3. CANCER IN EXPERIMENTAL ANIMALS

3.1 Diesel engine exhaust

The whole diesel exhaust used in the studies evaluated here was generated from fuels and diesel engines produced before the year 2000. Exhaust from these engines include three basic components: elemental carbon particles in respirable clusters; organic matter adsorbed onto the surface of the carbon particles, which is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using several animal species to evaluate the potential carcinogenicity of exposure to whole exhaust and to the components of exhaust from diesel engines. The studies were considered under three subcategories: (i) whole diesel engine exhaust; (ii) gas-phase diesel engine exhaust (with particles removed); and (iii) diesel engine exhaust particles or extracts of diesel engine exhaust particles.

Animal bioassays conducted with different diesel engine exhausts have been reviewed previously in the *IARC Monographs* (IARC, 1989). This section provides a summary of these and a detailed review of more recent studies.

3.1.1 Mouse

See Table 3.1

(a) Inhalation

Groups of 96 female NMRI mice, aged 8–10 weeks, were exposed to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (Volkswagen ; operated to simulate average urban driving) for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered and filtered exhausts were diluted 1:17 with air, and the unfiltered exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. Exposure to total diesel exhaust and filtered diesel exhaust significantly increased the number of animals with lung adenocarcinomas to 13 out of 76 (17%) and 18 out of 93 (19%), respectively, as compared with 2 out of 84 (2%) controls; no increase was seen in the numbers of animals with lung adenomas (Heinrich *et al.*, 1986a). [The incidence of lung tumours in 'historical' controls in this laboratory was reported to be 32% in untreated controls and 12.5% in inhalation controls (exposed to clean air only) (Heinrich *et al.*, 1986b).]

Two groups of 225 newborn male and female C57BL/N and 205 newborn male and female ICR mice each were exposed to either clean air (control) or diesel exhaust (from a 269-cm^3 displacement, small diesel engine run at idling speed), diluted 1:2–1:4 with clean air to give concentrations of 2–4 mg/m^3 of particulate matter and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week, starting within

Table 3.1 Studies of the carcinogenicity of diesel engine exhaust in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
NMRI (F) Up to 120 wk (lifetime) Heinrich *et al.* (1986a)	Inhalation Clean air (control), filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air, containing 4.24 mg/m^3 particles, and 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x (whole exhaust) and 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x (filtered exhaust); 19 h/d, 5 d/wk Groups of 96, aged 8–10 wk	Lung (adenoma): 9/84 (11%), 11/93 (12%), 11/76 (14%) Lung (adenocarcinoma): 2/84 (2%), 18/93 (19%)*, 13/76 (17%)*	*$P < 0.05$ compared with controls	Well conducted study; incidence of lung tumours in 'historical' controls in this laboratory reported to reach 32% in untreated controls and 12.5% in inhalation controls (exposed to clean air only) (Heinrich *et al.*, 1986b)
C57BL/6N and ICR (M, F) Up to 28 mo Takemoto *et al.* (1986)	Inhalation Clean air (control) or exhaust from a 269-cm^3 small diesel engine diluted 1:2–1:4 with clean air (2–4 mg/m^3 particulate matter, 2–4 ppm NO_2), 4 h/d, 4 d/wk for up to 24 mo; survivors held untreated for up to additional 4 mo Groups of 225 newborn C57BL/6N and 205 newborn ICR	C57BL/N Lung (adenocarcinoma): M+F–0/51, 5/150 (3%) Lung (adenoma): M+F–1/51 (2%), 12/150 (8%) ICR Lung (adenocarcinoma): 1/60, 4/56 (7%) Lung (adenoma): 6/60 (10%), 10/56 (18%)	NS	Daily duration and frequency of exposures were short
NMRI (F) Up to 23 mo Heinrich *et al.* (1995)	Inhalation Clean air (control), or filtered or unfiltered exhaust from two 40-kW 1.6-L diesel engines (VW; operated either according to the US 72 cycle or under constant load conditions), diluted 1:15 and 1:9 with clean air (average diesel soot particle concentrations, 4.5 and 7.0 mg/m^3, respectively); particle-free diesel exhaust diluted 1:15 with clean air and the particles removed by a heated filter system (whole exhaust: 0.5–0.6 ppm NO_2, 4.1–4.3 ppm NO_x; filtered exhaust: 0.5 ppm NO_2, 2.7 ppm NO_x), 18 h/d, 5 d/wk for 13.5 mo; kept untreated for up to additional 9.5 mo Groups of 80, aged 7 wk, exposed to (A) clean air, (B) diesel exhaust (7.0 mg/m^3 diesel soot); groups of 120, aged 8–10 wk, exposed to (C) clean air, (D) diesel exhaust (4.5 mg/m^3 diesel soot) or (E) filtered exhaust (particle-free)	Lung (adenoma): A, 25%; B, 21.8%; C, 25%; D, 18.3%, E, 31.7% Lung (adenocarcinoma): A, 15.4%; B, 15.4%; C, 8.8%; D, 5%; E, 15%	NS	Well conducted study; results, summarized in a general manner, no numbers of lung tumours given; pathology for organs other than the lung NR

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
C57BL/6N (F) Up to 30 mo Heinrich *et al.* (1995)	Inhalation Clean air (control), filtered or unfiltered exhaust from two 40-kW 1.6-L diesel engine (VW; operated either according to the US 72-cycle or under constant load conditions), diluted 1:15 with clean air (average diesel soot particles, 4.5 mg/m^3); particle-free diesel exhaust diluted 1:15 with clean air and particles removed by a heated filter system (whole exhaust: 0.5 ppm NO_2, 4.1 ppm NO_x; filtered exhaust: 0.5 ppm NO_2, 2.7 ppm NO_x); 18 h/d, 5 d/wk for 24 mo; kept untreated for up to additional 6 mo. Groups of 120, aged 7 wk	Lung (all tumours): 5.1%, 8.5%, 3.5%	NS	Well conducted study; results, summarized in a general manner, no numbers of lung tumours given; pathology for organs other than the lung NR
CD-1 (M, F) 24 mo Mauderly *et al.* (1996)	Inhalation Clean air (control), exhaust generated from a 1980 model 5.7-L V8 engine (operated according to US FTP cycles), concentrations reported as dilution of whole exhaust: measured soot of 0.35, 3.5 or 7.0 mg/m^3 (0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm NO_2, respectively); 7 h/d, 5 d/wk Numbers NR, aged 17 wk	Lung (bronchiolo-alveolar adenoma): M+F–10/157 (6%), 16/171 (9%), 8/155 (5%), 10/186 (5%) Lung (bronchiolo-alveolar carcinoma): M+F–9/157 (6%), 6/171 (3%), 7/155 (4%), 4/186 (2%)	NS	Well conducted study
C57BL/6N (F) 18 mo Kunitake *et al.* (1986)	Subcutaneous injection 0 (control), 10, 25, 50, 100, 200 or 500 mg/kg bw residue from dichloromethane extract of diesel particles from a V6 11-L heavy-duty diesel engine exhaust in olive oil containing 5% DMSO; once/wk for 5 wk Groups of 15–50, aged 6-wk	Soft-tissue (malignant fibrous histiocytoma): 0/38, 0/15, 1/15 (7%), 2/14 (14%); 3/30 (10%), 1/15 (7%), 5/22 (23%)*	*$P < 0.01$, Fisher's exact	

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
ICR and C57BL (M, F) 24 mo Kunitake *et al.* (1986)	Subcutaneous injection 0 (control), 2.5, 5 or 10 mg/mouse of residue from dichloromethane extract of diesel particles from a V6 11-L heavy-duty diesel engine exhaust in olive oil containing 5% DMSO; once 24 h after birth Groups of 12–36 newborn	ICR strain Liver (hepatoma): M–2/14 (14%), 0/13, 6/30 (20%), 3/12 (25%) F–0/16, 1/18 (6%), 2/36 (6%), 1/12 (8%) Lymphoma: M–2/14 (14%), 0/13, 4/30 (13%), 4/12 (33%) F–1/16 (6%), 1/18 (6%), 3/36 (8%), 1/12 (8%) Lung (all tumours [NR]): M–2/14 (14%), 4/13 (30%), 7/30 (23%), 2/12 (17%) F–3/16 (19%), 0/18, 5/36 (14%), 0/12	[NS]	Experiment with newborn C57BL mice performed only with 0 and 5 mg/mouse; the authors reported no increase in tumour incidence in treated mice versus controls
ICR (M) 12 mo Ichinose *et al.* (1997)	Intratracheal instillation 0 (control), 0.05, 0.1 or 0.2 mg/mouse diesel exhaust particles from exhaust emission of a diesel engine (2740 cm^3 exhaust volume, operated at 1500 rpm and 10 torque), collected on a glass filter and suspended in sterile 50 mM phosphate-buffered 0.9% saline (pH 7.4) containing 0.05% Tween 80; once/wk for 10 wk Groups of 120, aged 4 wk	Lung (adenoma): 18/116 (16%), 30/120 (25%), 31/119 (26%), 28/117 (24%) Lung (adenocarcinoma): 1/116 (1%), 6/120 (5%), 5/119 (4%), 5/117 (4%) Lymphoma: 13/116 (11%), 17/120 (14%), 17/119 (14%), 23/117 (20%)	NS	Short exposure period; high spontaneous lung tumour incidence; designed to investigate the involvement of oxygen radicals in lung carcinogenesis induced by diesel exhaust particles, i.e. the relationship between lung tumour response and formation of 8-OH-dG in lung DNA
SENCAR (M, F) 52 wk Nesnow *et al.* (1983)	Skin application 0 (control), 0.1, 0.5, 1.0, 2.0, or 4.0 mg/ mouse particulates from emissions of a 1973 Nissan Datsun 220C diesel engine, collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in 0.2 mL acetone, once/wk (4.0 mg dose given as 2.0 mg twice/wk) on shaved dorsal surface for 50–52 wk Groups of 40 M and 40 F, aged 7–9 wk	Skin (squamous cell carcinoma): M–0%, 0%, 0%, 0%, 0%, 3% F–0%, 0%, 0%, 0%, 0%, 5%	NS	Short duration of exposure

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Initiation-promotion				
ICR (F) [presumed to be 29 wk] Kunitake *et al.* (1986)	Skin application 0 (control), 0.5, 1.5 or 4.5 mg tar from a V6 11-L heavy-duty diesel engine exhaust in 0.1 mL acetone to shaved back, every other day for 20 days; 1 wk later, 2.5 µg TPA in 0.1 mL acetone, 3 × /wk for 25 wk Groups of 50, aged 8–9 wk	Skin (papilloma): 0/50, 0/49, 1/48, 4/50 Skin ('cancer'): 0/50, 0/49, 0/48, 0/50	[NS]	Study poorly reported, with short duration of treatment and implied lack of observation time after treatment
SENCAR (M, F) Up to 26 wk Nesnow *et al.* (1982a, b, 1983)	Skin application 0 (control), 0.1, 0.5, 1.0, 2.0 or 10.0 mg/mouse particulates from emissions of (A) a 1973 Nissan Datsun 220C, (B) a 1978 Oldsmobile 350, (C) a prototype VW turbo-charged Rabbit or (D) a 1972 heavy-duty Caterpillar 3304 diesel engine collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in acetone, 0.2 mL once (10 mg given as 5 × 2 mg/d) to shaved dorsal surface; 1 wk later, 2.0 µg TPA in 0.2 mL acetone twice/wk for up to 25 wk; B[*a*]P used as a positive control (highest dose, 101 µg/mouse) Groups of 40, aged 7–9 wk	Diesel engine A Skin (papilloma/mouse): M–0.08, 0, 0.34, 0.38, 1.1, 5.5 F–0.05, 0.03, 0.39, 0.53, 1.6, 5.7 Skin (squamous cell carcinoma): M–0/37 versus 12/38 (31%) high-dose F–1/38 versus 14/38 (36%) high-dose Diesel engines B, C and D Skin (papilloma/mouse): M+F– 0.1–0.5 compared with 0.05–0.08 in TPA controls B[a]P (101 µg/mouse) Skin (papilloma/mouse): M–10.2 F–7.9 Skin (squamous cell carcinoma): M–30% F–25%	[$P < 0.001$] [$P < 0.001$]	

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
NMRI (F) Up to 120 wk (lifetime) Heinrich *et al.* (1986a)	Intratracheal instillation/inhalation Instillations of 50 or 100 µg B[*a*]P for 20 and 10 wk, respectively, or 50 µg DB[*a*,*h*]A for 10 wk, followed by inhalation exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (VW; operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 64, aged 8–10 wk	20 B[*a*]P instillations induced a 71% lung tumour rate while 20 B[*a*]P instillations plus total diesel exhaust gave a 41% rate; results not reproduced in the group receiving the same total dose of B[*a*]P in 10 installations	NS	Authors reported that the various treatments with carcinogens gave no consistent results; no other data on tumours were reported
NMRI (F) 6 mo Heinrich *et al.* (1986a)	Subcutaneous injection/inhalation 5 or 10 µg DB[*a*,*h*]A 24– 48 h after birth followed by inhalation of clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 96 newborn	Lung tumour rate: DB[*a*,*h*]A–5 µg, 46%; 10 µg, 81% DB[*a*,*h*]A + unfiltered exhaust–10 µg, 63%*	*Significant decrease	Animals exposed for only 6 mo; authors reported that the various treatments with carcinogens gave inconsistent and erratic results; incidence of tumours not reported

B[*a*]P, benzo[*a*]pyrene; bw, body weight; d, day; DB[*a*,*h*]A, dibenz[*a*,*h*]anthracene; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NR, not reported; NS, not significant; rpm, revolutions per minute; 8-OH-dG, 8-hydroxydeoxyguanosine; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VW, Volkswagen; wk, week

24 hours of birth, for up to 24 months; survivors were maintained with no further treatment for up to an additional 4 months. A low, not significantly increased incidence of lung adenoma and/or adenocarcinoma was observed in mice of both strains exposed to diesel exhaust. Other tumours were observed in the liver, mammary gland and haematopoietic system (malignant lymphomas), but their incidence did not differ statistically significantly between the exposed and control groups (Takemoto *et al.*, 1986). [The Working Group noted that the duration and frequency of the daily exposures were short.]

Groups of 80 female NMRI mice, aged 7 weeks, were exposed to clean air (control) or diesel exhaust from a 1.6-L diesel engine (VW; operated according to the US 72-cycle or under constant load conditions) for 18 hours a day, 5 days a week for 13.5 months and then maintained with no further treatment for up to an additional 9.5 months. The exhaust was diluted 1:9 with air, and the unfiltered exhaust contained 7.0 mg/m^3 of particles. Levels of 0.5–0.6 ppm of nitrogen dioxide and 4.1–4.3 ppm of nitrogen oxides were found in the whole exhaust. Additional groups of 120 female NMRI mice, aged 8–10 weeks, or female C57BL/6N mice, aged 7 weeks, were exposed to either clean air (control), diesel exhaust containing 4.5 mg/m^3 of particles (0.5–0.6 ppm of nitrogen dioxide and 4.1–4.3 ppm of nitrogen oxides) or particle-free diesel exhaust for 18 hours a day, 5 days a week for either 13.5 months and then maintained without treatment for up to an additional 9.5 months (NMRI mice) or for 24 months and then maintained without treatment for up to an additional 6 months (C57BL/6N mice). The exhaust from the engine was diluted 1:15 with clean air, and the particles were removed by a heated filter system. Exposure to total or filtered diesel exhaust did not cause any increase in the number of animals with lung tumours (Heinrich *et al.*, 1995). [The study was well conducted, but the results were summarized in general manner. Histopathology for organs other than the lung and the lung tumour incidence were not reported.]

Groups of male and female CD1 mice [number unspecified], aged 17 weeks, were exposed to either clean air (control) or diesel engine exhaust generated from a 1980 model 5.7-L V8 engine operated according to US Federal Test Procedures (FTP) cycles at concentrations (reported as a dilution of the whole exhaust to measured soot concentrations) of 0.35, 3.5 or 7.0 mg/m^3 with levels of nitrogen dioxide of 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm, respectively, for 6 hours a day, 5 days a week for 24 months. Exposure to diesel exhaust did not affect survival or body weight and did not increase the incidence of bronchiolo-alveolar adenoma and/or carcinoma (Mauderly *et al.*, 1996).

(b) Subcutaneous administration

Groups of 15–50 female C57BL/6N mice, aged 6 weeks, received subcutaneous injections into the intrascapular region of 10, 25, 50, 100, 200 or 500 mg/kg body weight (bw) of residue from a dichloromethane extract of diesel particles (collected from the exhaust of a V6 11-L heavy-duty diesel engine) suspended in olive oil containing 5% dimethyl sulfoxide (DMSO) once a week for 5 weeks. A control group of 38 mice received injections of the vehicle only. The animals were killed 18 months after the beginning of the experiment. The first tumours were palpated at week 47 (25 mg/kg bw), week 30 (50 mg/kg bw), week 27 (100 mg/kg bw) and week 39 (200 and 500 mg/kg bw) in the five treated groups. A significant increase in the incidence of subcutaneous tumours, diagnosed as malignant fibrous histiocytomas, was observed in 5 out of 22 mice that receiving the 500-mg/kg bw dose ($P < 0.01$) in comparison with controls (0 out of 38) (Kunitake *et al.*, 1986).

In a second experiment, groups of 12–36 newborn male and female ICR mice received a single subcutaneous injection of 0, 2.5, 5 or 10 mg of residue from a dichloromethane extract

of diesel particles (collected from the exhaust of a V6 11-L heavy-duty diesel engine) suspended in olive oil containing 5% DMSO 24 hours after birth. The surviving animals were killed after 24 months. The incidence of lymphoma in male mice that received 10 mg of residue/mouse (4 out of 12 mice) was slightly increased compared with controls (2 out of 14 [not significant]). Overall, no statistically significant increase in the incidence of any tumours or of total tumours was observed in any of the treated groups. The authors reported that they also injected newborn C57BL mice with doses of 0 and 5 mg/mouse and observed no increase in the incidence of tumours in treated animals compared with controls (Kunitake *et al.*, 1986). [The Working Group noted that the study was limited by the small number of animals used.]

(c) Intratracheal instillation

Groups of 120 male ICR mice, aged 4 weeks, received intratracheal instillations 0, 0.05, 0.1 or 0.2 mg of diesel exhaust particles (obtained from the exhaust emission generated by a diesel engine, with a 2740-cm^3 exhaust volume, operated at 1500 revolutions per minute (rpm) and 10 torque, and collected on a glass filter) suspended in sterile 50 mM phosphate-buffered 0.9% saline (pH 7.4) containing 0.05% Tween 80 once a week for 10 weeks and were then killed 12 months after the first injection. A non-significant increase in the incidence of lymphoma was observed in the high-dose group compared with controls (23 out of 117 versus 13 out of 116) (Ichinose *et al.*, 1997). [The Working Group noted the high incidence of spontaneous lung tumours in control animals and the short observation period. This study was designed to investigate the role of oxygen radicals in lung carcinogenesis induced by diesel exhaust particles, i.e. the relationship between lung tumour response and the formation of 8-hydroxydeoxyguanosine in lung DNA, which explained the short exposure period.]

(d) Skin application

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received topical 0.2-mL applications of extracts of particles obtained from the emissions of a 1973 Nissan Datsun 220C diesel engine that were collected on Teflon-coated fibreglass filters, extracted with dichloromethane and then dissolved in acetone to give doses of 0, 0.1, 0.5, 1.0, 2.0 or 4.0 mg/mouse once a week (the 4.0-mg dose was given as two applications a week) to the shaved dorsal skin for 50–52 weeks. At that time, squamous cell carcinomas of the skin had developed in 3% of males and 5% of females given the 4.0-mg dose, which was not statistically significant compared with controls (Nesnow *et al.*, 1983). [The Working Group noted the short duration of exposure.]

(e) Initiation–promotion studies

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received a single topical 0.2-mL application of extracts of particles obtained from the emissions of (A) a 1973 Nissan Datsun 220C, (B) a 1978 Oldsmobile 350, (C) a prototype VW turbo-charged Rabbit or (D) a 1972 heavy-duty Caterpillar 3304 diesel engine that were collected on Teflon-coated fibreglass filters, extracted with dichloromethane and then dissolved in acetone to give doses of 0, 0.1, 0.5, 1.0 or 2.0 mg/mouse to the shaved dorsal skin; a 10-mg/mouse dose was administered as five daily applications of 2 mg. One week later the mice received topical applications of 2.0 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.2 mL of acetone twice a week for up to 25 weeks. Benzo[*a*]pyrene was used as a positive control. The sample from engine A produced a dose-related increase in the incidence of skin papillomas, with 5.5 and 5.7 papillomas/mouse at the highest dose; 12 out of 38 (31%) males [$P < 0.001$] and 14 out of 38 (36%) females [$P < 0.001$] treated with the highest dose developed squamous cell carcinomas of the skin compared with 0 out of 37

male and 1 out of 38 female controls. Responses of 0.1–0.5 papillomas/mouse were observed after treatment with samples from engines B, C and D, compared with 0.05–0.08 papillomas/mouse in TPA-treated controls (Nesnow *et al.*, 1982a, b, 1983).

Three groups of 50 female ICR mice, aged 8–9 weeks, received topical applications of extracts of diesel particles (collected from the exhaust of a V6 11-L heavy-duty displacement diesel engine) dissolved in acetone onto the shaved back skin every other day for 20 days (total doses: 5, 15 or 45 mg/animal). A further group of 50 mice treated with acetone only served as controls. Beginning 1 week after the last application of diesel extract, each animal received applications of 2.5 µg of TPA in 0.1 mL of acetone three times a week for 25 weeks [duration of the study presumed to be ~29 weeks]. No skin 'cancer' was found in the treated or control groups; skin papillomas were observed in 1 out of 48 and 4 out of 50 animals in the 15- and 45-mg dose groups, respectively (Kunitake *et al.*, 1986). [The Working Group noted that the limitations of the study included the short duration of treatment and the implied lack of observation time after treatment.]

(f) Administration with known carcinogens

Groups of 64 female NMRI mice, aged 8–10 weeks, received intratracheal instillations of 50 or 100 µg of benzo[*a*]pyrene once a week for 20 or 10 weeks, respectively, or 50 µg of dibenz[*a,h*]anthracene (DB[*a,h*]A) for 10 weeks, followed by exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (VW; operated to simulate average urban driving) for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered and filtered exhausts were diluted 1:17 with air, and the resulting whole exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The authors reported that the various treatments with carcinogens gave no consistent results and stated that the 20 instillations of benzo[*a*]pyrene induced a 71% lung tumour rate while the 20 instillations of benzo[*a*]pyrene plus exposure to total diesel exhaust produced a rate of only 41% that was not reproduced in the group that received the same total dose of benzo[*a*]pyrene in 10 instillations. No other data on tumours were reported (Heinrich *et al.*, 1986a).

In another experiment, groups of 96 newborn female NMRI mice received an initial subcutaneous injection of 5 or 10 µg of DB[*a,h*]A between 24 and 48 hours after birth followed by exposure to clean air (control), or filtered or unfiltered exhaust from a 1.6-L diesel engine (VW; operated to simulate average urban driving) for 19 hours a day, 5 days a week for up to 6 months. The unfiltered and filtered exhausts were diluted 1:17 with air, and the resulting whole exhaust contained 4.24 mg/m^3 of particles. Levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The authors reported that subcutaneous injection of the low dose of DB[*a,h*]A resulted in 46% of lung tumour-bearing animals and no significant variation was observed after exposure to diesel exhaust. The high dose of DB[*a,h*]A resulted in 81% of lung tumour-bearing animals, which was significantly reduced to 63% by exposure to whole diesel exhaust (Heinrich *et al.*, 1986a). [The Working Group noted that, although this type of inhibitory effect is not uncommon, the data obtained in this study were inconsistent and appeared to be erratic. The Working Group also noted that the animals were only exposed for 6 months and that the incidence of tumours was not reported.]

3.1.2 Rat

See Table 3.2

Table 3.2 Studies of the carcinogenicity of diesel engine exhaust in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Wistar (M) Up to 20 mo Karagianes *et al.* (1981)	Inhalation Clean air (control), 6.6 ± 1.9 mg/m^3 or 14.9 ± 6.2 mg/m^3 coal dust or exhaust from a 3-cylinder, 43-hp diesel engine run to simulate operating patterns of such engines in mines containing 8.3 ± 2.0 mg/m^3 soot or 8.3 ± 2.0 mg/m^3 diesel soot plus 5.8 ± 3.5 mg/m^3 coal dust, 6 h/d, 5 d/wk for up to 20 mo Groups of 24, aged 18 wk	No increase in the incidence of lung tumours in treated or control animals	NS	Study limited by very small number of animals exposed for 20 mo because groups of six rats per exposure group were killed after 4, 6, 16 and 20 mo of exposure, and use of male animals only.
Wistar (F) Up to 140 wk (lifetime) Heinrich *et al.* (1986a)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (4.24 mg/m^3 particles; whole exhaust: 1.5 ± 0.3 ppm NO_2, 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm (2.4 ± 0.5 mg/m^3) NO_2, 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 96, aged 8–10 wk	Lung (bronchiolo-alveolar adenoma): 0/96, 0/92, 8/95 (8%)* Lung (squamous cell tumour): 0/96, 0/92, 9/95 (9%, mainly benign)** Lung (all tumours): 0/96, 0/96, 15/95 (16%)***	*[$P = 0.003$] **[$P = 0.002$] ***[$P < 0.0001$]	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344/Jcl (M, F) 30 mo Ishinishi *et al.* (1986)	Inhalation Exhaust from (A) a light-duty 1.8-L, 4 cylinder diesel engine (particle concentrations, 0 (control), 0.11, 0.41, 1.08 or 2.32 mg/m^3; NO_2 concentrations, 0, 0.08, 0.26, 0.70 or 1.41 ppm; NO_x concentrations, 0, 1.24, 4.06, 10.14 or 20.34 ppm) or (B) a heavy-duty 11-L, 6 cylinder diesel engine (particle concentrations, 0 (control), 0.46, 0.96, 1.84 or 3.72 mg/m^3; NO_2 concentrations, 0, 0.46, 1.02, 1.68 or 3.00 ppm; NO_x concentrations, 0, 6.17, 13.13, 21.67 or 37.45 ppm), diluted ~10–15 times in volume with conditioned air, 16 h/d, 6 d/wk Groups of 64 M and 59 F, aged 5 wk	Exhaust A Lung (adenoma): M–0/64, 0/64, 1/64 (2%), 0/64, 0/64 F–1/59 (2%), 1/59 (2%), 0/61, 0/59, 1/60 (2%) Lung (carcinomas, all): M–2/64 (3%), 1/64 (2%), 0/64, 3/64 (5%), 2/64 (3%) F–1/59 (2%), 1/59 (2%), 0/61, 2/59 (3%), 0/60 Exhaust B Lung (adenoma): M–0/64, 0/64, 0/64, 0/64, 0/64 F–0/59, 0/59, 0/61, 0/59, 0/60 Lung (carcinomas, all): M–0/64, 1/64 (2%), 0/64, 3/64 (5%), 5/64 (8%) F–1/59 (2%), 0/59, 0/61, 1/59 (2%), 3/60 (5%)	See comments.	The incidence of lung carcinomas in high-dose M and F (combined) was significantly different ($P < 0.05$) from that in M and F controls (combined). Lung carcinomas were diagnosed as adenocarcinoma, squamous cell carcinoma or adenosquamous carcinoma
F344 (F) Up to 30 mo Iwai *et al.* (1986)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 2.4-L diesel truck engine diluted with conditioned air (particle concentration, 4.9 ± 1.6 mg/m^3; NO_2 concentration, 1.8 ± 1.8 ppm; NO_x concentration, 30.9 ± 10.9 ppm), 8 h/d, 7 d/wk for 24 mo and then held unexposed for up to 6 mo Groups of 24, aged 7 wk	Lung (adenoma or carcinoma combined): 1/22 (5%), 0/16, 8/19 (42%)* Lung (all carcinomas): 0/22, 0/16, 5/19 (26%) ** Spleen (lymphoma, with or without leukaemia): 2/24 (8%), 9/24 (37%)**, 6/24 (25%)	*$P < 0.01$ **$P < 0.05$	Small number of exposed animals; lung tumours (in animals surviving ≥ 2 years) in the group exposed to whole diesel exhaust were 3 adenomas, 1 adenocarcinoma, 2 adenosquamous carcinomas, 1 squamous cell carcinoma and 1 large cell carcinoma; lung tumour in the control group was 1 adenoma
F344 (M, F) 24 mo Lewis *et al.* (1986, 1989)	Inhalation Clean air (control), 2 mg/m^3 coal dust, exhaust from a 7.0-L Caterpillar Model 3304 diesel engine diluted 1:27 with clean air (particle concentration, 2 mg/m^3; NO_2 concentration, 1.5 ± 0.5 ppm; NO_x concentration, 8.7 ± 3.6 ppm), or 1 mg/m^3 coal dust plus 1 mg/m^3 diesel exhaust particles, 7 h/d, 5 d/wk Groups of 216 M and 72 F, aged 8–10 wk	Lung (adenoma): M+F–3/180 (2%), 7/175 (4%), 7/177 (4%), 7/171 (4%) Lung (carcinoma): M+F–3/180 (2%), 0/182, 1/183 (1%), 0/178	NS	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Up to 30 mo Mauderly *et al.* (1986, 1987)	Inhalation Clean air (control), or diesel engine exhaust from a 1980 model 5.7-L V8 engine (operated according to US FTP cycles); concentrations reported as a dilution of whole exhaust to measured soot concentrations (0, 0.35, 3.5 or 7.0 mg/m^3; 0, 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm NO_2, respectively), 7 h/d, 5 d/wk Groups of 221–230 M and F, aged 17 wk	Lung (total tumours): 0.9%, 1.3%, 3.6%*, 12.8%* Lung (bronchiolo-alveolar adenoma): 0%, 0%, 2.3%*, 0.4% Lung (adenocarcinoma and squamous cell carcinoma, combined): 0.9%, 1.3%, 0.5%, 7.5%* Lung (squamous cysts): 0%, 0%, 0.9%, 4.9%	*$P < 0.05$	Squamous cysts were mostly benign tumours
F344 (F) Up to 24 mo Takemoto *et al.* (1986)	Inhalation Clean air (control) or exhaust from a 269-cm^3 small diesel engine diluted 1:2–1:4 with clean air (particulate matter, 2–4 mg/m^3; NO_2, 2–4 ppm), 4 h/d, 4 d/wk for up to 24 mo Groups of 20–26, aged 5 wk	No lung tumours observed in either the 12 controls or 15 treated rats that survived 18–24 mo	NS	Small group sizes and short exposure durations
F344 (M, F) Up to 30 mo Brightwell *et al.* (1989)	Inhalation Conditioned air (control), (A) unfiltered exhaust emission from a VW Rabbit 1.5-L diesel engine, diluted with a constant volume of 800 m^3 of air (high dose), further diluted 1:3 (mid-dose) or 1:9 (low dose) in air (particle concentrations, 0.7, 2.2 or 6.6 mg/m^3, respectively; 0.9–2.8 ppm NO_x; 0.7–7 ppm NO_2) or (B) exhaust emission (A) passed through a stationary filtration system to remove 99.97% of the mass of particles, diluted 1:3 in air; 16 h/d, 5 d/wk for 2 yr and survivors maintained for an additional 6 mo; 8 animals per sex killed at 6, 12, 18 and 24 mo Treated groups of 72 M and 72 F, aged 6–8 wk; and control groups of 144 M and 144 F	Exhaust (A) Lung (all tumours): M–2/134 (1%), 1/72 (1%), 3/72 (4%), 16/71 (23%)* F–1/126 (1%) (C), 0/71, 11/72 (15%)*, 39/72 (54%)* Exhaust (B) No increase in the incidence of respiratory tract tumours	[*$P < 0.0001$] NS	Lung tumour incidence not reported by type; highest incidence seen in high-dose rats after the end of exposure (24–30 mo): 12/27 (M), 24/25 (F); malignant tumours: 10/27 (M), 19/25 (F); authors stated that the study showed a large and statistically significant increase in incidence of lung tumours in exposed F344 rats but provided no statistics. Tumours were mainly adenoma squamous cell carcinoma, adenocarcinoma, and mixed adenoma/adenocarcinoma/squamous cell carcinoma, and one mesothelioma

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Duration NR Takaki *et al.* (1989)	Inhalation Exhaust from a 1.8-L light-duty diesel engine diluted with filtered conditioned air (particle concentrations, 0 (control), 0.1, 0.4, 1.1 or 2.3 mg/m^3) or from an 11-L heavy-duty diesel engine diluted with filtered conditioned air (particle concentrations, 0 (control), 0.5, 1.0, 1.8 or 3.7 mg/m^3), 16 h/d, 6 d/wk Groups of 64 M and 59 F, aged 5 wk	Light duty Lung (carcinoma): M+F–3/123 (2%), 2/123 (2%), 0/125, 5/123 (4%), 2/124 (2%) Lung (adenoma) M+F–1/123 (1%), 1/123 (1%), 1/125 (1%), 0/123, 1/124 (1%) Heavy duty Lung (carcinoma): M+F–1/123 (1%), 1/123 (1%), 0/125, 4/123 (3%), 8/124 (1%) Lung (adenoma): M+F–0/123, 0/123, 0/125, 0/123, 0/124	NS	Study published as a poster presentation; lack of some experimental details; carcinomas were adenocarcinoma, adenosquamous carcinoma or squamous cell carcinoma.
F344 (M, F) Up to 30 mo (lifetime) Mauderly *et al.* (1994); Nikula *et al.* (1995)	Inhalation Conditioned air (control), aerosolized carbon black or diesel exhaust from two 1988 Model LH6 General Motors 6.2-L V8 diesel engines, diluted in filtered conditioned air (particulate concentrations, 2.5 or 6.5 mg/m^3; NO_2, 0.73 or 3.78 ppm; NO_x, 8.79 or 23.45 ppm), 16 h/d, 5 d/wk for 24 mo and then held for up to 6 mo Groups of approximately 100, aged 7–9 wk	Carbon black Lung (bronchiolo alveolar adenocarcinoma): M–1/109 (1%), 1/106 (1%), 1/106 (1%) F– 0/105, 6/107 (6%)*, 20/105 (19%)** Lung (squamous cell and adenosquamous carcinoma): M–control, 1/109 (1%), 0/106, 3/106 (3%) F–0/105, 0/107, 2/105 (2%) Lung (bronchiolo-alveolar adenoma): M–1/109 (1%), 1/106 (1%), 0/106 F–0/105, 2/107 (2%), 13/105 (12%)***	[*$P < 0.03$; **$P < 0.0001$; ***$P < 0.001$]	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (M, F) Up to 30 mo (lifetime) Mauderly et al. (1994); Nikula et al. (1995) (cont.)		Diesel exhaust Lung (bronchiolo alveolar adenocarcinoma): M–1/109 (1%), 1/105 (1%), 3/106 (3%) F–0/105, 3/105 (3%), 19/106 (18%)** Lung (squamous cell and adenosquamous carcinoma): M–1/109 (1%), 2/105 (2%), 2/106 (2%) F–0/105, 1/105 (1%), 2/106 (2%) Lung (bronchiolo-alveolar adenoma): M–1/109 (1%), 2/105 (2%), 4/106 (4%) F–0/105, 5/105 (5%), 19/106 (18%)**		
Wistar [Crl:(W1) BR] (F) Up to 30 mo Heinrich *et al.* (1995)	Inhalation Clean air (control) or exhaust from two 40-kW 1.6-L diesel engine (VW; operated either according to the US 72 cycle or under constant load conditions), diluted 1:80, 1:27 or 1:9 with clean air (average diesel soot particles, 0.8, 2.5 or 7.0 mg/m^3; 0.3–3.8 ppm NO_2; 4.7–33.1 ppm NO_x), 18 h/d, 5 d/wk for 24 mo and kept untreated for up to an additional 6 mo Groups of 100–220, aged 7 wk	Lung (all tumours): 1/217, 0/198, 11/200 (6%), 22/100 (22%) Lung (bronchiolo-alveolar adenoma): 0/217, 0/198, 2/200 (1%), 4/100 (4%)* Lung (adenocarcinoma): 1/217 (0.5%), 0/198, 1/200, 5/100 (5%)** Lung (squamous cell carcinoma): 0/217, 0/198, 0/200, 2/100 (2%) Lung (benign squamous cell tumour): 0/217, 0/198, 7/200 (4%)*, 14/100 (14%)***	*$P < 0.01$ **$P < 0.05$ *** $P < 0.001$	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) Up to 30 mo Iwai *et al.* (1997)	Inhalation *Experiment 1* Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine diluted 1:8 with conditioned air (particle concentration, 9.4 mg/m^3; 1.8 ppm NO_2) either directly or after particle exclusion through a HEPA filter, 8 h/d, 7 d/wk for 24 mo and survivors held unexposed for up to 6 mo. Groups of approximately 120 (clean air or filtered exhaust) and 24 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 8/19 (42%)*	*$P < 0.01$	Results of study poorly reported; small number of animals exposed to unfiltered exhaust; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
	Experiment 2 Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine diluted with conditioned air (particle concentration, 3.2 mg/m^3; 1.8 ppm NO_2), 8 h/d, 6 d/wk for 24 mo and then held unexposed for 6 mo Groups of approximately 120 (clean air or filtered exhaust) and 48 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 5/43 (12%)*	*$P < 0.01$	Results of study poorly reported; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
	Experiment 3 Clean air (control) or filtered or unfiltered exhaust from a 2.4-L diesel engine run on commercial light oil, diluted with conditioned air (particle concentration, 5.1 mg/m^3; 1.8 ppm NO_2), 18 h/d, 3 d/wk for 24 mo and then held unexposed for 6 mo Groups of approximately 120 (clean air or filtered exhaust) and 96 (unfiltered exhaust), aged 8 wk	Lung (all tumours): 5/121 (4%), 4/108 (4%), 40/96 (42%)*	*$P < 0.01$	Results of study poorly reported; lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) 30 mo Iwai *et al.* (2000)	Inhalation Clean air (control) or exhaust from a light-duty diesel engine (operating at 1050 rpm), diluted with filtered conditioned air (particle concentration, 3.5 ± 1.4 mg/m^3; 1.3 ± 1.0 ppm NO_2; 34.5 ± 10.8 ppm NO_x), 17 h/d, 3 d/wk for 3, 6, 9 or 12 mo, moved to a clean air room after each exposure period and maintained in clean air until end of 30 mo Control group of 50 and exposure groups of 48, aged 8 wk	Lung (all tumours): 1/48 (2%), 0/48, 6/43 (14%), 19/47 (40%)*, 10/44 (23%)**	*$P < 0.001$ **$P < 0.01$	Incidence of lung tumour types not reported; histological types of the lung tumours were bronchiolo-alveolar adenoma in 14 rats and adenocarcinoma in 22 rats, which were the major types observed, and squamous cell carcinoma in 3 rats, adenosquamous carcinoma in 1 rat and sarcoma in 1 rat
Wistar (M, F) 30 mo Stinn *et al.* (2005)	Inhalation (nose-only) Clean air (control) or unfiltered exhaust from a 1.6-L displacement VW diesel engine (operated under US FTP protocol), diluted 1:5 with air (3 mg/m^3 (low dose, obtained by further dilution) or 10 mg/m^3 (high dose) particles; 7 (low dose) or 23 (high dose) ppm NO; 9 (low dose) or 28 (high dose) ppm NO_x), 6 h/d, 7 d/wk for 24 mo Groups of 99 M and 99 F, aged 40 d	Lung (bronchiolo-alveolar adenoma): M–2/50 (4%), 3/50 (6%), 8/49 (16%)* F–0/51, 5/50 (10%)*, 21/51 (41%)* Lung (squamous cell carcinoma): M–0/50, 0/50, 1/49 (2%) F–0/51, 0/50, 4/51 (8%) Lung bronchiolo-alveolar carcinoma): M–0/50, 0/50, 3/49 (6%) F–0/51, 0/50, 1/51 (2%) Lung (all tumours): M–2/50 (4%), 9/50 (18%)*, 17/49 (35%)* F–0/51, 14/50 (28%)*, 29/51 (57%)*	*$P < 0.0$	

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Osborne-Mendel (F) Up to 140 wk Grimmer *et al.* (1987)	Intrapulmonary implantation Vehicle alone (control) or condensate from exhaust generated by a diesel passenger car (3.0-L, Daimler-Benz 300D), separated into hydrophilic (6.7 mg) and hydrophobic (20 mg) fractions (hydrophobic fraction separated by column chromatography into several subfractions): (A) non-aromatic compounds plus PAHs with two and three rings (19.22 mg), (B) PAHs with four or more rings (0.21 mg), (C) polar PAHs (0.29 mg), and (D) nitro-PAHs (0.19 mg) or a hydrophobic fraction reconstituted from subfractions A–D (19.9 mg) in beeswax:trioctanoin (1:1) Groups of 35, aged 3 mo	Lung (bronchiolo-alveolar adenoma): 1/35 (3%; control), 1/35 (3%; hydrophilic), 1/35 (3%; reconstituted hydrophobic) Hydrophobic fraction: Lung (squamous cell carcinoma): 0/35 (control), 5/35 (14%; hydrophobic)*, 6/35 (17%; PAHs 4–7 rings)*, 1/35 (3%; nitro-PAHs), 7/35 (20%; reconstituted hydrophobic)*	*[$P < 0.05$]	Study very poorly reported; details of results, including pathology and dosing regimen not clear; results summarized in a general manner and difficult to interpret
Wistar Crl:(WI) BR (F) Up to 800 d Dasenbrock *et al.* (1996)	Intratracheal instillation 0 (control) or 0.2–0.3 mL of particle suspensions from the 1:10 diluted exhaust of a 1.6-L VW diesel engine (Golf/Passat) out of a dilution tunnel on cellulose nitrate filters (pore size, 5 µm): A, diesel particles (total dose, 15 mg); B and C, diesel particles extracted with toluene (total doses, 15 and 30 mg); D, extracted diesel particles coated with B[*a*]P (total dose, 15 mg including 170 µg B[*a*]P); or E and F, B[*a*]P (total dose, 15 and 30 mg) in 0.25% Tween 80 saline, once/wk for 16–17 wk and observed for up to 800 d Groups of 50–52, aged 7 wk	Lung (cystic keratinizing epithelioma): 0/47, 8/48 (17%; A)*, 1/48 (2%; B), 8/48 (17%; C)*, 3/48 (6%; D), 3/48 (6%; E), 22/47 (47%; F)** Lung (bronchiolo-alveolar adenoma): 0/47, 0/48 (A), 1/48 (2%; B), 1/48 (2%; C), 0/48 (D), 2/48 (4%; E), 0/47 (F) Lung (squamous cell carcinoma): 0/47, 0/48 (A), 0/48 (B), 0/48 (C), 0/48 (D), 8/48 (17%; E)*, 38/47 (81%; F)** Lung (bronchiolo-alveolar carcinoma): 0/47, 0/48 (A), 0/48 (B), 2/48 (4%; C), 1/48 (2%; D),1/48 (2%; E), 2/47 (4%; F)	*$P < 0.01$, **$P < 0.001$	Early mortality (less than 50% survival after 2 yr) may have affected the outcome of the study

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
F344 (F) Up to 30 mo Iwai *et al.* (1997)	Intratracheal instillation 0.2 mL of 5 mg/[mL] suspension of particles from exhaust of a 2.4-L diesel engine (collected on each stage of a middle volume Andersen's sampler), suspended in a 0.05% Tween 80 or DMSO phosphate buffer (pH 7.4) solution, once/wk for 2–10 wk (total doses, 2, 4, 8 and 10 mg) and observed for up to 30 mo Groups of at least 50, aged 8 wk	Lung (all tumours): 6% (2% malignant), 20% (13% malignant), 43% (34% malignant), 74% (48% malignant)	NR	Study poorly described and designed, and results poorly reported; unclear whether there was a control group. No individual data on lung tumour incidence in exposed groups. Lung tumours were mainly bronchiolo-alveolar adenoma and adenocarcinoma
Wistar (HsdCpb:WU) (F) Up to 30 mo Pott & Roller (2005); Mohr *et al.* (2006)	Intratracheal instillation 0 (control) or 2.5 mg diesel soot suspended in 4.1 µL 0.5% Tween 80 phosphate buffer solution, once/wk for 3 wk, 3 mg suspended in 8.1 µL, once/wk for 5 wk, or 6 mg suspended in 16.2 µL, once/wk for 5 wk Groups of 48, aged 8–9 wk	Lung (adenocarcinoma or squamous cell carcinoma): 0/46, 1/45 (2%), 5/47 (11%), 6/45 (13%)* Lung (adenoma or epithelioma): 0/46, 1/45 (2%), 7/47 (15%)**, 12/45 (27%)***	[* $P = 0.03$, ** $P = 0.01$, *** $P = 0.0005$]	Control group not treated concurrently
Administration with known carcinogens				
F344 (F) Up to 24 mo Takemoto *et al.* (1986)	Intraperitoneal injection/inhalation Clean air (control) or exhaust from a 269 cm^3 small diesel engine, diluted 1:2 to 1:4 with clean air (D; particulate matter concentration, 2–4 mg/m^3; 2–4 ppm NO_2), 4 h/d, 4 d/wk for up to 24 mo; 1 mo later, two groups injected with 1 g/kg DiPN once/wk for 3 wk Groups of 20–35, aged 5 wk	Lung (carcinoma): 0/12, 0/15 (D), 7/18 (39%; D+DiPN), 4/21 (19%; DiPN) Lung (adenoma): 0/12, 0/15 (D), 12/18 (67%; D+DiPN), 10/21 (48%; DiPN)	NS (D+DiPN versus DiPN)	Small group size

B[*a*]P, benzo[*a*]pyrene; d, day; DiPN, *N*-nitrosodiisopropanolamine; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; HEPA, high-efficiency particulate air; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NR, not reported; NS, not significant; PAHs, polycyclic aromatic hydrocarbons; VW, Volkswagen; wk, week; yr, year

(a) Inhalation

Groups of 24 Wistar male rats, aged 18 weeks, were exposed for 6 hours a day, 5 days a week for 20 months to one of five experimental atmospheres: clean air (control); 8.3 ± 2.0 (standard deviation) mg/m^3 of soot from diesel exhaust; 8.3 ± 2.0 mg/m^3 of soot from diesel exhaust plus 5.8 ± 3.5 mg/m^3 of coal dust; 6.6 ± 1.9 mg/m^3 of coal dust; or 14.9 ± 6.2 mg/m^3 of coal dust. The diesel exhaust was produced by a three-cylinder, 43-brake horse power (hp) diesel engine. The fuel injection system of the engine was modified to simulate the operating patterns of such engines when used in mines and was operated on a variable duty cycle (dilution, approximately 35:1). Six rats per group were killed after 4, 8, 16 and 20 months of exposure, and grossly visible lesions were examined histopathologically. Significant non-neoplastic lesions were restricted primarily to the respiratory tract and increased in severity with duration of exposure. In the six rats examined from each group after 20 months of exposure, two bronchiolar adenomas [bronchiolo-alveolar adenomas] were observed: one in the group exposed to diesel exhaust only and one in the group exposed to diesel exhaust and coal dust. No tumours were observed in controls or in the two groups exposed to coal dust only (Karagianes *et al.*, 1981). [The Working Group noted the limited number of rats examined (24 rats per group, only six of which were exposed for 20 months) and the use of male animals only.]

Groups of 96 female Wistar rats, aged 8–10 weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving) diluted 1:17 with air for 19 hours a day, 5 days a week for life (up to 140 weeks). The unfiltered exhaust contained 4.24 mg/m^3 of particles; the levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. A significantly increased incidence of lung tumours [$P < 0.0001$] (identified histologically as eight bronchiolo-alveolar adenomas and nine squamous cell tumours [mainly benign]) was observed in rats exposed to unfiltered diesel exhaust (15 out of 95 (18%) versus 0 out of 96 controls). No lung tumours were reported in rats exposed to clean air or filtered exhaust (Heinrich *et al.*, 1986a).

Groups of 64 male and 59 female Fischer 344Jcl rats, aged 5 weeks, were exposed to diesel exhaust from either a light-duty 1.8-L displacement, four-cylinder engine (particle concentrations of 0.11, 0.41, 1.08 or 2.32 mg/m^3; nitrogen dioxide concentrations of 0.08, 0.26, 0.70 or 1.41 ppm; and nitrogen oxide concentrations of 1.24, 4.06, 10.14 or 20.34 ppm) or a heavy-duty 11-L displacement, six-cylinder engine (particle concentrations of 0.46, 0.96, 1.84 or 3.72 mg/m^3; nitrogen dioxide concentrations of 0.46, 1.02, 1.68 or 3.00 ppm; and nitrogen oxide concentrations of 6.17, 13.13, 21.67 or 37.45 ppm) for 16 hours a day, 6 days a week for up to 30 months. Separate control groups for the light-duty and heavy-duty engine exhaust-treated animals were exposed to clean air. The incidence of malignant lung tumours, diagnosed as adenocarcinoma, squamous cell carcinoma or adenosquamous carcinoma, was 5 out of 64 (8%) high-dose (3.72 mg/m^3) males and 3 out of 60 (5%) high-dose females exposed to heavy-duty engine diesel exhaust compared with 0 out of 64 control males and 1 out of 59 (2%) control females. The lung tumour incidence in males and females combined (6.5%) differed significantly ($P < 0.05$) from that in controls (0.8%). The incidence of malignant lung tumours in the groups exposed to 1.84 mg/m^3 heavy-duty engine diesel exhaust was 3 out of 64 (5%) males and 1 out of 59 (2%) females [and did not differ statistically significantly from that in the controls.] No statistically significant increase in the incidence of lung tumours was noted in the groups exposed to light-duty diesel engine exhaust (Ishinishi *et al.*, 1986).

Groups of 24 female Fischer 344 rats, aged 7 weeks, were exposed to clean air (control), diluted diesel exhaust or diluted filtered diesel exhaust for 8 hours a day, 7 days a week for 24 months, at which time some rats were killed and the remainder was returned to clean air for 6 months of observation. The diesel exhaust, produced by a 2.4-L small truck diesel engine, was diluted 1:10 in clean air and contained 4.9 ± 1.6 mg/m^3 of particles, 1.8 ± 1.8 ppm of nitrogen dioxide and 30.9 ± 10.9 ppm of nitrogen oxides. The incidence of lung tumours in the group exposed to whole diesel exhaust, with (n = 5) or without (n = 14) a subsequent observation period of 6 months, was 8 out of 19 (42%; three adenomas, one adenocarcinoma, two adenosquamous carcinomas, one squamous cell carcinoma and one large cell carcinoma) and was significantly higher (all tumours, $P < 0.01$; malignant tumours, $P < 0.05$) than that in the control group (1 (adenoma) out of 22, 5%). No lung tumours were observed in the group exposed to filtered exhaust (0 out of 16 rats). The incidence of lymphoma was increased ($P < 0.05$) in the group exposed to filtered exhaust (9 out of 24) compared with the controls (2 out of 24), but the incidence of tumours at other sites did not differ among the three groups (Iwai *et al.*, 1986). [The Working Group noted the small number of exposed animals.]

Groups of 72 male and 72 female Fischer 344 rats, aged 8–10 weeks, were exposed for 7 hours a day, 5 days a week for 24 months to: clean air (control); 2 mg/m^3 of coal dust; 2 mg/m^3 of diesel exhaust particles; or 1 mg/m^3 of coal dust plus 1 mg/m^3 of diesel exhaust particles. Additional groups of 144 male rats were also exposed to the same substances, and at least 10 animals from each group were killed at interim periods of 3, 6, 12 and 24 months. The diesel exhaust was generated by a 7.0-L displacement, four-cycle Caterpillar Model 3304 diesel engine and was diluted by a factor of 27:1 before the exposure; the nitrogen dioxide concentration in the exhaust was 1.5 ± 0.5 ppm. After 24 months of exposure, all survivors were killed. The numbers of rats necropsied and examined histologically in each of the four groups after 24 months of exposure were 120–121 males and 71–72 females. No statistically significant difference in tumour incidence was noted between the four groups (Lewis *et al.*, 1986, 1989).

Groups of 221–230 male and female Fischer 344 rats, aged 17 weeks, were exposed to one of three concentrations of diesel engine exhaust generated by a 1980 model 5.7-L V8 engine (operated according to US FTP cycles) for 7 hours a day, 5 days a week for up to 30 months. The exposure concentrations were reported as dilutions of the whole exhaust to measured soot concentrations of 0.35 (low), 3.5 (mid) or 7.0 (high dose) mg/m^3, for which the levels of nitrogen dioxide were 0.1 ± 0.1, 0.3 ± 0.2 and 0.7 ± 0.5 ppm, respectively. Sham-exposed controls received filtered air. Subgroups of animals were removed at 6, 12, 18 and 24 months for ancillary studies; all rats surviving after 30 months of exposure were killed. A total of 901 rats were necropsied and examined histologically for lung tumours. Four lung tumours types were found: bronchiolo-alveolar adenoma, adenocarcinoma, squamous cysts (mostly benign tumours) and squamous cell carcinoma. None of the tumours were found to have metastasized to other organs. The prevalence of lung tumours in males and females combined was 0.9% (adenocarcinoma and squamous cell carcinoma) in controls, 1.3% (adenocarcinoma and squamous cell carcinoma) in the low-dose, 3.6% (2.3% adenoma, 0.5% adenocarcinoma and squamous cell carcinoma, 0.9% squamous cysts) in the mid-dose and 12.8% (0.4% adenoma, 7.5% adenocarcinoma and squamous cell carcinoma, 4.9% squamous cysts) in the high-dose groups. Compared with controls, the prevalence of lung tumours at the mid-and high dose was significantly increased ($P < 0.05$) as was that of lung adenoma at the mid-dose and adenocarcinoma and squamous cell carcinoma (combined) at the high dose (Mauderly *et al.*, 1986, 1987).

Groups of 20–26 female Fischer 344 rats, aged 5 weeks, were exposed to clean air (control) or diesel exhaust from a 269-cm^3 displacement small diesel engine run at idling speed, diluted 1:2–1:4 with clean air to give a particulate matter concentration of 2–4 mg/m^3 and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week for up to 24 months. No lung tumours were observed in the 12 controls or 15 treated rats that survived for 18–24 months (Takemoto *et al.*, 1986). [The Working Group noted that the study was limited by the small number of exposed animals and the short duration of daily exposures.]

Groups of 72 male and 72 female Fischer 344 rats, aged 6–8 weeks, were exposed to one of three concentrations (0.7, 2.2 or 6.6 mg/m^3) of exhaust or particle-filtered exhaust from a 1.5-L VW Rabbit diesel engine (operated to simulate average urban driving conditions: US-72 FTP) for 16 hours a day, 5 days a week for 24 months, and survivors were maintained for a further 6 months in clean air. The exposure concentrations were reported as a dilution of the exhaust with a constant volume of 800 m^3 of air (high dose), a further 1:3 dilution of this mixture in air (mid-dose) or a further dilution of 1:9 (low dose). Levels of 0.9–2.8 ppm of nitrogen oxides and 0.7–7 ppm of nitrogen dioxide were reported in the different exposure chambers. Two control groups of 144 males and females were exposed to conditioned air. Eight animals per sex were killed at interim periods of 6, 12, 18 and 24 months. A significant increase in the incidence of lung tumours was observed in the high-dose males and high- and mid-dose females exposed to unfiltered exhaust. The highest incidence of tumours was seen in high-dose rats that died after the end of exposure (between 24 and 30 months) with a total lung tumour [mainly adenoma, squamous cell carcinoma, adenocarcinoma, and mixed adenoma/adenocarcinoma/squamous cell carcinoma, and one mesothelioma; individual incidences were not given] incidence of 12 out of 27 (44%) males (malignant lung tumours, 10 out of 27) and 24 out of 25 (96%) females (malignant lung tumours, 19 out of 25). No increase in the incidence of respiratory tract tumours was observed in rats exposed to the filtered exhaust (Brightwell *et al.*, 1989).

Groups of 64 male and 59 female Fischer 344 rats, aged 5 weeks, were exposed for 16 hours a day, 6 days a week [duration unspecified] to exhaust from a 1.8-L light-duty diesel engine or from an 11-L heavy-duty diesel engine diluted with filtered, conditioned air to give particle concentrations of 0, 0.1, 0.4, 1.1 or 2.3 mg/m^3 or 0, 0.5, 1.0, 1.8 or 3.7 mg/m^3, respectively. No significant increase in the incidence of lung tumours was observed in any of the treated animals (Takaki *et al.*, 1989). [The Working Group noted that the study was reported as a poster presentation, and some critical experimental details were absent, such as the duration of the experiment.]

To explore the importance of diesel exhaust soot-associated organic compounds in the induction of rat lung tumours, groups of approximately 100 male and female Fischer 344 rats, aged 7–9 weeks, were exposed to aerosolized carbon black or exhaust from two 1988 Model LH6 General Motors 6.2-L V8 diesel engines that was diluted with filtered conditioned air to obtain a particulate concentration of 2.5 or 6.5 mg/m^3 and levels of 0.73 or 3.78 ppm of nitrogen dioxide and 8.79 or 23.45 ppm of nitrogen oxides, respectively, for 16 hours a day, 5 days a week for 24 months, and then held for up to an additional 6 months. Both diesel exhaust soot and carbon black particles accumulated in the lungs of exposed rats, but the rate of accumulation was higher for diesel exhaust soot. Diesel exhaust and carbon black both induced concentration-related significant increases in the incidence of bronchiolo-alveolar adenoma and bronchiolo-alveolar adenocarcinoma in female rats only (Mauderly *et al.*, 1994; Nikula *et al.*, 1995).

Groups of 100–220 female Wistar rats, aged 7 weeks, were exposed for 18 hours a day, 5 days a week for 24 months to clean air (control) or diesel

exhaust from a 1.6-L diesel engine (VW; operated according to the US FTP 72-cycle or under constant load conditions) diluted 1:80, 1:27 or 1:9 with clean air to obtain average diesel soot particle concentrations of 0.8, 2.5 and 7.0 mg/m^3, respectively, and levels of 0.3–3.8 ppm of nitrogen dioxide and 4.7–33.1 ppm of nitrogen oxides in the diesel exhaust atmospheres. Surviving animals were maintained untreated for up to an additional 6 months. Exposure to diesel exhaust produced a statistically significant increase in the incidence of bronchiolo-alveolar adenoma (4 out of 100, 4%; $P < 0.01$) and adenocarcinoma (5 out of 100, 5%; $P < 0.05$) in high-dose animals and benign squamous cell tumours of the lung in the mid- (7 out of 200, 7%; $P < 0.01$) and high-dose (14 out of 100, 14%; $P < 0.001$) groups compared with controls (0 out of 217 adenoma or benign squamous cell tumours and 1 out of 217 adenocarcinoma) (Heinrich *et al.*, 1995).

Two groups of approximately 120 female Fischer 344 rats, aged 8 weeks, and another group of 24 females were exposed to clean air (control), diluted filtered diesel exhaust or diluted unfiltered diesel exhaust for 8 hours a day, 7 days a week for 24 months. Survivors were returned to clean air for up to 6 months of observation. The diesel exhaust produced by a 2.4-L diesel engine was diluted with conditioned air to give a particle concentration of 9.4 mg/m^3 and 1.8 ppm of nitrogen dioxide, and was delivered to the exposure chambers either directly or after excluding particles by passage through a high-efficiency particulate air filter. The incidence of lung tumours [mainly bronchiolo-alveolar adenoma and adenocarcinoma] in animals that survived at least 18 months was significantly higher in the group exposed to whole diesel exhaust (8 out of 19, 42%; including 5 out of 19 (26%) malignant tumours) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) (Iwai *et al.*, 1997). [The Working Group noted the small size of the group exposed to whole diesel exhaust.]

In a second, concurrent experiment, a group of 48 female Fischer 344 rats, aged 8 weeks, was exposed to diluted diesel exhaust produced by the system described above (2.4-L diesel engine) for 8 hours a day, 6 days a week for 24 months. The particle concentration was 3.2 mg/m^3 and the level of nitrogen dioxide was 1.8 ppm. After treatment, survivors were maintained in clean air for up to 6 months of observation. The incidence of lung tumours (mainly bronchiolo-alveolar adenoma and adenocarcinoma) was significantly higher in the group exposed to whole diesel exhaust (5 out of 43, 12%) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) from the previous experiment (Iwai *et al.*, 1997).

In a third, concurrent experiment, groups of 96 female Fischer 344 rats, aged 8 weeks, were exposed to diluted diesel exhaust for 18 hours a day, 3 days a week for 24 months. Survivors were maintained in clean air for up to 6 months of observation. The diesel exhaust was produced by a 2.4-L diesel engine and was diluted with conditioned air to give a particle concentration of 5.1 mg/m^3 and 1.8 ppm nitrogen dioxide. The incidence of lung tumours (mainly bronchiolo-alveolar adenoma and adenocarcinoma) was significantly higher in the group exposed to whole diesel exhaust (40 out of 96, 42%) than in the control group (5 out of 121, 4%; $P < 0.01$) or the group exposed to filtered exhaust (4 out of 108, 4%; $P < 0.01$) from the previous experiment (Iwai *et al.*, 1997).

Groups of 48–50 female Fischer 344 rats, aged 8 weeks, were exposed to either clean air (control) or diluted diesel exhaust for 17 hours a day, 3 days a week for 3, 6, 9 or 12 months, were moved to a clean-air room after each exposure period and were maintained in clean air until the end of the 30-month experimental period. The diesel exhaust was produced from a light-duty diesel engine (operated at 1050 rpm) and was diluted with filtered conditioned air to give a particle concentration of 3.5 ± 1.4 mg/m^3, 1.3 ± 1.0

ppm of nitrogen dioxide and 34.5 ± 10.8 ppm of nitrogen oxide. The incidence of lung tumours was significantly higher in the group exposed to whole diesel exhaust for 9 months (19 out of 47, 40%; $P < 0.001$) and 12 months (10 out of 44, 23%; $P < 0.01$) than that in the control group (1 out of 48, 2%). For all exposure periods, lung tumours were observed in treated rats only after the 18th experimental month. The histological types of lung tumour observed were bronchiolo-alveolar adenoma (14 rats) and adenocarcinoma (22 rats), which were the major types, and squamous cell carcinoma (three rats), adenosquamous carcinoma (one rat) and sarcoma (one rat) (Iwai *et al.*, 2000).

Groups of 99 male and 99 female Wistar rats, aged 40 days, were exposed by nose-only inhalation for 6 hours a day, 7 days a week for 24 months to exhaust from a 1.6-L displacement VW diesel engine (operated under US FTP conditions) that was diluted 1:5 with air and contained 3 mg/m^3 (low dose, obtained by further dilution) or 10 mg/m^3 (high dose) of particles. Levels of 7 (low dose) or 23 (high dose) ppm of nitrogen monoxide and 9 (low dose) or 28 (high dose) ppm of nitrogen oxides were found in the whole exhaust. Controls were exposed to clean air only. Surviving animals were maintained with no further treatment for an additional 6 months. A significantly increased incidence of lung tumours was observed in rats exposed to both the high dose (males, 17 out of 49; females, 29 out of 51) and low dose (males, 9 out of 50; females 14 out of 50) compared with controls (males, 2 out of 50 [adenomas only], 4%; females, 0 out of 51). A significant increase in the incidence of lung adenomas was also observed in the high-dose males (8 out of 49) and females (21 out of 51) and low-dose females (5 out of 50) (Stinn *et al.*, 2005).

(b) Intrapulmonary implantation

Groups of 35 female Osborne-Mendel rats, aged 3 months, received a single intrapulmonary implant of different fractions of organic material from a diesel exhaust condensate in beeswax:trioctanoin (1:1) and were observed for up to 140 weeks. The organic material was collected from a 3.0-L Daimler-Benz 300D diesel passenger car engine (operated under the first cycle of the European test cycle) and was separated by liquid–liquid distribution into a hydrophilic fraction (approximately 25% by weight of the total condensate) and a hydrophobic fraction (approximately 75% by weight). The latter was separated by column chromatography into several further fractions: (A) non-aromatic compounds plus polycyclic aromatic hydrocarbons (PAHs) with two and three rings (72% by weight of the total condensate), (B) PAHs with four to seven rings (0.8% by weight), (C) polar PAHs (1.1% by weight) and (D) nitro-PAHs (0.7% by weight). The animals received 6.7 mg of the hydrophilic fraction, 20 mg of the hydrophobic fraction, 19.22 mg of the hydrophobic subfraction containing non-aromatic compounds and two- and three-ring PAHs, 0.21 mg of the hydrophobic subfraction containing four to seven-ring PAHs, 0.29 mg of the hydrophobic subfraction containing polar PAHs, 0.19 mg of the hydrophobic subfraction containing nitro-PAHs or 19.9 mg of a reconstituted hydrophobic fraction (from subfractions A–D). A group of 35 females received implants of beeswax:trioctanoin only. Six lung tumours (17%; squamous cell carcinoma) were found in animals treated with the hydrophobic subfraction containing PAHs with four to seven rings. Similar carcinogenic potency was seen with the reconstituted hydrophobic subfraction (20%; seven squamous cell carcinomas) and the hydrophobic fraction (14%; five squamous cell carcinomas), whereas low carcinogenic potency was observed with the subfraction of nitro-PAHs (3%; one squamous cell carcinoma). The polar PAH subfraction induced no tumour; and one bronchiolo-alveolar adenoma (3%) was observed in animals treated with the non-aromatic subfraction with two- and three-ring PAHs. One bronchiolo-alveolar adenoma (3%)

occurred in the vehicle control group (Grimmer *et al.*, 1987). [The Working Group noted the poor reporting of the study, and that details of results, including pathology and dosing regimen, were not provided. The results were summarized in a general manner and were difficult to interpret.]

(c) Intratracheal instillation

Groups of 50–52 female Wistar Crl:(WI)BR rats, aged 7 weeks, received intratracheal instillations once a week for 16–17 weeks of 0.2–0.3 mL of suspensions of diesel particles that were collected on cellulose nitrate filters from the exhaust of a 1.6-L VW diesel engine (Golf/Passat) diluted 1:10 in a dilution tunnel and suspended in a 0.25% Tween 80 saline solution. Suspensions included: A, diesel particles (total dose, 15 mg); B and C, diesel particles extracted three times with toluene (total dose, 15 and 30 mg, respectively); D, extracted diesel particles coated with benzo[*a*]pyrene (total dose, 15 mg including 170 µg benzo[*a*]pyrene); and E and F, benzo[*a*]pyrene (total dose, 15 and 30 mg, respectively). Controls received 0.25% Tween 80 saline solution alone. Animals were then observed for up to 800 days after the first instillation. A significant increase in the incidence of lung cystic keratinizing epithelioma was observed in groups instilled with diesel particles (A: 8 out of 48, 17%; $P < 0.01$), with 30 mg of extracted diesel particles (C: 8 out of 48, 17%; $P < 0.01$), or with 30 mg benzo[*a*]pyrene (F: 22 out of 47, 47%; $P < 0.001$) compared with controls (0 out of 47) (Dasenbrock *et al.*, 1996). [The Working Group noted that early mortality (fewer than 50% survived more than 2 years) may have affected the outcome of the study.]

Groups of at least 50 female Fischer 344 rats, aged 8 weeks, received intratracheal instillations once a week for 2–10 weeks of 0.2 mL of a 5 mg/[mL] suspension (total doses, 2, 4, 8 and 10 mg) of diesel particulates collected from the exhaust of a 2.4-L diesel engine at each stage of a middle volume Andersen's sampler and suspended in 0.05% Tween 80 or DMSO phosphate buffer (pH 7.4) solution and were observed for up to 30 months after the first instillation. A dose-related increase in the incidence of lung tumours [mainly bronchiolo-alveolar adenoma and adenocarcinoma] was reported, reaching up to 74% (48% of malignant tumours) in rats instilled with 10 mg (Iwai *et al.*, 1997). [The Working Group noted that it was unclear whether a control group was used, the study was poorly described and designed, the results of the study were poorly reported, and no individual data on lung tumour incidence in exposed groups were available.]

Groups of 48 female Wistar HsdCpb:WU rats, aged 8–9 weeks, received intratracheal instillations of 2.5 mg of diesel soot suspended in 4.1 µL of a 0.5% Tween 80 phosphate buffer solution once a week for 3 weeks (A), 3 mg of diesel soot suspended in 8.1 µL of buffer solution once a week for 5 weeks (B), or 6 mg of diesel soot suspended in 16.2 µL of buffer solution once a week for 5 weeks (C) and were then observed for up to 30 months after the first instillation. A significant increase in the incidence of lung adenocarcinoma or squamous cell carcinoma (combined) in the high-dose group (C: 6 out of 45, 13%; $P = 0.03$) and significant increases in the incidence of lung adenoma or epithelioma (combined) in the mid- and high-dose groups (B: 7 out of 47, 15%; $P = 0.01$; C: 12 out of 45, 27%; $P = 0.0005$) were observed compared with the controls (0 out of 46) (Pott & Roller, 2005; Mohr *et al.*, 2006). [The Working Group noted that the control group was not treated concurrently.]

(d) Administration with known carcinogens

Groups of 20–35 female Fischer 344 rats, aged 5 weeks, were exposed to diesel exhaust from a 269-cm^3 displacement small diesel engine run at idling speed, diluted 1:2–1:4 with clean air to give concentrations of 2–4 mg/m^3 of particulate matter and 2–4 ppm of nitrogen dioxide, for 4 hours a day, 4 days a week for up to 24 months. After 1 month of exposure, two

groups were injected intraperitoneally with 1 g/kg bw of *N*-nitrosodiisopropanolamine (DiPN) once a week for 3 weeks. No lung tumours were observed in the groups treated with diesel exhaust alone or controls. In contrast, an increase in the incidence of lung tumours (adenoma and carcinoma) was observed in the groups treated with diesel exhaust plus DiPN and DiPN alone, but the difference in incidence between these groups was not significant (Takemoto *et al.*, 1986). [The Working Group noted the small group size.]

3.1.3 Hamster

See Table 3.3

(a) Inhalation

Groups of 48 female Syrian golden hamsters, aged 8 weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 2.4-L Daimler-Benz diesel engine, diluted 1:7 with air, for 7–8 hours a day, 5 days a week for life [up to 120 weeks]. The unfiltered exhaust contained 3.9 mg/m^3 of particles. The levels of nitrogen dioxide and nitrogen oxides were 1.2 ± 1.7 and 18.6 ± 5.8 ppm in whole exhaust and 1.0 ± 1.5 and 19.2 ± 6.1 ppm in filtered exhaust, respectively. Diesel exhaust had no effect on survival; the median lifespan was 72–74 weeks in all groups, and no lung tumours were reported in treated or control animals (Heinrich *et al.*, 1982).

Groups of 48 male and 48 female Syrian golden hamsters, aged 8–10-weeks, were exposed to clean air (control) or to filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air, for 19 hours a day, 5 days a week for life (up to 120 weeks). The unfiltered exhaust contained 4.24 mg/m^3 of particles. The levels of nitrogen dioxide and nitrogen oxides were 1.5 ± 0.3 and 11.4 ± 2.1 ppm in whole exhaust and 1.2 ± 0.26 and 9.9 ± 1.8 ppm in filtered exhaust, respectively. The median lifespan was not significantly influenced by exposure to diesel exhaust and was 75–80 weeks for females and 80–90 weeks for males. No lung tumour was observed in treated or control animals (Heinrich *et al.*, 1986a).

Groups of 104 male and 104 female Syrian golden hamsters, aged 6–8-weeks, were exposed to filtered or unfiltered exhaust emissions generated by a VW Rabbit 1.5-L diesel engine, diluted with clean air to give particle concentrations of 0.7, 2.2 or 6.6 mg/ m^3, for 16 hours a day, 5 days a week for 24 months. The filtered exhaust was passed through a stationary filtration system that removed 99.97% of the mass of particles before delivery to the animals. Further groups of 208 male and 208 female hamsters were exposed to clean air and served as controls. In addition, groups of 52 male and 52 female exposed hamsters and 104 male and 104 female controls were pretreated with 4.5 mg/kg of *N*-nitrosodiethylamine (NDEA) 3 days before the start of exposure. No significant increase in the incidence of respiratory tract (lung, trachea, larynx or nasal cavity) tumours was observed in any treated groups compared with their respective controls (Brightwell *et al.*, 1989).

(b) Intratracheal instillation

Groups of 59–62 male Syrian golden hamsters, aged 8 weeks, received intratracheal instillations of 0.1 mL of a suspension of 0 (control), 0.1, 0.5 or 1.0 mg of tar from a heavy-duty V6 11-L diesel engine exhaust suspended in 0.1 mL of a mixed solution of Tween 60:ethanol:phosphate buffer (1.5:2.5:30 mL, respectively) solution once a week for 15 weeks and were observed for life. Survival rates were 95%, 92%, 71% and 98% in the three treated groups and vehicle controls, respectively. [The duration of survival was not reported.] No significant difference in the incidence of tumours of the lung, trachea or larynx was observed between controls and treated groups (Kunitake *et al.*, 1986).

Table 3.3 Studies of the carcinogenicity of diesel engine exhaust in hamsters

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian golden (F) [Up to 120 wk] (lifetime) Heinrich *et al.* (1982)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 2.4-L Daimler-Benz diesel engine, diluted 1:7 with air (unfiltered exhaust: 3.9 mg/m^3 particles, 1.2 ± 1.7 ppm NO_2 and 18.6 ± 5.8 ppm NO_x; filtered exhaust: 1.0 ± 1.5 ppm NO_2 and 19.2 ± 6.1 ppm NO_x), 7–8 h/d, 5 d/wk Groups of 48, aged 8 wk	No lung tumours observed in treated or control animals	NS	Median lifespan was not influenced by exposure to diesel exhaust
Syrian golden (M, F) Up to 120 wk Heinrich *et al.* (1986a)	Inhalation Clean air (control), or filtered or unfiltered exhaust from a 1.6-L displacement diesel engine (operated to simulate average urban driving), diluted 1:17 with air (unfiltered exhaust: 4.24 mg/m^3 particles, 1.5 ± 0.3 ppm NO_2 and 11.4 ± 2.1 ppm NO_x; filtered exhaust: 1.2 ± 0.26 ppm NO_2 and 9.9 ± 1.8 ppm NO_x), 19 h/d, 5 d/wk Groups of 48 M and 48 F, aged 8–10 wk	No lung tumours observed in treated or control animals	NS	Median lifespan was not influenced by exposure to diesel exhaust
Syrian golden (M, F) 24 mo Brightwell *et al.* (1989)	Inhalation Clean air (control), or whole exhaust emissions generated by a VW Rabbit 1.5-L diesel engine, diluted with clean air (particle concentrations, 0.7 mg/m^3, 2.2 mg/m^3 or 6.6 mg/m^3) or exhaust passed through a stationary filtration system to remove 99.97% of the mass of particles, 16 h/d, 5 d/wk; additional groups pretreated with 4.5 mg/kg NDEA 3 days before start of exposure Treated groups of 104 M and 104 F; control groups of 208 M and 208 F; 52 M and 52 F treated and 104 M and 104 F control pretreated with NDEA; aged 6–8 wk	No significant increase in the incidence of respiratory tract (lung, trachea, larynx or nasal cavity) tumours observed in any diesel engine exhaust-treated groups compared with the respective controls	NS	Authors reported clinical evidence of 'wet-tail' disease in both NDEA-pretreated and non-pretreated hamsters in all experimental and control groups that resulted in significant mortality (~45%) between 10 and 12 mo of exposure. Oxytetracycline and dimetridazole added to the drinking-water of all hamsters at 400 mg/L and 5 g/L, respectively, from about 12 mo of exposure until 24 mo, effectively controlled the infection, and no further mortalities associated with the disease occurred up to the end of the 2-yr exposure period
Syrian golden (M) Lifetime Kunitake *et al.* (1986)	Intratracheal instillation 0 (control), 0.1, 0.5 or 1.0 mg tar from a heavy-duty V6 11-L diesel engine exhaust suspended in 0.1 mL Tween 60, ethanol and phosphate buffer solution (1.5:2.5:30), once/wk for 15 wk Groups of 59 or 62, aged 8 wk	No significant difference in the incidences of tumours of the lung, trachea or larynx observed between controls and treated groups	NS	Duration of survival was not reported

B[*a*]P, benzo[*a*]pyrene; d, day; F, female, h, hour; M, male; mo, month; NDEA, *N*-nitrosodiethylamine; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; NS, not significant; VW, Volkswagen; wk, week; yr, year

Table 3.4 Study of the carcinogenicity of diesel engine exhaust in monkeys

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Comments
Cynomolgus (*Macaca fascicularis*) (M) 24 mo Lewis *et al.* (1989)	Inhalation Clean air (control), exhaust from a 7.0-L Caterpillar Model 3304 diesel engine, diluted 1:27 with clean air (particle concentration, 1.95 ± 0.25 mg/m^3; 1.5 ± 0.5 ppm NO_2 and 8.7 ± 3.6 ppm NO), 2.00 ± 0.41 mg/m^3 coal dust or 2.02 ± 0.30 mg/m^3 coal dust plus diesel exhaust, 7 h/d, 5 d/wk Groups of 15 [age unspecified]	No statistical difference in tumour incidence between the four experimental groups	Study limited by the short observation period

d, day; h, hour; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; wk, week

3.1.4 Monkey

See Table 3.4

Inhalation

Groups of 15 male cynomolgus (*Macaca fascicularis*) monkeys were exposed for 7 hours a day, 5 days a week for 24 months to clean air or to the exhaust from a 7.0-L Caterpillar Model 3304 diesel engine, diluted 1:27 with clean air to give concentrations of 1.95 ± 0.25 mg/m^3 of particles, 1.5 ± 0.5 ppm of nitrogen dioxide and 8.7 ± 3.6 ppm of nitrogen oxide. Additional groups were exposed to 2.00 ± 0.41 mg/m^3 of coal dust or 2.02 ± 0.30 mg/m^3 of diesel exhaust plus coal dust. At the end of the exposure period, all survivors (59 out of 60) were necropsied and examined histologically. No significant difference in tumour incidence was reported among the four groups (Lewis *et al.*, 1989). [The Working Group noted that the study was limited by the short observation period.]

3.2 Gasoline engine exhaust

The gasoline engine exhausts used in the studies evaluated here were generated from fuels and engines produced before the year 2000. These exhausts include three basic components: particles composed primarily of elemental carbon and metallic compounds (especially lead, if present in the fuel); adsorbed organic material that is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using several animal species to evaluate the potential carcinogenicity of exposure to whole exhaust and to the components of exhaust from gasoline engines. The studies are considered under two subcategories: (i) whole gasoline engine exhaust; and (ii) condensates or extracts of gasoline engine exhaust.

Animal bioassays conducted with different gasoline engine exhausts have been reviewed previously in the *IARC Monographs* (IARC, 1989). This section provides a summary of these and a detailed review of more recent studies.

3.2.1 Mouse

See Table 3.5

(a) Inhalation

Groups of 37–38 male and female mice of 'mixed' [unspecified] strain, aged 3 months, were exposed to clean air (control) or to exhaust from either a four-cylinder, 23-hp car engine run on regular (unleaded) gasoline or a six-cylinder, 24-hp car engine run on leaded gasoline. The exhausts were initially diluted 1:145 with air for

Table 3.5 Studies of the carcinogenicity of gasoline engine exhaust in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
'Mixed' [strain NR] (M, F) Up to 25 mo (lifetime) Campbell (1936)	Inhalation Clean air (control) or exhaust from (A) a 4-cylinder, 23-hp gasoline car engine run on regular (unleaded) gas or (B) a 6-cylinder, 24-hp gasoline car engine run on leaded gas (1:1800), diluted 1:145 with air for 4 h and then diluted 1:83 for 3 h (particulate content and lead concentrations NR; average CO levels, 7.5% for A and 2.1% for B), 7 h/d on 5 d/wk Groups of 37 M and 38 F, aged 3 mo	Lung (all tumours): A (M+F)–8/74 (11%), 9/75 (12%) B (M+F)–6/70 (9%), 12/75 (16%)	NS	Study poorly reported; no details on survival or pathology; information on test atmosphere generation and chamber monitoring doubtful
ICR (F) 12 mo Yoshimura (1983)	Inhalation Exhaust emission generated by a small gasoline engine, diluted 1:250 with clean air to give concentration of 0.1 mg/m^3 (300 ± 50 ppm CO, 0.21 ppm NO and 0.08 ppm NO_2), 2 h/d on 3 d/wk for 6–12 mo [Initial numbers and age NR]	Lung (all tumours): 2/15 (13% adenoma only)	-	Study poorly designed and reported; short treatment period and lack of controls
C57BL [sex NR] [duration NR, > 390 days] Kotin *et al.* (1954)	Skin application Benzene alone (control) or an oil residue of the benzene extract of filter paper used to filter exhaust from an overhauled Ford V-8 gasoline engine [doses NR] in benzene, applied at frequent but irregular intervals Control groups of 42; treated groups of 86 [age NR]	Skin (all tumours): 0/42, 38/86 (44%)* Skin (squamous cell carcinoma): 0/42, 22/86** (26%)	[*$P < 0.0001$, **$P = 0.0005$]	The study appeared not to have been completed; authors state that "This 44% figure of positive tumour production is subject to upward revision in view of the possibility of tumour demonstration in 16 remaining mice." Study poorly reported: details on sex, age, dose used and dosing administration frequency NR
Swiss (F) Up to 18 mo Wynder & Hoffmann (1962)	Skin application 0 (control), 5, 10, 25, 33 or 50% of an oil residue of a benzene extract of condensed and filtered exhaust from a V8 gasoline engine in in acetone, applied with a No. 5 camel's hair brush dipped once in the 'tar' solution, 3 × /wk for 15 mo and held for an additional 3 mo Groups of 30–50, aged 6 wk	Skin (papilloma): 0%, 4%, 50%, 60%, 60%, 70% Skin (squamous cell carcinoma): 0%, 4%, 32%, 48%, 54%, 4%	NR	All animals in the high-dose group had died by 10 mo. Study poorly designed; dose application method gave inconsistent doses and high-dose animals were tested separately

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Swiss (F) 18 mo Hoffmann *et al.* (1965)	Skin application Tar [method of extraction NR] from exhaust of a V8 gasoline engine using approximately 1 L engine oil/200 miles [0.3 L/100 km] (A) or from exhaust of an engine using approximately 1 L oil/1600 miles [0.04 L/100 km] (B) in acetone, [frequency and method of application NR] Groups of 50 [age NR]	Skin (all tumours): 60% (48% carcinoma; A), 84% (52% carcinoma; B)	-	Study poorly designed and reported; method and frequency of exposure NR; no vehicle or untreated controls
CFLP (F) Lifetime Brune *et al.* (1978)	Skin application 0 (two control groups), 0.5, 1.6 or 4.7 µg of an exhaust condensate (C1, C2, C3) produced from a VW 1.5-L Otto gasoline engine during a European test cycle, nitromethane (two groups; N1, N2) and cyclohexane (two groups; CY1, CY2) fractions of the condensate, or reconstituted condensate (two groups; R1, R2) in 0.1 mL DMSO: acetone (3:1) on the shaved intrascapular region twice/wk for life Groups of 80 studied in Hamburg and groups of 40 studied in Heidelberg, aged 12 wk	Hamburg study Skin (squamous cell tumours): 0/76, 1/76 (1%), 3/77 (4%; C1), 26/74 (35%; C2)*, 60/78 (77%; C3)*, 11/67 (16%; N1)***, 51/74 (69%; N2)*, 10/73 (14%; CY1)****, 53/77 (69%; CY2)*, 6/76 (8%; R1), 44/75 (59%; R2)* Skin (squamous cell carcinoma): 0/76, 0/76, 1/77 (1%; C1), 22/74 (30%; C2)*, 56/78 (72%; C3)*, 9/67 (13%; N1)****, 48/74 (65%; N2)*, 10/73 (14%; CY1)****, 47/77 (61%; CY2)*, 3/76 (4%; R1), 40/75 (53%; R2)*	[*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$]	

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CFLP (F) Lifetime Brune et al. (1978) (cont.)		Heidelberg study Skin (squamous cell tumours): 0/30, 0/37, 1/31 (3%; C1), 3/37 (8%; C2), 19/38 (50%; C3)**, 3/34 (9%; N1), 14/37 (38%; N2)*, 0/34 (CY1), 11/38 (29%; CY2)*, 3/34 (9%; R1), 9/30 (30%; R2)** Skin (squamous cell carcinoma): 0/30, 0/37, 1/31 (3%; C1), 2/37 (5%; C2), 18/38 (47%; C3)*, 2/34 (6%; N1), 12/37 (32%; N2)*, 0/34 (CY1), 10/38 (26%; CY2)**, 3/34 (9%; R1), 7/30 (23%; R2)***** Lung (all tumours): 3/40 (7%), 3/40 (7%), 3/40 (7%; C1), 8/40 (20%; C2)*****, 9/40 (22%; C3)*****, 7/40 (17%; N1)***, 6/40 (15%; N2)****, 6/40 (15%; CY1)****, 4/40 (10%; CY2), 5/40 (12%; R1), 3/40 (7%; R2)	[*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$] [*$P < 0.0001$, **$P = 0.0002$, ***$P = 0.005$, ****$P = 0.009$, *****$P = 0.001$]	

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
CLFP (F) Lifetime Grimmer *et al.* (1983a, b)	Skin application 0 (control), 0.29, 0.87 or 2.6 mg/animal condensate (E1, E2, E3) from exhaust emissions generated by a passenger car gasoline engine (1500 cm^3, 50 hp); several fractions of condensate: 0.97 or 2.9 mg/animal PAH-free (A1, A2), 0.152 or 0.455 mg/animal PAH-containing (2 and 3 rings) (B1, B2), and 0.02 or 0.06 mg/animal PAH-containing (more than 3 rings) (C1, C2); or 0.003 or 0.009 mg/animal of a mixture of 15 PAHs (D1, D2) simulating those in automobile exhaust; dissolved in DMSO: acetone (3:1) and applied as 0.1 mL of the resultant solution, twice/wk for 104 wk Control groups of 65; treated groups of 80; aged 7 wk	Skin (mainly squamous cell carcinoma): 0/65, 6/80 (7%; E1)***, 34/80 (42%; E2)*, 65/80 (81%; E3)*, 4/80 (5%; A1), 11/80 (14%; A2)**, 3/80 (4%; B1), 1/80 (1%; B2), 7/80 (9%; C1)***, 50/80 (62%; C2)*, 1/80 (1%; D1), 29/80 (36%; D2)*	[*$P < 0.0001$, **$P = 0.003$, ***$P = 0.04$]	Clear dose–response relationships demonstrated for skin tumours in the groups treated with: condensate, the fraction containing PAHs with more than three rings, and the mixture of 15 PAHs
NMRI (F) [Duration NR] Pott et al. (1977	Subcutaneous injection 0 (control), 20 or 60 mg exhaust condensate, containing 0.163 µg/mg B[*a*]P, from a gasoline engine [specifics NR] in 0.5 mL tricaprylin, once or 60 mg, × 3 [frequency NR] Groups of 87–88 and 45 (3 injections) [age NR]	Skin (fibrosarcoma): 3/89 (3%), 10/87 (11%), 6/88 (7%), 5/45 (11%)	NR	Study poorly reported; no details of duration of study, age of animals or pathology; no statistics provided
Initiation-promotion				
SENCAR (M, F) 24–26 wk Nesnow *et al.* (1982a, 1983)	Skin application Single 0.2 mL application of 0 (control), 0.1, 0.5, 1.0, 2.0, or 3.0 mg/mouse particulates from the emissions of a 1977 Ford Mustang II-302 V-8 engine with catalyst collected on Teflon-coated fibreglass filters, extracted with dichloromethane removed by evaporation, dissolved in acetone to shaved dorsal skin followed 1 wk later by 2.0 µg TPA in 0.2 mL acetone, twice/wk for up to 25 wk Groups of 40 M and 40 F, aged 7–9 wk	Skin (papillomas/mouse): M–0.08 (8%), 0.05 (5%), 0.15 (13%), 0.18 (18%), 0.24 (22%), 0.24 (18%) F–0.05 (5%), 0.23 (13%), 0.24 (18%), 0.13 (10%), 0.23 (21%), 0.28 (23%)	NR	Authors stated that the responses at the higher doses were significantly greater than those of the TPA controls; no statistics provided; 20% of the females developed squamous cell carcinoma with the 3-mg dose

Table 3.5 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
Mouse, NMRI (F) [Duration NR] Pott *et al.* (1977)	Subcutaneous injection 10, 30, 90 or 270 µg B[*a*]P alone or together with 6.6, 20 or 60 mg of exhaust condensate (C) from a gasoline engine [specifics NR] in 0.5 mL tricaprylin, once Groups of 87–88 [age NR]	6.6 mg *C* + *10, 30 or* 90 µg *B[a]P* Skin (fibrosarcoma): 19/89 (21%), 25/88 (28%), 46/88 (52%) 20 mg *C* + *10, 30, 90 or* 270 µg *B[a]P* Skin (fibrosarcoma): 15/86 (17%), 24/88 (27%), 36/87 (41%), 45/87 (52%) 60 mg *C* + 270 µg *B[a]P* Skin (fibrosarcoma): 33/90 (37%) *3.3, 10, 30, 90 or* 270 µg *B[a]P* Skin (fibrosarcoma): 7/90 (8%), 18/90 (20%), 47/87 (54%), 67/88 (76%), 70/87 (80%)	Dose–response relationship reduced significantly by addition of both doses of the condensate.	Study poorly reported. No details on duration of study, age of animals or pathology
NMRI (F) 94 wk Heinrich *et al.* (1986c)	Inhalation/intratracheal instillation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 or 1:61 with air (mean concentrations: 350 ± 24 and 77.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates) for 18–19 h/d, 5 d/wk for 53 wk concomitantly with 10 instillations of 100 µg B[*a*]P, 20 instillations of 50 µg B[*a*]P or 10 instillations of 50 µg DB[*a,h*]A Groups of 60, aged 8–10 wk	Similar total numbers of lung tumour-bearing animals in clean air and exhaust-exposed groups	NS	Additional groups of 61–83 newborn NMRI mice received a single subcutaneous injection of 4 µg (M and F) or 10 µg (F only) DB[*a,h*]A followed by inhalation exposure to one of the two dilutions of gasoline exhaust for 6 mo, after which they were killed; the number of lung tumours per animal was not significantly different from that in controls exposed simultaneously to clean air

B[*a*]P, benzo[*a*]pyrene; CO, carbon monoxide; d, day; DB[*a,h*]A, diebenz[*a,h*]anthracene; DMSO, dimethyl sulfoxide; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; PAH, polycyclic aromatic hydrocarbon; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; VW, Volkswagen, wk, week

4 hours and then diluted 1:83 for the final 3 hours [particulate content and lead concentrations not provided] for a total exposure of 7 hours a day, 5 days a week for up to 25 months. Levels of carbon monoxide in the chambers averaged 7.5% for the unleaded gasoline engine and 2.1% for the leaded gasoline engine. Lung tumours [type unspecified] were observed in 9 out of 75 (12%) animals exposed to the unleaded gasoline exhaust compared with 8 out of 74 (11%) controls. The lung tumour incidence in animals exposed to the leaded gasoline exhaust was 12 out of 75 (16%) compared with 6 out of 70 (9%) controls (Campbell, 1936). [The Working Group noted the poor reporting of the study, in which no details were provided on survival or pathology, and the information on test atmosphere generation and chamber monitoring was doubtful.]

A group of female ICR mice [initial number and age unspecified] was exposed for 2 hours a day, 3 days a week for 12 months to exhaust emissions generated by a small gasoline engine, diluted 1:250 with clean air to give concentrations of 0.1 mg/m^3 of engine exhaust, 300 ± 50 ppm of carbon monoxide, 0.21 ppm of nitrogen oxide and 0.08 ppm of nitrogen dioxide. No unexposed controls were available. At 12 months, the incidence of lung adenoma was 2 out of 15 (13%), and no malignant lung tumour was observed (Yoshimura, 1983). [The Working Group noted that the study was poorly designed and reported because of the short treatment period and the lack of controls.]

(b) Skin application

Groups of 42 and 86 C57BL mice [sex and age unspecified] received topical applications of benzene alone or an oil residue [doses unspecified] of a benzene extract from filter paper used to filter of exhaust from an overhauled Ford V-8 gasoline engine dissolved in benzene "at frequent but irregular intervals" [duration of exposure unspecified]. Skin tumours were observed in 38 out of 86 (44%) animals (22 out of 86 carcinomas) surviving at the appearance of the first tumour (390 days). No skin tumours were reported in 0 out of 42 controls (Kotin *et al.*, 1954). [The Working Group noted that the study did not appear to have been completed, because the authors stated that "… this 44% figure of positive tumour production is subject to upward revision in view of the possibility of tumour demonstration in 16 remaining mice."]

Groups of 30–50 female Swiss mice, aged 6 weeks, received topical applications three times a week for 15 months of acetone containing 0 (control), 5, 10, 25, 33 or 50% of an oil residue of a benzene extract of condensed and filtered exhaust from a V8 gasoline engine using with a No. 5 camel's hair brush that was dipped once into the 'tar' solution. Survivors were then maintained with no further treatment for an additional 3 months. At 18 months, the incidence of skin tumours was 0, 4, 50, 60 and 60% papillomas and 0, 4, 32, 48 and 54% carcinomas in the control, 5-, 10-, 25- and 33%-dose groups, respectively. All of the mice in the high-dose group had died by 10 months; 70% had skin papillomas and 4% had skin carcinomas (Wynder & Hoffmann, 1962). [The Working Group noted the poor design of study, in which the method of application led to inconsistent doses and high-dose animals were tested separately.]

Groups of 50 Swiss female mice [age unspecified] received topical applications of tar from the exhaust of a V8 gasoline engine that used approximately 1 L of engine oil/200 miles [0.3 L/100 km] (A) or an engine that used approximately 1 L of oil/1600 miles [0.04 L/100 km] (B) in acetone [method of extraction not reported]. Skin tumours were reported in 60% (48% carcinomas) of the animals treated with exhaust from engine A and 84% (52% carcinomas) of the animals treated with exhaust from engine B (Hoffmann *et al.*, 1965). [The Working Group noted that the study was poorly designed and reported, the method and frequency of exposure

was not reported and no vehicle or untreated controls were available.]

Groups of 80 CFLP female mice, aged 12 weeks, studied in Hamburg (Germany), and groups of 40 CFLP female mice, aged 12 weeks, studied in Heidelberg (Germany), received topical applications onto the shaved interscapular region three times a week for life of 0.1 mL of DMSO:acetone (3:1) containing 0 (control), 0.5, 1.6 or 4.7 µg of an exhaust condensate produced by a VW 1.5-L Otto gasoline engine during a European test cycle, nitromethane or cyclohexane fractions of this condensate or reconstituted condensate, or were untreated. Results of the study performed in Hamburg indicated a linear relationship between the percentage of animals with local skin tumours (squamous cell papilloma or carcinoma combined) and treatment with fractions that contained PAHs with a nitromethane phase (tumour incidence: 16.4% with 0.179 mg and 68.9% with 0.537 mg) or a cyclohexane phase (tumour incidence: 13.7% with 0.358 mg and 68.8% with 1.07 mg), the reconstituted condensate (tumour incidence: 7.9% with 1.05 mg and 54.7% with 3.16 mg) and the total condensate (tumour incidence: 3.9, 35.1 and 76.9% with 0.5, 1.6 and 4.7 µg, respectively). Local skin tumour rates in mice treated with total condensate were higher than those in the two control groups (1.3 and 0%; $P < 0.0001$). The study performed in Heidelberg gave similar results; however, the incidence of local skin tumours was significantly higher in the study performed in Hamburg, probably due to minor differences in experimental techniques. In both studies, squamous cell tumours were mainly carcinomas (Brune *et al.*, 1978).

Groups of 65 (control) or 80 female CLFP mice, aged 7 weeks, received dermal applications twice a week for 104 weeks of 0 (control), 0.29, 0.87 and 2.6 mg/animal of a condensate from the exhaust emissions generated by a passenger car gasoline engine (1500 cm^3, 50 hp). The condensate was separated into several fractions that were also applied: (A) 0.97 or 2.9 mg/animal of a PAH-free fraction, (B) 0.152 or 0.455 mg/animal of a fraction containing PAHs with two and three rings and (C) 0.02 or 0.06 mg/animal of a fraction containing PAHs with more than three rings. In addition, a mixture of 15 PAHs simulating those found in automobile exhaust was applied at 0.003 or 0.009 mg/animal. All study materials were dissolved in DMSO:acetone (3:1) and applied as 0.1 mL of the resultant solution. All survivors were observed for life. A dose–response relationship was observed for the incidence of skin tumours (mainly squamous cell carcinomas) in the groups treated with total condensate (6 out of 80, 7%; 34 out of 80, 42%; and 65 out of 80, 81%; with the low, mid and high doses, respectively), in those given fraction C that contained PAHs with more than three rings (7 out of 80, 9%; and 50 out of 80, 62%; for the low and high doses, respectively) and in those given the mixture of 15 PAHs (1 out of 80, 1%; and 29 out of 80, 36%; for the low and high doses, respectively). An increased incidence of skin tumours was observed in the group treated with the high dose of fraction A. Fraction B did not produce a significant increase in the incidence of skin tumours, and no local skin tumours were seen in controls (0 out of 65) (Grimmer *et al.*, 1983a, b).

(c) Subcutaneous administration

Three groups of 87–88 female NMRI mice [age unspecified] received a single subcutaneous injection of 0 (control), 20 or 60 mg of an exhaust condensate (containing 0.163 µg/mg of benzo[*a*]pyrene) from a gasoline engine [unspecified] in 0.5 mL tricaprylin. A fourth group of 45 animals received three injections of the 60-mg dose. The mean survival time in the low- and mid-dose groups was similar to that of the controls (80–88 weeks), but was 57 weeks in the high-dose group. The number of animals with fibrosarcomas at the injection site was 3 out of 89 (3%), 10 out of 87 (11%), 6 out of 88 (7%) and 5 out of 45 (11%), respectively (Pott *et al.*, 1977). [The Working

Group noted that no details on the duration of the study or histopathology were reported.]

(d) Initiation–promotion studies

Groups of 40 male and 40 female SENCAR mice, aged 7–9 weeks, received a single topical application on the shaved dorsal skin of 0 (control), 0.1, 0.5, 1.0, 2.0 or 3.0 mg of a dichloromethane extract of the particulates from the emissions of a 1977 Ford Mustang II-302 V-8 engine with a catalyst that were collected on Teflon-coated fibreglass filters in 0.2 mL of acetone. One week later, the mice received topical applications of 2.0 µg of TPA in 0.2 mL of acetone twice a week for up to 25 weeks. At experimental week 26, the percentages of mice with skin papillomas and the numbers of papillomas/mouse in TPA-treated controls were 8% and 0.08 in males and 5% and 0.05 in females, respectively. In the groups treated with both TPA and the gasoline extract, the percentages and numbers of papillomas/mouse were: males – 5% and 0.05 (0.1 mg), 13% and 0.15 (0.5 mg), 18% and 0.18 (1 mg), 22% and 0.24 (2 mg) and 18% and 0.24 (3 mg); females – 13% and 0.23 (0.1 mg), 18% and 0.24 (0.5 mg), 10% and 0.13 (1 mg), 21% and 0.23 (2 mg) and 23% and 0.28 (3 mg). Of the high-dose females, 20% developed squamous cell carcinomas (Nesnow *et al.*, 1982a, 1983). [The Working Group noted that the authors stated that the responses at the higher doses were significantly greater than those of the TPA controls, but no statistics were reported.]

(e) Administration with known carcinogens

Groups of 86–90 female NMRI mice [age unspecified] received a single subcutaneous injection of 3.3, 10, 30, 90 or 270 µg of benzo[*a*]pyrene alone or together with 6.6, 20 or 60 mg of an exhaust condensate from a gasoline engine [unspecified] in 0.5 mL of tricaprylin. The dose–response relationship for local fibrosarcomas produced by benzo[*a*]pyrene (20, 54 and 76%, respectively) was significantly reduced by the addition of the condensate at all doses (Pott *et al.*, 1977). [The Working Group noted that no details on the duration of the study or histopathology were reported.]

Groups of 60 female NMRI mice, aged 8–10 weeks, received 10 intratracheal instillations of 100 µg of benzo[*a*]pyrene, 20 intratracheal instillations of 50 µg of benzo[*a*]pyrene or 10 intratracheal instillations of 50 µg of DB[*a*,*h*]A and were exposed concomitantly by inhalation for 18–19 hours a day, 5 days a week for 53 weeks to clean air or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L; operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of the exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3. The animals were then followed up during a 40-week observation period with no further treatment. The mean survival time of exhaust-exposed animals was 75–85 weeks. The total numbers of lung tumour-bearing animals in the clean air- and exhaust-exposed groups did not differ significantly. Additional groups of 61–83 newborn NMRI mice received a single subcutaneous injection of 4 µg (females and males) or 10 µg (females only) of DB[*a*,*h*]A followed by inhalation exposure to one of the two dilutions of gasoline exhaust for 6 months, after which they were killed. The number of lung tumours/animal did not differ significantly from that in controls exposed simultaneously to clean air (Heinrich *et al.*, 1986c).

3.2.2 Rat

See Table 3.6

Table 3.6 Studies of the carcinogenicity of gasoline engine exhaust in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
BOR,WISW (F) 30 mo Heinrich *et al.* (1986c)	Inhalation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:61 or 1:27 with air (mean concentrations, 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–83, aged 10–12 wk	Lung (all tumours): 1/78 (1%), 1/83 (1%), 3/78 (4%)	NS	The highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of CO
F344 (M, F) 30 mo Brightwell *et al.* (1989)	Inhalation Clean air (control) or exhaust emission generated from unleaded fuel in a Renault R18 1.6-L gasoline engine fitted with or without a three-way catalytic converter, diluted with a constant volume of 800 m^3 of air (high dose), or further dilution 1:3 in air (low dose) (low dose: 15 ppm NO, 15 ppm NO_2, 67 ppm CO; high dose: 44 ppm NO, 49 ppm NO_2, 224 ppm CO), 16 h/d, 5 d/wk for 24 mo. Control groups of 144 M and 144 F; treated groups of 72 M and 72 F; aged 6–8 wk	With catalytic converter Lung (all tumours): M–2/134 (1%), 0/37, 0/68 F–1/126 (1%), 0/36, 1/61 (2%) Without catalytic converter Lung (all tumours): M–2/134 (1%), 1/44 (2%), 0/65 F–1/126 (1%), 0/32, 1/63 (2%)	NS	Histological types NR
Osborne-Mendel (F) Lifetime Grimmer *et al.* (1984)	Intrapulmonary implantation 0 (control), 5.0 or 10.0 mg (A1, A2) condensate from the exhaust emission generated by a 1.5-L gasoline car engine (operated on the European test cycle), or one of several fractions: 4.36, 8.73 or 17.45 mg PAH-free (B1, B2, B3), 0.50, 0.99 or 1.98 mg PAHs with 2 or 3 rings (C1, C2, C3), or 0.14, 0.28 or 0.56 mg PAHs with more than 3 rings (D1, D2, D3) in beeswax:trioctanoin (1:1) into the left lobe of the lung, once Groups of 34–35, aged 3 mo	Lung (carcinoma): 0/34, 3/35 (9%; A1), 20/35 (57%; A2)**, 0/34 (B1), 3/34 (9%; B2), 1/34 (3%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 3/35 (9%; D1), 15/34 (44%; D2)*, 24/35 (69%; D3)** Lung (sarcoma): 0/34, 4/35 (11%; A1), 0/35 (A2), 0/34 (B1), 3/34 (9%; B2), 2/34 (6%; B3), 0/35 (C1), 0/35 (C2), 3/35 (9%; C3), 1/35 (3%; D1), 2/34 (6%; D2), 0/35 (D3)	[*P = 0.0002, **P < 0.001]	The authors reported that a lung tumour dose–response relationship was obtained with the condensate and with the fraction of PAHs with more than 3 rings

Table 3.6 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Administration with known carcinogens				
Sprague-Dawley (F) Up to 12 mo Yoshimura (1983)	Inhalation/oral administration Exhaust emissions from a small gasoline engine, diluted 1:250 with clean air (0.1 mg/m³ engine exhaust particulates, 300 ± 50 ppm CO, 0.21 ppm NO, 0.08 ppm NO_2), 2 h/d, 3 × / wk for 6–12 mo concomitantly with 0 or 0.01% DiPN in the drinking-water [Numbers and age NR]	Lung (all tumours): 0/23, 2/24 (8%), 11/37 (30%)*	[*P = 0.05]	Study limited by the lack of clean air controls; lung tumours observed following the combined treatment included one adenoma and 10 malignant tumours (undifferentiated carcinomas, squamous cell carcinomas, adenocarcinomas or mixed tumours)
Rat, BOR,WISW (F) 30 mo Heinrich *et al.* (1986c)	Subcutaneous injection/inhalation 0.25 or 0.5 g/kg bw NDPA, once/day for 25 days, followed by exposure to clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 (H) or 1:61 (L) with air (Mean concentrations: H– 350 ± 24 ppm CO, 28 ± 3 ppm NO, 1.9 ± 0.4 ppm NO_2, 95.8 ± 16.5 µg/m³ particulates; L–177.5 ± 12.5 ppm CO; 13.7 ± 1.5 ppm NO; 1.0 ± 0.2 ppm NO_2, 47.9 ± 20.2 µg/m³ particulates), 18–19 h/d, 5 d/wk for 24 mo. Groups of 60, aged 10–12 wk	0.5 g/kg NDPA Lung (all tumours): 44/49 (90%), 28/48 (58%; H)*, 33/49 (67%; L)* 0.25 g/kg NDPA Lung (all tumours): 27/48 (56%), 13/54 (24%; H)*, 15/47 (32%; L)*	*$P \leq 0.05$, decrease	

B[*a*]P, benzo[*a*]pyrene; CO, carbon monoxide; d, day; DiPN, *N*-nitrosodiisopropanolamine; F, female; FTP, Federal Test Procedures; h, hour; mo, month; NDPA, *N*-nitrosodipentylamine; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; wk, week

(a) Inhalation

Groups of 80–83 female Bor:WISW rats, aged 10–12 weeks, were exposed by inhalation for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel [0.3–0.56 g/L] (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. Animals surviving at the end of the 24-month exposure period were maintained in clean air for up to an additional 6 months. Neither concentration of gasoline exhaust produced a significant increase in the incidence of lung tumours: 1 out of 83 animals exposed to 1:61 had a squamous cell carcinoma; 3 out of 78 exposed to 1:27 had two squamous cell carcinomas and one adenoma; and 1 out of 78 controls had an adenoma (Heinrich *et al.* (1986c). [The Working Group noted that the highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of carbon monoxide.]

Groups of 72 male and 72 female Fischer 344 rats, aged 6–8 weeks, were exposed for 16 hours a day, 5 days a week for 24 months to exhaust emissions generated by a Renault R18 1.6-L gasoline engine run on unleaded fuel and fitted or not fitted with a three-way catalytic converter. The exhausts were diluted with a constant volume of 800 m^3 of air for the high dose, and further dilution of this mixture 1:3 with air produced the low dose; the levels of nitrogen oxide, nitrogen oxides and carbon monoxide were 15 and 44 ppm, 15 and 49 ppm and 67 and 224 ppm for the low and high doses, respectively. All survivors were maintained in clean air for up to an additional 6 months. No increase in the incidence of lung tumours was observed (Brightwell *et al.*, 1989).

(b) Intrapulmonary implantation

Groups of 34–35 female Osborne-Mendel rats, aged 3 months, received a single implantation into the left lobe of the lung of 0 (control), 5.0 and 10.0 mg in beeswax:trioctanoin (1:1) of a condensate from the exhaust emissions generated by a 1.5-L gasoline car engine operated on the European test cycle. The condensate was also separated into several fractions that were also implanted in beeswax:trioctanoin (1:1): 4.36, 8.73 or 17.45 mg/animal of a PAH-free fraction; 0.50, 0.99 or 1.98 mg/animal of a fraction containing PAHs with two to three rings; or 0.14, 0.28 or 0.56 mg/animal of a fraction of PAHs containing more than three rings. Animals were observed for life. Mean survival times for treated and controls ranged from 80 to 111 weeks. Only the fraction containing PAHs with more than three rings produced an increase in the incidences of lung tumours (carcinoma: 1 out of 35, 3%; 15 out of 34, 44% [$P = 0.0002$]; and 24 out of 35, 69% [$P < 0.0001$]; sarcoma: 1 out of 35, 3%; 2 out of 34, 6%; and 0 out of 35) comparable with that induced by total exhaust condensate (carcinoma: 3 out of 35, 9%; and 20 out of 35, 57% [$P < 0.0001$]; sarcoma: 4 out of 35, 11%; and 0 out of 35). No lung tumours were observed in the controls. The authors reported a dose–response relationship between the incidence of lung tumours and treatment with the total condensate and with the fraction of PAHs containing more than three rings (Grimmer *et al.*, 1984).

(c) Administration with known carcinogens

Groups of female Sprague-Dawley rats [numbers and age unspecified] were administered 0.01% DiPN in the drinking-water or were exposed by inhalation for 2 hours a day, 3 days a week to the exhaust emission generated by a small gasoline engine diluted 1:250 with clean air to give 0.1 mg/m^3 of engine exhaust particulates

(300 ± 50 ppm carbon monoxide, 0.21 ppm nitrogen oxide and 0.08 ppm nitrogen dioxide), or underwent both treatments concurrently for 6–12 months. In the animals killed between 7 and 12 months after the start of the experiment, the number of lung tumours (11 out of 37, 30%) in the combined treatment group (one adenoma and 10 malignant tumours including undifferentiated carcinomas, squamous cell carcinomas, adenocarcinomas and mixed tumours) was significantly greater than that in the group treated with DiPN alone (2 out of 24, 8% [$P < 0.05$]; two carcinomas). No lung tumours were observed in 23 animals exposed to gasoline exhaust alone (Yoshimura, 1983). [The Working Group noted that the study was limited by the lack of clean air controls.]

Groups of 60 female Bor:WISW rats, aged 10–12 weeks, received 25 daily subcutaneous injections of 0.25 or 0.5 g/kg bw of *N*-nitrosodipentylamine (NDPA) followed by exposure for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. All survivors were observed for up to an additional 6 months. A decrease in the incidence of benign and malignant lung tumours combined was observed in animals exposed to gasoline exhaust and NDPA compared with those exposed to NDPA alone (Heinrich *et al.*, 1986c).

3.2.3 Hamster

See Table 3.7

(a) Inhalation

Groups of 80–83 female Syrian hamsters, aged 10–12-weeks, were exposed by inhalation for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3. Exposure to gasoline engine exhaust had no effect on survival; the median lifespan was 70 weeks in all groups, and only one animal out of 75 in the high-dose group and three animals out of 80 in the low-dose group developed lung tumours; no lung tumours were observed in 83 controls (Heinrich *et al.*, 1986c). [The Working Group noted that the highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of carbon monoxide.]

Groups of 104 male and 104 female Syrian hamsters, aged 6–8 weeks, were exposed to exhaust emissions generated by a Renault R18 1.6-L gasoline engine run on unleaded fuel and fitted or not fitted with a three-way catalytic converter. The exhausts were diluted with a constant volume of 800 m^3 of air for the high dose, and further dilution of this mixture 1:3 in air produced the low dose; levels of nitrogen oxide, nitrogen dioxide and carbon monoxide were 15 and 44 ppm, 15 and 49 ppm and 67 and 224 ppm for the low and high dose, respectively. Further groups of 208 male and 208 female hamsters served as untreated controls. In addition, groups of 52 male and 52 female exposed hamsters and 104 male and 104 female controls were pretreated with 4.5 mg/kg of NDEA 3 days before the start of inhalation exposure. The authors reported

Table 3.7 Studies of the carcinogenicity of gasoline engine exhaust in hamsters

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian (F) 24 mo Heinrich *et al.* (1986c)	Inhalation Clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L)(operated according to US-72 FTP driving cycle), diluted 1:61 or 1:27 with air (mean concentrations: 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; and 95.8 ± 16.5 and 47.9 ± 20.2 μg/m^3 particulates, respectively), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–83, aged 10–12 wk	Lung (all tumours): 0/83, 3/80 (4%), 1/75 (1%)	NS	The highest levels of gasoline engine exhaust that could be tested in this study were limited by the toxicity of CO
Syrian (M, F) 24 mo Brightwell *et al.* (1989)	Inhalation Exposure to exhaust emission generated from a Renault R18 1.6-L gasoline engine run on unleaded fuel fitted with or without a three-way catalytic converter, diluted with a constant volume of 800 m^3 (high dose), or further diluted 1:3 in air (low dose) (15 and 44 ppm NO, 15 and 49 ppm NO_2 and 67 and 224 ppm CO for low and high doses, respectively), 16 h/d, 5 d/wk; additional groups were pretreated with 4.5 mg/kg NDEA 3 days before start of exposure Control groups of 208 M and 208 F; treated groups of 104 M and 104 F; pretreated control groups of 104 M and 104 F; pretreated treated groups of 52 M and 52 F; aged 6–8 wk	NDEA-pretreated groups With catalytic converter Lung (all tumours): M–4/101 (4%), 5/51 (10%), 1/51 (2%) F–3/101 (3%), 0/51, 3/51 (6%) Trachea (all tumours): M–14/101 (14%), 9/50 (18%), 5/48 (10%) F–18/103 (17%), 4/50 (8%), 10/51 (20%) Without catalytic converter Lung (all tumours): M–4/101 (4%), 5/51 (10%), 1/51 (2%) F–3/101 (3%), 2/52 (4%), 3/49 (6%) Trachea (all tumours): M–14/101 (14%), 8/52 (15%), 10/51 (20%) F–18/103 (17%), 12/50 (24%), 9/48 (19%)	[NS]	Authors reported that there was no evidence of any increase in respiratory tract tumours or tracheal tumours in hamsters (NDEA-pretreated and non-pretreated [data not shown here]) exposed to gasoline engine exhaust with or without a catalyst

Table 3.7 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Syrian (M) Lifetime Mohr *et al.* (1976); Reznik-Schüller & Mohr (1977)	Intratracheal instillation 0 (control), 2.5 or 5.0 mg of an automobile exhaust condensate from emissions of a common German passenger car (operating according to the European test cycle), containing 0.34 µg/mg B[*a*]P, dissolved in 0.2 m Tris-saline and EDTA buffer/saline, once/2 wk Groups of 6, aged 12 wk	Lung (adenoma): 0/6, 6/6 (100%)*, 6/6 (100%)*	*[$P < 0.05$]	Study poorly described; small number of animals per group
Syrian golden (M) Lifetime Künstler (1983)	Intratracheal instillation 0 (control), 0.5, 1.0 or 2.1 mg condensate (containing 0.41 µg/mg B[*a*]P) from exhaust emission generated by a VW 1500 Otto gasoline engine; condensate also separated into several fractions including a methanol phase, a cyclohexane phase, a nitromethane phase, and a reconstituted condensate from these fractions, dissolved in 0.2 mL of Tris-saline and EDTA buffer/saline, once/2 wk Groups of 30, aged 16 wk	No lung tumours observed in any animals treated with the condensate, its fractions or the reconstituted condensate	NS	
Administration with known carcinogens				
Syrian golden (F) 24 mo Heinrich *et al.* (1986c)	Subcutaneous injection/intratracheal instillation/inhalation Single subcutaneous injection of 3 mg/kg bw NDEA or 20 intratracheal instillations of 0.25 mg B[*a*]P followed by exposure to clean air (control) or exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to US-72 FTP driving cycle), diluted 1:27 or 1:61 with air (mean concentrations: 350 ± 24 and 177.5 ± 12.5 ppm CO; 28 ± 3 and 13.7 ± 1.5 ppm NO; 1.9 ± 0.4 and 1.0 ± 0.2 ppm NO_2; 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3 particulates), 18–19 h/d, 5 d/wk for 24 mo Groups of 80–81, aged 10–12 wk	The rates of NDEA- or B[*a*]P-induced tumours not increased by exposure to either dilution of exhaust	NS	Tumour rates in NDEA- and B[*a*]P-treated animals exposed to the 1:27 dilution of exhaust were approximately 50% lower than those in treated animals exposed to the 1:61 dilution or clean air

B[*a*]P, benzo[*a*]pyrene; bw, body weight; CO, carbon monoxide; d, day; EDTA, ethylenediaminetetraacetic acid; F, female; FTP, Federal Test Procedures; h, hour; M, male; mo, month; NDEA, *N*-nitrosodiethylamine; NO, nitrogen oxide; NO_2, nitrogen dioxide; NR, not reported; NS, not significant; PAH, polycyclic aromatic hydrocarbon; VW, Volkswagen; wk, week

no evidence of any increase in the incidence of respiratory tract tumours or tracheal tumours [type unspecified] in hamsters exposed to gasoline or gasoline catalyst engine exhaust with or without pretreatment with NDEA (Brightwell *et al.*, 1989).

(b) Intratracheal instillation

Groups of six male Syrian hamsters, aged 12 weeks, received intratracheal instillations once every two weeks for life of 0 (control), 2.5, or 5.0 mg of an automobile exhaust condensate prepared from the emissions of a common German passenger car (operated according to the European test cycle; containing 0.34 µg/mg of benzo[*a*]pyrene) dissolved in 0.2 mL Tris-hydrochloric acid and ethylenediaminetetraacetic acid (EDTA) buffer/saline. Survival of the treated animals ranged from 30 to 60 weeks. Pulmonary adenomas were observed in all treated animals but not in controls (Mohr *et al.*, 1976; Reznik-Schüller & Mohr, 1977). [The Working Group noted that the study was poorly described and the number of animals per group was small.]

Groups of 30 male Syrian hamsters, aged 16 weeks, received intratracheal installations once every two weeks for life of 0.2 mL of Tris-hydrochloric acid and EDTA buffer/saline containing 0 (control), 0.5, 1.0 or 2.1 mg of a condensate (containing 0.41 µg/mg of benzo[*a*]pyrene) from the exhaust emissions generated by a VW 1500 Otto gasoline engine. The condensate was also separated into several fractions including a methanol phase, a cyclohexane phase and a nitromethane phase, and another condensate was reconstituted from these fractions. Survival ranged from 68 to 87 weeks. No lung tumours were observed in any animals treated with the condensate, its fractions or the reconstituted condensate (Künstler, 1983).

(c) Administration with known carcinogens

Groups of 80–81 female Syrian golden hamsters, aged 10–12 weeks, received either a single subcutaneous injection of 3 mg/kg bw of NDEA or 20 intratracheal instillations of 0.25 mg of benzo[*a*]pyrene followed by inhalation exposure for 18–19 hours a day, 5 days a week for 24 months to clean air (control) or to exhaust from a 1.6-L displacement gasoline engine run on leaded fuel (0.3–0.56 g/L) (operated according to the US-72 FTP driving cycle), diluted 1:27 or 1:61 with air. Mean concentrations of exhaust components measured in the inhalation chambers were, respectively: carbon monoxide, 350 ± 24 and 177.5 ± 12.5 ppm; nitrogen oxide, 28 ± 3 and 13.7 ± 1.5 ppm; nitrogen dioxide, 1.9 ± 0.4 and 1.0 ± 0.2 ppm; and particulates, 95.8 ± 16.5 and 47.9 ± 20.2 µg/m^3. Administration of NDEA or benzo[*a*]pyrene to hamsters exposed to clean air resulted in basic rates of benign respiratory tract tumours of 12.8 and 6.5% of animals, respectively. The basic tumour rate was not significantly increased by exposure to either dilution of the exhaust (Heinrich *et al.*, 1986c).

3.2.4 Dog

See Table 3.8

Inhalation

Groups of 12–20 female Beagle dogs, aged 4 months, were exposed by inhalation for 16 hours a day for 68 months to clean air (control), to exhaust emissions generated from leaded fuel in a six-cylinder 2.4-L gasoline engine (operated to simulate urban driving) and/or to specific air pollutants: non-ultraviolet (UV)-irradiated exhaust, UV-irradiated exhaust, 0.5 ppm of sulfur dioxide (SO_2) + 100 µg/m^3 of sulfuric acid (H_2SO_4) mist in control air, non-UV-irradiated exhaust + 0.5 ppm of SO_2 + 100 µg/m^3 of H_2SO_4, UV-irradiated exhaust + 0.5 ppm of SO_2 + 100 µg/m^3 of H_2SO_4, 0.2 ppm of nitrogen oxide + 0.5–1.0 ppm of nitrogen dioxide or 1.5–2.0

Table 3.8 Study of the carcinogenicity of gasoline engine exhaust in dogs

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) and/or multiplicity of tumours	Significance
Beagle (F) 104 mo Stara *et al.* (1980)	Inhalation Clean air (control), UV-irradiated or non-irradiated exhaust emissions from a 6-cylinder, 2.4-L gasoline engine run on leaded fuel (operated to simulate urban driving) (non-irradiated exhaust: 100 ppm CO and 24–30 ppm hydrocarbon expressed as methane; UV-irradiated exhaust: 0.5–1.0 ppm NO_2, 0.1 ppm NO and 0.2–0.4 ppm oxygen expressed as O_3, lead concentrations, 14–26 µg/m³), 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4 mist in control air, non-UV-irradiated exhaust + 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4, UV-irradiated exhaust + 0.5 ppm SO_2 + 100 µg/m³ H_2SO_4, 0.2 ppm NO + 0.5–1.0 ppm NO_2, or 1.5–2.0 ppm NO + 0.2 ppm NO_2, 16 h/d for 68 mo Control group of 20; treated groups of 12; aged 4 mo	No lung tumours observed in 41 surviving dogs exposed to engine exhaust or 17 surviving controls	NS

CO, carbon monoxide; d, day; F, female; h, hour; H_2SO_4, sulfuric acid; mo, month; NO, nitrogen oxide; NO_2, nitrogen dioxide; NS, not significant; SO_2, sulfur dioxide; UV, ultraviolet

ppm of nitrogen oxide + 0.2 ppm of nitrogen dioxide. The exhaust contained 100 ppm of carbon monoxide and 24–30 ppm of hydrocarbon expressed as methane. The UV-irradiated exhaust contained 0.5–1.0 ppm of nitrogen dioxide, 0.1 ppm of nitrogen oxide and 0.2–0.4 ppm of oxygen expressed as O_3. The concentration of lead measured in the different exposure atmospheres was 14–26 µg/m³. The dogs were then maintained with no further treatment for up to an additional 36 months. No lung tumours were observed at necropsy in 41 surviving dogs from any of the groups exposed to the engine exhaust or 17 surviving controls (Stara *et al.*, 1980).

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