3.   Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1   Carcinogenicity studies in animals

Diesel engine exhaust

During the past decade, there has been worldwide interest in developing an improved data base for evaluating the potential carcinogenic effects of exposure to diesel exhaust. One of the earliest initiatives in this area was undertaken by the US Environmental Protection Agency (Pepelko & Peirano, 1983). The Working Group took cognizance of these preliminary studies which involved exposure by inhalation of SENCAR or strain A mice to whole diesel exhaust or by intraperitoneal injections of extracts of diesel exhaust particles.

Only increases in the incidence of pulmonary adenomas were measured as the end-point. In some cases, animals were also administered known carcinogens. The Working Group noted that the exposure and observation times in these studies were generally short as compared with those in later studies that yielded positive results.

(a) Inhalation exposure

Mouse: Heinrich *et al.* (1986a) exposed two groups of 96 female NMRI mice, eight to ten weeks old, to filtered or unfiltered exhaust from a 1.6-l displacement diesel engine operated according to the US-72 (FTP; see p. 80) test cycle to simulate average urban driving, or to clean air, for 19 h per day on five days per week for life. The unfiltered and filtered exhausts were diluted 1:17 with air and contained 4.24 mg/m³ particles. Levels of 1.5 ± 0.3 ppm $(3 \pm 0.6$ (SD) mg/m³) nitrogen dioxide and 11.4 ± 2.1 ppm nitrogen oxides were found in whole exhaust and 1.2 ± 0.26 ppm $(2.4 \pm 0.5$ mg/m³) nitrogen dioxide and 9.9 ± 1.8 ppm nitrogen oxides in filtered exhaust. Exposure to total diesel exhaust and filtered diesel exhaust significantly increased the number of animals with lung tumours (adenomas and carcinomas) to 24/76 (32%) and 29/93 (31%), respectively, as compared to 11/84 (13%) in controls. When the incidences of adenomas and carcinomas were evaluated separately, significantly higher numbers of animals in both diesel exhaust-exposed groups had adenocarcinomas (13 (17%) and 18 (19%), respectively) than in controls (2.4%); no increase was seen in the numbers of animals with adenomas. [The Working Group noted that the incidence of lung tumours in historical controls in this laboratory could reach 32% (Heinrich *et al.*, 1986b).]

Groups of ICR and C57Bl/6N mice (total number of treated and untreated animals combined alive at three months, 315 and 297, respectively) [initial numbers and sex distribution unspecified] were exposed to the exhaust from a small diesel engine (269 cm³ displacement, run at idling speed) used as an electric generator; the exhaust was diluted 1:2 to 1:4 in air (Takemoto *et al.*, 1986). The mice were exposed within 24 h after birth for 4 h per day on four days per week (2–4 mg/m³ particles; size, 0.32 μm; 2–4 ppm, 4–8 mg/m³ nitrogen dioxide). Between months 13 and 28, lung tumours (adenomas and adenocarcinomas) were found in 14/56 exposed ICR mice and in 7/60 controls and in 17/150 treated C57Bl/6N mice and 1/51 controls. The authors reported that the differences were not statistically significant. [The Working Group calculated that the difference in C57Bl/6N mice was statistically significant at $p < 0.05$.]

Rat: Karagianes *et al.* (1981) exposed groups of male specific-pathogen-free Wistar rats [numbers unspecified], 18 weeks old, for 6 h per day for 20 months to one of five experimental atmospheres: clean air (controls); 8.3 ± 2.0 (SD) mg/m³ soot from diesel exhaust; 8.3 ± 2.0 mg/m³ soot from diesel exhaust plus 5.8 ± 3.5 mg/m³ coal dust; 6.6 ± 1.9 mg/m³ coal dust; or 14.9 ± 6.2 mg/m³ coal dust. The diesel exhaust was produced by a three-cylinder, 43-brake horse power diesel engine driving a 15 kW electric generator. The fuel injection system of the engine was modified to simulate operating patterns of such engines in mines and was operated on a variable duty cycle (dilution, approximately 35:1). Six rats per group were killed after four, eight, 16 or 20 months of exposure. Complete gross necropsy was performed, and respiratory tract tissues, oesophagus, stomach and other

tissues with 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 were observed — one in the group exposed to diesel exhaust only and one in the group exposed to diesel exhaust and coal dust. None was observed in controls or in the two groups exposed to coal dust only. [The Working Group noted the limited number of animals studied at 20 months.]

Groups of 72 male and 72 female or 144 male Fischer 344 weanling rats were exposed for 7 h per day on five days per week for 24 months to either clean air (controls); 2 mg/m³ coal dust (<7 μm); 2 mg/m³ diesel exhaust particles, with specific limits on gaseous/vapour constituents; or 1 mg/m³ coal dust plus 1 mg/m³ diesel exhaust particles (Lewis et al., 1986). The nitrogen dioxide concentration in the diesel exhaust was 1.5 ± 0.5 ppm (3 ± 1 mg/m³); the exhaust was generated by a 7-l displacement, four-cycle, water-cooled, 'naturally aspirated' (open-chamber) diesel engine. The exhaust was diluted by a factor of 27:1 before entering the exposure chambers. Following three, six, 12 and 24 months of exposure, at least ten male rats per group were removed for ancillary studies. After 24 months of exposure, all survivors were killed. The numbers of rats necropsied and examined histologically in each of the four groups were 120–121 males and 71–72 females. No difference in survival was noted among treatment groups, chambers or sexes [data on survival unavailable]. No statistical difference in tumour incidence was noted among the four groups. [The Working Group noted that no detailed information on tumour incidence was available and that the animals were killed at 24 months, a shorter observation period than used in other inhalation studies with rats that gave positive results.][1]

Female specific-pathogen-free Fischer 344 rats [initial number unspecified], aged five weeks, were exposed to diesel exhaust from a small diesel engine (269 cm³ displacement) run at idling speed; rats were treated for 4 h per day on four days per week for 24 months, at which time they were killed or were left untreated (Takemoto et al., 1986). The exhaust was diluted 1:2 to 1:4 with air. The concentration of particulates (size, 0.32 μm) ranged from 2–4 mg/m³, and those of nitrogen dioxide were 2–4 ppm (3–8 mg/m³). No lung tumour was observed in either the 26 treated or 20 control rats; 15 and 12 rats in the two groups, respectively, survived 18–24 months. [The Working Group noted the small group sizes.]

Iwai et al. (1986) exposed two groups of 24 female specific-pathogen-free Fischer 344 rats, seven weeks of age, to either diluted diesel exhaust or diluted filtered diesel exhaust for 8 h per day on seven days a week for 24 months, at which time some rats were sacrificed and the remainder were returned to clean air for a further six months of observation. The diesel exhaust was produced by a 2.4-l displacement small truck engine; it was diluted ten times with clean air and contained 4.9 ± 1.6 mg/m³ particles, 1.8 ± 1.8 ppm (3.6 ± 3.6 mg/m³) nitrogen dioxide and 30.9 ± 10.9 ppm nitrogen oxides. Another group of 24 rats was exposed to fresh air only for 30 months. Incidences of lung tumours, diagnosed as adenomas, adenocarcinomas, squamous-cell carcinomas and adenosquamous carcinomas, were significantly higher in the group exposed to whole diesel exhaust, with or without a subsequent observation period (in 8/19 rats, including five with malignant tumours) than in

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[1]Subsequent to the meeting, a more detailed report of the study was published (Lewis et al., 1989).

the control group (one adenoma in 1/22 rats; $p < 0.01$). No lung tumour was observed in the group exposed to filtered exhaust (0/16 rats). Incidences of malignant lymphomas and tumours at other sites did not differ among the three groups. [The Working Group noted the small group sizes.]

Ishinishi *et al.* (1986a) exposed groups of 64 male and 59 female specific-pathogen-free Fischer 344 rats, four weeks of age, to diesel exhaust from either a light-duty 1.8-l displacement, four-cylinder engine (particle concentrations, 0.11, 0.41, 1.08 or 2.32 mg/m^3; nitrogen dioxide concentrations, 0.08, 0.26, 0.70 or 1.41 ppm (0.2, 0.5, 1.4 or 2.8 mg/m^3); nitrogen oxide concentrations, 1.24, 4.06, 10.14 or 20.34 ppm) or a heavy-duty 11-l displacement, six-cylinder engine (particle concentrations, 0.46, 0.96, 1.84 or 3.72 mg/m^3; nitrogen dioxide concentrations, 0.46, 1.02, 1.68 or 3.00 ppm (0.9, 2.1, 3.4 or 6 mg/m^3); nitrogen oxide concentrations, 6.17, 13.13, 21.67 or 37.45 ppm). Exposure was for 16 h per day on six days per week for up to 30 months. The diesel emissions were diluted about 10–15 times (v/v) with air. Separate control groups for the light-duty and heavy-duty series were exposed to clean air. The incidence of lung tumours diagnosed as adenocarcinomas, squamous-cell carcinomas or adenosquamous carcinomas was significantly increased only in the highest-dose group (in 5/64 males and 3/60 females) of the heavy-duty diesel exhaust-exposed series compared to controls (in 0/64 males and 1/59 females; $p < 0.05$). The incidences in the next highest-dose group in this series were 3/64 males and 1/59 females. [The Working Group noted that, although this incidence was not statistically different from that in the controls, it suggested an overall positive response for the two highest exposure levels.] No statistically significant increase in the incidence of lung tumours was noted in the groups exposed to light-duty diesel engine exhaust. [The Working Group noted that the highest level of exposure in the light-duty series was approximately one-half of the highest concentration used in the heavy-duty series, and that the incidence (3.3%) of lung tumours in the control animals of the light-duty diesel engine exhaust-exposed series was higher than that in the heavy-duty diesel controls (0.8%).]

Groups of 72 male and 72 female Fischer 344 rats, six to eight weeks old, were exposed to one of three concentrations of diesel engine exhaust or particle-filtered diesel engine exhaust from a 1.5-l displacement engine operated according to the US-72 (FTP) driving cycle which simulates average urban driving; exposure was for 16 h per day on five days per week for two years (Brightwell *et al.*, 1986). 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 dilution of this mixture in air of 1:3 (medium dose) and a dilution of 1:9 (low dose). The particle concentrations in the unfiltered diesel exhaust atmosphere were 0.7, 2.2 and 6.6 mg/m^3 for the low, medium and high doses (with 8 ± 2 ppm nitrogen oxides in the high dose), respectively. Two control groups of 144 rats of each sex were exposed to conditioned air. Following the exposure period, the animals were maintained for a further six months in clean air. An exposure concentration-related increase in the incidence of primary lung tumours [detailed histopathology unspecified] was reported only in groups exposed to unfiltered diesel exhaust. [The Working Group noted that no information of tumour incidence was given for rats exposed to filtered diesel exhaust.][1]

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[1]Subsequent to the meeting, a more detailed report of the study was published (Brightwell *et al.*, 1989; see also pp. 93, 98, 99, 104).

Heinrich *et al.* (1986a) exposed two groups of 96 female Wistar rats, eight to ten weeks old, to filtered or unfiltered exhaust, as described on p. 89. A significantly increased incidence of lung tumours (histologically identified as eight bronchioalveolar adenomas and nine squamous-cell tumours) was observed in rats exposed to unfiltered diesel exhaust (17/95 (18%) *versus* 0/96 controls). No lung tumour was reported in rats exposed to filtered exhaust.

Mauderly *et al.* (1986, 1987) exposed groups of 221–230 male and female specific-pathogen-free Fischer 344 rats, 17 weeks old, 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; exposure was for 7 h per day on five days per week for up to 30 months. The exposure concentrations were reported as a dilution of the whole exhaust to measured soot concentrations of 0.35 (low), 3.5 (medium) or 7.0 (high dose) mg/m³. Levels of nitrogen dioxide were 0.1 ± 0.1 (0.2 ± 0.2), 0.3 ± 0.2 (0.6 ± 0.4) and 0.7 ± 0.5 ppm (1.4 ± 1 mg/m³), respectively. Sham-exposed controls received filtered air. The soot particles were approximately 0.25 μm mass median diameter, and approximately 12% of their mass was composed of solvent-extractable organics. Subgroups of animals were removed at six, 12, 18 and 24 months for ancillary studies; all rats surviving after 30 months of exposure were killed. All rats that died or were killed were necropsied and examined histologically for lung tumours. Exposures did not significantly affect the survival of animals of either sex. The median survival time ranged from 880 (low) to 897 (medium) days of age for males and from 923 (high) to 962 (low) days for females. A total of 901 rats were examined for lung tumours; four types were found: bronchoalveolar adenomas, adenocarcinomas, squamous cysts (mostly benign) and squamous-cell carcinomas. None of the tumours was found to have metastasized to other organs. The incidences of lung tumours in males and females combined were 0.9% in controls, 1.3% in low-dose, 3.6% in medium-dose and 12.8% in high-dose groups. The authors noted that the prevalences at the medium and high levels were significantly increased ($p < 0.05$). A total of 42 rats developed 46 lung tumours; four females in the high-dose group had two lung tumours each. Lung tumours were found in two male controls, in one low-dose male, in four mid-dose males and in 13 high-dose males; in females, the respective incidences were zero, two, four and 20. Adenomas predominated in the medium-exposure group. Adenocarcinomas, squamous-cell carcinomas and squamous cysts were observed predominantly at the high dose. The tumours were observed late in the study: 81% after two years of exposure. The authors observed no exposure-related difference in cause of death; the tumours were found incidentally at death or at termination of the experiment.

Hamster: Groups of 48 female Syrian golden hamsters, eight weeks of age, were exposed to diluted (1:7 air) unfiltered diesel exhaust (mass median particle diameter, 0.1 μm; mean particle concentration, 3.9 ± 0.5 mg/m³; nitrogen dioxide, 1.2 ± 1.7 ppm (2.4 ± 3.4 mg/m³); nitrogen oxides, 18.6 ± 5.8 ppm) or filtered diesel exhaust (nitrogen dioxide, 1.0 ± 1.5 ppm (2 ± 3 mg/m³); nitrogen oxides, 19.2 ± 6.1 ppm); exposure was for 7–8 h per day on five days per week for life (Heinrich *et al.*, 1982). The exhaust was generated by a 2.4-l displacement engine operating at a steady state. A group of 48 hamsters inhaling clean air

served as controls. There was no effect of diesel exhaust on survival; median lifespan was 72–74 weeks in all groups, and no lung tumour was reported in treated or control animals.

Groups of 48 female and 48 male Syrian golden hamsters, eight to ten weeks of age, were exposed to diluted (1:17 air) filtered or unfiltered exhaust as described on p. 89 (Heinrich *et al.*, 1986a). A control group of 48 females and 48 males inhaled clean air. Median lifespan was not significantly influenced by diesel exposure and was 75–80 weeks for females and 80–90 weeks for males. No lung tumour was observed in treated or control animals.

Groups of 52 female and 52 male Syrian hamsters, six to eight weeks of age, were exposed to one of three concentrations of unfiltered or filtered exhaust as described on p. 91 (Brightwell *et al.*, 1986). Two control groups of 104 hamsters of each sex were exposed to clean air only. The authors reported that there was no increase in the incidence of respiratory-tract tumours in treated hamsters. [The Working Group noted the incomplete reporting of tumour incidence and survival.]

Monkey: Groups of 15 male cynomolgus monkeys (*Macaca fascicularis*) were exposed by Lewis *et al.* (1986) to coal dust and/or diesel exhaust particles for 7 h per day on five days per week for 24 months, as described on p. 90. Following the exposure period, all survivors (59/60) were necropsied and examined histologically. No significant difference in tumour incidence was reported among the four groups. [The Working Group noted the short duration and inadequate reporting of the study.]

(*b*) *Intratracheal or intrapulmonary administration*

Rat: Four groups of 31, 59, 27 and 53 female specific-pathogen-free Fischer 344 rats, six weeks of age, received ten weekly intrapulmonary instillations of 1 mg/animal activated carbon or 1 mg/animal diesel exhaust particles [source unspecified] in phosphate buffer with 0.05% Tween 80, 2 ml of buffer alone or were untreated (Kawabata *et al.*, 1986). Rats surviving 18 months constituted the effective numbers. The experiment was terminated 30 months after instillation. The survival rate was 71–83%, with the lowest value in the diesel particle-treated group. The numbers of animals with malignant lung tumours [histological type unspecified] were significantly higher ($p < 0.01$) in the groups treated with activated carbon (7/23) and with diesel particles (20/42) than in untreated (0/44) or vehicle controls (1/23). Similarly, the numbers of animals with benign and malignant lung tumours were also significantly increased in the groups treated with activated carbon (11/23) and diesel particles (31/42). [The Working Group noted the high incidence of pulmonary tumours observed after treatment with activated carbon, a material which is normally considered to be inert.]

Groups of 35 female inbred Osborne-Mendel rats, three months old, received lung implants of organic material from a diesel exhaust or a reconstituted hydrophobic fraction (Grimmer *et al.*, 1987). The organic material was collected from a 3-l diesel passenger car engine, operated under the first cycle of the European test cycle (see p. 80), 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 hydrophobic fraction was separated by column chromatography into several further

fractions: (i) nonaromatic compounds plus PAHs with two and three rings (72% by weight of the total condensate), (ii) PAHs with four or more rings (0.8% by weight), (iii) polar PAHs (1.1% by weight) and (iv) nitro-PAHs (0.7% by weight). Animals received 6.7 mg hydrophilic fraction, 20 mg hydrophobic fraction, 19.2, 0.2, 0.3 or 0.2 mg of the four hydrophobic fractions, respectively, or 19.9 mg of reconstituted hydrophobic fraction. Two groups of 35 animals were untreated or received implants of the vehicle (beeswax:tri-octanoin, 1:1) only. All animals were observed until spontaneous death (mean survival time, 24–140 weeks). Six lung tumours (squamous-cell carcinomas) 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 subfractions (seven carcinomas) and with the hydrophobic fraction (five carcinomas). A low carcinogenic potential was observed with the subfraction of nitro-PAHs (one carcinoma); the polar PAH produced no tumour; and one bronchiolar-alveolar adenoma was observed in animals treated with the nonaromatic subfraction with two- and three-ring PAHs. One adenoma of the lung occurred in the vehicle control group.

Hamster: Shefner *et al.* (1982) gave groups of 50 male Syrian golden hamsters, 12–13 weeks of age, intratracheal instillations once a week for 15 weeks of 1.25, 2.5 or 5 mg diesel particles (obtained from US Environmental Protection Agency; 90% by mass <10 μm) or diesel particles plus the same amounts of ferric oxide in 0.2 ml propylene glycol/gelatine/-saline; or a dichloromethane extract of diesel particles plus ferric oxide in 0.2 ml propylene glycol/saline once a week for 15 weeks. Ten animals in each group were sacrificed at 12 months. At the time of reporting (61 weeks), one lung adenoma had been found in the group receiving the high dose of diesel particles and one in the group receiving the high dose of diesel particle extract plus ferric oxide. No lung tumour was reported in various untreated or solvent-treated controls. [The Working Group noted the short observation period and the preliminary reporting of the experiment.]

Three groups of 62 male Syrian golden hamsters, eight weeks of age, were given intratracheal instillations of 0.1 ml of a suspension of 0.1, 0.5 or 1 mg of an exhaust extract in Tween 60:ethanol:phosphate buffer (1.5:2.5:30 v/v) from a heavy-duty diesel engine (V6 11-l) once a week for 15 weeks and observed for life (Kunitake *et al.*, 1986). A control group of 59 animals received instillations of the vehicle only and a positive control group of 62 animals received 0.5 mg benzo[*a*]pyrene weekly for 15 weeks. Survival rates were 95%, 92%, 71% and 98% in the three treated groups and the vehicle controls, respectively. No significant difference in the incidences of tumours of the lung, trachea or larynx was observed between untreated control and treated groups; respiratory tumours occurred in 88% benzo[*a*]pyrene-treated hamsters. [The Working Group noted that length of survival was not reported.]

(c) Skin application

Mouse: A group of 12 male and 40 female C57Bl mice [age unspecified] received skin applications of 0.5 ml of an acetone extract of particles collected from a diesel engine [unspecified] running at zero load during the warm-up phase; treatment was given three times a week for life (Kotin *et al.*, 1955). Groups of 50 male and 25 female strain A mice [age

unspecified] received similar applications of an extract of particles derived from the warmed-up engine running at full load. Of the mice in the first group, 16 had died by ten weeks; 33 mice survived to the appearance of the first skin tumour (13 months), and two skin papillomas developed. Of the male strain A mice, eight survived to the appearance of the first skin tumour (16 months), and one papilloma and three squamous-cell carcinomas were observed. Of the female strain A mice, 20 survived to the appearance of the first skin tumour (13 months), and 17 skin tumours [unspecified] were observed between 13 and 17 months. Both experiments were terminated after 22–23 months. No skin tumour occurred in 69 C57Bl controls (37 alive after 13 months) or in 34 (24 female and 10 male) strain A controls.

In a study reported before completion (Depass et al., 1982), groups of 40 male C3H / HeJ mice [age unspecified] received skin applications of 0.25 ml of a 5 or 10% solution in acetone or 5, 10, 25 or 50% dichloromethane extracts of diesel particles collected from a [5.7-l] diesel engine; treatment was given three times per week for life. A positive control group received 0.2% benzo[a]pyrene in acetone, and a negative control group received acetone only. One squamous-cell carcinoma of the skin was observed in the group treated with the highest dose of dichloromethane extract after 714 days of treatment. All 38 mice receiving benzo[a]-pyrene developed skin tumours. [The Working Group noted the inadequate reporting of the study.]

In a series of promotion-initiation studies (Depass et al., 1982), groups of 40 male C3H / HeJ mice received a single initiating dose of 0.025 ml 1.5% benzo[a]pyrene in acetone, followed one week later by repeated applications of the 10% solution of diesel particles in acetone described above, 50% dichloromethane extract, 25% dichloromethane extract, acetone only or 0.015 µg phorbol 12-myristyl 13-acetate (TPA) five times per week for life. An additional group received no further treatment after the initiating dose of benzo[a]-pyrene. In initiation studies, a single initiating dose of 0.025 ml of the 10% solution of diesel particles in acetone, 50% dichloromethane extract, acetone or TPA was followed after one week by of 0.015 µg TPA three times per week. The concentration of TPA used in the initiation and promotion studies was changed after eight months to 1.5 µg. In the promotion study, one mouse receiving the 50% dichloromethane extract had a squamous-cell carcinoma and two mice receiving the 25% extract had one squamous-cell carcinoma and one papilloma. In the initiation study, three (two papillomas, one carcinoma), three (two papillomas, one fibrosarcoma), one (papilloma) and two (one carcinoma, one papilloma) tumours were observed in the groups that received diesel particles, dichloromethane extract, acetone and TPA, respectively. [The Working Group noted the preliminary reporting of the study.]

Nesnow et al. (1982a,b) gave skin applications to groups of 40 male and 40 female SENCAR mice, seven to nine weeks of age, of 0.1, 0.5, 1.0, 2 or 10 mg of dichloromethane extracts of particles obtained from the exhausts of five diesel engines, A, B, C, D and E (E being a heavy duty engine) in 0.2 ml acetone; the 10-mg dose was given in five daily doses. The benzo[a]pyrene content ranged from 1173 ng/mg in the exhaust from engine A to 2 ng/mg in that from engines B and E. One week later, all mice received 2 µg TPA in 0.2 ml acetone twice a week for 24–26 weeks. A control group was treated with TPA only. The sample from engine A produced a dose-related increase in the incidence of skin papillomas,

with 5.5 and 5.7 papillomas/mouse, 31% of males and 36% of females at the highest dose having skin carcinomas. With samples from engines B, C and D, responses of 0.1—0.5 papilloma/mouse were observed compared to 0.05—0.08 papilloma/mouse in TPA controls. The sample from engine E produced a response similar to that in controls (0.05—0.2 papilloma/mouse).

Similar groups of 40 male and 40 female SENCAR mice received weekly skin applications of 0.1, 0.5, 1, 2 or 4 mg extracts of particles from the emissions of engines A, B and E for 50—52 weeks (Nesnow et al., 1982b, 1983). The high dose was given in two split doses. At that time, skin carcinomas had occurred in 3% of male and 5% of female mice given the 4-mg dose of the sample from engine A, in 3% of males given the 0.5-mg dose of the sample from engine B and in 3% of females given the 0.1-mg dose of the sample engine E. Doses of 12.6—202 μg per week benzo[a]pyrene produced skin carcinoma responses of 10—93%.

Groups of 50 female specific-pathogen-free ICR mice, aged eight to nine weeks, received skin applications of extracts of diesel particles collected from a V6 11-1 heavy-duty displacement diesel engine in 0.1 ml acetone onto shaved back skin every other day for 20 days (total doses, 5, 15 or 45 mg/animal; Kunitake et al., 1986). A further group of 50 mice treated with acetone only served as controls. Beginning one week after the last diesel extract treatment, each animal received applications of 2.5 μg TPA in 0.1 ml acetone three times a week for 25 weeks, at which time they were autopsied. No skin 'cancer' was found in either treated or control groups; skin papillomas were seen in 1/48 and 4/50 surviving animals in the 15- and 45-mg dose groups, respectively [The Working Group noted the short duration of both the treatment and observation time.]

(d) Subcutaneous administration

Mouse: Groups of 15—30 female specific-pathogen-free C57Bl/6N mice, six weeks of age, received subcutaneous injections into the intrascapular region of suspensions in olive oil containing 5% dimethyl sulfoxide of 10, 25, 50, 100, 200 or 500 mg/kg bw of diesel particles collected from a V6 11-1 heavy-duty displacement diesel engine; the treatment was given once a week for five weeks (Kunitake et al., 1986). A control group of 38 mice received injections of the vehicle only. Animals were killed 18 months after the beginning of the experiment. The first tumours were palpated in week 47 (a total dose of 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, respectively. A significant increase in the incidence of subcutaneous tumours, diagnosed as malignant fibrous histiocytomas, was observed only in 5/22 mice receiving the 500-mg/kg bw dose ($p < 0.05$) in comparison with controls (0/38). [The Working Group noted the high dose required to produce a carcinogenic effect.]

(e) Administration with known carcinogens

Rat: Two groups of female specific-pathogen-free Fischer 344 rats [initial number unspecified], five weeks of age, were exposed to diesel exhaust, as described on p. 89 or to clean air for 4 h per day on four days per week for 24 months (Takemoto et al., 1986). One month after the beginning of treatment, both groups received three weekly intraperitoneal

injections of 1 g/kg bw *N*-nitrosodipropanolamine. Rats were killed at six, 12, 18 and 24 months after the start of treatment. A slight but nonsignificant increase in the incidences of lung adenomas and adenocarcinomas was observed in rats exposed to both exhaust and the nitrosamine compared to those exposed to the nitrosamine alone. After 12–24 months of observation, 16 lung tumours (12 adenomas and four carcinomas) were observed in 29 *N*-nitrosodipropanolamine-treated rats and 34 tumours (24 adenomas and 10 carcinomas) were observed in 36 rats exposed to both exhaust and *N*-nitrosodipropanolamine. The authors interpreted this result as an 'overadditive' effect on lung tumour incidence.

Heinrich *et al.* (1986a) gave groups of 48 female specific-pathogen-free Wistar rats, eight to ten weeks of age, 25 weekly subcutaneous injections of 250 or 500 mg/kg bw *N*-nitrosopentylamine during the first 25 weeks of exposure by inhalation to unfiltered diesel engine exhaust, to filtered diesel engine exhaust or to clean air, as described on p. 89. Significant increases in the incidences of squamous-cell carcinomas of the lung were observed in animals treated with the nitrosamine and exposed to total exhaust (22/47 low-dose nitrosamine; 15/48 high-dose nitrosamine compared to 2/46 and 8/48 clean air controls, respectively), although overall lung tumour rates were comparable in the groups exposed to the nitrosamine and to engine exhaust or clean air. The incidence of benign tumours (papillomas) of the upper respiratory tract was significantly reduced in nitrosamine-treated rats exposed to unfiltered or filtered diesel exhaust compared to controls exposed to nitrosamine and clean air.

Hamster: Heinrich *et al.* (1982) gave groups of 48–72 female Syrian golden hamsters, eight weeks old, weekly intratracheal instillations of 0.1 or 0.3 mg dibenzo[*a,h*]anthracene for 20 weeks or a single subcutaneous injection of 1.5 or 4.5 mg/kg bw *N*-nitrosodiethyl-amine (NDEA) and exposed them concomitantly by inhalation to unfiltered or filtered diesel exhaust or clean air, as described on p. 92. The incidence of tumours in the larynx/trachea was increased in animals treated with the higher dose of NDEA and exposed concomitantly to total exhaust (70.2%) or filtered exhaust (66%) as compared to controls (44.7%). The lower dose of NDEA and treatment with dibenzo[*a,h*]anthracene resulted in a lower incidence of these tumours. Only two lung tumours were found: one with the high dose of dibenzo[*a,h*]anthracene and filtered exhaust, the other with the low dose of NDEA and total exhaust.

Groups of 48 male and 48 female Syrian golden hamsters, eight to ten weeks of age, received a single subcutaneous injection of 4.5 mg/kg bw NDEA or 20 intratracheal instillations of 0.25 mg benzo[*a*]pyrene with concomitant exposure by inhalation to filtered or unfiltered diesel engine exhaust or to clean air, as described on p. 89 (Heinrich *et al.*, 1986a). Treatment with NDEA or benzo[*a*]pyrene produced respiratory tract tumour incidences of 10% or 2%, respectively, in animals exposed to clean air; rates were not significantly increased by concomitant exposure to filtered or unfiltered diesel engine exhaust.

Groups of 52 female and 52 male Syrian hamsters, six to eight weeks old, received a single subcutaneous injection of 4.5 mg/kg bw NDEA three days prior to exposure by inhalation to unfiltered or filtered diesel engine exhaust, as described on p. 91 (Brightwell *et al.*, 1986). The authors reported a nonsignificantly increased incidence of tracheal

papillomas. [The Working Group noted that no information on tumour incidence was given.]

Gasoline engine exhaust

(a) Inhalation exposure

Mouse: Campbell (1936) exposed two groups of 37 male and 38 female mice [strain unspecified], three months old, by inhalation for 7 h per day on five days per week for about two years to one of two gasoline engine exhaust emissions: A was from a four-cylinder, 23-horse power, ordinary gasoline engine and B from a six-cylinder, 24-horse power engine run on gasoline with tetraethyllead 1:1800. Exposure was to a dilution of 1:145 in air for 4 h in the morning and to a dilution of 1:83 for 3 h in the afternoon. [The total particulate content of the exhaust and the lead concentration were not specified.] Of the animals exposed to exhaust emissions from car A, 9/75 had primary lung tumours compared to 8/74 controls; of those exposed to emissions from car B, primary lung tumours were seen in 12/75 animals compared to 6/70 controls. [Survival data not given.] Other types of tumours observed included mammary tumours and skin cancers among both treated groups and controls. [The Working Group noted the inadequate reporting of the study.]

Two groups of female ICR mice [initial numbers and age unspecified] were either exposed by inhalation to 0.1 mg/m³ gasoline exhaust (1:250 dilution of emission from a small gasoline engine; carbon monoxide, 300 ± 50 ppm (350 ± 60 mg/m³); nitric oxide, 0.21 ppm (0.3 mg/m³); nitrogen dioxide, 0.08 ppm (0.16 mg/m³) [total particulate concentration unspecified]) for 2 h per day on three days per week for six to 12 months, or were administered urethane (0.01%) in the drinking-water until sacrifice (Yoshimura, 1983). No untreated control group was included. Lung adenomas were found in 2/19 exposed mice killed between seven and 12 months; the incidence of tumours (adenomas and adenocarcinomas) in the urethane-treated group was 21/25. [The Working Group noted the short period of treatment, the short observation time and the absence of a control group.]

Rat: Groups of 72 male and 72 female Fischer 344 rats, six to eight weeks old, were exposed to one of three dilutions of gasoline engine exhaust from a 1.6-l displacement engine operated according to the US-72 (FTP) driving cycle; exposure was for 16 h per day on five days per week for two years (Brightwell *et al.*, 1986). Further groups were exposed to exhaust from a gasoline engine fitted with a three-way catalytic converter. The exhaust was diluted by a constant volume of 800 m³ air or at further dilutions of 1:3 or 1:9 of this mixture in air; the particulate concentration was less than the detection limit of 0.2 mg/m³. The concentration of nitrogen oxides in the high dose of exhaust from the engine without a converter was 49 ± 5 ppm and that of carbon monoxide was 224 ± 32 ppm (260 ± 36 mg/m³). Two control groups of 144 rats of each sex were exposed to conditioned air. After the exposure period, animals were maintained for a further six months in clean air. No increase in lung tumour incidence was reported among rats exposed to gasoline engine exhaust as compared with controls. [The Working Group noted the inadequate reporting of the study.]

Three groups of 80—83 female Bor:WISW rats, ten to 12 weeks old, were exposed by inhalation to 1:61 or 1:27 dilutions with clean air of leaded gasoline engine exhaust generated by a 1.6-l engine operated according to the US-72 (FTP) driving cycle or to clean air (Heinrich et al., 1986c). The lead content of the fuel was 0.3—0.56 g/l. Mean concentrations of exhaust components measured in the inhalation chambers were (high [low]): carbon monoxide, 350 ± 24 [177.5 ± 12.5] mg/m³; nitric oxide, 28 ± 3 [13.7 ± 1.5] mg/m³; nitrogen dioxide, 1.9 ± 0.4 [1.0 ± 0.2] mg/m³; particles, 95.8 ± 16.5 [47.9 ± 20.2] 25g/m³. About 35% of the particulate mass was lead. Exposure was for 18—19 h per day on five days per week for two years, followed by a maximal observation period of six months in clean air. Mean survival time of exposed and control animals was 105 weeks. Exposure to either concentration (1:61 or 1:27) of gasoline exhaust did not produce a significant increase in lung tumour incidence: 1/83 exposed to 1:61 had a squamous-cell carcinoma and 3/78 exposed to 1:27 had two squamous-cell carcinomas and one adenoma; 1/78 controls had an adenoma. In addition, one animal in each of the three exposure groups showed a tumour in the nasal cavities. [The Working Group noted that the nonlead particulate concentration was less than 1/20 the lowest level of particulates that produced an excess of lung tumours in the studies of diesel exhaust. The highest levels of gasoline engine exhaust that can be tested are limited by the toxicity of carbon monoxide.]

Hamster: Three groups of 80—83 female Syrian golden hamsters, ten to 12 weeks old, were exposed to gasoline engine exhaust, as described above but without the six-month observation period (Heinrich et al., 1986c). Median survival in treated and control groups was 70 weeks. One of 75 animals exposed to the high concentration of exhaust (1:27) and three of 80 exposed to the low concentration (1:61) had a tumour of the respiratory tract. No respiratory tract tumour occurred in the 83 controls. [The Working Group noted that the nonlead particulate concentration was less than 1/20 the lowest level of particulates that produced an excess of lung tumours in the studies of diesel exhaust. The highest levels of gasoline engine exhaust that can be tested are limited by the toxicity of carbon monoxide.]

Brightwell *et al.* (1986) exposed groups of 52 male and 52 female Syrian hamsters, six to eight weeks of age, to gasoline engine exhaust, as described on p. 98. Two control groups of 104 hamsters of each sex were exposed to conditioned air only. The authors reported that respiratory tract tumours in treated hamsters were rare and not related to treatment. [The Working Group noted the inadequate reporting of the data.]

Dog: Stara *et al.* (1980) exposed seven groups of 12 female beagle dogs, four months of age, to exhaust from a six-cylinder, 2.4-l gasoline engine run on leaded fuel and operated to simulate urban driving, and to specific pollutants found in gasoline engine exhaust (dilution, 1:570 in air). The groups were exposed to nonirradiated exhaust, to exhaust irradiated with ultra-violet, to sulfur dioxide and sulfuric acid, to nonirradiated exhaust plus sulfur dioxide and sulfuric acid, to exhaust irradiated with ultra-violet plus sulfur dioxide and sulfuric acid, to nitrogen oxides with high nitrogen dioxide and to nitrogen oxides with high nitric oxide. A group of 20 dogs was exposed to clean air. The exhaust contained 100 ppm (115 mg/m³) carbon monoxide and 24—30 ppm hydrocarbon expressed as methane. The irradiated exhaust contained 0.5—1.0 ppm (1—2 mg/m³) nitrogen dioxide, 0.1 ppm (0.12 mg/m³) nitric oxide and 0.2—0.4 ppm oxygen expressed as O_3. The

concentration of lead measured in the different exposure atmospheres was 14–26 $\mu g/m^3$. The dogs were exposed for 16 h per day for 68 months and then held in clean air for 29–36 months. Complete necropsies were performed on 85 dogs. No lung tumour was observed in the 40 exposed or 17 control dogs. [The Working Group noted that the concentrations of particles in the exposure atmospheres were not given.]

(b) Intratracheal or intrapulmonary administration

Rat: Groups of 34–35 inbred female Osborne-Mendel rats, three months old, received a single implantation of 5.0 or 10.0 mg/animal of gasoline engine exhaust condensate, 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 of PAHs with two to three rings, 0.14, 0.28 or 0.56 mg/animal of a fraction of PAHs with more than three rings, or 0.03, 0.10 or 0.30 mg benzo[a]pyrene in beeswax:trioctanoin (1:1) into the left lobe of the lung and were observed until natural death (Grimmer *et al.*, 1984). The exhaust was produced by a 1.5-l passenger car engine operated on the European test cycle. One control group of 34 rats received an injection of the vehicle only, and another control group of 35 animals remained untreated. At death, animals were autopsied and lungs were examined histopathologically. Mean survival times in the treated groups and controls were similar, ranging from 80–111 weeks. Only the fraction containing PAHs with more than three rings produced lung tumour (carcinomas and sarcomas) incidences comparable to those induced by total exhaust condensate (4/35, 17/34 and 24/35 *versus* 7/35 and 20/35). No lung tumour was observed in the untreated or vehicle controls. A dose-response relationship was obtained with the total condensate and with the fraction of PAHs with more than three rings.

Hamster: In an experiment by Mohr *et al.* (1976) and Reznik-Schüller and Mohr (1977), two groups of six male Syrian golden hamsters, 12 weeks old, each received intratracheal instillations of 2.5 or 5 mg gasoline exhaust condensate, prepared from emissions of a common German passenger car operating according to the European test cycle and containing 340 $\mu g/g$ benzo[a]pyrene, in Tris-HCl and EDTA solution. Treatment was every two weeks for life. Moribund animals were killed and their lungs examined histologically for tumours. A further group of six animals was treated with solvent only and were sacrificed after the last exhaust condensate-treated animal had died. Survival times ranged from 30–60 weeks, during which time animals had received 15–30 instillations of condensate. All condensate-treated animals developed pulmonary adenomas.

Groups of 30 male Syrian golden hamsters, 16 weeks of age, received intratracheal instillations of 0.2 ml of a gasoline exhaust condensate from a 1.5-l engine, its fractions, including the methanol phase, the cyclohexane phase II and the nitromethane phase, a reconstitution product of these fractions, a synthetic mixture of pure carcinogenic PAHs or 40 μg benzo[a]pyrene in Tris-buffer/saline; treatment was every two weeks until natural death (Künstler, 1983). One group of 30 untreated animals and one group of 30 solvent-treated animals served as controls. Tracheas and lungs of all hamsters were examined histologically by light microscopy. Survival time was 68–87 weeks. No lung tumour was found in animals treated with the condensate or its fractions. In the benzo[a]pyrene-treated group, one mucoepidermoidal carcinoma of the respiratory tract and one lung adenoma

were found; one animal treated with cyclohexane phase II (0.13 mg/animal; 10.7 μg benzo[a]pyrene equivalents) had a lung adenoma.

(c) Skin application

Mouse: A group of 108 C57Bl mice [age and sex unspecified] received skin applications of a concentrated benzene extract of particles from a V8 gasoline engine [procedures unspecified] (Kotin et al., 1954). Among 86 mice surviving at the appearance of the first skin tumour (390 days), 38 developed 68 skin tumours, including 22 skin carcinomas. Among 69 benzene-treated controls, 42 survived to the time of appearance of the first skin tumour in treated mice; no skin tumour was reported.

Wynder and Hoffmann (1962) gave groups of 50 female Swiss (Millerton) mice, six weeks of age, skin applications of 5, 10, 25, 33 or 50% solutions in acetone of the 'tar' from a V8 gasoline engine (Hoffmann & Wynder, 1962b) exhaust extracted with benzene. Treatment was given three times a week for 15 months; the mice were observed for a further three months, at which time they were killed. Thirty mice painted with acetone served as controls. The numbers of mice with skin papillomas at 18 months were 0, 4, 50, 60 and 60% in the control, 5, 10, 25 and 33% dose groups, respectively; the corresponding incidences of skin carcinomas were 0, 4, 32, 48 and 54, respectively. In the high-dose group, all mice had died by ten months; 70% had skin papillomas and 4% had skin carcinomas.

In similar studies by Hoffmann et al. (1965), the incidence of skin papillomas and carcinomas was higher in 20 Swiss ICR mice treated with extracts of exhaust from a V8 engine that used approximately 1 l of engine oil/200 miles (0.3 l/100 km) than in those treated with exhausts from an engine that used approximately 1 l of oil/1600 miles (0.04 l/ 100 km).

Brune et al. (1978) gave groups of 50 or 80 female random-bred CFLP mice, approximately 12 weeks of age, skin applications of an exhaust condensate produced from a 1.5-l gasoline engine during a European test cycle, fractions of this condensate or benzo[a]pyrene in 0.1 ml dimethyl sulfoxide:acetone (3:1) twice a week for life. The groups treated with the total condensate received doses of 0.526, 1.579 or 4.737 mg/animal (0.15, 0.45 or 1.35 μg/animal benzo[a]pyrene equivalents) per treatment; the two groups treated with the methanol phase (66% of the total condensate) received doses of 1.389 or 4.168 mg/animal (0.60 or 1.80 μg/animal benzo[a]pyrene equivalents); those treated with the cyclohexane phases I and II (34% and 17% of the total condensate), the nitromethane phase (17% of the total condensate) and a reconstitution of the fractions received 0.30 and 0.90 μg/animal benzo[a]pyrene equivalents. Three further groups of 50 mice received applications of 1.92, 3.84 or 7.68 μg/animal benzo[a]pyrene. One control group received applications of the vehicle alone and another remained untreated. Animals with advanced malignant tumours were killed; all other animals were observed until natural death. Statistical analysis of the results revealed a linear relationship between the percentage of animals with local tumours (squamous-cell papillomas or carcinomas) and dose for the nitromethane phase (16.4 and 68.9%), the cyclohexane phase I (13.7 and 68.8%), the reconstitution (7.9 and 54.7%) and the total condensate (3.9, 35.1 and 76.9%). Local tumour rates in mice treated with total

condensate were significantly higher than those in mice treated with benzo[a]pyrene (19.5, 15.2 and 60%) or the PAH-free fractions (methanol phase (2.6 and 5.9%) and cyclohexane phase II (2.8 and 1.5%)), which did not differ significantly from controls (1.3 and 0%). A second experiment by the same group using 40 mice per group gave similar results; however, local tumour incidences were significantly higher in the first experiment, probably due to minor differences in experimental techniques.

Grimmer *et al.* (1983a) gave groups of 65 or 80 female CFLP mice, seven weeks old, dermal applications of extracts of an exhaust condensate from a 1.5-l gasoline engine run on the European test cycle, its fractions or benzo[a]pyrene in 0.1 ml dimethyl sulfoxide:acetone (1:3) solvent; treatment was given twice a week for 104 weeks. Doses administered were: total condensate — 0.292, 0.875 or 2.626 mg/animal (0.12, 0.36 or 1.09 μg/animal benzo[a]pyrene equivalents); benzo[a]pyrene, 0.0039, 0.0077 or 0.0154 mg/animal; the methanol phase (PAH-free fraction), 0.97 or 2.9 mg/animal (0.48 or 1.45 μg/animal benzo[a]pyrene equivalents); the PAH-fraction containing PAHs with two and three rings, 0.152 or 0.455 mg/animal (0.46 or 1.39 μg/animal benzo[a]pyrene equivalents); the PAH-fraction containing PAHs with more than three rings, 0.02 or 0.06 mg/animal (0.24 or 0.73 μg/animal benzo[a]pyrene equivalents); and a mixture of 15 PAHs in a ratio corresponding to that of the automobile exhaust, 0.003 or 0.009 mg/animal (0.24 or 0.73 μg/animal benzo[a]pyrene equivalents). One group treated with 0.1 ml of the solvent only and one untreated group served as controls. Animals with advanced tumours were killed; the remaining animals were observed until natural death. The PAH-free fraction (methanol phase) and the fraction of PAHs with two or three rings produced low rates of skin tumours (carcinomas and papillomas): 11 [13.9%] and one [1.3%] animals with local tumours, respectively, in the high-dose groups. Clear dose-response relationships were demonstrated for tumour incidence in the groups treated with total condensate (six [7.7%], 34 [44.3%] and 65 [83.3%]), in those given the fraction containing PAHs with more than three rings (seven [8.9%] and 50 [63.5%]), in those given the mixture of 15 PAHs (one [1.3%] and 29 [38.7%]) and in benzo[a]pyrene-treated animals (22 [34.4%], 39 [60.9%] and 56 [89.1%]). No local skin tumour was seen in controls. Similar results were obtained by Grimmer *et al.* (1983b).

Groups of 40 male and 40 female SENCAR mice, seven to nine weeks of age, received single skin applications in 0.2 ml acetone of 0.1, 0.5, 1, 2 or 3 mg of dichloromethane extracts of particulates collected from the emission of an unleaded gasoline engine (of a 1977 model passenger car [engine volume unspecified]) with a catalytic converter (Nesnow *et al.*, 1982a). One week later, all mice received 2 μg TPA in 0.2 ml acetone twice weekly for 24–26 weeks. At that time, the percentages of mice with 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 respective percentages and numbers 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).

(d) Subcutaneous administration

Mouse: Groups of 87 or 88 female NMRI mice [age unspecified] received a single subcutaneous injection in 0.5 ml tricaprylin of 20 or 60 mg exhaust condensate from a gasoline engine [unspecified] (Pott *et al.*, 1977). A third group of 45 mice was injected three times with 60 mg condensate containing 0.163 μg/mg benzo[a]pyrene. A group of 89 mice that received 0.5 ml tricaprylin alone and a further group of 87 untreated mice served as controls. Animals that developed tumours up to 10 mm in diameter at the application site were killed. The mean survival time in the low- and medium-dose groups was in the range of that of the control groups (80—88 weeks), but was 57 weeks in the high-dose group. The numbers of animals with sarcomas at the injection site were 10/87 (11.5%), 6/88 (6.8%) and 5/45 (11.1%) in the condensate-treated groups and 3.4% in the tricaprylin-treated group.

(e) Administration with known carcinogens

Mouse: Groups of 60 female NMRI mice, eight to ten weeks old, received ten intra-tracheal instillations of 100 μg benzo[a]pyrene, 20 intratracheal instillations of 50 μg benzo[a]pyrene or ten intratracheal instillations of 50 μg dibenzo[a,h]anthracene, with concomitant exposure to gasoline engine exhaust, as described on p. 99, for 53 weeks only and were observed for a further 40 weeks (Heinrich *et al.*, 1986c). Administration of benzo[a]pyrene or dibenzo[a,h]anthracene with clean air induced a high basic lung tumour rate of 70—90% (adenomas and adenocarcinomas). Mean survival times (75—85 weeks) of exhaust-exposed animals were clearly shorter, with the exception of the groups treated ten times with 100 μg benzo[a]pyrene, in which gasoline exhaust exposure induced a higher incidence of adenocarcinomas (22/38 and 28/40 in the 1:27 and 1:61 dilution groups) but a significantly reduced incidence of adenomas (4/38 and 3/40) compared to clean air controls (20/42 adenocarcinomas, 16/42 adenomas). The total numbers of tumour-bearing animals in clean air and exhaust-exposed groups were not, however, significantly different. In the groups exposed 20 times to 50 μg benzo[a]pyrene, adenocarcinoma induction by the exhaust was inhibited significantly (3/35, 5/36, 15/42 in the 1:27, 1:61 and control groups, respectively). Additional groups of 61—83 newborn NMRI mice received a single subcutaneous injection of 4 μg (females and males) or 10 μg (females only) dibenzo[a,h]anthracene followed by inhalation exposure to one of the two dilutions of gasoline exhaust for six months, 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.

Groups of 86—90 female NMRI mice [age unspecified] were injected subcutaneously with 10, 30 or 90 μg benzo[a]pyrene alone or together with 6.6 or 20 mg exhaust condensate from a gasoline engine [unspecified] (Pott *et al.*, 1977). The dose-response relationship for local sarcomas produced by benzo[a]pyrene (20%, 54%, 76%) was reduced significantly by the addition of both doses of the condensate. The difference was seen most clearly 30 weeks after treatment.

Rat: Two groups of female Sprague-Dawley rats [initial numbers unspecified] were either administered N-nitrosodiisopropanolamine in the drinking-water (0.01%) or were exposed concomitantly by inhalation for 2 h per day on three days per week to gasoline

engine (generator EM300) exhaust diluted 1:250 in air for six to 12 months, at which time the animals were killed (Yoshimura, 1983). In animals killed between seven and 12 months, the number of lung tumours (11/37) in the combined treatment group (one adenoma and ten undifferentiated carcinomas, squamous-cell carcinomas, adenocarcinomas and mixed tumours) was significantly greater than that in the 24 nitrosamine controls (two carcinomas; $p < 0.05$).

Groups of 60 female Bor:WISW rats, ten to 12 weeks old, received 25 daily subcutaneous injections of 0.25 or 0.5 g/kg bw N-nitrosodipentylamine and were exposed to gasoline engine exhaust, as described on p. 99 (Heinrich et al., 1986c). The treatments induced significant increases in the incidences of benign tumours of the whole respiratory tract (in 9/47 and 14/48 rats given the 1:27 and 1:61 dilutions of exhaust and receiving 0.5 g/kg bw nitrosamine, and in 15/50 and 14/45 rats given the 1:27 and 1:61 dilutions and receiving 0.25 g/kg bw nitrosamine, respectively) compared with clean air controls (5/48 and 4/46 rats), but decreases in the incidences of malignant tumours (33/47 and 34/48, respectively, compared to 43/48 controls; and 13/50 and 18/45 rats, compared to 29/46 in the groups receiving 0.5 and 0.25 g/kg bw nitrosamine). When lung tumour rates were evaluated separately, the incidences of malignant tumours (mostly squamous-cell carcinomas and adenocarcinomas) were also reduced in nitrosamine-treated rats by exposure to either concentration of exhaust (in 24/48, 25/49 and 40/49 rats in the 0.5 g/kg bw groups and in 11/54, 14/47 and 26/48 rats in the 0.25 g/kg bw groups exposed to 1:27 and 1:61 dilutions and clean air, respectively), whereas the incidence of benign tumours remained unchanged. Rats given the low dose of N-nitrosodipentylamine exposed to 1:61 or 1:27 dilutions of gasoline exhaust showed overall lung tumour rates of 15/47 and 13/54, respectively, versus 27/48 rats treated with nitrosamine but exposed to clean air. In animals given the high dose of N-nitrosodipentylamine, these rates were 33/49 and 28/48, respectively, versus 44/49 controls.

Hamster: Groups of 80–81 female Syrian golden hamsters, ten to 12 weeks old, received a single subcutaneous injection of 3 mg/kg bw N-nitrosodiethylamine (NDEA) or 20 intratracheal instillations of 0.25 mg benzo[a]pyrene and were exposed to gasoline engine exhaust, as described on p. 99 (Heinrich et al., 1986c). 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; one malignant tumour of the paranasal cavity was also seen in the group exposed to benzo[a]pyrene. The basic tumour rate was not significantly increased by exposure to either dilution of exhaust. Tumour rates in NDEA- and benzo[a]pyrene-treated animals inhaling the 1:27 dilution of exhaust were approximately 50% lower than those in treated animals inhaling the 1:61 dilution or clean air.

Groups of 52 male and 52 female Syrian hamsters, six to eight weeks old, received a single subcutaneous injection of 4.5 mg/kg bw NDEA three days prior to exposure by inhalation to gasoline engine exhaust, as described on p. 98 (Brightwell et al., 1986). The authors reported that NDEA-treated hamsters had a nonsignificantly increased incidence of tracheal papillomas. [The Working Group noted the inadequate reporting of the data.]