: 45/58 (78%; 20 P, 14 polyp, 10 A, 31 SCC, 11 AdC, 2 oat-cell C, 4 undifferentiated C, 1 carcinoS, 6 fibroS, 1 haemangioS), LFU: 31/53 (59%; 16 P, 10 polyp, 2 A, 36 SeC, 16 AdC, 2 ASC, 2 oat-cell C, 2 undifferentiated C, 5 fibroS, 2 chondroS, 2 lymphoma, 5 reticulum-cell S)	+	Site of respiratory tract T NS	Smith *et al.* (1975b)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
						High-vitamin A group: CH: 43/58 (74%; 19 P, 11 polyp, 18 A, 37 SCC, 8 AdC, 7 fibroS) LFU: 28/49 (57%; 21 P, 9 polyp, 7 A, 22 SCC, 14 AdC, 7 AdSC, 14 oat-cell C, 2 fibroS, 2 lymphoma, 2 reticulum-cell S) **Forestomach P (CH + LFU combined)** *Low-vitamin A group* [55/109] (50%) (multiplicity, 2.9 ± 0.2) *Mid-vitamin A group* [28/111] (25%) (multiplicity, 2.2 ± 0.3) *High-vitamin A group* [28/107] (26 %) (multiplicity, 3.1 ± 0.2)			Smith *et al.* (1975b) (contd)
Hamster, Syrian golden	M, F	48 or 90	NS (0.2% saline solution); ferric oxide, magnesium oxide	0 (untreated), 2 mg + 1 mg magnesium oxide/animal, 1×/wk, 20 wk, 3 mg + 3 mg ferric oxide/animal, 1×/wk, 15 wk	Lifespan (up to 120 wk)	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, 1 adenoSC), 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)	+		Stenbäck *et al.* (1975)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	50 untreated controls	>99% (saline, 0.5% gelatine in saline); manganese dioxide, silicon dioxide	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, 1×/wk, 20 wk	Lifespan (up to 100 wk)	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)	+		Stenbäck & Rowland (1979)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamsters, Syrian golden	M, F	40	NS (saline); ferric oxide	10 mg + 8 mg ferric oxide/animal, 1×/2wk for 10 wk to animals kept on a low- (600 IU/kg) [M only], medium- (4000 IU/kg) or high- (100 000–200 000 IU/kg) vitamin A diet	M, up to 512 days, F, up to 380 days	**Respiratory tract T** M: 16/36 (44%; 21 T: 4 epidermoid P, 11 epidermoid C, 4 epidermoid/AdC, 1 AdC, 1 S), 14/39 (36%; 20 T: 4 epidermoid P, 7 epidermoid C, 5 epidermoid/AdC, 1 AdC, 3 S), 13/37 (35%; 17 T: 6 epidermoid P, 6 epidermoid C, 3 epidermoid/AdC, 2 S) F: 14/38 (37%; 22 T: 12 epidermoid P, 1 C *in situ*, 8 epidermoid C, 1 epidermoid/AdC), 12/37 (32%; 15 T: 4 epidermoid P, 6 epidermoid C, 2 epidermoid/AdC, 3 S)	+	No control; site of respiratory tract T NS	Beems (1984)
Hamster, Syrian golden	M, F	40	NS (saline); ferric oxide	4 mg + 4 mg ferric oxide/ animal, 1×/wk, 15 wk to animals on control (low-fat diet), high-saturated fat diet or high-unsaturated fat diet	656 days for M, 512 days for F	**Respiratory tract T** M: 10/33 (30%; 2 P, 6 epidermoid C, 1 epidermoid/AdC, 1 A, 1 S), 14/32 (44%; 7 P, 6 epidermoid C, 3 epidermoid/AdC, 1 AdC, 1 A, 1 S), 17/27 (63%; 9 P, 11 epidermoid C, 3 epidermoid AdC) F: 16/40 (40%; 5 P, 9 epidermoid C, 3 epidermoid/AdC, 1 A), 20/38 (53%; 13 P, 13 epidermoid C, 1 epidermoid/AdC, 1 A, 1 S), 22/37 (59%; 12 P, 10 epidermoid C, 9 epidermoid/AdC)	+	No control; site of respiratory tract NS	Beems & van Beek (1984)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	35	NS (saline, gelatine); glass microfibres (code 104; glasswool); size distribution by number: length, 95% <20 μm, 89% <12 μm, 59% <5 μm; diameter, 25% <2 μm, 88% <1 μm, 60% <0.5 μm, 31% <0.25 μm	0, 1 mg glass fibres, 1 mg, 1 mg + 1 mg glass fibres/animal, 1×/2 wk, 52 wk	85 wk	**Respiratory tract T** M: 0/31, 0/34, 6/34 (18%; 3 tracheal P, 1 SCC, 1 S, 1 bronchial P), 3/35 (9%; 2 tracheal P, 1 S) F: 0/28, 0/30, 1/29 (3%; 1 laryngeal C *in situ*), 1/31 (3%; 1 tracheal S)	±	Statistics NS	Feron *et al.* (1985)
Hamster, Syrian golden	M, F	35	NS (0.9 % saline solution); ferric oxide	0, 8 mg + 6 mg ferric oxide/animal, 1×/wk, 6 wk	82 wk	**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)	+		Reynders *et al.* (1985)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamsters, Syrian golden	M, F	40 or 60	NS (saline); ferric oxide	8 mg + 8 mg ferric oxide, 1×/2wk, 16 wk given to animals on control (low-selenium/low-fat) diet, high-selenium/low-fat diet, low-selenium/high-fat diet or high-selenium/high-fat diet	429 days for M; 374 days for F	**Respiratory tract T** M: 34/57 (60%; 7 P, 4 C *in situ*, 15 epidermoid C, 13 epidermoid/AdC, 1 AdC, 5 lung A, 2 S), 27/38 (71%; 6 P, 1 C *in situ*, 14 epidermoid C, 8 epidermoid/AdC, 8 lung A, 1 S), 31/38 (82%; 6 P, 3 C *in situ*, 13 epidermoid C, 12 epidermoid/AdC, 1 AdC, 10 lung A, 1 S), 28/39 (72%; 4 P, 1 C *in situ*, 16 epidermoid C, 10 epidermoid/AdC,10 lung A) F: 37/57 (65%; 5 P, 2 C *in situ*, 14 epidermoid C, 19 epidermoid/AdC, 2 lung A, 3 S), 30/35 (86%; 11 P, 2 C *in situ*, 11 epidermoid C, 11 epidermoid/AdC, 4 lung A, 1 S), 28/36 (78%; 4 P, 5 C *in situ*, 11 epidermoid C, 9 epidermoid/AdC, 1 AdC, 5 lung A), 22/34 (65%; 5 P, 2 C *in situ*, 10 epidermoid C, 7 epidermoid/AdC, 1 AdC, 3 lung A)	+	No control; site of respiratory tract T NS	Beems (1986)

Table 3.15 (contd)

Species and strain	Sex	No./ group at start	Purity (vehicle); particles/fibres	Dose and duration of exposure	Duration of study	Incidence of tumours	Result[a]	Comments	Reference
Hamster, Syrian golden	M, F	40 or 60	NS (sterile saline); ferric oxide	8 mg + 8 mg ferric oxide, 1×/2wk, 16 wk given to animals on control diet or diet supplemented with 56 mg/kg carotene	429 days for M; 374 days for F	**Respiratory tract T** M: 34/57 (60%; 7 P, 4 C *in situ*, 11 epidermoid C, 13 epidermoid/AdC, 1 AdC, 5 lung A, 2 S), 26/38 (68%; 7 P, 3 C *in situ*, 10 epidermoid C, 10 epidermoid/AdC, 3 lung A, 1 S) F: 37/57 (65%; 5 P, 2 C *in situ*, 14 epidermoid C, 19 epidermoid/AdC, 2 lung A, 3 S), 25/36 (69%; 7 P, 3 C *in situ*, 10 epidermoid C, 14 epidermoid/AdC, 3 lung A, 1 S)	+	No control; site of respiratory tract T NS	Beems (1987)

A, adenoma; AdC, adenocarcinoma; AdSC, adenosquamous carcinoma; C, carcinoma; F, female; M, male; NS, not specified; P, papilloma; S, sarcoma; SCC, squamous-cell carcinoma; T, tumour; vs, versus; wk, week

[a] –, negative; +, positive; ±, equivocal

In Syrian golden hamsters given 20 weekly intratracheal instillations of a suspension of silicon dioxide mixed with benzo[*a*]pyrene in saline (3 mg benzo[*a*]pyrene + 3 mg silicon dioxide/instillation), a clearly higher incidence of respiratory tract tumours was observed than that in hamsters similarly treated with benzo[*a*]pyrene alone (23 tumours in 48 animals given the combination versus six tumours in 46 animals that received benzo[*a*]pyrene alone and no tumours in 48 saline-treated controls). Manganese dioxide did not elicit such an effect (Stenbäck & Rowland, 1979). Magnesium oxide was found to be as effective as ferric oxide as a carrier for benzo[*a*]pyrene suspended in saline in the induction of respiratory tract tumours in Syrian golden hamsters following repeated intratracheal instillation of such suspensions (Stenbäck *et al.*, 1975).

Repeated intratracheal instillation of benzo[*a*]pyrene mixed with glasswool microfibres suspended in gelatine/saline induced a few tracheal tumours in male and female Syrian hamsters; the incidence was comparable with that found after instillation of benzo[*a*]pyrene in gelatine/saline alone (Feron *et al*., 1985).

The tumour response in the respiratory tract (16/48 or 33%; mainly laryngeal and tracheobronchial papillomas and carcinomas) of Syrian golden hamsters administered 45 weekly intratracheal instillations of 850 µg atmospheric urban dust mixed with 340 µg benzo[*a*]pyrene and suspended in 2% Tween 60 in saline (total doses, 38 mg dust and about 15 mg benzo[*a*]pyrene) was not appreciably different from that (14/48 or 29%) of animals similarly treated with benzo[*a]*pyrene alone (Pott *et al*., 1973b).

Benzo[*e*]pyrene

Previous evaluation

Benzo[*e*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated two bioassays in which benzo[*e*]pyrene was administered dermally to mice (repeatedly and initiation–promotion protocols). On the basis of the available data, the Working Group concluded that benzo[*e*]pyrene was not an initiator of skin carcinogenesis in mice. Benzo[*e*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the bioassays considered previously, plus two studies in which benzo[*e*]pyrene was administered intraperitoneally to neonatal mice and one study in which it was administered by pulmonary injection into rats. All of the studies are summarized in Tables 3.2, 3.4 and 3.7. On the basis of the available studies, the Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of benzo[*e*]pyrene to experimental animals. No new studies were available.

Chrysene

Previous evaluation

Chrysene was considered in December 1972 (IARC, 1973) and February 1983 (IARC, 1983) by working groups that evaluated bioassays in which chrysene was administered dermally (repeatedly and initiation–promotion protocols) to mice, subcutaneously to mice and rats and intraperitoneally to mice. Chrysene was active when applied to mouse skin, when given subcutaneously to mice and when administered to newborn mice. On the basis of the available data, the working groups concluded that there was *limited evidence* for the carcinogenicity of chrysene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

In mouse skin initiation–promotion studies in which TPA was subsequently applied as a promoter, total initiating doses of 0–1.5 μmol chrysene applied to the skin of female CD-1 mice increased the incidence of skin papillomas (Chang *et al.*, 1983; Rice *et al.*, 1988). A total initiating dose of 1.0 mg chrysene resulted in papillomas in 92% of animals (Rice *et al*., 1985). However, female CD-1 mice initiated with a single dose of 33 nmol chrysene (Amin *et al.*, 1990), and male and female SENCAR mice initiated with a single application of 1600 nmol (Bhatt & Coombs, 1990) did not develop skin tumours.

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn male and female Swiss-Webster BLU:Ha (ICR) mice, intraperitoneal injection of chrysene at doses of 0–1.4 μmol significantly increased the incidence of lung and liver tumours in male mice (Chang *et al.*, 1983). In another study, intraperitoneal injection of chrysene at total doses of 0, 0.03 or 0.92 μmol did not increase the incidence of lung tumours in male or female mice (Busby *et al.*, 1989). In newborn male and female CD-1 mice, intraperitoneal injection of total doses of 700 nmol chrysene increased the incidence of liver adenomas and carcinomas combined in male mice (Wislocki *et al.,* 1986), and the highest dose (2800 nmol) significantly increased the incidence of liver carcinomas and lung adenomas in male mice (Wislocki *et al.,* 1986).

Intrapulmonary administration (see also Table 3.4)

Rat

Intrapulmonary implantation of 0, 1 or 3 mg chrysene into Osborne-Mendel rats induced dose-related increases (0%, 14.3% and 28.6%) in the incidence of pulmonary squamous-cell carcinomas (Wenzel-Hartung *et al.*, 1990).

Coronene

Previous evaluation

Coronene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which coronene was administered dermally to mice (repeatedly) and one initiation–promotion assay in mice. The initiation–promotion assay gave positive results; the repeated application gave negative results. On the basis of these studies, the Working Group concluded that these data were inadequate to permit an evaluation of the carcinogenicity of coronene to experimental animals. No new data were available.

4*H*-Cyclopenta[*def*]chrysene

Dermal initiation–promotion (see also Table 3.2)

In an initiation–promotion study in female CD-1 mice, an initiating dose of 1.0 mg 4*H*-cyclopenta[*def*]chrysene prior to thrice-weekly applications of TPA resulted in 100% of the animals bearing skin papillomas with an average of 5.63 papillomas/animal (Rice *et al.*, 1985). In another initiation–promotion study in female CD-1 mice, total initiating doses of 0.15, 0.5 or 1.5 μmol 4*H*-cyclopenta[*def*]chrysene prior to thrice-weekly applications of TPA resulted in increased incidences of skin papillomas (13/20 (65%), 19/19 (100%) and 19/19 (100%)) in treated mice, with averages of 1.10, 6.84 and 8.47 tumours/mouse, respectively; papillomas were observed in 4/20 (20%) of the acetone controls (Rice *et al.*, 1988).

Cyclopenta[*cd*]pyrene

Previous evaluation

Cyclopenta[*cd*]pyrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which cyclopenta[*cd*]pyrene was administered dermally to mice, results were positive in one study. It also gave positive results as an initiator in three initiation–promotion studies on mouse skin. The Working Group concluded that there was *limited evidence* for the carcinogenicity of cyclopenta[*cd*]pyrene

in experimental animals. Animal bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

Cyclopenta[*cd*]pyrene applied to the skin of female Swiss mice at doses of 0, 22.2, 66.6 or 200 nmol twice a week resulted in dose-related increases in the incidence of skin tumours (Cavalieri *et al.*, 1983).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies of newborn male and female Swiss-Webster BLU:ha (ICR) mice, intraperitoneal injection of cyclopenta[*cd*]pyrene at doses of 0, 1.55, 3.09, 4.64, 6.19 or 7.73 μmol increased the incidence of lung adenomas and adenocarcinomas (males: 8%, 62%, 56%, 86%, 77% and 89%; females: 8%, 60%, 70%, 93%, 100% and 100%) and number of tumours/animal (males: 0.08, 1.12, 2.78, 9.29, 4.08 and 7.33; females: 0.08, 2.20, 3.20, 6.71, 13.57 and 5.33) in males and females combined (Busby *et al.*, 1988). Intraperitoneal injection of male Strain A/J mice with single doses of 0, 10, 50, 100 or 200 mg/kg bw cyclopenta[*cd*]pyrene resulted in an increased incidence of lung adenomas per mouse at doses above 10 mg/kg (Nesnow *et al.*, 1994, 1995; Ross *et al.*, 1995; Nesnow *et al.*, 1998a,b).

5,6-Cyclopenteno-1,2-benzanthracene

Dermal application (see also Table 3.1)

Mouse

Groups of 10 mice [strain, sex, age and body weight unspecified] were treated dermally with solutions of 5,6-cyclopenteno-1,2-benzanthracene in benzene [volume and number of treatments unspecified]. Three groups were treated with a 0.3 % solution (two groups) or a 0.1% solution (one group) of ‘pure’ compound (purity determined by melting-point); an additional group was treated with a 0.3% solution of compound that was purified through recrystallization of the corresponding picrate. No control group was used. The time of death of the last animal in each group was 224–321 days in the group treated with 0.3% 5,6-cyclopenteno-1,2-benzanthracene and 339 days in the group treated with the 0.1% dose. Among the groups treated with the 0.3% solution, the tumour incidence was 2/10, 1/10 and 0/10 skin papillomas and 1/10, 4/10 and 8/10 skin epitheliomas (the last value in each series corresponded to the tumours obtained with the

material purified as the picrate). The group treated at the 0.1% dose developed 0/10 skin papillomas and 4/10 skin epitheliomas (Cook, 1932). A subsequent, similarly designed experiment using 0.03, 0.1 and 0.3% solutions of 5,6-cyclopenteno-1,2-benzanthracene [purity unspecified] resulted in an incidence of 1/20, 0/10 and 5/40 skin papillomas and 1/20, 5/10 and 14/40 skin epitheliomas, respectively (Barry *et al*., 1935).

Dibenz[*a*,*c*]anthracene

Previous evaluation

Dibenz[*a*,*c*]anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which dibenz[*a*,*c*]anthracene was administered dermally to mice, either *per se* or as part of initiation–promotion protocols. These studies are summarized in Tables 3.1 and 3.2, respectively. On the basis of the available data, the Working Group concluded that there was *limited evidence* that dibenz[*a*,*c*]anthracene was carcinogenic to experimental animals. Additional studies that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 39–40 female CD-1 white mice, approximately 45 days of age [body weight unspecified], received a single dermal application of 0, 25 or 50 μg dibenz[*a*,*c*]-anthracene [purified by chromatography and recrystallized] in 100 μL acetone. One week later, promotion began with twice-weekly applications of 0.64 μg TPA in 100 μL acetone for 29 weeks, followed by doses of 1 μg for the subsequent 38 weeks. Systematic post-mortem examinations were conducted on all animals and tumours were examined histologically. At the end of the experiment, 5/39 mice in the 25-μg dose group and 8/40 mice in the 50-μg dose group had developed skin papillomas compared with 3/40 mice in the TPA-treated control group (Chouroulinkov *et al*., 1983).

Subcutaneous administration (see also Table 3.3)

Mouse

Groups of 30 male and female C57BL/6J, DBA/2J and B6D2F$_1$ mice, approximately 5 weeks of age [body weight unspecified], received a single subcutaneous injection of 150 or 300 μg dibenz[*a*,*c*]anthracene [purity unspecified] in 50 μL trioctanoin. Controls in all groups (10 mice/group) received trioctanoin alone. The mice were monitored for 12 months. At the end of the experiment, no tumours were observed in any of the groups treated with 150 μg dibenz[*a*,*c*]anthracene. The incidence of subcutaneous fibrosarcoma

in the 300-μg dose groups was 1/30, 0/30 and 1/30 for C57BL/6, DBA/2 and B6D2F_1 mice, respectively. Tumour incidences were not reported for the control groups (Kouri *et al*., 1983).

Intraperitoneal administration (see also Table 3.7)

Mouse

A group of 24 newborn male B6C3F_1 mice received intraperitoneal injections of a total dose of 400 nmol [111 μg] dibenz[*a*,*c*]anthracene (purity >99% by HPLC) in 35 μL DMSO [1/7, 2/7 and 4/7 of the dose on days 1, 8 and 15 of life, respectively]. The mice were monitored for 12 months. At the end of the experiment, necropsies were performed and liver and lung masses were subjected to histology. The incidence of liver tumour was 9/24 (adenomas) dibenz[*a*,*c*]anthracene-treated mice compared with 2/24 (adenomas) and 1/24 (carcinoma) solvent-treated control mice. Lung tumours were not observed in either of the two groups (von Tungeln *et al*., 1999b).

Dibenz[*a,h*]anthracene

Previous evaluations

Dibenz[*a,h*]anthracene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenz[*a*,*h*]anthracene was tested by oral administration to mice, dermal application in mice and hamsters, by intratracheal injection into hamsters, by intramammary injection into rats, by subcutaneous injection into mice, rats and guinea-pigs, by intramuscular injection into pigeons and chickens, by intravenous injection into mice, pulmonary administration to mice and rats and by intrarenal injection into frogs. These studies are summarized in Tables 3.1–3.4, 3.6, 3.9, 3.10, 3.12 and 3.14. On the basis of the available data, the Working Group concluded that dibenz[*a*,*h*]anthracene was carcinogenic in experimental animals. Dibenz[*a,h*]anthracene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenz[*a,h*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal application (see also Table 3.1)

Mouse

Groups of 50 female NMRI mice [age unspecified] were treated three times a week with 17 μL acetone that contained 0.1, 0.4 or 1.1 μg dibenz[*a,h*]anthracene (>99 % pure

by gas chromatography (GC) and HPLC) for 112 weeks (total doses, 37.8, 125 and 378 μg). A control group of 50 female mice was treated with acetone alone. In the treated groups, skin tumours were observed in 3/50 low-dose, 4/50 mid-dose and 16/50 high-dose mice; 2/48 mice treated with acetone developed skin tumours (Platt *et al*., 1990).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 16 female NMRI mice [age unspecified] received a single dermal application of 100 μL acetone that contained 83.5 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC) or 100 μL tetrahydrofuran that contained 167 μg dibenz[*a*,*h*]-anthracene. A control group of 30 female mice was treated with 100 μL acetone alone. One week later, all mice received twice weekly dermal applications of 10 nmol TPA in 100 μL acetone. The experiment was terminated after 24 weeks. In treated groups, skin tumours were observed in 6/16 (38%) low-dose and 15/16 (93%) high-dose mice; no skin tumour was observed in mice treated with acetone alone (Platt *et al*., 1990).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 50 male and female C57BL mice [age unspecified] received a single subcutaneous injection of 20 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 500 μL tricaprylin. Mice surviving more than 4 months were included in the final tumour count. A control group of 304 mice was treated with tricaprylin alone. The experiment was continued for 22 months. Sarcomas occurred in 28/48 mice treated with dibenz[*a*,*h*]-anthracene compared with 3/280 control mice (Steiner & Falk, 1951).

Groups of 100 female NMRI mice [age unspecified] received a single subcutaneous injection of 2.35, 4.7, 9.3, 18.7, 37.5 or 75.0 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 500 μL tricaprylin. A control group of 100 mice was available. The experiment was continued for 114 weeks. The incidence of sarcomas was 37/100, 39/100, 44/100, 56/100, 65/100 and 69/100 in the groups treated with 2.35, 4.7, 9.3, 18.7, 37.5 and 75.0 μg dibenz[*a*,*h*]anthracene, respectively. The incidence in the control group was not reported (Pfeiffer, 1977).

Groups of male and female B6 (30 mice), D2 (30 mice) or B6D2F_1 (57 or 60) mice [age unspecified] received a single subcutaneous injection of 150 or 300 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 50 μL tricaprylin. Masses >1 cm were considered to be positive. Control mice (10 per group) were treated with tricaprylin alone. The experiment was terminated after 12 months. In the treated groups, the incidence of fibrosarcomas was 16/30 low-dose and 14/30 high-dose B6 mice; 1/30 low-dose and 0/30

high-dose D2 mice; and 8/57 low-dose and 33/60 high-dose B6D2F$_1$ mice. The tumour incidence in the control group was not reported (Kouri *et al*., 1983).

Groups of 30 male C3H/HeJ, C57BL/6J, AKR/J and DBA/J2 mice [age unspecified] received single subcutaneous injections of 150 μg dibenz[*a*,*h*]anthracene [purity unspecified] in 50 μL trioctanoin. Control groups of 10 mice were treated with the solvent alone. The experiment was continued for 9 months. The incidence of fibrosarcomas in the treated groups was 24/30 C3H/HeJ, 16/30 C57BL/6J, 0/30 AKR/J and 1/30 DBA/J2 mice. No fibrosarcomas were observed in the control groups (Lubet *et al*., 1983).

Groups of 50 female NMRI mice [age unspecified] received single subcutaneous injections into the interscapular region of 500 μL tricaprylin that contained 10, 11, 30, 86 or 112 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC). Two control groups of 50 female mice were treated with tricaprylin alone. Masses >1 cm were considered to be positive. The experiment was terminated after 112 weeks. The incidence of fibrosarcomas in dibenz[*a*,*h*]anthracene-treated groups was: 10 μg, 25/48; 11 μg, 16/47; 30 μg, 25/50; 86 μg, 31/49; and 112 μg, 38/48; the incidence in controls was 3/49 and 1/50 (Platt *et al*., 1990).

Groups of 40 male and 44 female newborn NMRI mice, 2 days of age, received a single subcutaneous injection of 50 μL of an aqueous solution (1% gelatine, 0.9% saline, 0.4% Tween 20) that contained 11 or 111 μg dibenz[*a*,*h*]anthracene (>99 % pure by GC and HPLC). A control group of 49 male and female mice was treated with the solvent alone. In the treated groups, the incidence of pulmonary tumours was 6/13 (tumour multiplicity, 3.3 tumours/mouse) low-dose and 16/17 (30.6 tumours/mouse) high-dose females and 6/22 (3.8 tumours/mouse) low-dose and 19/21 (27.3 tumours/mouse) high-dose males. The incidence in controls was 1/19 (1.0 tumours/mouse) females and 1/14 (2.0 tumours/mouse) males (Platt *et al*., 1990).

Rat

Groups of 12 female Sprague-Dawley rats, 30 days of age, received thrice weekly subcutaneous injections for a total of 20 times of 100 μL sesame oil:DMSO (9:1) that contained 300 μg dibenz[*a*,*h*]anthracene (single peak by GC/mass spectrometry (MS) and HPLC). A control group of 12 rats was treated with the solvent alone. The experiment was continued for 37 weeks. The incidence of sarcomas in rats treated with dibenz[*a*,*h*]-anthracene was 12/12 compared with 0/12 in the solvent-treated controls (Flesher *et al*., 2002).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 20 male A/J mice, 5–6 weeks of age, received single intraperitoneal injections of 1.25, 2.5, 5 or 10 mg/kg bw dibenz[*a*,*h*]anthracene (purity, 97%) in 100 or

200 μL tricaprylin. A control group of 20 mice was treated with the solvent alone. The experiment continued for 8 months (survival, >90%), at which time gross lung adenomas were counted. The incidence of lung adenoma was 100% at doses above 2.5 mg/kg dibenz[*a*,*h*]anthracene. Tumour multiplicity (adenomas/mouse) was 1.44, 3.05, 13.1 and 32.1 at 1.25, 2.5, 5 and 10 mg/kg dibenz[*a*,*h*]anthracene, and was significantly different ($p < 0.05$) from the solvent-treated control (0.6 lung adenomas/mouse) at doses above 1.25 mg/kg (Nesnow *et al*., 1995; Ross *et al*., 1995; Nesnow *et al*., 1998a,b).

Intrapulmonary administration (see also Table 3.4)

Rat

Groups of 35 inbred female Osborne-Mendel rats [age unspecified] received a single pulmonary implantation of 100 μg dibenz[*a*,*h*]anthracene (purity, 99.3%) in a mixture of beeswax and tricaprylin or the solvent alone. The animals were monitored for 123 weeks. Lung carcinomas were found in 20 rats administered dibenz[*a*,*h*]anthracene, but not in solvent-treated control rats (Wenzel-Hartung *et al*., 1990).

Intramammary administration (see also Table 3.5)

Rat

Groups of 20 female Sprague-Dawley rats, 50 days of age, received a single intramammary injection of 1.1 or 4.5 mg finely powdered dibenz[*a*,*h*]anthracene (purity >99%). The untreated contralateral breast served as the control. All rats were killed after 20 weeks. No tumours were observed in either the treated or contralateral control mammary glands (Cavalieri *et al*., 1988a).

Intravenous administration (see also Table 3.9)

Mouse

A group of equal numbers of male and female strain A mice [age unspecified] received a single intrapulmonary injection of 250 μg dibenz[*a*,*h*]anthracene (melting-point, 266.0–266.8 °C) suspended in 250 μL water. The control group received the solvent alone. Twenty weeks after treatment, the incidence of pulmonary tumours was 10/10 (30.5 tumours/mouse) in the experimental group compared with 4/19 (1.0 tumours/mouse) in the controls (Andervont & Shimkin, 1940; Shimkin & Stoner, 1975).

Intratracheal injection (see also Table 3.12)

Hamster

Groups of 48 male Syrian golden hamsters [age unspecified] received a total of 30 weekly intratracheal administrations of 50 or 250 μg dibenz[*a*,*h*]anthracene (purity >99% by thin-layer chromatography; TLC) mixed with an equal amount of haematite dust (Fe_2O_3) in 200 μL saline and were monitored for 120 weeks. The incidence of respiratory tract tumours was 0/46 low-dose animals and 2/46 high-dose animals. No respiratory tract tumour was observed in 82 untreated controls (Sellakumar & Shubik, 1974).

Groups of Syrian golden hamsters [initial number, sex and age not specified] received weekly intratracheal injections of 300 or 900 μg dibenz[*a*,*h*]anthracene in a saline solution that contained 0.4% Tween 80 for 20 weeks. Control animals were treated with the solvent alone. The incidence of respiratory tract tumours was 55% in low-dose animals, 65% in high-dose animals and 3% in animals treated with the solvent (Pott *et al*., 1978).

Dibenz[*a,j*]anthracene

Previous evaluation

Dibenz[*a,j*]anthracene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which dibenz[*a,j*]anthracene was administered dermally and subcutaneously to mice. These studies are summarized in Tables 3.1 and 3.3. On the basis of the available data, the Working Group concluded that there was *limited evidence* that dibenz[*a,j*]anthracene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 400 or 800 nmol [111 or 223 μg] benz[*a,j*]anthracene [purity not specified] in 200 μL acetone. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA [solvent unspecified] twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence [no histology] was 70% (1.3 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]-anthracene and 97% (3.0 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]-anthracene compared with 19% (0.19 papillomas/mouse) in acetone-treated control mice (Sawyer *et al.*, 1987).

Groups of 24 shaved female SENCAR mice [age unspecified] received a single dermal application of 400 nmol [111 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA in 200 μL acetone twice a week for 14 weeks. After 14 weeks of promotion, the skin tumour incidence (histology on random samples) was 29% (0.58 papillomas/mouse) in mice treated with dibenz[*a,j*]anthracene compared with 5% (0.05 papillomas/mouse) in the tetrahydrofuran-treated control mice (p <0.05) (Harvey *et al*., 1988).

Groups of 30 shaved female SENCAR mice [age unspecified] received a single dermal application of 400 or 800 nmol [111 or 223 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence [no histology] was 70% (1.27 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]-anthracene and 97% (3.00 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]-anthracene compared with 16% (0.16 papillomas/mouse) in acetone-treated control mice (Sawyer *et al*., 1988). [The Working Group noted that these appear to be the same data as those given in Sawyer *et al*. (1987).]

Groups of 24 shaved female SENCAR mice [age unpecified] received a single dermal application of 400 or 800 nmol [111 or 223 μg] dibenz[*a,j*]anthracene [purity unspecified] in 200 μL peroxide-free tetrahydrofuran. Control mice were treated with the solvent alone. Two weeks later, all mice were administered 3.4 nmol [2.1 μg] TPA twice a week for 20 weeks. After 20 weeks of promotion, the skin tumour incidence was 39% (0.86 papillomas/mouse) in mice treated with 400 nmol dibenz[*a,j*]anthracene and 65% (1.83 papillomas/mouse) in those treated with 800 nmol dibenz[*a,j*]anthracene compared with 5% (0.05 papillomas/mouse) in the tetrahydrofuran-treated control mice (Sawyer *et al*., 1988).

Dibenzo[*a,e*]fluoranthene

Previous evaluation

Dibenzo[*a,e*]fluoranthene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one study in which dibenzo[*a,e*]fluoranthene was applied dermally to mice and one initiation–promotion study in mice. Both studies gave positive results. On the basis of these data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of dibenzo[*a,e*]fluoranthene in experimental animals. No new studies were available.

13*H*-Dibenzo[*a,g*]fluorene

Dermal application (see also Table 3.1)

Rat

In two experiments that explored the anti-carcinogenic activity of certain organic compounds, a group of 10 male and 10 female CF1 mice, 12 weeks of age, received dermal applications of one drop (0.02 mL) of a 0.3% solution of 13*H*-dibenzo[*a,g*]-fluorene [purity unspecified] [~ 60 µg] in acetone on the shaved interscapular region twice a week for 31 weeks. The experiments were terminated after 48 weeks. In one of the two experiments, 6/20 animals (30%) developed skin tumours (six squamous-cell carcinomas and one sarcoma). In the other experiment, 9/20 animals (45%) had skin tumours (nine squamous-cell carcinomas and two sarcomas) (Riegel *et al*., 1951). [The Working Group noted the absence of controls.]

Dibenzo[*h,rst*]pentaphene

Previous evaluation

Dibenzo[*h,rst*]pentaphene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated one bioassay in mice that were injected subcutaneously with dibenzo[*h,rst*]pentaphene, this is summarized in Table 3.3. On the basis of this study, another Working Group (IARC, 1987) concluded that there was *limited evidence* in experimental animals for the carcinogenicity of dibenzo[*h,rst*]pentaphene. No new studies were available.

Dibenzo[*a,e*]pyrene

Previous evaluations

Dibenzo[*a,e*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*e*]pyrene was administered dermally and subcutaneously to mice. These studies, which are summarized in Tables 3.1 and 3.3, indicated the induction of skin papillomas and epitheliomas after dermal application and sarcomas after subcutaneous injection. Dibenzo[*a*,*e*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*e*]-pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female Ha/ICR/Mil (Swiss albino) mice [age and body weight unspecified] received 10 dermal applications of 25 μL 0.1% dibenzo[*a*,*e*]pyrene [25 μg; recrystallized and chromatographically pure; purity confirmed by determination of melting-point] in dioxane at 2-day intervals [total dose, 250 μg/animal]. On day 28, dermal applications of 2.5% (2.3 mg) croton oil in acetone [volume, number, frequency and duration of treatments unspecified] were begun. A group of 30 mice was treated with croton oil solution alone. All survivors were killed after 6 months, at which time mortality rates were 2/28 and 4/30 and tumour incidence (skin papillomas) was 10/28 and 2/30 in the dibenzo[*a*,*e*]pyrene-treated and croton oil control groups, respectively. The total number of papillomas/mouse was 0.83 and 0.1 in the dibenzo[*a*,*e*]pyrene-treated and croton oil control mice, respectively (Hoffmann & Wynder, 1966; LaVoie *et al.*, 1979).

A group of 21 female SENCAR mice, 8 weeks of age, [weight unspecified] received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*e*]pyrene (purity >99% by HPLC) in 100 μL dioxane/DMSO (75:25). A vehicle control group was treated with 100 μL dioxane/DMSO alone. One week later, all mice were treated with 4.26 nmol (2.6 μg) TPA in 100 μL acetone twice a week for 25 weeks and were killed after the 25th week of promotion. Complete necropsies were performed. At the end of the experiment, 5/21 mice in the dibenzo[*a*,*e*]pyrene-treated group had developed seven skin papillomas (0.3 papillomas/mouse) and 2/23 mice in the control group had developed one skin papilloma (0.1 papilloma/mouse). The first skin papillomas appeared after 15 weeks in the dibenzo[*a*,*e*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

Intramamillary administration (see also Table 3.5)

Rat

A group of 19 female Sprague-Dawley rats, 8 weeks of age, [weight unspecified] received a single intramamillary injection of 4 μmol (1.2 mg)/gland dibenzo[*a*,*e*]pyrene (purity >99% by HPLC) dissolved in 100 μL trioctanoin (total dose, 32 μmol [9.6 mg]). One control group of 21 rats was treated with 100 μL solvent and another control group of 20 rats remained untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter; all the remaining animals were killed at 40 weeks. Complete necropsies were performed. Survival rates (mean ± SD) were 38 ± 0, 37 ± 4 and 40 ± 0 weeks in the dibenzo[*a*,*e*]pyrene-treated, untreated and vehicle-control groups, respectively, and tumour latencies were 35 ± 0 and 25 ± 13 weeks in the dibenzo[*a*,*e*]pyrene-treated and untreated groups, respectively. At the end of the study, 1/19 rats in the dibenzo[*a*,*e*]pyrene-treated group and 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma in the

dibenzo[*a*,*e*]pyrene-treated rat; one adenofibroma and one adenocarcinoma in the untreated rats). No tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Dibenzo[*a,h*]pyrene

Previous evaluations

Dibenzo[*a,h*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*h*]pyrene was administered dermally and subcutaneously to mice and by intramuscular injection to rats. These studies are summarized in Tables 3.1, 3.3 and 3.10. On the basis of the available data, the Working Group concluded that dibenzo[*a*,*h*]pyrene was carcinogenic in experimental animals. Dibenzo[*a*,*h*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*h*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or were not considered in an earlier monograph are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 40 random-bred female Swiss mice [age unspecified] received dermal applications of 119 μg dibenzo[*a*,*h*]pyrene (purity, 99.6%) in 16.7 μL acetone twice weekly for 30 weeks. A control group of 40 mice was treated with acetone alone. Tumours were recorded when they persisted for 4 weeks or more. All dibenzo[*a*,*h*]-pyrene-treated mice had died or were removed by 45 weeks and 35/39 had skin tumours (papillomas, keratoacanthomas and carcinomas). No skin tumours were observed in 29 control mice after a period of 70 weeks (Cavalieri *et al*., 1977).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 31 female CD-1 mice [age unspecified] received a single dermal application of 200 μg dibenzo[*a*,*h*]pyrene [purity unspecified] in acetone. A control group of 32 mice was treated with acetone alone. One week later, all mice received twice weekly dermal applications of 10 μg TPA for 26 weeks. Skin tumours occurred in 26/28 mice treated with dibenzo[*a*,*h*]pyrene compared with 2/32 solvent-treated control mice (Sardella *et al*., 1981).

Groups of 30 female CD-1 mice, 7–8 weeks of age, received a single dermal application of 15 μg (50 nmol) dibenzo[*a*,*h*]pyrene ('essentially' pure by chromatography, mass spectrometry (MS) and nuclear magnetic resonance (NMR)) in 200 μL 10% DMSO in tetrahydrofuran or the solvent alone. Seven days later, all mice received twice-weekly applications of 16 nmol TPA in 200 μL acetone. Papillomas >2 mm in diameter that persisted for ≥2 weeks were included in the cumulative total. After 16 weeks of TPA promotion, 55% of the dibenzo[*a*,*h*]pyrene-treated mice had papillomas (1.41 tumours/mouse) compared with 0% in the solvent-treated controls; after 24 weeks of TPA promotion, the respective values were 72% (3.97 tumours/mouse) and 0%. In a second experiment, groups of 30 female CD-1 mice received a single dermal application of either 60 μg (200 nmol) or 180 μg (600 nmol) dibenzo[*a*,*h*]pyrene followed by applications of TPA. After 16 weeks of TPA promotion, papillomas had occurred in 79% (4.72 tumours/mouse) and 72% (5.52 tumours/mouse) low- and high-dose animals, respectively, compared with 10% (0.01 tumours/mouse) of the solvent-treated controls (Chang *et al*., 1982).

A group of 24 female SENCAR mice, 8 weeks of age, received a single dermal application of 240 μg (800 nmol) dibenzo[*a*,*h*]pyrene (purity >99% by HPLC) in 100 μL dioxane: DMSO (75:25); a control group of 23 mice was treated with the solvent alone. One week later, all mice received twice weekly dermal applications of 4.26 nmol TPA in 100 μL acetone for 25 weeks. Papillomas occurred in 18/24 mice treated with dibenzo[*a*,*h*]pyrene (5.3 tumours/mouse) compared with 2/23 solvent-treated control mice (0.1 tumours/mouse) (Cavalieri *et al*., 1989).

Intraperitoneal administration (see also Table 3.7)

Mouse

60 Newborn male and female Swiss-Webster mice (BLU:Ha(ICR)) received intraperitoneal injections on days 1, 8 and 15 of life of 3.8, 7.6 and 15.2 μg dibenzo[*a*,*h*]-pyrene ('essentially' pure by chromatography, MS and NMR) in 5, 10 and 20 μL DMSO. Control mice were treated with the solvent alone. The experiment continued until the mice were 49–54 weeks old. Pulmonary tumours occurred in 13/14 treated females (4.78 tumours/mouse), 25/25 treated males (5.20 tumours/mouse), 11/39 control females (0.44 tumours/mouse) and 7/32 control males (0.80 tumours/mouse). Hepatic tumours occurred in 1/14 treated females (0.07 tumours/mouse) and 11/25 treated males (0.88 tumours/mouse), but not in solvent-treated controls. Two female mice treated with dibenzo[*a*,*h*]pyrene had skin sarcomas and one had an adenocarcinoma of the small intestine (Chang *et al*., 1982).

Intramamillary administration (see also Table 3.5)

Rat

A group of 20 female Sprague-Dawley rats, 8 weeks of age, received a single intramamillary injection of 1.2 μg (4 μmol)/gland dibenzo[*a*,*h*]pyrene (purity >99% by HPLC) in 100 μL tricaprylin (total dose, 9.6 μg for eight glands). A group of 21 control rats was treated with tricaprylin alone. Animals were killed when tumours were ≥2 cm in diameter. The experiment lasted 40 weeks. Fibrosarcomas occurred in 19/20 rats treated with dibenzo[*a*,*h*]pyrene compared with 0/21 control rats. Mammary gland adenofibromas and adenocarcinomas occurred in 4/20 rats treated with dibenzo[*a*,*h*]pyrene compared with 0/21 control rats (Cavalieri *et al.*, 1989).

Dibenzo[*a*,*i*]pyrene

Previous evaluations

Dibenzo[*a*,*i*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated bioassays in which dibenzo[*a*,*i*]pyrene was administered dermally to mice and subcutaneously to mice and hamsters. The transfer of injection-site tissues following subcutaneous injection to mice was also analysed. These studies, which are summarized in Tables 3.1 and 3.3, indicated the induction of skin papillomas and epitheliomas [the Working Group considered these tumours to be squamous-cell carcinomas] after repeated dermal application and sarcomas after subcutaneous injection; in addition, it was shown that transfer of injection-site tissues to secondary hosts shortened the latent period. Dibenzo[*a*,*i*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that dibenzo[*a*,*i*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time or that were not considered in an earlier monograph are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female Ha/ICR Swiss albino mice, 50–55 days of age [body weight unspecified], received 10 dermal applications of dibenzo[*a*,*i*]pyrene (95% pure by HPLC) in 100 μL acetone once on alternate days (total doses, 100 μg and 500 μg) or acetone alone. Ten days after initiation had been completed, all animals received thrice-weekly applications of 2.5 μg TPA in 100 μL acetone for 20 weeks. Tumours were counted weekly and at autopsy, and were examined histologically; however, skin tumour types were not reported. Mice treated with 100 μg dibenzo[*a*,*i*]pyrene had a 40% skin tumour

incidence (average of 0.5 skin tumours/mouse), whereas the group treated with 500 μg dibenzo[*a*,*i*]pyrene had an 85% skin tumour incidence (average of 5.8 skin tumours/mouse). No skin tumours were observed in the vehicle-control group (Hecht *et al.*, 1981).

Groups of 30 female CD-1 mice, 7–8 weeks of age [body weight unspecified], were treated with a single dermal application of 15, 60 or 180 μg dibenzo[*a*,*i*]pyrene ('essentially pure' on the basis of chromatography, MS and NMR) in 200 μL 10% DMSO in tetrahydrofuran or the solvent alone. One week later, the mice received twice-weekly applications of 10 μg TPA in 200 μL acetone. Papillomas >2 mm in diameter were included in the cumulative total when they persisted for ≥2 weeks. After a 16-week period of promotion, skin tumour incidence was 28, 67 and 79% in the low-, mid- and high-dose groups, respectively. The corresponding numbers of skin papillomas/mouse [no histology] were 0.52, 5.33 and 5.25. In a separate experiment, promotion with TPA was continued for 24 weeks in a group of 30 female mice previously treated with 15 μg dibenzo[*a*,*i*]pyrene; skin tumour incidence was 69%, and the number of skin papillomas/mouse was 2.07 ± 0.44 (mean ± SE). No skin tumours were observed in the solvent control groups for both 15-μg dose groups; the skin tumour incidence was 10% (0.10 papillomas/mouse) in the controls for the 60- and 180-μg dose groups (Chang *et al.*, 1982).

Groups of 24 female SENCAR mice, 8 weeks of age [body weight unspecified], received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*i*]pyrene (purity >99% by HPLC) in 100 μL dioxane:DMSO (75:25) or dioxane:DMSO only. One week later, all mice were treated with TPA (4.26 nmol [2.6 μg]/100 μL acetone] twice a week for 25 weeks, and were killed after the 25th week of promotion. At the end of the experiment, 15/24 mice in the dibenzo[*a*,*i*]pyrene-treated group had developed 63 skin papillomas (2.6 papillomas/mouse) and 2/23 mice in the control group had developed a skin papilloma (0.1 papillomas/mouse). The first skin papillomas appeared after 12 weeks in the dibenzo[*a*,*i*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

Subcutaneous administration (see also Table 3.3)

Mouse

A group of 50 mice [strain, sex, age and weight unspecified] received a single subcutaneous injection of 100 μg dibenzo[*a*,*i*]pyrene [purity unspecified] in tricaprylin [volume unspecified]. A control group of 25 mice was treated with tricaprylin alone. Survival rates after 75 weeks were 41/50 dibenzo[*a*,*i*]pyrene-treated animals and 24/25 controls. Tumour incidences (local sarcomas) at 75 weeks were 40/50 treated mice and 0/25 controls, respectively (Sardella *et al.*, 1981).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of newborn male and female Swiss-Webster [BLU:Ha(ICR)] mice received three intraperitoneal injections of 12.5, 25 and 50 nmol (3.8, 7.6 and 15.1 μg) dibenzo[*a,i*]pyrene ('essentially pure' on the basis of chromatography, MS and NMR) dissolved in 5, 10 and 20 μL DMSO, respectively, on days 1, 8 and 15 of life (total dose, 87.5 nmol [26.5 μg]). Control mice received injections of DMSO alone. The mice were weaned at 25 days of age, and killed at 49–54 weeks. At the end of the experiment, the incidence of pulmonary tumours was 38/39 (3.64 tumours/mouse) treated males, 7/32 (0.80 tumours/mouse) control males, 21/21 (5.80 tumours/mouse) treated females and 11/39 (0.44 tumours/mouse) control females. In addition, hepatic tumours were observed in 21/39 treated males [0.82 tumours/mouse], but not in the other groups. A representative number of pulmonary tumours and all hepatic tumours were examined histologically. Most of the hepatic tumours were type A or neoplastic nodules (Chang *et al*., 1982).

Intratracheal administration (see also Table 3.12)

Hamster

A group of 36 male Syrian golden hamsters, 9–10 weeks of age and weighing approximately 98 g, received four weekly intratracheal doses of 2 mg dibenzo[*a,i*]pyrene (purity > 99% by TLC; total dose, 8 mg), ground to a finely aggregated dust (1:1, w:w) with haematite (particle size <1 μm) and then suspended in 200 μL saline (0.9% aqueous). A second group of 48 male hamsters of the same strain and age was treated similarly with 24 weekly doses of 500 μg dibenzo[*a,i*]pyrene (total dose, 12 mg), and an additional group of 90 hamsters was untreated. No control group received intratracheal instillation of the vehicle. The animals were monitored daily, weighed once a week and died spontaneously or were killed when moribund. At 100 weeks, all treated animals and 71/90 controls had died. Respiratory insufficiency, due to extensive tumour involvement in the respiratory tract, accounted for the increased mortality rates in the treated groups. In the group treated with four doses of 2 mg dibenzo[*a,i*]pyrene, the incidence of tumours of the respiratory tract was 16/34; specific incidences were one tumour of the larynx, two of the trachea, 13 of the bronchi and one of the lung; no tumours were found at other sites. In the group treated with 24 doses of 500 μg dibenzo[*a,i*]pyrene, the incidence of tumours of the respiratory tract was 39/44; specific incidences were six tumours of the trachea, 37 of the bronchi and one of the lung. In addition, two malignant lymphomas occurred in this group. A total of 19 treatment-induced respiratory tract tumours developed in the group treated with four doses of 2 mg, and a total of 95 tumours in the group treated 24 times with 500 μg; squamous-cell carcinomas were the predominant histological type. No

tumours of the respiratory tract were observed in the untreated group, which had an incidence of 11/82 tumours at other sites (Sellakumar & Shubik, 1974).

Two groups of 24 male and 24 female Syrian golden hamsters, 6–7 weeks of age [weight unspecified], were treated intratracheally with dibenzo[*a*,*i*]pyrene (purity >99% by TLC) finely suspended in distilled water (particle size <25 μm). One group received 1 mg once a week for 12 weeks (total dose, 12 mg) and the other group received 500 μg once a week for 17 weeks (total dose, 8.5 mg). No control group was available. Animals were monitored weekly and those in poor condition were isolated and allowed to die spontaneously or were killed when moribund [follow-up time unspecified]; death was most frequently attributed to respiratory insufficiency due to extensive neoplastic involvement of the respiratory system. The incidence of respiratory tumours (males and females combined) was 36/48 in the group that received 12 doses of 1 mg and 39/48 in the group that received 17 doses of 500 μg; the earliest tumours appeared in the larynx and trachea at 8 weeks. Main bronchi tumours (62% at the 12 × 1-mg dose level and 82% at the 17 × 500-μg dose level) predominated, followed by tracheal tumours (19% at the 12 × 1-mg dose level and 13% at the 17 × 500-μg dose level). Tumours of the larynx, lung and pleura were observed at lower incidences. Morphologically, the most common tumours were squamous-cell carcinomas (Stenbäck & Sellakumar, 1974).

Intramamillary administration (see also Table 3.5)

Rat

A group of approximately 20 female Sprague-Dawley rats, 8 weeks of age, [weight unspecified], received a single intramamillary injection of 4 μmol (1.2 mg)/gland dibenzo[*a*,*i*]pyrene (purity >99% by HPLC) dissolved in 100 μL tricaprylin (eight glands; total dose, 32 μmol [9.6 mg]). One control group was treated with 100 μL solvent and another was untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter. All the remaining animals were killed at 40 weeks and complete necropsies were performed. Survival rates (mean ± SD) were 30 ± 5, 37 ± 4 and 40 ± 0 weeks in the dibenzo[*a*,*i*]pyrene-treated, untreated and vehicle-control groups, respectively. Tumour latencies were 19 ± 2 and 25 ± 13 weeks in the dibenzo[*a*,*i*]pyrene-treated and untreated groups, respectively. At the end of the study, 18/19 rats in the dibenzo[*a*,*i*]pyrene-treated group had developed fibrosarcomas (2.4 tumours/tumour-bearing rat), 11/19 rats had mammary adenocarcinomas (1.4 tumours/tumour-bearing rat) and 1/19 had mammary adenofibromas (two tumours). In contrast, 2/20 rats in the untreated group had developed mammary epithelial tumours (one adenofibroma and one adenocarcinoma) but no fibrosarcomas. No tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Intrauterine administration (see Table 3.14)

Mouse

A group of 30 female mice [strain, age and weight unspecified] received a single intrauterine injection of 0.5 mg dibenzo[*a,i*]pyrene [vehicle, purity and volume unspecified]. After 32 weeks, none of the 30 treated animals developed tumours (Homburger & Tregier 1960).

Dibenzo[*a,l*]pyrene

Previous evaluations

Dibenzo[*a,l*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that restricted its evaluation to work published after 1966, since earlier data reported for dibenzo[*a,l*]pyrene had in fact been obtained from experiments that used dibenzo[*a,e*]fluoranthene. A single study was analysed that showed the induction of sarcomas following subcutaneous administration of dibenzo[*a,l*]pyrene to mice (Table 3.3). Dibenzo[*a,l*]pyrene was also assessed in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassay as that considered previously, and an additional study of dermal application to mice that resulted in the induction of skin tumours (see Table 3.1). On the basis of these data, the Working Group concluded that there was *sufficient evidence* that dibenzo[*a,l*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Oral administration (see also Table 3.6)

Fish

A group of 65 Japanese medaka fish (*Oryzias latipes*), 2 months of age, was fed a diet containing 100 ppm dibenzo[*a,l*]pyrene (purity >97% by HPLC; dissolved in menhaden oil before mixing) *ad libitum* once a day on 5 days per week for 28 days, followed by basal diet for an additional 9 months. A control group of 75 fish was fed basal diet only. At the end of the experiment, tumour incidence was 17/65 (26%) in the dibenzo[*a,l*]pyrene-treated group compared with 6/75 (8%) in the control group (p <0.05). Hepatic neoplasia (12/65 (18%) versus 0/75; p <0.001) predominated, followed by hepatocellular carcinoma (7/65 (11%) versus 0/75). Other types of neoplasia observed included bile duct adenoma, ovarian dysgerminoma, testicular neurofibrosarcoma and paravertebral ganglioneuroma (1/65 (1.5%), 2/65 (3%), 0/65 and 1/65 (1.5%), respectively, compared with 0/75, 5/75 (7%), 1/75 (1%) and 0/75 in the control group) (Reddy *et al*., 1999a).

A group of 260 Shasta strain rainbow trout (*Oncorhynchus mykiss*), 19 weeks of age, was fed a diet containing 500 ppm dibenzo[*a,l*]pyrene [purity unspecified] for 2 weeks

and then returned to basal diet for 11 months. Since the 500-ppm dose induced a high mortality rate [level unspecified] at the end of the treatment period, a second group of 260 fish, 21 weeks of age, was fed a diet containing a lower dose of dibenzo[*a*,*l*]pyrene (200 ppm) for an extended exposure period (4 weeks); this group was then fed basal diet for 9 months. Control groups were fed basal diet only. At the end of the study, tumour incidence in the 500-ppm group was 61% (liver), 91% (stomach) and 53% (swimbladder) (2.58, 5.67 and 2.25 tumours/tumour-bearing fish, respectively). The corresponding numbers in the 200-ppm group were 36% (liver), 48% (stomach) and 30% (swimbladder) (2.00, 3.80 and 2.40 tumours/tumour-bearing fish, respectively). No tumours were observed in the control groups. Stomach and swimbladder tumours were papillary adenomas in both dose groups. Among the liver tumours, hepatocellular carcinomas (64% relative incidence) predominated in the 500-ppm dose group. In the 200-ppm dose group, the relative incidences of hepatocellular carcinomas (43%) and adenomas (44%) were comparable. Cholangiocellular adenomas and mixed hepato/cholangiocellular carcinomas were also observed, at lower incidences, in both groups (Reddy *et al*., 1999b).

Dermal application (see also Table 3.1)

Mouse

Groups of 22–24 female SENCAR mice, 8 weeks of age, were treated with repeated applications of 1, 4 or 8 nmol [0.3, 1.2 or 2.4 μg] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL acetone twice a week for 40 weeks. The highest dose was the maximum dose tolerated without the development of erythema. A control group of 27 mice was treated with 100 μL acetone alone. Mice were killed when tumours reached 2 cm in diameter. All surviving mice were killed by 48 weeks, and complete necropsies were performed; all tumours were histologically verified. At the end of the study, skin tumour incidence was 1/24 (three squamous papillomas) low-dose group, 19/23 (82%; 16/23 carcinomas; 1.8 tumours/tumour-bearing animal; 9/23 squamous papillomas; 1.9 tumours/tumour-bearing animal; and 2/23 sebaceous gland adenomas; 1.5 tumours/tumour-bearing animal) mid-dose group and 20/22 (90%; 20/22 carcinomas; 2.6 tumours/tumour-bearing animal; 16/22 squamous papillomas; 1.9 tumours/tumour-bearing animal; and 3/22 sebaceous gland adenomas; 1.7 tumours/tumour-bearing animal) high-dose group. In contrast, skin tumours were not observed in the control group. In addition, tumours were observed at other sites (totals of 5/24 (21%), 9/23 (39%) and 15/22 (68%) low-, mid- and high-dose animals and 1/27 (4%) controls). These tumours included lung adenomas, malignant lymphomas of the spleen and malignant lymphomas with multiple organ involvement; lung adenomas predominated in the low-dose group, as opposed to lymphomas of the spleen in the mid- and high-dose groups (Higginbotham *et al*., 1993).

A recent study compared the response of aryl hydrocarbon receptor-deficient ($AhR^{-/-}$) and -proficient ($AhR^{+/+}$) C57BL/6J mice to repeated dermal applications of dibenzo[*a*,*l*]-pyrene. A group of 15 $AhR^{-/-}$ and a group of 17 $AhR^{+/+}$ mice [sex, age and body weight

unspecified] received a single dermal application of 30 μg dibenzo[*a*,*l*]pyrene [purity unspecified; solvent and volume unspecified]. The initial treatment was followed by successive applications of 6 μg dibenzo[*a*,*l*]pyrene [solvent and volume unspecified] once a week for 20 weeks [the time between the first and second treatment was unspecified]. No vehicle control group was used. Skin tumours were first detected in $AhR^{+/+}$ mice at 11 weeks; a tumour incidence of 17/17 was reached in this group by 24 weeks. In contrast, skin tumours were first detected in $AhR^{-/-}$ mice at 21 weeks; a tumour incidence of 5/15 was reached in this group by 24 weeks and did not increase further during the follow-up period (up to 2 years). Multiplicities of skin tumours were 2.7 ± 1.4/mouse in the $AhR^{+/+}$ group (76% papillomas and 24% squamous-cell carcinomas) and 0.46 ± 0.83/mouse in the $AhR^{-/-}$ group (100% papillomas). The differences between the two groups were significant, both for tumour incidence (p <0.001) and tumour multiplicity (p <0.005) (Nakatsuru *et al.*, 2004).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 24 female SENCAR mice, 8 weeks of age, [body weight unspecified], received a single dermal application of 800 nmol [242 μg] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL dioxane:DMSO (75:25). A vehicle-control group was treated with 100 μL dioxane:DMSO alone. One week after initiation, most of the mice in the dibenzo[*a*,*l*]pyrene-treated group had developed erythema at the site of application, which resolved 2 weeks later. One (control group) and 3 (dibenzo[*a*,*l*]pyrene-treated group) weeks after initiation, all mice were treated with 4.26 nmol [2.6 μg] TPA in 100 μL acetone twice a week for the duration of the experiment. The mice were killed 26 weeks after initiation. Complete necropsies were performed. At the end of the experiment, 22/24 (92%) mice in the dibenzo[*a*,*l*]pyrene-treated group had developed 92 skin papillomas (3.8 papillomas/mouse) and 2/23 (9%) mice in the control group had developed a skin papilloma (0.1 papillomas/mouse). The first skin papilloma appeared after 5 weeks in the dibenzo[*a*,*l*]pyrene-treated group compared with 20 weeks in the control group (Cavalieri *et al.*, 1989).

In another similarly designed initiation–promotion study, groups of 24 female SENCAR mice, 8 weeks of age, were treated dermally with 0, 33.3, 100 or 300 nmol [10.1, 30.2 or 90.7 μg] dibenzo[*a*,*l*]pyrene in 100 μL acetone followed 1 week later by promotion with 3.24 nmol [2.0 μg] TPA in 100 μL acetone. The mice treated with dibenzo[*a*,*l*]pyrene developed erythemas after the first treatment with TPA, and the severity of the reaction was directly related to the initiating dose. Due to this reaction, promotion was stopped until the 4th experimental week and was then resumed for 12 weeks. The mice were killed 15 weeks after initiation, and complete necropsies were performed. At the end of the experiment, 23/24 (96%), 22/24 (92%) and 24/24 (100%) mice in the low-, mid- and high-dose groups had developed skin tumours (6.75, 7.92 and

8.50 tumours/mouse, respectively), which were predominantly papillomas; a small number of suspected carcinomas were not verified histologically. In contrast, no skin tumours were observed in the control group. The first skin papilloma appeared at 4 weeks in the mid- and high-dose groups, and at 8 weeks in the low-dose group (Cavalieri *et al.*, 1991).

In a separate experiment, doses of 4, 20 or 100 nmol [1.2, 6.0 or 30.2 μg] dibenzo[*a*,*l*]pyrene were applied dermally to groups of 24 female NMRI mice at 8 weeks of age; promotion with 2.0 μg TPA in 100 μL acetone was begun 2 weeks later. An additional group of mice was treated once with 100 nmol dibenzo[*a*,*l*]pyrene and received no TPA. The experiment lasted 27 weeks. In the group that was not treated with TPA, 7/24 (29%) mice developed skin tumours (1 tumour/tumour-bearing animal), of which [4/7] were skin papillomas and [3/7] were squamous-cell carcinomas. Among animals treated with TPA, the incidence of skin tumours was 22/24 (92%), 20/24 (83%) and 20/24 (83%) (6.96, 5.29 and 3.29 tumours/mouse) in the low-, mid- and high-dose groups, respectively; no skin tumours were observed in the control group. Most of the tumours were skin papillomas; squamous-cell carcinomas represented <2.5% of the total. The inverse relationship between dose and tumorigenic response was attributed to interference in tumour initiation by the toxicity of the compound (Cavalieri *et al.*, 1991).

Lower doses of initiator and promoter also effectively induced skin tumours. Groups of 24 female SENCAR mice, 8 weeks of age, received single dermal applications of 0.25 or 1 nmol [75.5 or 302 ng] dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) in 100 μL acetone or the solvent alone. One week later, promotion was begun with twice-weekly dermal applications of 2.16 nmol [1.3 μg] TPA in 100 μL acetone; this dose was chosen to prevent the appearance of erythema in the dibenzo[*a*,*l*]pyrene-treated group. The mice were killed 27 weeks after initiation, at which time approximately 30% of mice in the 0.25-nmol group and 80% of mice in the 1-nmol group had developed skin papillomas; in addition, two skin carcinomas were observed in the high-dose group. The first tumour developed in this group after 5 weeks. Tumour incidence was not reported for the control group (Higginbotham *et al*., 1993).

In another study that involved multiple dermal applications of dibenzo[*a*,*l*]pyrene, groups of 20 female CD-1 mice, 50–55 days of age [body weight unspecified], received total doses of 1, 4, 10 and 25 nmol [0.3, 1.2, 3.0 and 7.6 μg] dibenzo[*a*,*l*]pyrene [purity unspecified] dissolved in 100 μL acetone applied to the skin as 10 subdoses on alternate days. One control group was treated with acetone alone. Ten days after the last treatment, promotion was begun by applying 2.5 μg TPA in 100 μL acetone three times a week for 20 weeks. The incidence of skin tumours was 18/19 (95%; 5.0 tumours/mouse), 20/20 (100%; 17.8 tumours/mouse), 18/20 (90%; 11.3 tumours/mouse) and 20/20 (100%; 15.0 tumours/mouse) in the lowest- to the highest-dose groups compared with 3/20 (15%; 0.15 tumours/mouse) in the control group (p <0.001 at all doses) (LaVoie *et al.*, 1993).

Other similarly designed initiation–promotion studies in female SENCAR mice entailed single dermal applications of 1.33–12 nmol [0.4–3.6 μg] dibenzo[*a*,*l*]pyrene

followed by twice-weekly applications of 1.62 nmol [1 μg] TPA for 25–28 weeks. High tumour incidence and multiplicity were observed (Gill *et al.*, 1994; Marston *et al.*, 2001), which increased in a dose-related manner (Gill *et al.*, 1994).

Additional initiation–promotion studies were conducted with other strains of mice. In one study, a group of 16 female outbred NMRI mice, 7 weeks of age [body weight unspecified], received a single dermal application of 40 nmol [12 μg] dibenzo[*a*,*l*]pyrene (purity ≥99.7%) in 100 μL acetone, followed 1 week later by twice-weekly applications of 10 nmol [6.2 μg] TPA in 100 μL acetone for 30 weeks. A strong increase in skin tumour rates was observed 8–9 weeks after exposure to dibenzo[*a*,*l*]pyrene. At 18 weeks, the incidence of skin tumours (papillomas and carcinomas combined) in the dibenzo[*a*,*l*]pyrene-treated group was 15/16 (94%; 6.5 tumours/mouse). No skin tumours were observed in a vehicle-control group (Luch *et al.*, 1999).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 30–35 male A/J mice, 5–6 weeks of age and weighing approximately 20 g, received a single intraperitoneal injection of 0.3, 1.5, 3.0 or 6.0 mg/kg bw [6, 30, 60 or 120 μg] dibenzo[*a*,*l*]pyrene (analytical grade) suspended in tricaprylin or tricaprylin alone [volume unspecified]. In a separate experiment, groups of mice of the same strain, sex and age were given higher doses (12, 18 or 24 mg/kg bw [240, 360 or 480 μg]) in a similar manner. The mice were killed 8 months after treatment, and lungs were removed and examined for the presence of tumours. The treatment was associated with an increased incidence of hepatocellular necrosis and inflammation, although no dose-related effect was apparent. Lung adenomas were observed in 14/33 (42%), 33/34 (97%), 34/34 (100%) and 35/35 (100%) mice treated with 6–120 μg dibenzo[*a*,*l*]pyrene at increasing dose levels compared with 15/30 (50%) vehicle controls. The corresponding numbers of lung adenomas/mouse (0.42 ± 0.56, 4.30 ± 2.86, 7.50 ± 3.79 and 16.1 ± 7.26 (mean ± SD)) increased in a dose-dependent manner in the dibenzo[*a*,*l*]pyrene-treated groups; 0.67 ± 0.80 lung adenomas/mouse were observed in the control group (p <0.001 for doses ≥30 μg compared with the control group). Treatment at the three highest doses (240–480 μg) did not affect survival up to 8 months, but a 22% weight loss was observed in the group treated with 480 μg dibenzo[*a*,*l*]pyrene; 5/5 mice in this group developed lung adenomas (36.67 ± 10.64 tumours/mouse) (Prahalad *et al.*, 1997).

A similarly designed study of groups of 20 mice of the same strain and age that received doses of 0–6 mg/kg dibenzo[*a*,*l*]pyrene gave comparable results (Nesnow *et al.*, 1998a).

Groups of 25–40 male and female newborn Crl:CD-1(ICR)BR mice received three intraperitoneal injections of dibenzo[*a*,*l*]pyrene [purity unspecified] (total doses, 40 or 400 nmol [12.1 or 121 μg]). The mice received 1/8 (12%), 1/4 (25%) and 5/8 (62%) of the total dose dissolved in 10, 20 and 50 μL DMSO on days 1, 8 and 15 of life,

respectively. One group of control mice was left untreated and another was administered DMSO only. The mice were weaned at 3–4 weeks of age and killed at 51 ± 1 weeks, except for the high-dose dibenzo[*a*,*l*]pyrene-treated group, in which high mortality imposed termination of the experiment after 17 weeks. At the end of the experiment, the incidence of pulmonary tumours was 84.8% in low-dose males (2.85 ± 0.44 tumours/mouse (mean ± standard error of the mean [SEM])), 41.2% in high-dose males (0.65 ± 0.21 tumours/mouse), and 31.4% (0.54 ± 0.17 tumours/mouse) and 25.0% (0.33 ± 0.14 tumours/mouse) in male untreated and solvent-treated mice, respectively. In addition, 84.8% of low-dose males had hepatic tumours (5.67 ± 0.86 tumours/mouse) and 30.3% had tumours at other sites (0.58 ± 0.17 tumours/mouse); 35.3% of high-dose males had hepatic tumours (1.00 ± 0.38 tumours/mouse) and 23.5% had tumours at other sites (0.35 ± 0.19 tumours/mouse); extrapulmonary tumours were not detected in any of the control groups. In female mice, the incidence of pulmonary tumours at 17 weeks was 89.5% in the low-dose (2.95 ± 0.67 tumours/mouse), 35.7% in the high-dose (0.57 ± 0.29 tumours/mouse), and 37.0% (0.67 ± 0.19 tumours/mouse) and 10.0% (0.67 ± 0.19 tumours/mouse) in the untreated and solvent-treated groups, respectively; the incidence of hepatic tumours was 10.5% in the low-dose (0.11 ± 0.07 tumours/mouse) and 14.3% in the high-dose groups (21 ± 0.15 tumours/mouse). Tumours at other sites were observed in 47.4% of low-dose (0.53 ± 0.14 tumours/mouse) and 42.9% of high-dose females (0.50 ± 0.17 tumours/mouse). Female controls did not develop any extrapulmonary tumours. Lung and liver tumours were predominantly adenomas; in addition to the lung and liver, the kidneys, intestine, ovaries and skin were found to be prone to dibenzo[*a*,*l*]pyrene-induced tumorigenicity (Platt *et al.*, 2004).

Intramamillary administration (see also Table 3.5)

Rat

A group of 19 female Sprague-Dawley rats, approximately 8 weeks of age [body weight unspecified], received a single intramamillary injection of 4 μmol [1.2 mg]/gland dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) dissolved in 100 μL trioctanoin (eight glands; total dose, 32 μmol [9.6 mg]). One control group of 21 rats was treated with 100 μL solvent and another control group was untreated. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter. Of the dibenzo[*a*,*l*]pyrene-treated rats, 10/19 (53%) died within the first 9 weeks of treatment apparently as a result of toxicity; the remaining rats in this group were killed after 15 weeks because of poor health. Survival rates (mean ± SD) were 37 ± 4 and 40 ± 0 weeks in the untreated and vehicle-control groups, respectively. Complete necropsies were performed. Tumour latencies were 9 ± 1 and 25 ± 13 weeks in the dibenzo[*a*,*l*]-pyrene-treated and untreated groups, respectively. At the end of the study, tumour incidence was 100% in the dibenzo[*a*,*l*]pyrene-treated group. Within this group, 7/9 (78%) rats developed fibrosarcomas (2.1 tumours/tumour-bearing rat), 8/9 (89%) rats had

mammary adenocarcinomas (3.8 tumours/rat) and 8/9 (89%) rats had squamous-cell carcinomas of the skin (2.4 tumours/rat); mammary adenofibromas were not observed in this group. In contrast, 2/20 (10%) rats in the untreated group developed mammary epithelial tumours (one adenofibroma and one adenocarcinoma) but no fibrosarcomas or skin tumours. Similarly, no tumours were observed in the vehicle-control group (Cavalieri *et al*., 1989).

Groups of 20 female Sprague-Dawley rats, 8 weeks of age [body weight unspecified], received a single intramamillary injection of 0.25 or 1 μmol [75.6 or 302 μg]/gland dibenzo[*a*,*l*]pyrene (purity >99% by HPLC) dissolved in 50 μL trioctanoin (eight glands; total doses, 2 or 8 μmol [605 or 2420 μg]). One control group of 19 rats was treated with 50 μL solvent. The animals were monitored weekly for tumour development and were killed when tumours were ≥2 cm in diameter; surviving animals were killed after 24 weeks. Complete necropsies were performed on all animals. Survival rates (mean ± SD) were 17 ± 2, 20 ± 2 and 24 ± 0 weeks in the high- and low-dose dibenzo[*a*,*l*]pyrene-treated and control groups, respectively; the corresponding tumour latencies were 11 ± 1, 14 ± 2 and 22 ± 0 weeks. At the end of the study, 19/19 (100%) and 20/20 (100%) rats in the high- and low-dose groups had developed mammary adenocarcinomas (9.1 and 6.3 tumours/rat, respectively), but no mammary adenofibromas, compared with 0/18 (adenocarcinomas) and 1/18 (5%) (adenofibromas) controls. In addition, 14/19 (74%) rats in the high-dose group and 4/20 (20%) rats in the low-dose group developed fibrosarcomas (2.4 and 1.3 tumours/tumour-bearing animal, respectively), and one animal from each treatment group developed a squamous-cell carcinoma of the skin; fibrosarcomas and squamous-cell carcinomas were not observed in the control group (Cavalieri *et al*., 1991).

Tongue application (see also Table 3.14)

Hamster

A group of seven female Syrian hamsters [age and body weight unspecified] received applications on the tongue of 0.01 μmol (3 μg) dibenzo[*a*,*l*]pyrene [purity unspecified] in acetone five times a week for 30 weeks, at which time 6/7 (86%) animals had developed oral cavity carcinomas (2.6 tumours/animal) compared with 0/4 solvent-treated controls. (Schwartz *et al.*, 2004).

Intragastric administration (see also Table 3.14)

Mouse

A group of 18 female wild-type (mixed genetic background of C57Bl/6 and 129/Sv) and 13 female P450 1B1-null mice, 7 weeks of age, was treated intragastrically with daily doses of 1.07 mg/kg (~32 μg/mouse) dibenzo[*a*,*l*]pyrene (99.8 % pure [criteria for purity

unspecified]) dissolved in 100 μL corn oil on 5 days a week for 3 weeks. An additional group of mice of each genotype was treated with corn oil alone. The mice were monitored twice weekly and were killed whenever a sudden weight loss (>20% in a week) or the appearance of tumours (>1 cm) were detected. All surviving mice were killed at 12 months. Complete autopsies and histopathological analyses were performed on 17 of the wild-type mice and all of the P450 1B1-null mice. Survival rates at the end of the experiment were 61% (11/18) in the wild-type mice and 92% (12/13) in the P450 1B1-null mice. Tumour incidence was 18/18 (100%) wild-type mice and 8/13 (61%) P450 1B1-null mice compared with 4/27 (15%) solvent-control mice of both genotypes combined (one lung adenoma, one liver adenoma, one follicular lymphoma and one endometrial cystic hyperplasia in four wild-type mice [numbers of control mice of each genotype unspecified]). Wild-type mice treated with dibenzo[*a,l*]pyrene developed ovarian tumours (12/17; predominantly (83%) granulosa-cell tumours), lymphomas (5/17; 60% lymphoblastic and 40% follicular), a liver adenoma (1/17), verruciform hyperplasia of the skin (8/17) and uterine tumours (5/17; 60% endometrial cystic hyperplasia and 40% haemangiosarcomas); lung tumours were not observed in this group. P450 1B1-null mice developed a follicular lymphoma (1/13; 8%), a liver haemangioma (1/13; 8%), endometrial cystic hyperplasia (5/13; 38%) and lung adenomas (5/13; 38%); tumours of the ovary were not observed in this group. The differences between the two genotypes were statistically significant for tumours of the ovary, skin and lung (Buters *et al*., 2002).

Dibenzo[*e,l*]pyrene

Dermal application (see also Table 3.1)

Mouse

Two groups of 20 female Swiss albino Ha/ICR/Mil mice, 7–8 weeks of age [body weight unspecified], received thrice-weekly dermal applications of either a 0.05% or a 0.07% solution [volume unspecified] of dibenzo[*e,l*]pyrene (purified by column chromatography and recrystallization; purity verified by determination of melting-point in dioxane) for 12 months. An additional group of 20 mice was treated with dioxane only. Skin tumours were not observed in any of the groups at 15 months, when the study was terminated (Hoffmann & Wynder, 1966).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Two groups of 30 Swiss albino Ha/ICR/Mil mice [sex, age and body weight unspecified] received 10 dermal applications of 25 μL of 0.1% chromatographically pure (recrystallized) dibenzo[*e,l*]pyrene in dioxane [25 μg; total dose, 250 μg/animal] at 2-day

intervals. On day 28, 2.5% (2.3 mg) croton oil in acetone was applied to the skin [volume, number, frequency and duration of treatments not given]. A group of 30 mice was treated with croton oil solution alone. All survivors were killed after 6 months, at which time mortality rates were 7/30 (23%) and 4/30 (13%), and the number of skin papilloma-bearing mice was 0/30 and 2/30 (7%) in the dibenzo[*e*,*l*]pyrene-treated and control groups, respectively (Hoffmann & Wynder, 1966).

1,2-Dihydroaceanthrylene

Subcutaneous administration (see also Table 3.3)

Mouse

A group of nine female mice [strain, age and body weight unspecified] received a single subcutaneous injection of 5 mg 1,2-dihydroaceanthrylene (crystalline) [solvent and volume unspecified]. No control group was used in the study. All mice were alive and tumour-free after 14 months. By 17 months, five mice were still alive with no evidence of tumours. The last two mice were killed after 20 months with no evidence of tumours (Shear, 1938).

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of 31 male and 23 female newborn CD-1 mice received intraperitoneal injections of 1,2-dihydroaceanthrylene (purified by HPLC; purity assessed by GC–MS) in DMSO three times over 2 weeks (total volume, 35 μL; total doses, 0.86, 2.14 and 4.29 μmol [175, 437.5 and 875 μg]). Injections given were on postnatal days 1 [1/7 of the total dose in 5 μL], day 8 [2/7 of the total dose in 10 μL] and 15 [4/7 of the total dose in 20 μL]. A group of 24 male and 34 female control mice was treated in the same manner with DMSO alone. The mice were killed at 9 months of age and necropsied, and tumours were evaluated histologically. The incidence of liver tumours in male mice was 0/24, 1/17 (6%), 1/25 (4%) and 0/31 at the 0-, 175-, 437.5- and 875-μg dose levels, respectively. Liver tumours were not observed in female mice at any of the dose levels (groups of 34, 23, 21 and 13 mice, respectively). Lung tumour incidence was 0/24 (adenomas) and 1/24 (4%; adenocarcinomas) at 0 μg, 2/17 (12%; adenomas) and 2/17 (12%; adenocarcinomas) at 175 μg, 2/25 (8%; adenomas) and 0/25 (adenocarcinomas) at 437.5 μg and 5/31 (16%; adenomas) and 1/31 (3%; adenocarcinomas) at 875 μg in male mice; that in female mice was 1/34 (3%; adenomas) and 0/34 (adenocarcinomas) at 0 μg, 0/23 (adenomas) and 1/23 (4%; adenocarcinomas) at 175 μg, 1/21 (5%; adenomas) and 0/21 (adenocarcinomas) at 437.5 μg and 1/13 (8%; adenomas) and 1/13 (8%; adenocarcinomas) at 875 μg. When the data from male and female mice were combined, the incidence of combined adenoma and

adenocarcinoma (8/44; 18%) in the 875-μg group was significantly different (p <0.025) from that in the control group (2/58), as was the number of tumours/mouse (0.03 ± 0.02 versus 0.20 ± 0.07; p <0.01) (Wang *et al*., 1999).

In a second experiment, a group of 10 male and 23 female newborn BLU:Ha mice was treated with 1,2-dihydroaceanthrylene, using the protocol described above, at a total dose of 0.86 μmol (175 μg). A group of 22 male and 25 female control mice was treated with DMSO alone. The mice were killed at 6 months of age and necropsied, and tumours were evaluated histologically. The incidence of lung tumours was 0/10 treated male and 1/23 (4%; lung adenomas) treated female mice, and 1/22 (5%; adenomas) control males and 1/25 (4%; adenomas) control females (Wang *et al*., 1999).

1,4-Dimethylphenanthrene

Previous evaluation

1,4-Dimethylphenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two initiation–promotion bioassays in which 1,4-dimethylphenanthrene was applied dermally to mice; these are summarized in Table 3.2.

The Working Group concluded that the available data were inadequate to permit an evaluation of the carcinogenicity to experimental animals of 1,4-dimethylphenanthrene *per se*. No new studies were available.

Fluoranthene

Previous evaluation

Fluoranthene was evaluated by earlier working groups in February 1983 (IARC, 1983) and March 1987 (IARC, 1987). Fluoranthene alone was not carcinogenic in experimental species following exposure via dermal or subcutaneous routes. The study of subcutaneous administration was considered to be inadequate. Fluoranthene had no carcinogenic effect in two studies of dermal application and was inactive as an initiator in the mouse skin initiation–promotion assay. However, when co-administered with benzo[*a*]pyrene, fluoranthene significantly increased the incidence of tumour-bearing mice and produced an excess number of skin tumours (primarily squamous-cell carcinomas) compared with that induced by the same dose of benzo[*a*]pyrene alone. The Working Group of March 1987 (IARC, 1987) concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of fluoranthene. Additional bioassays that have been published since 1983 are summarized below.

Dermal application (see also Table 3.1)

Mouse

In a co-carcinogenicity study, groups of 20 male C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of a solution of 0.1% fluoranthene in combination with 0.001% benzo[*a*]pyrene in toluene for 104 weeks. The combined treatment induced skin tumours in one (8%) mouse; mice treated with fluoranthene alone or benzo[*a*]pyrene alone and vehicle controls did not develop skin tumours (Warshawsky *et al.*, 1993).

Intraperitoneal administration (see also Table 3.7)

Mouse

In newborn male and female Swiss-Webster BLU:ha (ICR) mice, intraperitoneal injections of total doses of 3.46 or 17.30 μmol fluoranthene on postnatal days 1, 8 and 15 increased the incidence of lung tumours (primarily adenomas) at 24 weeks in male mice at both doses and in female mice at 17.30 μmol (Busby *et al*., 1984).

Similar intraperitoneal injection of a total dose of 1.27 μmol fluoranthene increased the incidence of lung tumours (primarily adenomas) in both sexes at 26 weeks (Busby *et al*., 1989).

Newborn male and female CD-1 mice received intraperitoneal injections of 1/7, 2/7 and 4/7 of total doses of 3.46, 8.65 and 17.30 μmol fluoranthene dissolved in 5 μL DMSO on postnatal days 1, 8 and 15, respectively, and were killed at 6 and 9 months. At that time, the incidence of lung tumours (primarily adenomas) was increased in both sexes. A low incidence of liver tumours was noted in male mice at 6 months, whereas the incidence of liver adenomas was increased in male mice at 9 months (Wang & Busby, 1993).

In newborn male and female CD-1 mice, similar intraperitoneal injection of total doses of 3.46 or 17.30 μmol fluoranthene induced a significantly increased incidence of lung tumours (primarily carcinomas) in both males and females at 6 months. At both doses, the incidence of liver adenoma in male mice was increased (LaVoie *et al*. 1994b).

Fluorene

Previous evaluation

Fluorene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated studies in which fluorene was administered to mice by dermal application and subcutaneous injection and to female rats in the diet. The studies were considered to be inadequate for evaluation. No new studies were available.

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

Previous evaluations

Indeno[1,2,3-*cd*]pyrene was considered in December 1972 (IARC, 1973) by a Working Group that evaluated a bioassay in which indeno[1,2,3-*cd*]pyrene was administered to mice by dermal application (repeatedly and initiation–promotion). Positive responses were obtained. Indeno[1,2,3-*cd*]pyrene was also administered by subcutaneous injection to mice in an experiment that did not include control animals and induced sarcomas. On the basis of these data, the Working Group concluded that indeno[1,2,3-*cd*]pyrene was a complete carcinogen. Indeno[1,2,3-*cd*]pyrene was also considered in February 1983 (IARC, 1983) by a Working Group that evaluated the same bioassays as those considered previously and concluded that there was *sufficient evidence* that indeno[1,2,3-*cd*]pyrene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

A total initiating dose of 1.0 mg indeno[1,2,3-*cd*]pyrene in acetone applied to the skin of 25 female outbred albino Crl:CD-1 (ICR) BR mice followed by thrice-weekly applications of 2.5 μg TPA for 20 weeks induced squamous-cell papillomas in 90% of the animals, with an average of 2.83 skin tumours/mouse; papillomas occurred in fewer than 5% of TPA controls (Rice *et al.* 1986). In a similar experiment with a total initiating dose of 4.0 μmol [1105 μg] indeno[1,2,3-*cd*]pyrene, 72% of treated female CD-1 mice developed papillomas; TPA controls did not develop skin tumours (Rice *et al.* 1990).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female mice CD-1, intraperitoneal injection of total doses of 0 or 2.1 μmol indeno[1,2,3-*cd*]pyrene on postnatal days 1, 8 and 15 induced lung adenomas in 9.1% [1/11] of treated male mice; DMSO control mice did not develop tumours (LaVoie *et al.*, 1987).

Intrapulmonary administration (see also Table 3.4)

Rat

In Osborne-Mendel rats, intrapulmonary implantation of indeno[1,2,3-*cd*]pyrene at doses of 0, 0.16, 0.83 or 4.15 mg induced dose-related increases in the incidence of

pulmonary squamous-cell carcinomas and sarcomas (0% [0/35], 11.4% [4/35], 22.9% [8/35] and 60.0% [21/35]) (Deutsch-Wenzel *et al.*, 1983).

1-Methylchrysene

Previous evaluation

1-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 1-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 1-Methylchrysene was considered to be active as a tumour initiator but not when applied repeatedly to mouse skin. The Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of 1-methylchrysene to experimental animals. No new studies were available.

2-Methylchrysene

Previous evaluation

2-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 2-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 2-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 2-methylchrysene to experimental animals. No new studies were available.

3-Methylchrysene

Previous evaluation

3-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 3-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Table 3.1 and 3.2. 3-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 3-methylchrysene to experimental animals. No new data were available.

4-Methylchrysene

Previous evaluation

4-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 4-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 4-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 4-methylchrysene to experimental animals. No new studies were available.

5-Methylchrysene

Previous evaluation

5-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated numerous positive bioassays in which 5-methylchrysene was administered dermally (repeatedly and initiation–promotion protocols) and by subcutaneous injection to mice. Selected studies are summarized in Tables 3.1 and 3.2. On the basis of the available data, the Working Group concluded that there was *sufficient evidence* for the carcinogenicity of 5-methylchrysene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal initiation–promotion (see also Table 3.2)

Mouse

Application of total initiating doses of 0–100 nmol and 0–1.5 μmol 5-methylchrysene to the skin of female CD-1 mice followed by thrice-weekly applications of 2.5 μg TPA significantly increased the incidence (65–100%) and multiplicity of skin papillomas. Controls treated with solvent and TPA alone had a low incidence (0–10%) of skin papillomas (Amin *et al.*, 1985c; Hecht *et al.*, 1985; El-Bayoumy *et al.*, 1986, Hecht *et al.*, 1987; Rice *et al.*, 1988; Amin *et al.*, 1990, 1992).

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female ICR mice, intraperitoneal injection of 5-methylchrysene at total doses of 0 or 56 nmol on postnatal days 1, 8 and 15 significantly increased the incidence of lung (males, 4% and 20%; females, 7% and 21%) and liver (males, 2% and 23%; females, 2% and 12%) adenomas at 35 weeks (Hecht *et al.*, 1985).

Male strain A/J mice injected intraperitoneally with 0, 10, 50, 100 or 200 mg/kg bw 5-methylchrysene had dose-related increases in the incidence (55%, 65%, 100%, 100% and 100%) and multiplicity (0.6, 1.8, 39.0, 93.1 and too numerous to count) of lung adenomas (You *et al.*, 1994; Nesnow *et al.*, 1995; Ross *et al.*, 1995; Nesnow *et al.,* 1998a).

6-Methylchrysene

Previous evaluation

6-Methylchrysene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated one bioassay in which 6-methylchrysene was applied dermally (repeatedly) to mice and one initiation–promotion study in mice. These data are summarized in Tables 3.1 and 3.2. 6-Methylchrysene was considered to be active in both assays. On the basis of the available data, the Working Group concluded that there was *limited evidence* for the carcinogenicity of 6-methylchrysene to experimental animals. No new studies were available.

2-Methylfluoranthene

Previous evaluation

2-Methylfluoranthene was considered by a Working Group in February 1983 (IARC, 1983). On the basis of the available data (see Tables 3.1 and 3.2), the Working Group concluded that there was *limited evidence* that 2-methylfluoranthene was carcinogenic to experimental animals. Additional bioassays that have been published since that time are summarized below.

Intraperitoneal administration (see also Table 3.7)

Mouse

Groups of newborn male and female CD-1 mice received intraperitoneal injections of 2-methylfluoranthene in DMSO on postnatal days 1, 8 and 15 (total doses, 3.46 or 17.3 μmol). Controls received DMSO alone. The bioassay was terminated when the mice were 1 year old. At the end of the study, the incidence of lung tumours in male (23/24; 96%) and female (11/16; 69%) mice and the number of tumours per mouse were significantly increased following treatment with 17.3 μmol. The incidence of liver tumours in male mice (22/24; 92%) was significantly increased by the high dose compared with controls (3/29; 10%); high-dose female mice had a lower incidence of liver tumours (5/16; 31%) than high-dose males. Alterations of hepatic foci were noted in males at the low dose (LaVoie *et al.*, 1994b).

3-Methylfluoranthene

Previous evaluation

3-Methylfluoranthene was considered by a Working Group in February 1983 (IARC, 1983). On the basis of the available data (see Table 3.2), the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of 3-methylfluoranthene to experimental animals. Additional bioassays that have been published since that time are summarized below.

Intraperitoneal injection (see also Table 3.7)

Mouse

Groups of newborn male and female CD-1 mice received intraperitoneal injections of 3-methylfluoranthene in DMSO on postnatal days 1, 8 and 15 (total doses, 3.46 or 17.3 μmol). Controls received DMSO alone. The bioassay was terminated when the mice were 1 year old. At the end of the study, the incidence of lung tumours in control and treated male (5/29, 17%; 6/24, 25%; and 5/26; 19%) and female (4/34, 12%; 5/33, 15%; and 6/28, 21%) mice was similar. The incidence of liver tumours in male mice (15/26; 55%) was significantly increased at the high dose compared with controls (3/29; 10%) (LaVoie *et al.*, 1994b).

1-Methylphenanthrene

Previous evaluation

1-Methylphenanthrene was considered by a Working Group in February 1983 (IARC, 1983) and was classified as having inadequate evidence of carcinogenicity. In one bioassay in which 1-methylphenanthrene was applied dermally to mice (initiation–promotion protocol), it was inactive as an initiator (Table 3.2). No new studies were available to the Working Group.

Naphtho[1,2-*b*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Total initiating doses of 0, 1.0 or 4.0 μmol naphtho[1,2-*b*]fluoranthene [purity unspecified] applied to the skin of female CD-1 mice followed by thrice-weekly applications of 2.5 μg TPA induced squamous-cell papillomas in 65% and 100% of the treated animals with averages of 2.5 and 6.6 skin tumours/mouse, respectively.

Papillomas occurred in 10% of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Naphtho[2,1-*a*]fluoranthene

Dermal initiation–promotion (see also Table 3.2)

Mouse

Total initiating doses of 0, 1.0 or 4.0 μmol naphtho[2,1-*a*]fluoranthene [purity unspecified] applied to the skin of female CD-1 mice followed by thrice weekly applications of 2.5 μg TPA induced squamous-cell papillomas in 90% and 100% of the treated animals with averages of 5.9 and 7.3 skin tumours/mouse, respectively. Papillomas occurred in 10% of TPA controls with an average of 0.1 tumours/mouse (Weyand *et al*., 1990).

Naphtho[2,3-*e*]pyrene

Dermal application (see also Table 3.1)

Mouse

A group of 20 female Swiss-Albino Ha/ICR/Mil mice [age unspecified] received dermal applications of 100 μg naphtho[2,3-*e*]pyrene in dioxane three times a week [duration of treatment not specified]. No tumours developed (Hoffmann & Wynder, 1966; LaVoie *et al.,* 1979).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 30 female Swiss-Albino Ha/ICR/Mil mice [age unspecified] received 10 dermal applications of 25 μg naphtho[2,3-*e*]pyrene in dioxane every other day (total dose, 250 μg). A control group received dioxane alone. Ten days after the last application, all mice received dermal applications of 2.5% croton oil in acetone for 20 weeks. After 20 weeks of promotion, the incidence of mice bearing skin tumours was 33% in the treated group compared with 7.6% in the control group (Hoffman & Wynder, 1966; LaVoie *et al.,* 1979).

Perylene

Previous evaluation

Perylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which perylene was applied dermally to mice (repeated dose and initiation–promotion protocols), both of which gave negative results; these are summarized in Tables 3.1 and 3.2. On the basis of the available studies, the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of perylene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application

Mouse

Female CD-1 mice [number and age not specified] received dermal applications of 1% perylene [purity, solvent and volume not specified] three times a week for 1 year. The incidence of skin tumours [number not specified] did not differ from that in the control group (Anderson & Anderson, 1987).

Dermal initiation–promotion (see also Table 3.2)

Mouse

Groups of 20 female Crl/CD-1 (ICR)Br mice [age unspecified] received a total of 10 dermal applications of 0 or 100 μg perylene (purity >99% by HPLC) in 100 μL acetone on alternate days. Ten days after the last application, all mice received thrice weekly dermal applications of 2.5 μg TPA for 25 weeks. At the end of the treatment period, the incidence of skin tumours (5%) in mice treated with perylene (0.1 tumours/mouse) did not differ from that in the control mice (0.1 tumours/mouse) (El-Bayoumy *et al*., 1982).

Intraperitoneal administration

Mouse

Female strain A mice [number and age not specified] received intraperitoneal injections 0, 200, 500 or 1000 mg/kg bw perylene [purity not specified] three times a week for 8 weeks and the incidence of lung tumours was determined 16 weeks after the last injection. None of the treatments with perylene affected the number of lung tumours [incidence not given] (Anderson & Anderson, 1987).

Phenanthrene

Previous evaluation

Phenanthrene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated bioassays in which phenanthrene was fed to rats, administered dermally and by subcutaneous injection to mice or given by intraperitoneal injection to neonatal mice. One study on tumour initiation gave positive results; all of the other studies gave either negative results or were considered to be inadequate; these are summarized in Tables 3.1–3.3, 3.6 and 3.7. On the basis of the available studies, the Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of phenanthrene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.1)

Mouse

A group of 20 male C3H/HeJ mice, 6–8 weeks of age, received twice-weekly skin applications of 50 μL of a 0.1% toluene solution of phenanthrene (>99% pure by HPLC; 50 μg per treatment) for 104 weeks. A control group of 50 male mice was treated with toluene alone. Lesions (≥1 mm^3) that persisted for at least 1 week were diagnosed as papillomas. After 104 weeks, one benign skin tumour was observed in the 12 surviving experimental mice and no benign or malignant skin tumours in the 39 surviving control mice. Gross examination of internal organs indicated no tumours in either the experimental or control groups (Warshawsky *et al*., 1993).

Intrapulmonary administration (see also Table 3.4)

Rat

Groups of 35 inbred female Osborne-Mendel rats, 3 months of age and weighing on average 256 g, received a single pulmonary implantation of 1, 3 or 10 mg phenanthrene (purity, 99.9%) in a mixture of beeswax and tricaprylin. The animals were monitored for 132–135 weeks. A single lung carcinoma was found in the high-dose phenanthrene-treated group. No lung carcinomas developed in solvent-treated control rats (Wenzel-Hartung *et al*., 1990).

Picene

Dermal application (see also Table 3.1)

Mouse

In early experiments, picene was applied dermally to mice. The results were negative (Kennaway, 1924a,b, 1930; Kennaway & Hieger, 1930). [The experiments are poorly described; the data are not included in the Table.]

Groups of 50 female NMRI mice [age unspecified] received thrice-weekly dermal applications of 0.4, 1.33 or 4.04 nmol [0.1, 0.4 or 1.1 μg] picene (>99% pure) in 17 μL acetone for 112 weeks (total doses, 38, 125 or 378 μg). A control group of 50 female mice was treated with the solvent alone. Skin tumours were observed in 3/49 mice treated with 0.1 μg picene, 11/48 mice treated with 0.4 μg picene, 11/50 mice treated with 1.1 μg picene and 2/48 mice treated with the solvent [no histopathology] (Platt *et al*., 1990).

Dermal initiation–promotion (see also Table 3.2)

Mouse

A group of 30 female CD-1 mice, 8 weeks of age, received a single skin application of 10 μmol [2.8 mg] picene (purified by preparative layer chromatography) in benzene [volume unspecified]. One week later, the mice received twice-weekly skin applications of 10 μmol TPA for 34 weeks. [The dose of TPA was probably 10 μg.] A control group of 30 female mice of the same strain and age was treated twice weekly with 10 μmol TPA only for 34 weeks. At the end of the promotion period, all mice in the picene-treated and control groups were still alive. At that time, 8/30 (27%) picene-treated mice had developed skin papillomas (0.60 tumours/mouse [no histology]). One skin tumour occurred in 1/30 (3%) TPA controls by week 25, but it had regressed by the end of the study, when no tumours were found in the control group (Scribner, 1973).

Groups of female NMRI mice [age unspecified] received a single dermal application of 300 nmol [83.5 μg] picene (>99% pure) in 100 μL acetone (16 mice), 600 nmol [167 μg] picene in 100 μL tetrahydrofuran (16 mice) or 10000 nmol [2800 μg] picene in 100 μL benzene (30 mice). A control group of 30 female mice was treated with 100 μL acetone alone. One week later, all mice received twice-weekly dermal applications of 10 nmol (6.2 μg) TPA in 100 μL acetone. The experiment was terminated after 24 weeks. Skin tumours were not observed in mice treated with 83.5 or 167 μg picene or in the acetone-treated control mice; the incidence in mice treated with 2800 μg picene was 19% (0.29 tumours/mouse) [no histopathology] (Platt *et al*., 1990).

Subcutaneous administration (see also Table 3.3)

Mouse

Groups of 50 female NMRI mice [age unspecified] received a single subcutaneous injection into the interscapular region of 36, 40, 108, 308 or 399 nmol [10, 11, 30, 86 or 111 μg] picene (>99 % pure) in 500 μL tricaprylin. Two control groups of 50 female mice were treated with tricaprylin alone. Animals were palpated weekly; masses >1 cm were considered to be positive. The experiment was terminated after 112 weeks. [No histopathology appeared to be performed.] The incidence of fibrosarcomas was: 10 μg picene, 9/50 (18%); 11 μg picene, 12/46 (26%); 30 μg picene, 17/49 (35%); 86 μg picene, 31/49 (63%); 111 μg picene, 29/50 (58%); and controls, 3/49 (6%) and 1/50 (2%) (Platt *et al*., 1990).

Groups of 45 male and 50 female newborn NMRI mice, 2 days of age, received a single subcutaneous injection of 40 or 400 nmol [11 or 111 μg] picene (>99% pure) in 50 μL of an aqueous solution (1% gelatin, 0.9% saline, 0.4% Tween 20). A control group of 49 male and female mice was treated with the solvent alone. The mice were separated by sex at 30 days of age and housed for a total of 40 weeks. A necropsy was performed, lung nodules were counted and histopathology was performed. The incidence of pulmonary tumours [type unspecified] was 4/16 (25%; 11 μg picene; 2.8 tumours/tumour-bearing mouse) and 8/23 (35%; 111 μg picene; 2.1 tumours/tumour-bearing mouse) in females, and 1/22 (4%; 11 μg picene; 1.0 tumours/tumour-bearing mouse) and 2/13 (15%; 111 μg picene; 1.5 tumours/tumour-bearing mouse) in males. The incidence in controls was 1/19 (5%; 1.0 tumours/tumour-bearing mouse) in females and 2/14 (14%; 2.0 tumours/tumour-bearing mouse) in males (Platt *et al*., 1990).

Rat

Groups of 12 female Sprague-Dawley rats, 30 days of age, received a total of 20 thrice-weekly subcutaneous injections of 300 μg picene (recrystallized, sharp melting-point, single peak by GC/MS and HPLC) in 100 μL sesame oil: DMSO (9:1). A control group of 12 rats was treated with the solvent alone. The experiment was continued for 37 weeks. The incidence of sarcomas in the rats treated with picene was 0/12 compared with 0/12 solvent-treated controls (Flesher *et al*., 2002).

Pyrene

Previous evaluation

Pyrene was first considered in February 1983 (IARC, 1983) by a Working Group that evaluated several bioassays in which pyrene was applied dermally to mice; no tumors were observed. It was also tested in initiation–promotion studies in mice with

inconclusive results. A study of subcutaneous injection into mice was considered to be inadequate, and a study of intratracheal administration in hamsters gave negative results. On the basis of the available studies, a Working Group in 1987 (IARC, 1987) concluded that the data were inadequate to permit an evaluation of the carcinogenicity of pyrene in experimental animals. Additional bioassays that have been published since that time are summarized below.

Dermal application (see also Table 3.16)

Mouse

In a co-carcinogenicity study in male C3H/HeJ mice, a solution of 0.1% pyrene applied to the skin simultaneously with 0.001% benzo[*a*]pyrene did not induced skin tumours; one mouse (8%) treated with pyrene alone developed a skin tumour; mice treated with benzo[*a*]pyrene alone and vehicle (toluene) controls did not develop skin tumours (Warshawsky *et al.*, 1993).

Table 3.16. Incidence of benign and malignant skin tumours in mice treated with some PAHs and mixtures of PAHs

Compound	Malignant skin tumours	Benign and malignant skin tumours combined
Toluene	0/20	0/39
Benzo[*a*]pyrene	19/20 (95%)	0/14
Benzo[*a*]pyrene + chrysene	13/16 (81%)	3/13 (23%)
Benzo[a]pyrene + phenanthrene + pyrene	18/19 (95%)	19/19 (100%)
Benzo[*a*]pyrene + anthracene	1/13 (8%)	1/13 (8%)
Benzo[*a*]pyrene + fluoranthene		1/12 (8%)
Benzo[*a*]pyrene + phenanthrene		1/17 (6%)
Benzo[*a*]pyrene + pyrene		0/13
Benzo[*a*]pyrene + anthracene, chrysene, fluoranthene, phenanthrene and pyrene		8/17* (47%)
Anthracene		0/14
Chrysene		1/15 (7%)
Fluoranthene		0/15
Phenanthrene		1/12 (8%)
Pyrene		1/13 (8%)
Anthracene, chrysene, fluoranthene, phenanthrene and pyrene		3/13 (23%)

From Warshawsky *et al.* (1993)
PAH, polycyclic aromatic hydrocarbon
*Significantly different from benzo[*a*]pyrene alone

Intraperitoneal administration (see also Table 3.7)

Mouse

In studies with newborn male and female CD-1 and Swiss-Webster BLU:Ha (ICR) mice and 5–6-week-old male strain A mice, intraperitoneal injection of pyrene at doses of 0–9.65 μmol did not significantly increase the incidences of lung adenomas (Wislocki *et al.,* 1986; Busby *et al.*, 1989; Ross *et al.*, 1995). However, in one study, the highest initiating dose (2800 nmol) significantly increased the incidence of liver adenomas (Wislocki *et al.,* 1986).

Triphenylene

Previous evaluation

Triphenylene was considered in February 1983 (IARC, 1983) by a Working Group that evaluated two bioassays in which triphenylene was applied dermally to mice and these are summarized in Table 3.1. The Working Group concluded that the data were inadequate to permit an evaluation of the carcinogenicity of triphenylene to experimental animals. No new studies were available.

Laboratory mixtures of PAHs

Dermal application

Mouse

Groups of IRC mice [number and age unspecified] received dermal applications of 0.005% benzo[*a*]pyrene alone or in combination with 0.1% pyrene, 0.5% fluoranthene, 0.1% phenanthrene or 0.015% benz[*a*]anthracene [number of applications, volume applied and treatment period unspecified]. The total number of tumour-bearing mice appeared to be decreased by phenanthrene and benz[*a*]anthracene, and the total number of carcinoma-bearing mice appeared to be decreased by benz[*a*]anthracene and increased by pyrene and fluoranthene [given the limited documentation, the data are very difficult (if not impossible) to evaluate] (Hoffmann & Wynder, 1963).

Groups of male Swiss mice [age unspecified] received dermal applications every 4th day of two drops of a benzene solution that contained 0.3% benzo[*a*]pyrene [purity unspecified] (40 mice), 0.3% perylene [purity unspecified] (20 mice) or a mixture of 0.3% benzo[*a*]pyrene and 0.3% perylene (40 mice). The experiment was continued for ~22 weeks, at which time ~50% of the animals treated with benzo[*a*]pyrene alone or with the mixture had died. Tumours with a diameter >0.5 mm were scored, but these were not verified histologically. The incidence of skin tumours was: benzo[a]pyrene alone, 36/40 (90%); perylene alone, 0/20 (0%); and benzo[*a*]pyrene + perylene, 13/40 (33%) (Finzi *et*

al., 1968). [The addition of perylene to benzo[*a*]pyrene significantly reduced the tumorigenic response observed with benzo[*a*]pyrene alone (Fisher's exact two-tailed test).]

Groups of 50 female ICR/Ha mice [age unspecified] received thrice-weekly dermal applications for 52 weeks of 100 µL acetone that contained 5 µg benzo[*a*]pyrene alone or in combination with 15 µg benzo[*e*]pyrene, 12 µg pyrene or 21 µg benzo[*ghi*]perylene. Each of the compounds was 'highly pure' as assessed by TLC, multichannel ultraviolet-visible (UV-VIS), spectrophotometery, fluorescence and MS. Additional groups [number unspecified] were treated in a similar manner with 15 µg benzo[*e*]pyrene, 12 µg pyrene, 21 µg benzo[*ghi*]perylene or the solvent alone. The incidence of skin papilloma and squamous-cell carcinoma (verified histologically) after 52 weeks was, respectively: benzo[*a*]pyrene, 13/42 (31%) and 10/42 (24%); benzo[*a*]pyrene + benzo[*e*]pyrene, 34/39 (87%) and 27/39 (69%); benzo[*a*]pyrene + pyrene, 27/41 (66%) and 19/41 (46%); and benzo[*a*]pyrene + benzo[*ghi*]perylene, 20/37 (54%) and 17/37 (46%) (Van Duuren *et al.*, 1973). [Each mixture significantly increased the incidence of papilloma and squamous-cell carcinoma compared with benzo[*a*]pyrene alone, with the exception of squamous-cell carcinomas following treatment with benzo[*ghi*]perylene (Fisher's exact two-tailed test).]

Groups of 50 female ICR/Ha mice, 6–8 weeks of age, received thrice-weekly dermal applications for 368 or 440 days of 100 µL acetone that contained 5 µg benzo[*a*]pyrene alone or in combination with 4, 12 or 40 µg pyrene, 7 or 21 µg benzo[*ghi*]perylene, 5 or 15 µg benzo[*e*]pyrene or 40 µg fluoranthene. Fluoranthene was purified by zone refinement and the other compounds were purified by recrystallization. Each was characterized by its melting-point. Additional groups of 50 mice were treated similarly with 12 or 40 µg pyrene, 21 µg benzo[*ghi*]perylene, 15 µg benzo[*e*]pyrene, 40 µg fluoranthene or acetone alone. The incidence of skin papilloma and squamous-cell carcinoma (verified histologically) is given in Table 3.17 (Van Duuren & Goldschmidt, 1976). [Each of the following mixtures significantly increased the incidence of papilloma and squamous-cell carcinoma compared with benzo[*a*]pyrene alone: 12 µg pyrene + benzo[*a*]pyrene (368 days), 40 µg pyrene + benzo[*a*]pyrene (440 days), 15 µg benzo[*e*]pyrene + benzo[*a*]pyrene (368 days) and 40 µg fluoranthene + benzo[*a*]pyrene (440 days) (Fisher's exact two-tailed test).]

Groups of 30 female Swiss mice [age unspecified] received twice-weekly dermal applications for 48 weeks of 2.2, 6.6 or 20 nmol benzo[*a*]pyrene [purity unspecified], 22.2, 66.6 or 200 nmol cyclopenta[*cd*]pyrene [purity unspecified], 2.2 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene, 6.6 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 20 nmol benzo[*a*]pyrene + 200 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 6.6 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene + 200 nmol cyclopenta[*cd*]-pyrene or 20 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta[*cd*]pyrene in 50 µL acetone. The experiment lasted 61 weeks. The following mixtures gave a tumour incidence that was significantly greater than the sum of the individual components at the same doses: 6.6 nmol benzo[*a*]pyrene + 66.6 nmol cyclopenta[*cd*]pyrene, 2.2 nmol benzo[*a*]pyrene +

Table 3.17. Incidence of skin tumours in female ICR/Ha mice following dermal application of benzo[*a*]pyrene and binary mixtures of benzo[*a*]pyrene and some PAHs

Compound	Papilloma	Squamous-cell carcinoma
B[*a*]P (368 days)	14/50 (28%)	10/50 (20%)
B[*a*]P + 4 μg pyrene (368 days)	12/50 (24%)	6/50 (12%)
B[*a*]P + 12 μg pyrene (368 days)	25/50 (50%)*	20/50 (40%)*
B[*a*]P +7 μg benzo[*g,h,i*]perylene (368 days)	19/50 (38%)	10/50 (20%)
B[*a*]P + 21 μg benzo[*g,h,i*]perylene (368 days)	20/50 (40%)	18/50 (36%)
B[*a*]P + 5 μg B[*e*]P (368 days)	24/50 (50%)	9/50 (18%)
B[*a*]P + 15 μg B[*e*]P (368 days)	33/50 (66%)*	27/50 (54%)*
B[*a*]P (440 days)	16/50 (32%)	12/50 (24%)
B[*a*]P + 40 μg pyrene (440 days)	35/50 (70%)*	26/50 (52%)*
B[*a*]P + 40 μg fluoranthene (440 days)	39/50 (78%)*	37/50 (74%)*

From Van Duuren & Goldschmidt (1976)
B[*a*]P, benzo[*a*]pyrene; B[*e*]P, benzo[*e*]pyrene; PAH, polycyclic aromatic hydrocarbon
*Significantly different from benzo[*a*]pyrene alone at 368 or 440 days

66.6 nmol cyclopenta[*cd*]pyrene and 6.6 nmol benzo[*a*]pyrene + 22.2 nmol cyclopenta-[*cd*]pyrene (Table 3.18; Cavalieri *et al*., 1983).

Groups of 20 shaved male C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of 0.2% benzo[*a*]pyrene (purity, 99.5%), a mixture of 0.2% benzo[*a*]pyrene and 0.2% chrysene (purity, 99.5%) or a mixture of 0.2% benzo[*a*]pyrene, 0.2% phenanthrene (purity >99%) and 0.26% pyrene (purity, 99.5%) in toluene, or toluene alone. [The volume was not specified. Histopathology appeared to be performed. There was no statistical analysis. None of the treatments resulted in a tumour incidence that differed from that induced by benzo[*a*]pyrene alone (Fisher's exact two-tailed test). Tumour latency appeared to be shortened with the mixtures, but this was not examined statistically] (Table 3.16; Warshawsky *et al*., 1993).

Groups of 20 shaved male C3H/HeJ mice, 6–8 weeks of age, received twice-weekly dermal applications of 50 μL toluene solutions that contained 0.1% anthracene (purity, 99.5%), 0.1% chrysene, 0.1% fluoranthene (purity, >99%), 0.2% phenanthrene or 0.1% pyrene, or a mixture of 0.1% anthracene, 0.1% chrysene, 0.1% fluoranthene, 0.1% phenanthrene and 0.1% pyrene. Additional groups were treated in an identical manner with 0.001% benzo[*a*]pyrene (18 mice), each of the solutions in combination with 0.001% benzo[*a*]pyrene (20 mice per group) or the solvent alone (50 mice). The incidence of benign and malignant skin tumours after treatment for 14 weeks is given in Table 3.16 (Warshawsky *et al*., 1993). [None of the mixtures, with the exception of the

mixture of anthracene, chrysene, fluoranthene, phenanthrene, pyrene and benzo[*a*]pyrene, resulted in a tumour incidence that differed from that induced by benzo[*a*]pyrene alone. The tumour incidence induced by the mixture of anthracene, chrysene, fluoranthene, phenanthrene and pyrene did not differ from that induced by the mixture of anthracene, chrysene, fluoranthene, phenanthrene, pyrene and benzo[*a*]pyrene (Fisher's exact two-tailed test).]

Table 3.18. Tumour incidence and multiplicity in mice treated with benzo[*a*]pyrene, cyclopenta[*cd*]pyrene and combinations of the two compounds

Benzo[*a*]pyrene (nmol)	Cyclopenta[*cd*]-pyrene (nmol)	Tumour incidence (%)	Squamous-cell carcinoma/tumour-bearing animal
2.2		7	1.0
6.6		7	1.0
20		57	2.6
0	22.2	7	0.0
0	66.6	7	1.0
0	200	83	2.9
2.2	22.2	3	5.0
2.2	66.6	30*	1.0
2.2	200	97	3.8
6.6	22.2	31*	1.8
6.6	66.6	69*	7.2
20	22.2	83	3.8
20	200	87	5.1

From Cavalieri *et al.* (1983)
*Significantly greater that the sum of tumour incidence for the individual compounds at the same doses

Dermal initiation–promotion

Mouse

Groups of 30 shaved female Charles River CD-1 mice [age unspecified] received a single dermal application of 200 μL acetone that contained 100 μg benzo[*e*]pyrene (>99% pure), 100 μg pyrene [purity unspecified] or 100 μg fluoranthene [purity unspecified] or the solvent alone. After 5 min, the mice received a dermal application of 200 nmol benzo[*a*]pyrene [purity, solvent and volume unspecified]. One week later, the mice received twice-weekly dermal applications of 10 μg TPA for 30 weeks. The incidence of papillomas per mouse (± SD) was: benzo[*a*]pyrene, 100 ± 12; benzo[*e*]pyrene + benzo-[*a*]pyrene, 130 ± 13; pyrene + benzo[*a*]pyrene, 135 ± 18; and fluoranthene + benzo-

[*a*]pyrene, 123 ± 12 (Slaga *et al*., 1979). [Each of the mixtures significantly increased the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's test). Since the compounds were not tested individually, it cannot be ascertained whether the increase is due to an additive response or to an effect of the compounds on benzo[*a*]pyrene.] Additional experiments were conducted with 7,12-dimethylbenz[*a*]anthracene (DMBA) instead of benzo[*a*]pyrene and the opposite effect was observed, i.e. the number of papillomas per mouse decreased in the mixture-treated group compared with groups treated with DMBA alone.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 nmol benzo[*e*]pyrene [purity unspecified] in 200 μL acetone or the solvent alone. After 5 min, the mice received a single application of 200 nmol benzo[*a*]pyrene [purity unspecified] in 200 μL acetone and, 1 week later, twice-weekly dermal applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 3.3 for benzo[*a*]pyrene-treated mice (88% papilloma incidence) and 4.3 for benzo[*e*]pyrene + benzo[*a*]pyrene-treated mice (94% tumour incidence). The standard deviation for each of these values was <16%. Random papillomas were verified histologically (DiGiovanni *et al*., 1982). [Benzo[*e*]pyrene caused a statistically significant increase in the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's test). Since benzo[*e*]pyrene was not tested individually, it cannot be ascertained whether the increase was due to an additive response or to an effect of benzo[*e*]pyrene on benzo[*a*]pyrene.] Additional experiments were conducted using DMBA, and the opposite effect was observed, i.e. the number of papillomas per mouse decreased in the mixture-treated group compared with the group treated with DMBA alone. Further experiments were conducted with 3-methylcholanthrene instead of benzo[*a*]pyrene and the results were inconclusive.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 or 400 nmol dibenz[*a,c*]anthracene [purity unspecified] in 200 μL acetone or the solvent alone. After 5 min, the mice received an application of 200 nmol benzo[*a*]pyrene in 200 μL acetone and, 1 week later, twice-weekly dermal applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 3.3 for benzo[*a*]pyrene (88% papilloma incidence) [the Working Group noted that this was the same group of animals as that used in the previous experiment], 3.2 for 200 nmol dibenz[*a,c*]anthracene + benzo[*a*]pyrene (97% tumour incidence) and 2.9 for 400 nmol dibenz[*a,c*]anthracene + benzo[*a*]pyrene (88% tumour incidence). The standard deviation for each of these values was <20%. Dibenz[*a,c*]-anthracene had 'little to no' tumour-initiating activity (DiGiovanni *et al*., 1982). [Treatment with 400 nmol dibenz[*a,c*]anthracene caused a statistically significantly increase in the number of papillomas per mouse compared with that observed with benzo[*a*]pyrene alone (ANOVA, followed by Dunnett's).] Additional experiments were conducted with DMBA and 3-methylcholanthrene. Dibenz[*a,c*]anthracene appeared to

inhibit the tumour multiplicity induced by these compounds to an even greater extent than for tumours induced by benzo[*a*]pyrene.

Groups of 30 shaved female SENCAR mice, 7–9 weeks of age, received a single dermal application of 200 nmol benzo[*e*]pyrene or dibenzo[*a,c*]anthracene in 200 μL acetone or the solvent alone. After 5 min, the mice received a single dermal application of 200 nmol 5-methylchrysene [purity unspecified] or dibenz[*a,h*]anthracene [purity unspecified] in 200 μL acetone, followed 1 week later by twice-weekly applications of 3.4 nmol TPA in 200 μL acetone for 16 weeks. The incidence of papillomas per mouse was 2.9 for 5-methylchrysene (63% papilloma incidence), 2.6 for 5-methylchrysene + benzo[*e*]pyrene (64% tumour incidence) and 2.9 for 5-methylchrysene + dibenz[*a,c*]-anthracene (69% tumour incidence); 6.8 for dibenz[*a,h*]anthracene (91% papilloma incidence), 3.5 for dibenz[*a,h*]anthracene + benzo[*e*]pyrene (74% tumour incidence) and 4.1 for dibenz[*a,h*]anthracene + dibenz[*a,c*]anthracene (71% tumour incidence). The standard deviation for each of these values was <20% (DiGiovanni *et al*., 1982). [Benzo[*e*]pyrene and dibenzo[*a,c*]anthracene caused a statistically significantly increase in the number of papillomas per mouse compared with that observed with dibenzo[*a,h*]-anthracene alone, but had no effect upon the tumour multiplicity induced by 3-methyl-cholanthrene (ANOVA, followed by Dunnett's test)]. Additional experiments were conducted with 7- and 12-methylbenz[*a*]anthracene. The tumour multiplicity with 7-methylbenz[*a*]anthracene was decreased, but that with 12-methylbenz[*a*]anthracene was not affected.

Subcutaneous administration

Mouse

Groups of 50 male and 50 female C57 black mice, 3–4 months of age, received a single subcutaneous injection of 5.0 mg chrysene [purity unspecified], 5.0 mg benz[*a*]anthracene [purity unspecified], 20 μg dibenz[*a,h*]anthracene [purity unspecified] or mixtures of 2.5 mg chrysene + 2.5 mg benz[*a*]anthracene or 5.0 mg benz[*a*]anthracene + 20 μg dibenz[*a,h*]anthracene in 500 μL tricaprylin. A total of 304 control mice were given a single injection of 0.2–2.0 mL tricaprylin. Mice that survived 4 months after the treatment were evaluated for tumour incidence and the experiment was continued for 22 months. The incidence of sarcomas (histologically verified) is given in Table 3.19 (Steiner & Falk, 1951). [The addition of benz[*a*]anthracene to dibenz[*a,h*]anthracene significantly decreased (Fisher's exact two-tailed test) the response observed with dibenz[*a,h*]anthracene alone based upon the number of mice alive 4 months after treatment. The difference was not significant when the comparison was made based upon the number of animals alive when the first tumour occurred. The response observed with chrysene and benz[*a*]anthracene appeared to be additive, and was also marginally greater than that observed with benz[*a*]anthracene alone when the comparison was made based upon the number of animals alive 4 months after treatment. It was significantly greater

when the comparison was made based upon the number of animals alive when the first tumour appeared.]

Table 3.19. Incidence of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma[a]	Sarcoma[b]
Chrysene (5 mg)	4/39 (10.3%)	4/24 (16.7%)
Benz[*a*]anthracene (5.0 mg)	8/46 (17.4%)	8/44 (18.2%)
Dibenz[*a,h*]anthracene (20 μg)	28/48 (58.3%)	28/48 (58.3%)
Chrysene (2.5 mg) + benz[*a*]anthracene (2.5 mg)	14/41 (36.6%)	15/34 (44.1%)*
Benz[*a*]anthracene (5.0 mg) + dibenz[*a,h*]anthracene (20 μg)	11/39 (28.2%)**	11/30 (36.7%)
Tricaprylin (0.2–2.0 mL)	3/280 (1.1%)	3/233 (1.3%)

From Steiner & Falk (1951)
PAH, polycyclic aromatic hydrocarbon
[a]Based upon those alive 4 months after treatment
[b]Based upon those alive when the first tumour occurred
*Significantly different from benz[*a*]anthrancene alone
**Significantly different from dibenz[*a,h*]anthrancene alone

Groups of 40–50 male and 40–50 female C57 black mice [age unspecified] received a single subcutaneous injection of 20 or 40 μg dibenz[*a,h*]anthracene [purity unspecified], 5 or 10 mg benz[*a*]anthracene [purity unspecified], 90 μg benzo[*a*]pyrene [purity unspecified], 5 mg chrysene [purity unspecified], 5 mg anthracene [purity unspecified] or 5 mg phenanthrene [purity unspecified], or mixtures of 20 or 40 μg dibenz[*a,h*]anthracene + 5 or 10 mg benz[*a*]anthracene, 90 μg benzo[*a*]pyrene + 5 mg benz[*a*]anthracene, 20 μg dibenz[*a,h*]anthracene + 5 mg chrysene, 20 μg dibenz[*a,h*]anthracene + 5 mg anthracene or 20 μg dibenz[*a,h*]anthracene and 5 mg phenanthrene. The number of mice alive when the first sarcoma appeared was used to calculate the tumour incidence. The experiments were continued for 22–28 months. The incidence of sarcomas (histologically verified) is given in Table 3.20 (Steiner, 1955). [The addition of benz[*a*]anthracene, chrysene, anthracene or phenanthrene to dibenz[*a,h*]anthracene did not significantly affect the incidence of sarcomas observed with treatment with dibenz[*a,h*]anthracene alone (Fisher's exact two-tailed test). Similarly, the addition of benz[*a*]anthracene to benzo[*a*]-pyrene did not significantly affect the indicence of sarcomas observed with benzo[*a*]-pyrene alone.]

In a subsequent experiment, mice were administered 50, 200 or 1000 μg benz[*a*]-anthracene with or without 20 μg dibenz[*a,h*]anthracene (Steiner, 1955) (see Table 3.20). [The only significant difference occurred when dibenz[*a,h*]anthracene was combined with 50 μg benz[*a*]anthracene.]

Groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 275 μg dibenz[*a,h*]anthracene [purity unspecified] in ethyl laurate [volume

unspecified] either alone or in combination with phenanthrene [purity unspecified] in molar ratios (phenanthrene:dibenz[*a*,*h*]anthracene) of 12:1, 24:1 or 48:1. The incidence of sarcomas 30 months after treatment is given in Table 3.21 (Falk *et al*., 1964). [Although there was a decrease in the incidence of sarcomas with the addition of phenanthrene, the difference was not significant (Fisher's exact two-tailed test). There was also a decreasing trend when the ratio of phenanthrene:dibenzo[*a*,*h*]anthracene was increased, but this was not significant either.]

Table 3.20. Incidence of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma
Experiment 1	
Dibenz[*a,h*]anthracene (20 μg)	7/21 (33%)
Dibenz[*a,h*]anthracene (40 μg)	6/18 (33%)
Benz[*a*]anthracene (5 mg)	20/36 (56%)
Benz[*a*]anthracene (10 mg)	5/16 (31%)
Benzo[*a*]pyrene (90 μg)	16/21 (76%)
Chrysene (5 mg)	5/22 (23%)
Anthracene (5 mg)	0/26 (0%)
Phenanthrene (5 mg)	0/27 (0%)
Dibenz[*a,h*]anthracene (20 μg) + benz[*a*]anthracene (5 mg)	14/35 (40%)
Dibenz[*a,h*]anthracene (40 μg) + benz[*a*]anthracene (10 mg)	16/27 (59%)
Benzo[*a*]pyrene (90 μg) + benz[*a*]anthracene (5 mg)	16/27 (59%)
Dibenz[*a,h*]anthracene (20 μg) + chrysene (5 mg)	9/25 (36%)
Dibenz[*a,h*]anthracene (20 μg) + anthracene (5 mg)	13/29 (45%)
Dibenz[*a,h*]anthracene (20 μg) + phenanthrene (5 mg)	14/26 (54%)
Experiment 2	
Dibenz[*a,h*]anthracene (20 μg)	7/21 (33%)
Benz[*a*]anthracene (50 μg)	5/44 (11%)
Benz[*a*]anthracene (200 μg)	11/45 (24%)
Benz[*a*]anthracene (1000 μg)	15/44 (34%)
Benz[*a*]anthracene (50 μg) + dibenz[*a,h*]anthracene (20 μg)	18/28 (64%)*
Benz[*a*]anthracene (200 μg) + dibenz[*a,h*]anthracene (20 μg)	14/22 (64%)
Benz[*a*]anthracene (1000 μg) + dibenz[*a,h*]anthracene (20 μg)	13/25 (52%)

From Steiner (1955)
PAH, polycyclic aromatic hydrocarbon
*Significantly different from dibenz[*a,h*]anthracene (20 μg) alone

Additional groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 60 μg dibenz[*a*,*h*]anthracene in ethyl laurate either alone or in combination with phenanthrene in a molar ratio (phenanthrene:dibenz[*a*,*h*]anthracene) of 24:1. The incidence of sarcomas 30 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al*., 1964). [Although there was a decrease in incidence of

sarcomas with the addition of phenanthrene, the difference was not significant (Fisher's exact two-tailed test).]

Table 3.21. Incidences of sarcomas in mice after subcutaneous injection of some PAHs and binary mixtures of PAHs

Compound	Sarcoma
Experiment 1	
Dibenzo[*a*,*h*]anthracene (275 μg)	~16/30[a] (53%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (12:1)	~14/30[a] (47%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (24:1)	~9/30[a] (30%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (48:1)	~10/30[a] (33%)
Experiment 2	
Dibenzo[*a*,*h*]anthracene (60 μg)	~9/30[a] (30%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (12:1)	~3/30[a] (10%)
Experiment 3	
Dibenzo[*a*,*h*]anthracene (30 μg × 4)	~12/30[b] (40%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	~13/30[b] (43%)
Dibenzo[*a*,*h*]anthracene (60 μg × 2)	19/30[b] (63%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	16/30[b] (53%)
Dibenzo[*a*,*h*]anthracene (120 μg × 1)	24/30[b] (80%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1)	14/30*[b] (47%)
Dibenzo[*a*,*h*]anthracene (150 μg)	16/30[b] (53%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1) 100 μL triethylene glycol	28/30*[b] (93%)
Phenanthrene + dibenzo[*a*,*h*]anthracene (15:1) 200 μL triethylene glycol	30/30*[b] (100%)
Benzo[*a*]pyrene (400 μg)	27/30[b] (90%)
Benzo[*a*]fluorene + benzo[*a*]pyrene (0.1:1)	2/30*[b] (67%)
Perylene + benzo[*a*]pyrene (0.1:1)	5/30*[b] (17%)
Chrysene + benzo[*a*]pyrene (0.15:1)	3/30*[b] (10%)
Benzo[*k*]fluoranthene + benzo[*a*]pyrene (1:1)	5/30*[b] (17%)
Benzo[*mno*]fluoranthene + benzo[*a*]pyrene (1:1)	3/30*[b] (10%)
Anthracene + phenanthrene + pyrene + benzo[*a*]pyrene (10:10:10:1)	1/30*[b] (3%)

From Falk *et al.* (1964)
PAH, polycyclic aromatic hydrocarbon
* Significantly different from the control group
[a] After 30 months
[b] After 18 months

In a subsequent experiment, groups of 30 male C57BL mice [age unspecified] received four subcutaneous injections of 30 μg dibenz[*a*,*h*]anthracene in ethyl laurate at 2-month intervals, two subcutaneous injections of 60 μg dibenz[*a*,*h*]anthracene in ethyl laurate at 4-month intervals or a single subcutaneous injection of 120 μg dibenz[*a*,*h*]-anthracene in ethyl laurate, either alone or in combination with phenanthrene in a molar ratio (phenanthrene:dibenz[*a*,*h*]anthracene) of 15:1. The incidence of sarcomas 18 months

after treatment is given in Table 3.21 (Falk *et al*., 1964). [There was a significant decrease in the incidence of sarcomas with the addition of phenanthrene in a single injection (Fisher's exact two-tailed test).]

In a further experiment, groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 500 μg dibenz[*a,h*]anthracene in 100 μL triethylene glycol either alone or in combination with phenanthrene in a molar ratio (phenanthrene: dibenz[*a,h*]anthracene) of 15:1. One additional group was administered the mixture in 200 μL triethylene glycol. The incidence of sarcomas 18 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al.*, 1964). [There was a significant increase in the incidence of sarcomas with the addition of phenanthrene when triethylene glycol was used as the vehicle (Fisher's exact two-tailed test).]

In another experiment, groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 400 μg benzo[*a*]pyrene [purity unspecified] in tricaprylin [volume unspecified] either alone or in combination with benzo[*a*]fluorene [purity unspecified] (molar ratio of benzo[*a*]fluorene:benzo[*a*]pyrene, 0.1:1), perylene [purity unspecified] (molar ratio of perylene:benzo[*a*]pyrene, 0.1:1), chrysene [purity unspecified] (molar ratio of chrysene:benzo[*a*]pyrene, 0.15:1), benzo[*k*]fluoranthene [purity unspecified] (molar ratio of benzo[*k*]fluoranthene:benzo[*a*]pyrene, 1:1), benzo-[*mno*]fluoranthene [purity unspecified] (molar ratio of benzo[*mno*]fluoranthene: benzo[*a*]pyrene, 1:1) or a mixture of anthracene, phenanthrene and pyrene [purities unspecified] (molar ratio of anthracene:phenanthrene:pyrene:benzo[*a*]pyrene, 10:10:10:1). The incidence of sarcomas 18 months after treatment (verified histologically) is given in Table 3.21 (Falk *et al*., 1964). [There was a significant decrease in the incidence of sarcomas with the addition of all compounds (Fisher's exact two-tailed test).]

Additional groups of 30 male C57BL mice, 3–4 months of age, received a single subcutaneous injection of 400 μg benzo[*a*]pyrene in tricaprylin [volume unspecified] in combination with acenaphthylene [purity unspecified] (molar ratio of acenaphthylene:benzo[*a*]pyrene, 5:1), fluorene [purity unspecified] (molar ratio of fluorene: benzo[*a*]pyrene, 1:1), coronene [purity unspecified] (molar ratio of coronene: benzo[*a*]pyrene, 1:1), benzo[*ghi*]perylene [purity unspecified] (molar ratio of benzo[*ghi*]-perylene:benzo[*a*]pyrene, 0.3:1), dibenzo[*b,e*]pyrene [purity unspecified] (molar ratio of dibenzo[*b,e*]pyrene:benzo[*a*]pyrene, 0.15:1), dibenzo[*a,l*]pyrene [purity unspecified] (molar ratio of dibenzo[*a,l*]pyrene:benzo[*a*]pyrene, 0.1:1), dibenz[*a,h*]anthracene [purity unspecified] (molar ratio of dibenz[*a,h*]anthracene:benzo[*a*]pyrene, 0.1:1), indeno[1,2,3-*cd*]pyrene [purity unspecified] (molar ratio of indeno[1,2,3-*cd*]pyrene:benzo[*a*]pyrene, 0.1:1) or anthanthrene [purity unspecified] (molar ratio of anthanthrene:benzo[*a*]pyrene, 0.1:1). The observation period and resultant tumour data were not reported; however, the authors stated that "no inhibitory effects on benzo[*a*]pyrene carcinogenesis could be observed" (Falk *et al*., 1964).

Groups of 100 female C57BL mice [age unspecified] received a single subcutaneous injection of 3.12, 6.25, 12.5, 25.0, 50.0 or 100.0 μg benzo[*a*]pyrene [purity unspecified] in

500 μL tricaprylin (Series A). Additional groups (Series B) were similarly treated with 2.35, 4.7, 9.3, 18.7, 37.5 or 75.0 μg dibenz[*a,h*]anthracene [purity unspecified]. Further groups of mice (Series C) were treated with a mixture of 10 PAHs that included benzo[*e*]pyrene, benz[*a*]anthracene, phenanthrene, anthracene, pyrene, fluoranthene, chrysene, perylene, benzo[*ghi*]perylene and coronene [purities unspecified]. Additional groups were treated with mixtures of benzo[*a*]pyrene and dibenz[*a,h*]anthracene (Series D) and mixtures of these two compounds and the other 10 PAHs (Series E), at the same mass ratios as those used in the preceding studies. The study was continued for 114 weeks, at which time fewer than 10% of the control mice were alive. The incidence of sarcomas increased in a dose–responsive manner in mice treated with benzo[*a*]pyrene, from 9/100 at 3.12 μg to 83/100 at 100 μg. Dibenz[*a,h*]anthracene also increased the incidence of sarcomas in a dose–responsive manner, from 37/100 at 2.35 μg to 69/100 at 75 μg. In mice treated with the mixture of 10 PAHs, the incidence of sarcomas varied between 4/100 and 13/100, and a dose–response was not evident. The mixture of benzo[*a*]pyrene and dibenz[*a,h*]anthracene was 1.4 times more effective at inducing sarcomas than dibenz[*a,h*]anthracene alone, a difference that was not statistically significant. The shape of the dose–response curve for benzo[*a*]pyrene and dibenz[*a,h*]-anthracene was similar to that for dibenz[*a,h*]anthracene alone. Administration of the mixture of benzo[*a*]pyrene and dibenz[*a,h*]anthracene and the 10 PAHs gave a sarcoma response that was similar to that induced by dibenz[*a,h*]anthracene alone (Pfeiffer, 1973, 1977).

Intraperitoneal administration

Mouse

Groups of 20 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 200 μL tricaprylin that contained a mixture of five PAHs: 30 or 75 mg/kg benzo[*a*]pyrene (purity, ≥98%), 30 or 75 mg/kg bw benzo[*b*]fluoranthene (purity, 99%), 2.5 or 10 mg/kg bw dibenz[*a,h*]anthracene (purity, 97%), 10 or 30 mg/kg bw 5-methyl-chrysene (purity, 99%) and 30 or 100 mg/kg bw cyclopenta[*cd*]pyrene (purity, 99%) using a 2^5 factorial design. Eight months after treatment, the number of lung adenomas was assessed. No histopathology was carried out. Survival in the 32 groups ranged from 70 to 100% (mean, 85%). Body weights were initially affected by treatment. The incidence of lung adenomas was 100% in each group, with the exception of the solvent-treated control group, which had a 26% incidence. Tumour multiplicity ranged from 16.8 to 63.8 lung adenomas per mouse, compared with 0.32 lung adenomas per mouse in the tricaprylin controls, a difference that was significant (p <0.01). Two groups exhibited a statistically significant (p <0.05) increase in tumour multiplicity compared with that expected from summation of the tumour response of the individual PAHs. [The identity of these mixtures was not given]. Thirteen groups exhibited a statistically significant (p<0.05) decrease in tumour multiplicity compared with that expected from summation of

the tumour response of the individual PAHs. [The identity of these mixtures was not given] (Nesnow *et al*., 1998a).

In a subsequent experiment, the effect of pyrene on a quintary mixture was examined. Specifically, a group of 20 male A/J mice, 6–8 weeks of age, received a single intraperitoneal injection of 100 mg/kg bw pyrene (purity, 99.7%), 30 mg/kg bw benzo[*a*]-pyrene, 30 mg/kg bw benzo[*b*]fluoranthene, 2.5 mg/kg bw dibenz[*a,h*]anthracene, 30 mg/kg bw 5-methylchrysene and 100 mg/kg bw cyclopenta[*cd*]pyrene. After 8 months, all of the mice had lung adenomas, with a mean multiplicity of 30.5 adenomas per mouse. This was a statistically significant reduction compared with the multiplicity of 47.1 adenomas per mouse in mice treated with the mixture of 5 PAHs. Mice administered pyrene (10–200 mg/kg) had a 15–40% incidence of adenomas, with a multiplicity of 0.3–0.6 adenomas per mouse, which did not differ significantly from those in the control group (Nesnow *et al*., 1998a).

Environmental mixtures of PAHs

Dermal application

Mouse

Groups of 50 male Swiss CD-1 and C3H/HeJ mice [age unspecified] received twice-weekly dermal applications of two types of asphalt and two types of coal-tar pitch used on roofs for 78 weeks. The materials, which were chosen on the basis of their common use and extremes of classification, were Type I ('dead level') and Type III ('steep') asphalt and Type I ('regular roofing') and Type III ('low fuming' or 'low burn') coal-tar pitch (see Table 3.22 for PAH content). Fumes were collected at two temperatures (232 °C and 316 °C), and condensed material was collected using a glass cryogenic system. The collections at 316 °C gave 9–16 times more volatile material from the asphalts and 2–7 times more volatile material from the coal-tar pitches as compared with the yield from 232 °C collections. The materials were applied in 50 μL of a mixture of cyclohexane and acetone (1:1). The coal-tar pitch condensate solutions were adjusted to give a concentration of benzo[*a*]pyrene of approximately 0.01% (100 μg/mL), which resulted in 30–84 mg/mL of condensed fumes being applied, depending upon the specific coal-tar pitch. The asphalt condensate solutions were adjusted to give 50% total solids (500 mg/mL). The benzo[*a*]pyrene content of these solutions was ≤3 μg/mL. Benzo[*a*]pyrene [purity not specified] (100 μg/mL) was used as a positive control; and other control mice were treated with the solvent. Additional groups were exposed on alternate weeks to Type I coal-tar pitch condensate and Type III asphalt condensate. One arm of the experiment included exposure to simulated solar light. This arm is not considered further. The incidence of malignant tumours (squamous-cell carcinoma and fibrosarcoma) was much lower in CD-1 mice (~5%) compared with C3H/HeJ mice (~60%), and fibrosarcomas were more common in C3H/HeJ mice. C3H/HeJ mice treated

with the condensates had decreased survival compared with the controls, except for those exposed to 232 °C Type I asphalt condensate. CD-1 mice treated with 232 °C Type I and 232 °C Type III coal-tar pitch condensate had lower survival than the control group. C3H/HeJ mice exposed to the 316 °C asphalt condensates had a decreased tumour latency compared with those exposed to 232 °C asphalt condensates. The temperature of the coal-tar pitch condensates had no effect on tumour latency in C3H/HeJ mice. The temperature of the asphalt or coal-tar pitch condensates had no effect on tumour latency in CD-1 mice. Compared with C3H/HeJ mice exposed to benzo[*a*]pyrene alone, C3H/HeJ mice treated with each of the condensates (except for 232 °C Type I and Type III asphalt) had a decreased tumour latency. In CD-1 mice, treatment with 232 °C Type I and Type III asphalt increased tumour latency, while 316 °C Type III coal-tar pitch condensate decreased tumour latency (Niemeier *et al.*, 1988).

Table 3.22. Concentration of PAHs (μg/mL) in solutions for dermal application

PAH	Asphalt[a]				Pitch[a]			
	Type I		Type III		Type I		Type III	
	232 °C	316 °C	232 °C	316 °C	232 °C	316 °C	232 °C	316 °C
Naphthalene	22	4	17	49	>1800	1770	288	620
Fluorene	26	22	39	28		740		
Anthracene/phenanthrene	180	53	300	69	>960	2960	>2580	>5200
Fluoranthene	86	20	97	7	>2940	2350	>960	>2800
Pyrene	70	9	63	8	>2070	1790	>720	>2300
Benz[*a*]anthracene	11	10	8	6	570	330	330	800
Chrysene/Triphenylene	25	19	13	14	460	300	290	710
Benzofluoranthenes	3	4	5	–	230	230	250	260
Benzo[*e*]pyrene	6	8	4	1	42	51	45	45
Benzo[*a*]pyrene	2	2	3	–	96	85	102	90
Indeno[*cd*]pyrene	3	3	2	–	33	2	11	7
Benzo[*ghi*]perylene	1	2	1	–	28	2	7	1
Dibenzanthracenes	2	–	2	–	12	–	4	–
Coronene	–	–	–	–	–	–	–	–
Dibenzopyrenes	–	–	–	–	–	–	–	–

From Niemeier *et al*. (1988)
PAH, polycylic aromatic hydrocarbon
[a] –, not tested

Groups of 30 shaved male C3H/HeJ mice, 8 weeks of age, received twice-weekly dermal applications for 104 weeks of 50 μL cyclohexane:acetone (1:1) that contained 25 mg standard commercial Type III 'steep' asphalt. Additional groups were similarly treated with 25 mg asphalt that had been heated to 316 °C, 25 mg of a combination of asphalt that had been heated to 316 °C and the fumes that were released and collected by condensation and 25 mg of the fumes that resulted from heating asphalt to 316 °C. There

was no consistent effect of treatment on survival. Histopathology was conducted. After 104 weeks, the only exposure that resulted in a significant induction of skin tumours was asphalt fumes, which gave a total of 12 skin papillomas and 25 skin carcinomas in 21 tumour-bearing mice compared with no tumours in the solvent-treated controls (see Table 3.23). The collected fume condensates were then fractionated into five fractions, designated A, B, C, D and E, and these were applied to additional groups of C3H/HeJ mice (either alone or in combination) in amounts corresponding to their original contribution to the fume condensate. Only fraction B (or mixtures containing fraction B), which contained benzothiophenes, anthracenes and/or phenanthrenes, fluorenes, pyrenes and/or fluoranthenes, benzofurans and fluorenones, and fraction C (or mixtures containing fraction C), which contained various ketones, pyrenes and/or fluoranthenes, chrysenes and fused-ring thiophenes, demonstrated significant activity (see Table 3.23). In an additional experiment, groups of 30 male SENCAR mice, 8 weeks of age, received twice-weekly dermal applications for 104 weeks of 50 μL cyclohexane:acetone (1:1) that contained 25 mg condensed asphalt fume or the solvent alone. After 104 weeks, the condensed fume exposure resulted in a significant induction of skin tumours with a total of 21 skin papillomas and 18 skin carcinomas in 20 tumour-bearing mice compared with no tumours in the solvent-treated controls (see Table 3.23) (Sivak *et al.*, 1997).

Table 3.23. Tumour incidence in male C3H/HeJ and SENCAR mice exposed dermally to asphalts

Compound	No. surviving 104 weeks	Total no. of papillomas/ group	Total no. of carcinomas/ group	No. of TBAs	No. of tumours/ TBA
Experiment 1: C3H/HeJ					
Type III 'steep asphalt'	15/30 (50%)	1	3	4	1.0
Heated asphalt (less fumes)	18/30 (60%)	0	0	0	0
Heated asphalt (plus fumes)	21/30 (70%)	0	0	0	0
Heated asphalt fumes	2/30 (7%)	12*	25*	21	1.8
Solvent control	11/30 (37%)	0	0	0	0
Fraction A	19/30 (63%)	0	0	0	0
Fraction B	12/30 (40%)	2	10*	11	1.1
Fraction C	6/30 (20%)	4	18*	20	1.1
Fraction D	7/30 (23%)	0	0	0	0
Fraction E	13/30 (43%)	0	0	0	0
Experiment 2: SENCAR					
Heated asphalt fume	5/30 (17%)	21*	18*	20	2.0
Solvent control	18/30 (60%)	0	0	0	0

From Sivak *et al.* (1997)
TBA, tumour-bearing animal
*Significantly different from the control group

Dermal initiation–promotion studies

Mouse

Groups of 30 shaved female CD-1 mice [age unspecified] received dermal applications of narrow temperature range distillates from solvent-refined coal (SRC) heavy-end coal liquids. Specifically, 17 mg of 800–850 °F+ [427–454 °C+] distillate from SRC-I process solvent, 17 mg of 800–850 ºF [427–454 °C+] distillate from SRC-II process solvent or 17 mg >850 ºF [>454 °C+] distillate from SRC-II process solvent (see Table 3.24 for PAH content) in 50 μL of a mixture of acetone and methylene chloride (1:1) once to the back. Additional mice were treated with subfractions of the materials, which were obtained by chromatographing the materials on alumina to give fractions that contained primarily aliphatic and olefinic compounds (A1), neutral PAHs (A2), nitrogen-containing PAHs (A3) and hydroxy PAHs (A4). These subfractions were applied in amounts corresponding to their original contribution to the mixture. Control mice were treated with the acetone and methylene chloride mixture. Positive-control mice were given a single dose of 50 μg benzo[*a*]pyrene in 50 μL of the same solvent. Two weeks later, all mice were given twice-weekly dermal applications of 5 μg TPA in 50 μg acetone for 24 weeks. After 24 weeks of TPA, the incidence of skin tumours (no histopathology) was 76% (2.24 tumours/mouse) for 800–850 ºF+ [427–454 °C+] distillate from SRC-I process solvent, 72% (1.43 tumours/mouse) for 800–850 ºF [427–454 °C+] distillate from SRC-II process solvent and 79% (4.59 tumours/mouse) for >850 ºF [>454 °C+] distillate from SRC-II process solvent. The control group had an incidence of 14% (0.17 tumours/mouse; see Table 3.25). Subfraction A2 showed the greatest tumour-initiating activity, followed by A3, then A1 and A4 (Mahlum *et al.*, 1984; Springer *et al.*, 1988). [Each of the crude distillates was more active than controls based upon incidence (Fisher's exact one-sided test) or tumours/mouse (ANOVA, Dunnett's test). Fractions A2 and A3 also tended to be significantly different from controls based upon incidence and multiplicity. The >850 ºF [>454 °C+] SRC-II was significantly different from the other two fractions based upon tumours/mouse.]

Groups of 30 or 40 shaved female SENCAR mice [age unspecified] received dermal applications of either coal tar-based paints or petroleum asphalt-based paints. Three coal tar-based paints (designated E, F and G) were used and these typically contained measurable quantities (>1 mg/g) of naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, 2-methylphenanthrene, fluoranthene, pyrene, chrysene + benz[*a*]anthracene and benzo[*a*]pyrene + benzo[*e*]pyrene. Four petroleum asphalt-based paints (designated A, B, C and D) were used; two of these (A and D) were assessed for their PAH content and, with the exception of naphthalene, none (<0.01 mg/g) was detected. The coal tar-based paints were applied once at 0.2–20 μL/animal. Two weeks later, animals were treated thrice weekly for 20 weeks with 1.0 μg TPA in 200 μL acetone. Control mice were treated with acetone alone, and benzo[*a*]pyrene (10 μg) was used as a positive control. Each of the coal tar-based paints caused an [significant; Fisher's exact one-tailed

test] increase in the incidence of papilloma and carcinoma, typically at doses ≥0.6 μL. Benzo[*a*]pyrene caused an [significant; Fisher's exact one-tailed test] increase in the incidence of papilloma. The petroleum asphalt-based paints were applied once at 200 μL/animal. Two weeks later, animals were treated thrice weekly for 20 weeks with 1.0 μg TPA in 200 μL acetone. Control mice were treated with acetone alone. None of the petroleum asphalt-based paints caused an increase in the incidence of papilloma and carcinoma, with one exception. In an additional experiment, groups of 40 mice were treated weekly for 30 weeks with 2 μL coal tar E or 200 μL petroleum asphalt D. Control mice were treated with the solvent (200 μL mineral spirits) alone. Coal tar caused an [significant; Fisher's exact one-tailed test] increase in the incidence of carcinoma (Robinson *et al.*, 1984).

Table 3.24. Levels of several PAHs in the neutral PAH fractions (ppm)

Compound	SRC-I[a] (800–850 °F+ [427–454 °C+])	SRC-II[a] (800–850 °F [427–454 °C+])	SRC-II[a] (>850 °F [>454 °C+])
Naphthalene	–	–	–
Acenaphthylene	–	10	12
Acenaphthene	–	–	–
Fluorene	–	41	–
Phenanthrene	60	211	58
Fluoranthene	–	232	30
Pyrene	30	2275	235
9,10-Dimethylanthracene	–	22	–
2,4-Methylpyrene or benzo[*i*]fluorene	–	5742	–
1-Methylpyrene	–	1307	–
Benz[*a*]anthracene	387	2997	–
Chrysene	563	7324	31
Methylchrysene	3372	3320	215
Benzo[*b*]fluoranthene	2316	9592	2361
Benzo[*k*]fluoranthene	306	86	3972
Dimethylbenzanthracene	95	4532	–
Benzo[*e*]pyrene	2305	6135	5755
Benzo[*a*]pyrene	1707	3530	3637
Indeno[1,2,3-*cd*]pyrene	428	–	7232
Dibenz[*a,c*]anthracene or dibenz[*a,h*]anthracene	7232	–	1997
Benzo[*ghi*]perylene	366	–	15311
Coronene	–	–	1072

From Mahlum *et al* (1984); Springer *et al.* (1988)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal
[a] –, not determined by gas chromatography because of their low volatility

Groups of 20 shaved female SENCAR mice, 6 weeks of age, received a single application of 200 μL standard crude coal-tar solution. Additional groups of 20 mice received 39 nmol 7,12-dimethylbenz[*a*]anthracene [purity unspecified], 746 nmol 3-methylcholanthrene [purity unspecified], 396 nmol benzo[*a*]pyrene [purity unspecified] or 352 nmol 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene [purity unspecified] in 200 μL acetone or 200 μL acetone alone. Seven days later, all mice were treated twice weekly with 3.24 nmol TPA. The experiment lasted 11 weeks. [Histopathology was not performed, and statistical analyses were not conducted]. All animals developed skin tumours, and the average number of tumours/mouse was 24.3, 15.0, 9.8, 6.6 and 3.3 in those treated with 7,12-dimethylbenz[*a*]anthracene, 3-methylcholanthrene, benzo[*a*]-pyrene, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene and coal-tar solution, respectively. A dose of 500 μL coal-tar solution gave a tumour incidence similar to that of the 200 μL treatment (Mukhtar *et al*., 1986).

Table 3.25. Skin tumour incidence in female CD-1 mice treated dermally with SRC[a]

Compound	Incidence (%)	Tumours/mouse
Control	14	0.17 ± 0.09
800–850 °F+ [427–454 °C+], SRC-I	76*	2.24 ± 0.39*
A1	20	0.30 ± 0.13
A2	88*	3.04 ± 0.52*
A3	60*	0.76 ± 0.16*
A4	12	0.12 ± 0.07
800–850 °F [427–454 °C+], SRC-II	72*	1.43 ± 0.26*
A1	27	0.27 ± 0.08
A2	92*	1.93 ± 0.23*
A3	61*	0.97 ± 0.20*
A4	25	0.27 ± 0.10
>850 °F [>454 °C+], SRC-II	79*	4.59 ± 0.62*
A1	43*	0.93 ± 0.34
A2	100*	6.89 ± 0.59*
A3	83*	2.07 ± 0.30*
A4	41*	0.40 ± 0.12

From Mahlum *et al.* (1984); Springer *et al.* (1988)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal
[a] A1, fractions containing primarily aliphatic and olefinic compounds; A2, neutral PAHs; A3, nitrogen-containing PAHs; A4, hydroxy-PAHs
* Significantly different from control

Groups of 30 shaved female CD-1 mice [age unspecified] received dermal applications of complex mixtures obtained from an SRC-II process. Specifically, five distillates were obtained from distillation of a full boiling-range (300–850 ºF+ [149–

454 °C+]) blend of atmospheric flash bottoms and recycle process solvent. The fractions were 300–700 ºF [149–371 °C+], 700–750 ºF [371–399 °C+], 750–800 ºF [399–427 °C+], 800–850 ºF [427–454 °C+] and >850 ºF [>454 °C+] (see Table 3.26 for distribution of PAHs). The distillates (5 mg) were applied once in 50 μL of a mixture of acetone and methylene chloride (1:1) to the shaved backs of the mice. Additional groups of 30 mice were treated with subfractions of the materials, obtained by chromatographing on alumina to give fractions that contained primarily aliphatic hydrocarbons (AH), PAH, nitrogen-containing PAH (NPAH) and hydroxy PAH (HPAH). These subfractions were applied in amounts corresponding to their original contribution to the mixture. Control mice were treated with the acetone and methylene chloride mixture alone. Positive-control mice were given a single dose of 50 μg benzo[*a*]pyrene in 50 μL of the same solvent. Additional groups of 30 mice were treated with each of the distillates in combination with 50 μg benzo[*a*]pyrene. Two weeks later, all mice were given twice-weekly dermal applications of 5 μg TPA in 50 μg acetone for 24 weeks. After 24 weeks of TPA, the incidence of skin tumours from the 300–700 °F [149–399 °C+] mixture did not result in a statistically significant increase in tumour multiplicity compared with controls (see Table 3.27). The other mixtures caused significant tumour-initiating activity with the order increasing as follows: 700–750 °F [371–399 °C+], 750–800 °F [399–427 °C+], 800–850 °F [427–454 °C+], >850 °F [>454 °C+]. When the mixtures were co-administered with benzo[a]pyrene, the 700–750 °F [371–399 °C+], 750–800 °F [399–427 °C+] and 800–850 °F [427–454 °C+] distillates decreased tumour multiplicity

Table 3.26. Concentrations of selected PAH components in SRC-II distillates (mg/g)

Compound	700–750 °F [371–399 °C]	750–800 °F [399–427 °C]	800–850 °F [427–454 °C]	>850 °F [>454 °C]
Fluoranthene	15	1.3		
Pyrene	140	18	1.2	
Benzo[*b*]fluorene	130	37	2.9	
1-Methylpyrene	17	8.0	0.53	
Benz[*a*]anthracene	2.0	3.1	1.5	
Chrysene	1.0	3.8	3.7	0.014
6- or 4-Methylchrysene	0.081	4.7	18	0.010
Benzo[*j* or *b*]fluoranthene			4.8	1.1
Benzo[*k*]fluoranthene			2.0	0.14
Benzo[*e*]pyrene			3.1	2.6
Benzo[*a*]pyrene			1.8	1.7
Indeno[1,2,3-*cd*]pyrene				3.3
Benzo[*ghi*]perylene				7
Coronene				0.49

From Springer *et al.* (1989)
PAH, polycyclic aromatic hydrocarbon; SRC, solvent-refined coal

compared with mice treated with benzo[*a*]pyrene alone. When AH, PAH, NPAH and HPAH subfractions from the 700–750 ºF [371–399 °C+] distillate were applied to the mouse in an amount similar to their contribution to the original mixture, only the PAH subfraction induced a significant increase in tumour multiplicity. When the subfractions from the 700–750 °F [371–399 °C+] distillate were co-administered with benzo[*a*]pyrene, the PAH and NPAH subfractions decreased tumour multiplicity compared with mice treated with benzo[*a*]pyrene alone (Springer *et al.*, 1989).

Table 3.27. Tumour incidence in female CD-1 mice exposed dermally to complex mixtures of PAHs

Compound	No. of mice/group	No. of tumours/mouse (± SEM)
Solvent	30	0.17 ± 0.07
300–700 °F [149–371 °C]	30	0.37 ± 0.13
700–750 °F [371–399 °C]	30	0.57 ± 0.14[a]
750–800 °F [399–427 °C]	30	0.60 ± 0.18[a]
800–850 °F [427–454 °C]	30	1.23 ± 0.43[a]
>850 °F [>454 °C]	29	4.52 ± 0.43[a]
B[*a*]P	30	7.07 ± 0.67
300–700 °F [149–371 °C] + B[*a*]P	30	6.63 ± 0.50
700–750 °F [371–399 °C] + B[*a*]P	29	4.14 ± 0.49[b]
750–800 °F [399–427 °C] + B[*a*]P	29	2.93 ± 0.33[b]
800–850 °F [427–454 °C] + B[*a*]P	30	3.00 ± 0.36[b]
>850 ºF [>454 °C] + B[*a*]P	30	6.33 ± 0.75
Solvent	29	0.24 ± 0.07
750–800 °F [399–427 °C]	29	0.69 ± 0.09[c]
AH from 750–800 °F [399–427 °C]	30	0.13 ± 0.07
PAH from 750–800 °F [399–427 °C]	30	0.67 ± 0.08[c]
NPAH from 750–800 °F [399–427 °C]	30	0.27 ± 0.06
HPAH from 750–800 °F [399–427 °C]	30	0.23 ± 0.07
B[*a*]P	29	7.21 ± 0.65
750–800 °F [399–427 °C] + B[*a*]P	30	2.23 ± 0.29[d]
AH from 750–800 °F [399–427 °C] + B[*a*]P	30	5.73 ± 0.78
PAH from 750–800 °F [399–427 °C] + B[*a*]P	30	2.50 ± 0.32[d]
NPAH from 750–800 °F [399–427 °C] + B[*a*]P	27	2.81 ± 0.51[d]
HPAH from 750–800 °F [399–427 °C] + B[*a*]P	27	6.44 ± 0.53

From Springer *et al.* (1989)
AH, aliphatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; HPAH, hydroxy PAH; NPAH, nitrogen-containing PAH; PAH, polycyclic aromatic hydrocarbon; SEM, standard error of the mean
[a] Significantly different from control (as reported by authors)
[b] Significantly different from B[*a*]P (ANOVA, followed by Dunnett's test)
[c] Significantly different from control (ANOVA, followed by Dunnett's test)
[d] Significantly different from B[*a*]P (ANOVA, followed by Dunnett's test)

Groups of 30 shaved female CD-1 mice, 7–8 weeks of age, received dermal applications on 5 days per week for 2 weeks of 50 mg of a 1.5% coal-tar ointment (Lorinden). Beginning 1 week after the coal-tar treatment, one group of mice received thrice-weekly dermal applications for 40 weeks of 50 mg of a 0.1% dithranol cream (promotion), while the second group of mice remained untreated. An additional group of mice was treated with dithranol only. After 40 weeks of treatment, skin papillomas (histologically verified) occurred in four mice administered coal tar followed by dithranol, in no mice treated with coal tar only and in no mice treated with dithranol only. The incidence of tumours in mice treated with coal tar and dithranol was significantly different (log-rank test) from that in mice treated with only coal tar or only dithranol (see Table 3.28). Benzo[*a*]pyrene, with dithranol promotion, which was used as a positive initiation control, gave papillomas in 14 mice (Phillips & Alldrick, 1994).

Table 3.28. Tumour incidence in female CD-1 mice treated dermally

Compound	No. of survivors at 40 weeks	No. of mice with skin tumours
Coal tar + dithranol	27/30 (90%)	4*
B[*a*]P + dithranol	28/30 (93%)	14*
Coal tar	30/30 (100%)	0
Dithranol	28/30 (93%)	0

From Phillips & Alldrick (1994)
B[*a*]P, benzo[*a*]pyrene
*Significantly different from coal tar alone

Groups of shaved female SENCAR mice, 6–7 weeks of age, received dermal applications of 1 mg medium crude coke-oven coal tar (standard reference material (SRM) 1597) that contained 10.4 μg benzo[*a*]pyrene in 125 μL toluene (30 mice) [the 10.4 μg benzo[*a*]pyrene/1 mg SRM 1597 is an error; the correct value is 95.8 ng (see Table 3.29)], 200 nmol (50.4 μg) benzo[*a*]pyrene [purity not specified] in 200 μL toluene (35 mice), 1 mg SRM 1597 + 200 nmol benzo[*a*]pyrene in 100 μL toluene (35 mice) or 200 μL toluene alone (10 mice). In a separate experiment, groups of shaved female SENCAR mice received dermal applications of 1 mg SRM 1597 in 125 μL toluene (30 mice), 2 nmol (600 ng) dibenzo[*a,l*]pyrene [purity, solvent and volume not specified] (35 mice), 1 mg SRM 1597 + 2 nmol dibenzo[*a,l*]pyrene [solvent and volume not specified] (35 mice) or 200 μL toluene alone (10 mice). Two weeks later, all mice received twice-weekly dermal applications of 1 μg TPA in 200 μL acetone for 25 weeks. Tumours were subjected to routine histopathology. After 25 weeks of TPA, 24/26 (92%) mice (4.92 tumours/mouse) treated with SRM 1597, 27/30 (90%) mice (8.03

tumours/mouse) treated with benzo[a]pyrene, 29/29 (100%) mice (8.72 tumours/mouse) treated with SRM 1597 + benzo[*a*]pyrene and 0/8 mice (0.12 tumours/mouse) treated with toluene developed skin tumours that were almost exclusively papillomas. Mice administered benzo[*a*]pyrene and SRM 1597 + benzo[*a*]pyrene had a significantly greater tumour multiplicity than those treated with SRM 1597 alone. There was no significant difference between treatment with benzo[*a*]pyrene and SRM 1597 + benzo[*a*]pyrene. After 25 weeks of TPA, 26/27 (96%) mice (3.41 tumours/mouse) treated with SRM 1597, 30/30 (100%) mice (7.87 tumours/mouse) treated with dibenzo[*a,l*]pyrene, 29/30 (97%) mice (4.67 tumours/mouse) treated with SRM 1597 + dibenzo[*a,l*]pyrene and 2/9 (22%) mice (0.25 tumours/mouse) treated with toluene developed skin tumours that were almost exclusively papillomas. Mice administered dibenzo[*a,l*]pyrene had a significantly greater tumour multiplicity than those treated with SRM 1597 + dibenzo[*a,l*]pyrene. Mice administered SRM 1597 + dibenzo[*a,l*]pyrene had a significantly greater number of tumours/mouse than those treated with SRM 1597. The authors concluded that SRM 1597 inhibited dibenzo[*a,l*]pyrene tumorigenicity but had no effect upon that of benzo[*a*]pyrene. Their mechanistic data did not provide a reason for this because SRM 1597 inhibited DNA adduct formation with both compounds, but did not induce cytochrome P450 1A1 or 1B1 (Marston *et al.*, 2001).

Table 3.29. Certified concentrations of selected PAHs in SRM 1597

Compound	Concentration (mg/kg)
Naphthalene	1160
Phenanthrene	462
Anthracene	101
Fluoranthene	322
Pyrene	235
Benz[*a*]anthracene	98.6
Chrysene	71.7
Triphenylene	12.1
Benzo[*a*]pyrene	95.8
Perylene	26.1
Indeno[1,2,3-*cd*]pyrene	60.2
Benzo[*ghi*]perylene	53.7

From Marston *et al.* (2001)
PAH, polycyclic aromatic hydrocarbon; SRM, standard reference material

Oral administration

Mouse

Groups of 40 female A/J mice, 8 weeks of age, were administered 1.0, 10.0 or 55.0 mg coal-tar paint in 200 μL 2% Emulphor by gavage thrice weekly for 8 weeks. Due to the viscosity of the solution, the 55.0 mg dose was given twice daily at 27.5 mg per treatment. The coal-tar paint consisted of 67% coal-tar pitch and 33% xylene. The coal-tar paint had been applied to glass panels that were kept in a dust-free chamber for 4 months. The paint was scraped from the panels and particulate suspensions were made in the Emulphor solution. Benzo[*a*]pyrene (250 μg; 99% pure) was used as a positive control. Groups of 20 control mice were treated with the solvent either once a day or twice a day. The experiment lasted 7 months. Histopathogy was conducted. The two highest doses of coal-tar paint resulted in a significant induction of lung tumours, based upon both incidence and multiplicity (see Table 3.30). Benzo[*a*]pyrene also resulted in a significant induction of lung tumours. The two highest doses of coal-tar paint resulted in a significant induction of forestomach papilloma and carcinoma (see Table 3.31), as did benzo[*a*]pyrene. The benzo[*a*]pyrene content of the coal-tar paint could account for the induction of forestomach tumours, but not of the lung tumours (Robinson *et al*., 1987).

Table 3.30. Lung tumour incidence in female A/J mice treated orally with coal-tar paint (CTP)

Compound	No. of survivors	% of mice with lung tumours	Multiplicity
Solvent control	38/40	29	0.32 ± 0.09
CTP 1.0 mg	37/40	35	0.46 ± 0.13
CTP 10.0 mg	37/40	97*	4.27 ± 0.39**
CTP 55.0 mg	36/40	72*	4.33 ± 2.70**
Benzo[*a*]pyrene	36/40	61*	1.42 ± 0.40**

From Robinson *et al.* (1987)
Significantly different from control *p <0.01; **p <0.001

Groups of approximately 30 female A/J mice, 6 weeks of age, were fed a gel diet that contained 0.10 or 0.25% manufactured gas plant (MGP) residue (coal tar) for 260 days. Additional groups were fed gel diets that contained 16 or 98 ppm benzo[*a*]pyrene. During the 260-day feeding period, the mice fed MGP diet consumed 0.65 and 1.53 g coal tar, which contained 1.8 and 4.2 mg benzo[*a*]pyrene. The mice fed benzo[*a*]pyrene consumed a total of 11 and 67 mg benzo[*a*]pyrene. Lungs and forestomachs were examined histologically. After 260 days of feeding, the incidence of lung tumours was 19/27 (70%) mice fed 0.10% MGP diet (1.19 tumours/mouse), 29/29 (100%) mice fed 0.25% MGP diet (12.17 tumours/mouse), 9/25 (36%) mice fed 16 ppm benzo[*a*]pyrene diet

(0.48 tumours/mouse) and 14/27 (52%) mice fed 98 ppm benzo[*a*]pyrene diet (0.59 tumours/mouse). Control mice fed the gel diet had a lung tumour incidence of 4/19 (21%) (0.19 tumours/mouse). The lung tumour incidence in the mice fed MGP diet and those fed 98 ppm benzo[*a*]pyrene was significantly different from that in the control group. The lung tumour multiplicity in mice fed MGP diet was significantly different from that in the control group, whereas that in mice fed benzo[*a*]pyrene did not differ from that in the control group. Forestomach tumours did not occur in mice fed MGP diet or control mice, whereas they were induced in mice fed 16 ppm (5/25 (20%); 0.22 tumours/mouse) and 98 ppm (27/27 (100%); 4.22 tumours/mouse) benzo[*a*]pyrene (Weyand *et al*., 1995). [Benzo[*a*]pyrene does not seem to be responsible for the induction of lung tumours, and the quantity of benzo[*a*]pyrene in the MGP diet was insufficient to induce forestomach tumours.]

Table 3.31. Incidence of forestomach tumours in female A/J mice treated orally with coal-tar paint (CTP)

Compound	No. of survivors	% of mice with forestomach tumours	% of mice with forestomach papilloma	% of mice with forestomach carcinoma
Solvent control	38/40 (95%)	0	0	0
CTP 1.0 mg	37/40 (92%)	0	0	0
CTP 10.0 mg	37/40 (92%)	0	0	0
CTP 55.0 mg	36/40 (90%)	42**	36**	19*
Benzo[*a*]pyrene	36/40 (90%)	92**	67**	61*

From Robinson *et al.* (1987)
Significantly different from control *p <0.01; **p <0.001

Groups of 48 female B6C3F$_1$ mice, 5 weeks of age, were fed diets that contained 0.01, 0.03, 0.1, 0.3, 0.6 or 1.0% of a coal-tar mixture designated Coal Tar Mixture 1 (CT-1) for 104 weeks. This mixture was a composite from seven MGP waste sites and had a benzo[*a*]pyrene concentration of 1837 mg/kg (see Table 3.32). Additional groups of female mice were fed diets that contained 0.03, 0.1 or 0.3% of a coal-tar mixture designated Coal Tar Mixture 2 (CT-2) for 104 weeks. This mixture was a composite from two of the seven waste sites plus a third site that had a very high benzo[*a*]pyrene content. The benzo[*a*]pyrene concentration of this coal tar mixture was 2760 mg/kg. For comparison, additional mice were fed diets that contained 0.0005, 0.0025 or 0.01% benzo[*a*]pyrene. None of the mice fed 0.6 and 1.0% CT-1 survived the 104-week feeding; survival in mice fed 0.3% CT-1 and CT-2 was ~15–20%. All other groups had a survival similar to the control groups (~70%). Liver neoplasms (hepatocellular adenomas, carcinomas or both) did not occur in the control group but occurred in all groups of mice fed the coal tar, and the incidence was significant in mice fed 0.3% CT-1 and 0.3% CT-2 (see Table 3.33); all tumours were verified by histopathology. Alveolar/bronchiolar

Table 3.32. Polycyclic aromatic hydrocarbon composition of coal tar mixtures (CT)

Compound	CT-1 (mg/kg)	CT-2 (mg/kg)
Acenaphthene	2049	1270
Acenaphthylene	390	5710
Anthracene	2524	2900
Benz[*a*]anthracene	2374	3340
Benzo[*b*]fluoranthene	2097	2890
Benzo[*k*]fluoranthene	699	1010
Benzo[*ghi*]perylene	1493	2290
Benzo[*a*]pyrene	1837	2760
Chrysene	2379	2960
Dibenz[*a,h*]anthracene	267	370
Dibenzofuran	1504	1810
Fluoranthene	965	6370
Fluorene	3692	4770
Indan	1133	490
Indeno[1,2,3-*cd*]pyrene	1353	1990
1-Methylnaphthalene	6550	5660
2-Methylnaphthalene	11 289	10 700
Naphthalene	22 203	32 300
Phenanthrene	7640	10 100
Pyrene	5092	7220

From Culp *et al.* (1998)

Table 3.33. Tumour incidence in female B6C3F$_1$ mice fed coal-tar mixtures (CT)

Compound	Hepatocellular adenoma and/or carcinoma	Alveolar/bronchiolar adenoma and/or carcinoma	Forestomach papilloma and/or carcinoma	Small intestine adenocarcinoma
Control	0/47	2/47 (4%)	0/47	0/47
CT-1 0.01%	4/48 (8%)	3/48 (6%)	2/47 (4%)	0/46
CT-1 0.03%	2/46 (4%)	4/48 (8%)	6/45 (13%)	0/45
CT-1 0.1%	3/48 (6%)	4/48 (8%)	3/47 (6%)	0.47
CT-1 0.3%	14/45* (31%)	27/47* (57%)	14/46* (30%)	0/42
CT-1 0.6%	1/42 (2%)	25/47* (53%)	15/45* (33%)	22/36* (64%)
CT-1 1.0%	5/43 (12%)	21/45* (47%)	6/41 (15%)	36/41* (88%)
CT-2 0.03%	7/47 (15%)	4/48 (8%)	3/47 (6%)	0/47
CT-2 0.1%	4/47 (8%)	10/48* (21%)	2/47 (4%)	0/47
CT-2 0.3%	10/45* (22%)	23/47* (49%)	13/44* (30%)	1/37 (3%)

From Culp *et al.* (1998)
*Significantly different from controls, $p < 0.05$

adenomas, carcinomas or both were present in all groups fed coal tar. The incidence was significant in those fed 0.3%, 0.6% and 1.0% CT-1 and 0.1% and 0.3% CT-2 compared

with the control group. Papillomas and/or carcinomas of the forestomach squamous epithelium occurred in all groups fed coal tar, and the incidence was significant in those fed 0.3% CT-1, 0.6% CT-1 and 0.3% CT-2 compared with the control group. Adenocarcinoma of the small intestine was present in mice fed 0.6% and 1.0% CT-1, and the incidence was significant compared with the control group. The coal-tar mixtures also induced significant dose-related increases in haemangiosarcomas, histiocytic sarcomas and sarcomas. Mice fed benzo[*a*]pyrene had an increased incidence of papillomas and/or carcinomas of the tongue, oesophagus and forestomach. A comparison of the results obtained from mice fed benzo[*a*]pyrene indicated that the benzo[*a*]pyrene in coal-tar mixtures could be responsible for the forestomach tumours. The lung and liver tumours appeared to be due to other components contained in the coal-tar mixtures (Culp *et al.*, 1998).

Intraperitoneal injection

Mouse

Groups of approximately 30 male and 30 female B6C3F1 mice, 15 days of age, were administered a single intraperitoneal injection of MGP residue (coal tar). One group received 7.98 mg MGP-4, a product obtained from a single MGP site (see Table 3.34) for characterization), in corn oil [volume not specified]. The benzo[*a*]pyrene content of this mixture was 1.56 g/kg. Additional groups received 1.995, 3.99 or 7.98 mg MGP-7 (see Table 3.31 for characterization), a product formulated by mixing equal amount of residues from seven different MGP sites, including MGP-4, in corn oil. The benzo[*a*]pyrene content of this mixture was 1.84 g/kg. Further groups of mice received 125, 250 or 375 µg benzo[*a*]pyrene [purity not specified] in corn oil, or corn oil alone (approximately 60 of each sex). Tumorigenicity was assessed at 26, 39 and 52 weeks. Livers, lungs and forestomachs were examined histologically. Forestomach tumours were not detected and there was only a very low incidence of pulmonary tumours. With one exception, liver tumours occurred only in male mice. When assessed 26 weeks after treatment, only a low incidence of liver tumours was observed in male mice treated with MGP residue, and none were detected in male mice treated with benzo[*a*]pyrene. Thirty-nine weeks after treatment, the incidence of liver tumours in male mice was 4/34 (9%), 0/33 and 23/28 (82%) for groups that received 1.995, 3.99 and 7.98 mg MGP-7, 10/22 (45%) for the group that received 9.98 mg MGP-4, 6/26 (23%), 13/34 (38%) and 15/23 (65%) for the mice that received 125, 250 and 375 µg benzo[*a*]pyrene and 0/59 for corn oil controls. Fifty-two weeks after treatment, the incidence of liver tumours in male mice was 4/34 (12%), 8/32 (25%) and 17/29 (59%) for groups that received 1.995, 3.99 and 7.98 mg MGP-7, 12/28 (43%) for the group that received 9.98 mg MGP-4, 13/29 (45%), 14/27 (52%) and 19/24 (79%) for the mice that received 125, 250 and 375 µg benzo[a]pyrene and 3/63 (5%) for corn oil controls (Rodriguez *et al*., 1997). [At 39 weeks, the low and high doses of MGP gave significantly different results from the corn oil control. At

52 weeks, the medium and high doses gave significantly different results from the control. The benzo[*a*]pyrene was administered in great excess of that found in the coal tar (125–375 μg as compared with <15 μg benzo[*a*]yrene in coal tar); thus, the liver tumour response observed with coal tar may be due to compounds other than its benzo[*a*]pyrene content.]

Groups of 30 male B6C3F$_1$ mice, 15 days of age, were administered a single intraperitoneal injection of a mixture of 17 PAHs including indan (0.2% of the mixture), naphthalene (23.8%), 2-methylnaphthalene (23.2%), 1-methylnaphthalene (13.3%), acenaphthylene (7.7%), acenaphthene (0.6%), dibenzofuran (0.7%), fluorene (4.3%), phenanthrene (10.5%), anthracene (3.4%), fluoranthene (2.4%), pyrene (4.3%), benz[*a*]anthracene (1.4%), chrysene (1.5%), benzo[*b*]fluoranthene (0.8%), benzo[*k*]fluoranthene (0.9%) and benzo[*a*]pyrene (0.9%). This mixture was modelled on the PAHs identified in a MGP residue (coal tar) designated MGP-4, a product obtained from a single MGP site. Absence from the mixture (but present in MGP-4; see Table 3.34) were indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*]anthracene and benzo[*ghi*]perylene. The mixture was given at a level of 193 mg/kg in the presence of 26.75–126.75 μg benzo[*a*]-pyrene. Additional mice were treated with 193, 535 or 1041 mg/kg of the mixture. Fifty-two weeks after treatment, none of the mice had liver tumours (Goldstein *et al*., 1998).

Table 3.34. Composition of coal tar mixtures (% of quantified total aromatic compounds)

Compound	MGP-4	MGP-7
Indan	0.2	1.4
Naphthalene	29.8	27.3
2-Methylnaphthalene	21.4	13.9
1-Methylnaphthalene	12.3	8.1
Acenaphthylene	6.9	3.9
Acenaphthene	0.5	2.5
Dibenzofuran	0.6	1.8
Fluorene	3.9	4.5
Phenanthrene	9.7	9.4
Anthracene	3.1	3.1
Fluoranthene	2.7	6.1
Pyrene	3.9	6.3
Benz[*a*]anthracene	1.3	2.9
Chrysene	1.3	2.9
Benzo[*b*]fluoranthene	0.8	2.6
Benzo[*k*]fluoranthene	0.3	0.9
Benzo[*a*]pyrene	0.9	2.3
Indeno[1,2,3-*cd*]pyrene	2.8	1.6
Dibenz[*a,h*]anthracene	1.0	0.4
Benzo[*ghi*]perylene	3.9	1.8

From Goldstein *et al.* (1998)

Inhalation exposure

Mouse

Groups of female NMRI mice [number and age unspecified] were exposed by inhalation to coal oven flue gas for 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven. Since the PAH-content of the flue gas was very low, a tar pitch was added to the coal embers in the oven, which resulted in an increase to 0.3 μg benzo[*a*]pyrene/m^3 (Table 3.35). Subsequently, the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. These modifications resulted in the following exposures: adult NMRI mice, coal oven gas mixed with pyrolysed pitch (COP) with a benzo[*a*]pyrene content of ~0.3 μg/m^3 for 9 months, followed by COP with a benzo[*a*]pyrene content of ~60 μg/m^3 for 15 months (Series 1); adult NMRI mice, COP with a benzo[*a*]pyrene content of ~50 μg/m^3 for 12 months (Series 2); and neonatal NMRI mice, COP with a benzo[*a*]pyrene content of ~90 μg/m^3 for 10 months (Series 3). Adult NRMI mice exposed to COP with a low benzo[*a*]pyrene content (~0.3 μg/m^3) followed by a high benzo[*a*]pyrene content (~60 μg/m^3) (Series 1) had a lung tumour incidence of 79.0% (7.0 ± 7.9 tumours/mouse) compared with an incidence of 32.0% (0.7 ± 1.7 tumours/mouse) in clean air control mice (p <0.05). Adult NRMI mice exposed to COP with a benzo[*a*]pyrene content ~50 μg/m^3 (Series 2) had a lung tumour incidence of 70.0% (3.8 ± 5.2 tumours/mouse) compared with an incidence of 12.5% (0.2 ± 0.5 tumours/mouse) in clean air control mice (p <0.05). NRMI mice exposed, beginning as neonates, to COP with a benzo[*a*]pyrene content ~90 μg/m^3 (Series 3) had a lung tumour incidence of 85.7% (7.9 ± 8.8 tumours/mouse) compared with an incidence of 3.5% (0.03 ± 0.19 tumours/mouse) in clean air control mice (p <0.05) (Heinrich *et al*., 1986a,b).

Table 3.35. Concentration of particle-bound PAH in diluted coal oven/pyrolysed pitch exhaust

Compound	Concentration (μg/mL)
Fluoranthene	47.5
Pyrene	33.8
Benz[*a*]anthracene	18.8
Chrysene	18.3
Benzofluoranthenes	27.2
Benzo[*e*]pyrene	11.2
Benzo[*a*]pyrene	14.7
Indeno[1,2,3-*cd*]pyrene	9.1
Benzo[*ghi*]perylene	10.1
Coronene	2.1

Heinrich *et al* (1986b)

Rat

Groups of female Wistar rats [number and age unspecified] were exposed by inhalation to coal oven flue gas for an average of 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven. Since the PAH-content of the flue gas was very low, for some exposures, a tar pitch was added to the coal embers in the oven, which resulted in an increase of PAH content to 0.3 μg benzo[*a*]pyrene/m^3. Subsequently, the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. These modifications resulted in female Wistar rats being exposed to COP with a benzo[*a*]pyrene content of ~0.3 μg/m^3 for 10 months, followed by COP with a benzo[*a*]pyrene content of ~90 μg/m^3 for 12 months. Wistar rats exposed to COP as described above, followed by clean air for up to 8 months had an incidence of 16/116 (14%) benign lung tumours and 5/116 (4%) malignant lung tumours. Control rats (115) that had been exposed to only clean air did not have any benign or malignant tumours [benign tumours, p <0.0001; malignant tumours, p <0.0306; combined benign and malignant tumours (21/116; 18%), p <0.0001] (Heinrich *et al*., 1986a,b).

Seventy-two groups of female Wistar rats [age unspecified] were exposed for 18 h per day on 5 days per week for 43 weeks to an aerosol that contained PAHs that was produced by heating pitch to ~750 °C under a nitrogen atmosphere, diluting the high-temperature vapour with 200 °C clean air and then 12 °C clean air. The mass concentration of the condensation aerosol was ~2.5 mg/m^3 and the benzo[*a*]pyrene content was 50 μg/m^3. The exposure period was followed by 57 weeks of exposure to clean air, at which time the PAH-exposed rats had a lung tumour incidence of 31% compared with 0% in the clean-air control rats. Additional groups of rats were exposed for 43 weeks to aerosols that contained PAHs with a benzo[*a*]pyrene content of 20 or 90 μg/m^3 [particle mass not specified]. After 57 additional weeks of exposure to clean air, the lung tumour incidence was 3 and 56%, respectively (Heinrich, 1989).

Groups of 72 female Wistar rats, 10 weeks of age, were exposed to a tar-pitch condensation aerosol for 17 h per day on 5 days per week for 43 or 86 weeks. The animals were then maintained on clean air for an additional 86 or 43 weeks. The tar-pitch condensation aerosol was generated by heating hard coal-tar pitch to 750 °C under a nitrogen atmosphere and diluting the high-temperature tar-pitch vapour with clean air at 12 °C. The resulting PAH-rich material, which was free of any carbon core, was administered to the rats by inhalation at concentrations of 1.1 and 2.6 mg/m^3, an amount that contained 20 and 50 μg benzo[*a*]pyrene/m^3. At the end of the 129-week experimental period, the incidence of lung tumours (histologically verified, primarily squamous carcinoma) was 4% and 33% in rats exposed to 1.1 and 2.6 mg tar-pitch/m^3 for 43 weeks, and 39% and 97% in rats exposed to 1.1 and 2.6 mg tar pitch/m^3 for 86 weeks, respectively. No lung tumours occurred in animals exposed to clean air only (Heinrich *et al*., 1994a,b). [With the exception of 20 μg benzo[*a*]pyrene at 43 weeks, all the exposures were significant (Fisher's exact one-tailed test)].

Hamster

Groups of female Syrian golden hamsters [number and age unspecified] were exposed by inhalation to coal oven flue gas for an average of 16 h per day on 5 days per week. The flue gas was obtained from a domestic coal oven in which the PAH-content was raised further by mixing the coal oven exhaust with the gaseous components developed by continuous heating of pitch at approximately 750 °C in a nitrogen atmosphere. This modification resulted in the exposure of Syrian golden hamsters to COP with a benzo[*a*]pyrene content of ~50 μg/m^3 for 18 months. This was followed by 11 months of clean air. Syrian golden hamsters exposed to COP with a benzo[*a*]pyrene content ~50 μg/m^3 did not develop lung tumours; however, 50% of the animals had bronchiolo-alveolar nodular squamous metaplasia and 14% had papillomas in the larynx/trachea region. These lesions did not occur in hamsters exposed to clean air (Heinrich *et al*., 1986a,b).

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