3.2 Other relevant data

(a) *Experimental systems*

(i) *Deposition, clearance, retention and metabolism*

Engine exhaust contains material in gaseous, vapour and particulate phases, and the absorption, distribution and excretion of individual constituents is influenced by the phase in which they occur and by the properties of each compound. After inhalation, highly soluble compounds in the gaseous phase, such as sulfur dioxide, are absorbed in the upper airways and do not penetrate significantly beyond the level of the bronchioles. Compounds that interact biochemically with the body are also retained in significant quantities; thus, processes such as binding of carbon monoxide to haemoglobin normally occur in the gas-exchange (pulmonary) region of the lung. Retention characteristics of materials not associated with the particulate phase are highly compound-specific. The factors affecting the uptake of a wide variety of vapours and gases have been summarized (Davies, 1985).

As described on p. 47, a proportion of a compound in the vapour phase condenses onto the particulate material produced in the engine exhaust. The association of a compound with the particulate phase modifies the deposition pattern and affects its lung retention; the lung burden of a compound following continuous exposure to that compound coated on particles may be many times that of continuous exposure to the compound alone (Bond *et al.*, 1986).

Deposition in the respiratory tract is a function of particle size. The median particle size in a variety of long-term exposure systems has been between 0.19 and 0.54 μm (Yu & Xu, 1986), representative of that in an urban environment (Cheng *et al.*, 1984). However, some of the carbonaceous mass in environmental samples results from airborne suspension of material collected in automobile exhaust pipes and is >5 μm in size (Chamberlain *et al.*, 1978); such particles are unlikely to be produced in a static exposure system. Dilution has little effect on the size distribution of particles used in long-term studies (0.3–7 mg/m^3; Cheng *et al.*, 1984), although rapid dilution (<1 sec) can lead to a smaller size (0.10–0.15 μm; Chan *et al.*, 1981). The presence of sulfates in the particulate phase (Lies *et al.*, 1986) may lead to enlargement of individual particles in the high humidity of the respiratory tract, thereby altering the deposition pattern (Pritchard, 1987).

Diesel engine exhaust

Deposition: Studies of the deposition of diesel engine exhaust, representative of fresh urban exhaust, are summarized in Table 24; the particle sizes used were in the lower part of the range found in long-term exposure chambers. Deposition following nose-only exposure was measured by radiotracer technique. Data are quoted as a proportion of the amount of inhaled aerosol, which is based on estimates of ventilation rates. [The Working Group noted that the data on deposition of diesel particles in rats are in broad agreement with data for other particulate materials of similar size (Raab *et al.*, 1977; Wolff *et al.*, 1984).]

Table 24. Experimental deposition in the respiratory tract of diesel engine exhaust particles

Species	Mass median particle diameter (μm)	% total deposition of inhaled exhaust particles	Reference
Rat	0.1–0.15	15–17	Chan *et al.* (1981)
Rat	0.16–0.19	10–17	Dutcher *et al.* (1984)
Rat	0.12	17[a] (calculated) 20[a] (estimated)	Lee *et al.* (1983)
Guinea-pig	0.12	20[a] (initial deposition)	Lee *et al.* (1983)

[a]Mean values

A model for the deposition of diesel exhaust particles predicts that, as the median size increases from 0.08 to 0.30 μm, total deposition in rats falls from 25 to 15%, tracheobronchial deposition from 5 to 2% and pulmonary deposition from 12 to 5%; upper respiratory tract deposition remains constant at 8% (Yu & Xu, 1986). The model predicts that pulmonary deposition will vary only with (body weight)$^{-0.14}$, since diffusion is the predominant mechanism (Xu & Yu, 1987). [The Working Group noted that this model is in good agreement with the observed deposition of other particles (e.g., Raab *et al.*, 1977; Wolff *et al.*, 1981, 1984)].

Following exposure of rats for six, 12, 18 and 24 months to 0.4, 3.5 and 7.1 mg/m^3 diesel exhaust particles, there was no significant effect of length of exposure or exposure concentration on the deposition of 0.1 μm gallium oxide particles (Wolff *et al.*, 1987).

Mucociliary clearance: The clearance of particles from the lung following a single exposure to radiolabelled diesel particles is summarized in Table 25. The fast phase of clearance is conventionally assumed to be due to mucociliary action, the remainder (slow phase) to pulmonary clearance. The variation in the fraction of the lung deposit cleared by mucociliary action (i.e., the tracheobronchial deposit) is linked to particle size and hence deposition pattern. [The Working Group noted that Gutwein *et al.* (1974) give no information on particle size and that, without this, the high tracheobronchial deposit cannot be accounted for.]

In rats exposed for short periods (4–100 h) to diesel exhaust with particulate concentrations in the range 0.9–17 mg/m^3, a dose-dependent reduction in mucociliary clearance occurred, although the effect was less marked on exposure to the gas phase alone (Battigelli *et al.*, 1966). No such effect occurred in sheep exposed for 30 min to concentrations of 0.4–0.5 mg/m^3 of resuspended diesel particles, i.e., in the absence of the gas phase (Abraham *et al.*, 1980). Exposure-related differences in tracheal mucociliary clearance have also been reported over 1–12 weeks in rats exposed to 1 and 4.4 mg/m^3 particulates in diesel exhaust. However, in another study, there was no effect on tracheal

Table 25. Clearance of diesel exhaust particles from rat lung following single exposures

Fraction of lung deposit clearance (%)		Half-time of slow phase (days)	Reference
Fast phase	Slow phase		
34	66	62	Chan *et al.* (1981)
6	35	6[a]	Lee *et al.* (1983)
	59	80[a]	
b	*b*	77	Chan *et al.* (1984)
75	25	*b*	Gutwein *et al.* (1974)

[a]Three clearance phases are given: fast clearance with a half-time of one day (cf. Chan *et al.*, 1981), a clearance phase with a half-time of six days and a slow phase with a half-time of 80 days.

[b]Data not available

mucociliary clearance of exposures of six to 24 months to particulate concentrations of 0.4–7.1 mg/m³ (Wolff *et al.*, 1987). [The Working Group noted that there may be some impairment of mucociliary clearance, possibly caused by the gas phase of engine exhaust, but that its effect is of limited significance in the long term.]

Pulmonary (alveolar) clearance: The pulmonary clearance of diesel particles is very much slower than the mucociliary clearance (see Table 25). On the basis of these data, the lung burden of rats during protracted exposure should tend exponentially toward an equilibrium value at 12 months. In rats exposed to diesel exhaust with a particulate concentration of 0.3 mg/m³, there was evidence of equilibration after 12 months (only a 2.5-fold increase over 24 months); however, with exposures of 3.5 and 7.0 mg/m³, lung burdens increased steadily (five to 11 fold) over 24 months. This has been referred to as the 'overload' phenomenon (Wolff *et al.*, 1987). The clearance rate of insoluble particles following prolonged exposure to diesel exhaust at a variety of concentrations and durations also indicates impaired long-term clearance (Wolff *et al.*, 1984). Thus, it appears that the normal clearance mechanisms become seriously impaired, leading to very long-term retention of material in the lung, usually referred to as 'sequestration'.

Results of studies on particulate clearance in rats following repeated exposures to diesel exhaust are summarized in Table 26. Lung clearance was estimated either by exposure to a pulse of ^{14}C-labelled diesel exhaust particles at the end of the cumulative exposure (Chan *et al.*, 1984; Lee *et al.*, 1987) or by measuring the lung burden of soot spectrophotometrically (Griffis *et al.*, 1983). Also included are data on the clearance of a pulse of radiolabelled fused aluminosilicate particles following exposure to diesel exhaust for two years at particulate concentrations between 0.4 and 7.0 mg/m³ (Wolff *et al.*, 1987). [The Working Group noted that pulse techniques measure only the clearance of the material that has most recently entered the lung. Since there is no difference between this and total soot measurements,

Table 26. Pulmonary clearance in rats of insoluble particles following exposure to diesel exhaust

Exposure			Pulmonary clearance		Reference
Concentration (mg/m³)	Duration (weeks)	h per day × days/week	Material studied	Half-time (days)	
0	0	0	Diesel exhaust	77	Chan *et al.* (1984)
0.25	7	20 × 7		90	
0.25	16	20 × 7		92	
6.00	1	20 × 7		166	
6.00	9	20 × 7		562	
6.00	16	20 × 7		[>1000]	
0.15	18	7 × 5	Diesel exhaust	87	Griffis *et al.* (1983)
0.94	18	7 × 5		99	
4.10	18	7 × 5		165	
6.00	1	20 × 7	Diesel exhaust	61	Lee *et al.* (1987)
6.00	3	20 × 7		124	
6.00	6	20 × 7		192	
0	0	0	FAP[a]	79	Wolff *et al.* (1987)
0.35	104	7 × 5		81	
3.50	104	7 × 5		264	
7.00	104	7 × 5		240	

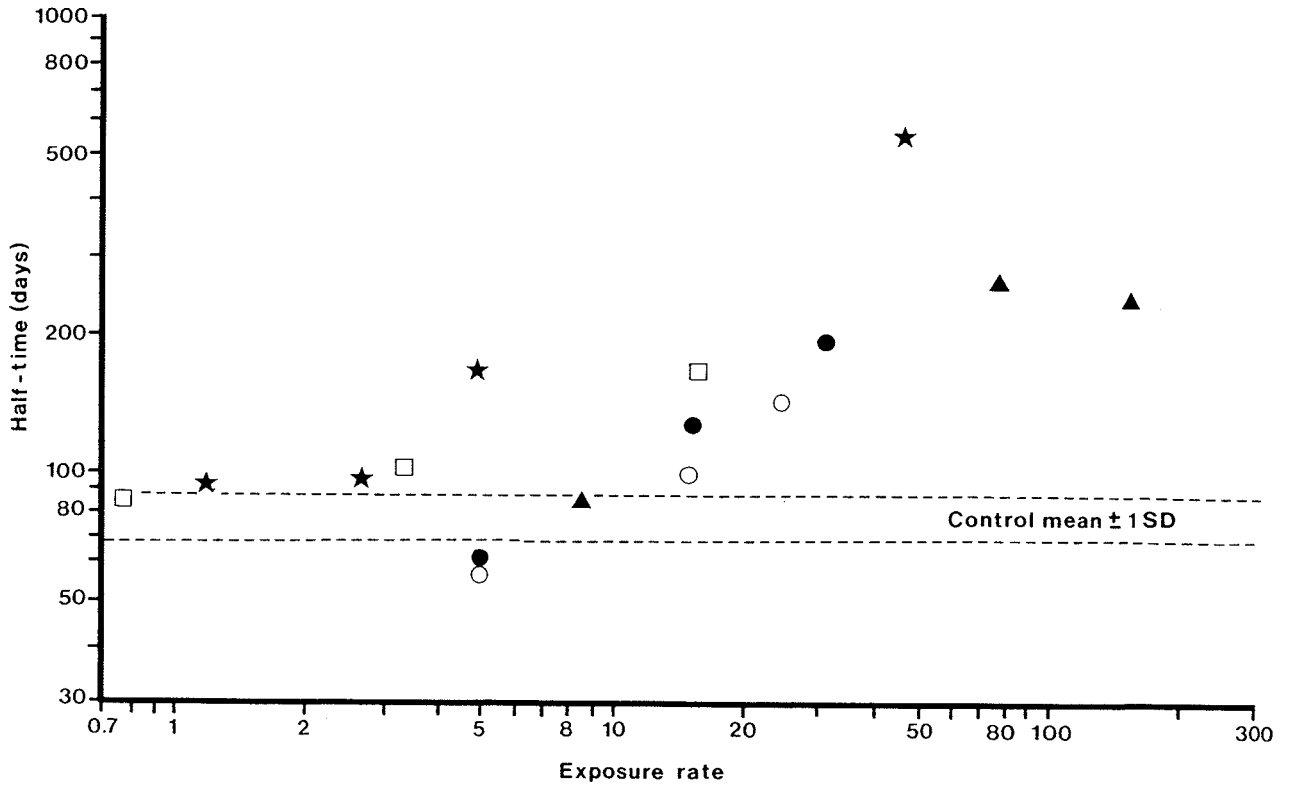
[a]FAP, radiolabelled (^{134}Cs) fused aluminosilicate particles

deposition, and hence ventilation, must continue to occur in those areas where clearance is impaired, confirming the findings of Wolff *et al.* (1987) that deposition is unaffected by prolonged exposure to diesel exhaust.]

Pulmonary clearance as a function of contemporary lung burden has also been considered (McClellan, 1986). In an analysis of the published data, Wolff *et al.* (1986) concluded that in rats sequestration becomes a significant burden at a certain level [about 1 mg/lung] and is related to the rate of accumulation; i.e., short exposure to a high concentration produces an effect at a lower lung burden than more protracted exposure at a lower concentration. Thus, there is no strong relationship between the half-time of clearance and cumulative exposure.

[The relationship between half-times for pulmonary clearance of diesel exhaust particles and other insoluble particles in the rat following exposure to diesel exhaust and an 'exposure rate', calculated by the Working Group from cumulative exposure, mg/m³ × weeks × (h/week)/168, is plotted in Figure 8. The Working Group noted that there is an effect on clearance at 'exposure rates' above 10 mg/m³ × week (i.e., continuous exposure to 0.2 mg/m³ or exposure for 40 h per week to 0.8 mg/m³ for one year) and a strong suggestion that impaired clearance occurs over the whole range of 'exposure rates' studied.]

Fig. 8. Pulmonary clearance in rats of diesel exhaust particles and other insoluble particles following exposure to diesel exhaust



□, Griffis *et al.* (1983), spectrophotometric technique; ★, Chan *et al.* (1984), pulse technique; •, Lee *et al.* (1987), pulse technique; o, Lee *et al.* (1987), carbon black; ▲, Wolff *et al.* (1987), radiolabelled (^{134}Cs) fused aluminosilicate particles. Exposure rate = (mg × week/m^3) × (h/week)/168

Studies in rats on the effect of exposure to diesel exhaust on the clearance of metal oxide particles containing a γ-emitting isotope are summarized in Table 27 (Bellmann *et al.*, 1983; Heinrich *et al.*, 1986a; Lewis *et al.*, 1986; Wolff *et al.*, 1987). The control animals cleared the metal oxide particles much faster than they did diesel particles or fused aluminosilicate particles (see Table 26; Wolff *et al.*, 1987). [The Working Group noted that this suggests that clearance of metal oxides involves a significant soluble component.]

[The relationship between half-times for pulmonary clearance of metal oxide particles in rats following exposure to diesel exhaust and an 'exposure rate' calculated by the Working Group is plotted in Figure 9. The Working Group noted that impaired clearance of metal oxide particles does not become apparent until significantly higher values of 'exposure rate' than in the studies on diesel and fused aluminosilicate particles and considered that the differences in the results could be explained by continuing solubility masking an impairment in mechanical clearance, implying that sequestration is primarily a mechanical effect. For comparison, data for gasoline from Bellmann *et al.* (1983) have been added.]

After only two months' exposure of rats to a diesel exhaust particulate concentration of 2 mg/m^3, clearance of metal oxide particles was significantly faster than in controls,

Table 27. Pulmonary clearance in rats of metal oxide particles following exposure to diesel engine exhausts

Exposure			Pulmonary clearance		Reference
Concentration (mg/m³)	Duration (weeks)	h/day × days/week	Material	Half-time (days)	
0	0	0	$^{59}Fe_2O_3$	50[a]	Bellmann *et al.* (1983)
0	0	0		47[b]	
0	0	0		43[c]	
3.90	52	7 × 5		127	
3.90	78	7 × 5		92	
3.90	104	7 × 5		54	
0	0	0	$^{59}Fe_3O_4$	47	Lewis *et al.* (1986)
2	9	7 × 5		37	
0	0	0	$^{67}Ga_2O_3$	36[d]	Wolff *et al.* (1987)
0	0	0		48[a]	
0	0	0		47[b]	
0	0	0		36[c]	
0.35	26	7 × 5		53	
0.35	52	7 × 5		36	
0.35	78	7 × 5		72	
0.35	104	7 × 5		40	
3.50	26	7 × 5		37	
3.50	52	7 × 5		60	
3.50	78	7 × 5		82	
3.50	104	7 × 5		79	
7.00	26	7 × 5		151	
7.00	52	7 × 5		121	
7.00	78	7 × 5		84	
7.00	104	7 × 5		121	
0	0	0	Fe_20_3	49[e]	Heinrich *et al.* (1986a)
4	13	19 × 5		170	
4	35	19 × 5		170	
4	52	19 × 5		95	
4	82	19 × 5		125	

[a]Control animals (17 weeks of age at start) after 26 weeks

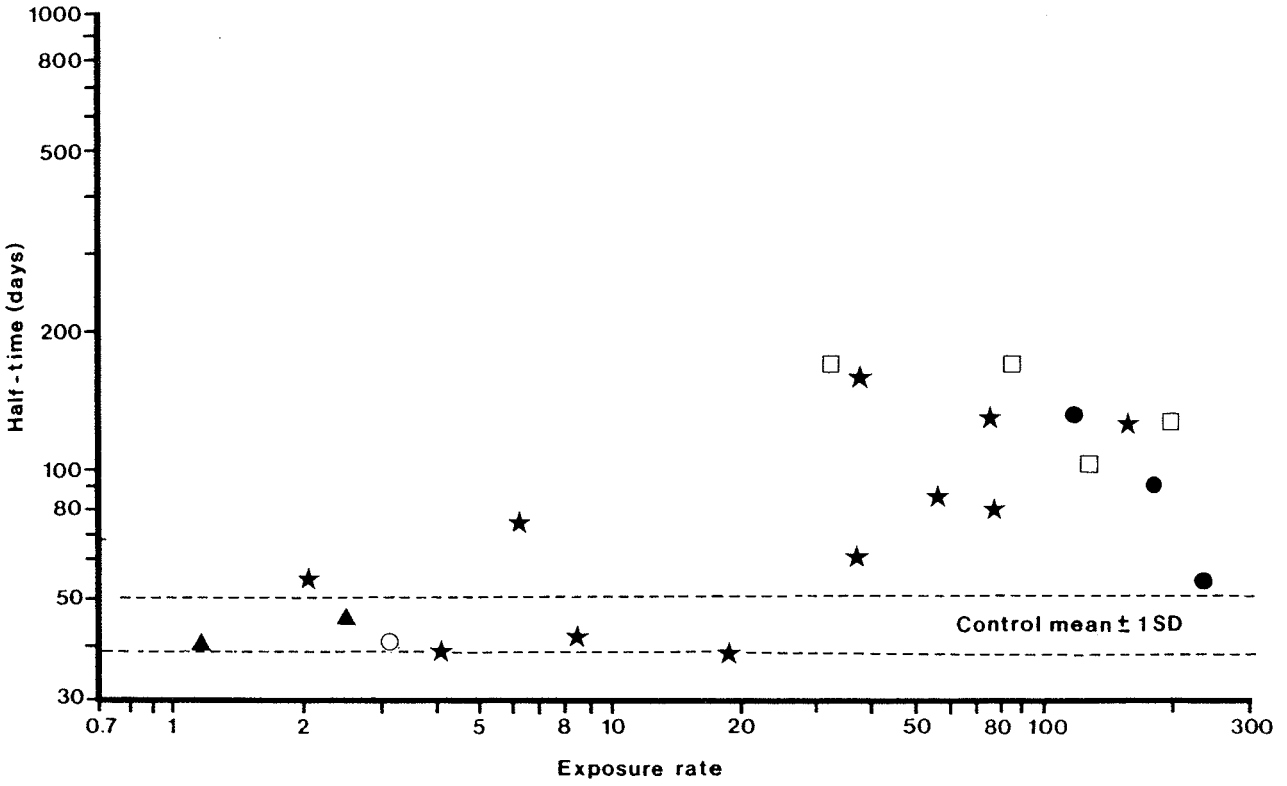
[b]Control animals after 52 weeks

[c]Control animals after 78 weeks

[d]Control animals after 164 weeks

[e]Average of controls aged 26–104 weeks

Fig. 9. Pulmonary clearance of metal oxide particles in rats following exposure to engine exhaust



□, Heinrich *et al.* (1986a), diesel exhaust; ★, Wolff *et al.* (1987), diesel exhaust; •, Bellmann *et al.* (1983), diesel exhaust; o, Lewis *et al.* (1986), diesel exhaust; Δ, Bellmann *et al.* (1983), gasoline exhaust. Exposure rate = (mg × week/m³) × (h/week)/168

suggesting a stimulated lung response; no such effect was observed subsequently (Oberdoerster *et al.*, 1984; Lewis *et al.*, 1986). [The Working Group noted that overloading had probably occurred.]

The rate of clearance of ferric oxide in hamsters was slightly lower (75 ± 40 days) following one year's exposure to diesel exhaust particles (4 mg/m³) than that in clean-air controls (55 ± 17 days; Heinrich *et al.*, 1986a). In another study, only 10% clearance of ^{14}C-labelled diesel particles was observed 400 days after a single exposure of guinea-pigs (Lee *et al.*, 1983). Six months after a three-month exposure of mice, rats and hamsters to diesel exhaust (particles, 1.5 mg/m³), the mice appeared to have a slower clearance than rats and hamsters (Kaplan *et al.*, 1982).

The gas phase alone appears to have no effect on pulmonary clearance in rats or hamsters (Heinrich *et al.*, 1986a). Clearance of diesel particles following prolonged exposure to a carbon black aerosol of similar size showed a pattern of impairment similar to that observed after diesel exposure (see Fig. 8), strongly suggesting that dust overloading *per se* impairs mechanical clearance (Lee *et al.*, 1987). [The Working Group noted that the half-time lung clearance of carbon black is shorter than that of diesel exhaust at similar

'exposure rates'. This may reflect a local effect of diesel particles on the alveolar macrophages which mediate mechanical clearance; diesel particles depress the phagocytic capacity of macrophages, whereas coal dust activates them (see below and Castranova *et al.*, 1985).]

The majority of the particles that are cleared by macrophages from the pulmonary region leave *via* the ciliated epithelium and are excreted *via* the gut. However, a proportion penetrate the lymphatic system, borne by macrophages, and are filtered by the lymph nodes to form aggregates of particles (Vostal *et al.*, 1981). It has been estimated that one-third of clearance occurred *via* this route during the first 28 days after exposure of rats to diesel exhaust (Chan *et al.*, 1981). [The Working Group noted that there is no information on how this proportion changes with time or with prolonged exposure.]

Retention: The retention of the organic compounds associated with exhaust particles has been reviewed (McClellan *et al.*, 1982; Vostal *et al.*, 1982; Holmberg & Ahlborg, 1983; Vostal, 1983; Wolff *et al.*, 1986). Organic compounds adsorbed on exhaust particles can be extracted by biological fluids, as has been observed in assays for mutagenesis (Claxton, 1983; Lewtas & Williams, 1986; see p. 121). The half-time of the slow phase of lung clearance for ^{14}C derived from labelled diesel exhaust was 25 days in rats (Sun & McClellan, 1984), and that for ^{3}H-benzo[*a*]pyrene coated on diesel particles was 18 days (Sun *et al.*, 1984). The retention of 1-nitropyrene adsorbed onto diesel exhaust particles is described in the monograph on that compound.

No data were available on changes in the retention of individual compounds after prolonged exposure to diesel exhaust.

Metabolism: The metabolism of several components of engine exhausts has been reported previously: some polycyclic aromatic hydrocarbons (IARC, 1983), formaldehyde (IARC, 1982a), lead (IARC, 1980), nitroarenes (IARC, 1984) and benzene (IARC, 1982b). The metabolism of 1-nitropyrene associated with diesel exhaust particles is described in the monograph on that compound.

The metabolism of benzo[*a*]pyrene coated on diesel exhaust particles has been studied in different experimental systems. Fischer 344 rats were exposed for 30 min by nose-only inhalation to ^{3}H-benzo[*a*]pyrene adsorbed onto diesel engine exhaust particles. The majority (65–76%) of the radioactivity retained in the lungs (as determined by high-performance liquid chromatography) 30 min and 20 days after exposure was associated with benzo[*a*]pyrene. Smaller amounts of benzo[*a*]pyrene-phenols (13–18%) and benzo[*a*]-pyrene-quinones (5–18%) were also detected. No other metabolite was found (Sun *et al.*, 1984).

The pulmonary macrophages of dogs metabolized 1 μM ^{14}C-benzo[*a*]pyrene, either in solution or coated on diesel particles, into benzo[*a*]pyrene-7,8-, -4,5- and -9,10-dihydrodiols (major metabolites) as well as into benzo[*a*]pyrene-phenols and benzo[*a*]pyrene-quinones (minor metabolites). The total quantity of metabolites did not differ when macrophages were incubated with either benzo[*a*]pyrene in solution or benzo[*a*]pyrene coated on diesel particles (Bond *et al.*, 1984).

Fischer 344 rats were exposed to diesel engine exhaust (7.1 mg/m^3 particles) for about 31 months. After sacrifice, DNA was extracted from the right lung lobe and analysed for adducts by ^{32}P-postlabelling: more DNA adducts were found in the exhaust-exposed group than in the unexposed group (Wong *et al.*, 1986).

Fischer 344 rats and Syrian golden hamsters were exposed to different dilutions of diesel engine exhaust for six months to two years, when blood samples were analysed for levels of haemoglobin adducts (2-hydroxyethylvaline and 2-hydroxypropylvaline) by gas chromatography-mass spectrometry. A dose-dependent increase in the level of haemoglobin adducts was found, corresponding to the metabolic conversion of about 5–10% of inhaled ethylene and propylene to ethylene oxide and propylene oxide, respectively (Törnqvist *et al.*, 1988).

Gasoline engine exhaust

Deposition: In a study on the deposition of particles from inhaled gasoline exhausts (mass median diameter, 0.5 μm) in rats, mean total deposition of particles was 30.5%. Most deposition occurred in the alveolar region and in the nasal passages (Morgan & Holmes, 1978). In this study, the concentration of carbon monoxide in the gasoline exhaust was reduced before inhalation, and the particles were larger than those of the diesel exhausts reported. [The Working Group noted that the greater deposition of gasoline exhaust particles is consistent with the larger size of the particles and does not imply any fundamental difference in deposition between diesel and gasoline exhausts particles.]

Clearance: The results of a study on pulmonary clearance of ferric oxide by rats and hamsters following exposure to gasoline engine particles (0.04 and 0.09 mg/m^3) for two years are summarized in Table 28 (Bellmann *et al.*, 1983). Clearance was similar to that in controls and in animals exposed to diesel exhaust (see Table 27). [The Working Group noted that, on the basis of the data concerning exposure to diesel exhaust, clearance of metal oxide particles would not be impaired by exposures to such low concentrations.]

Table 28. Pulmonary clearance by rats and hamsters of ferric oxide particles following exposure to gasoline engine exhausts[a]

Exposure			Pulmonary clearance (half-time in days)	
Concentration (mg/m^3)	Duration (months)	h/day × days/week	Rats	Hamsters
0	0	0	34	86
0.04	12	19 × 5	39	64
0.09	(US–72 cycle)	19 × 5	44	86

[a]From Bellmann *et al.* (1983)

Metabolism: As reported in an abstract, crude extracts of gasoline exhaust were applied topically to male BALB/c mice over a period of one to two weeks and DNA was isolated from the treated skin for analysis by ^{32}P-postlabelling. The major DNA adduct derived from benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide was found in exposed mice (Randerath *et al.*, 1985).

Fischer 344 rats and Syrian golden hamsters were exposed to different dilutions of gasoline engine exhaust for six months to two years, and blood samples were analysed for levels of 2-hydroxyethylvaline and 2-hydroxypropylvaline in haemoglobin by gas chromatography-mass spectrometry. A dose-dependent increase in the level of haemoglobin adducts was found, corresponding to the metabolic conversion of about 5–10% of inhaled ethylene and propylene to ethylene oxide and propylene oxide, respectively (Törnqvist *et al.*, 1988).

(ii) *Toxic effects*

Diesel engine exhaust

After about 480 days, NMRI mice exposed to unfiltered, diluted (1:17) diesel exhaust (particles, 4 mg/m^3; carbon monoxide 14.3 ± 2.5 mg/m^3) had lost body weight in comparison with animals exposed to filtered exhaust (carbon monoxide, 12.7 ± 2.2 mg/m^3) or with controls. Under the same circumstances, rats had a lower weight increase (Heinrich *et al.*, 1986a).

The livers of Syrian golden hamsters exposed for five months to diesel exhaust diluted 1:5 and 1:10 in air had enlarged sinusoids with activated Kupffer's cells. Nucleoli were frequently fragmented or irregularly shaped. Fat deposition was observed in the sinusoids. Mitochondria from animals exposed to the 1:5 dilution had frequently lost cristae. Giant microbodies were observed in hepatocytes, and gap junctions between hepatocytes were disturbed (Meiss *et al.*, 1981).

In an initiation-promotion assay in rat liver using induction of γ-glutamyl transpeptidase-positive foci as the endpoint, Pereira *et al.* (1981a) exposed partially hepatectomized Sprague-Dawley rats to diesel exhaust (particles, 6 mg/m^3) for up to six months. The animals were also fed choline-supplemented or choline-deficient diets. Exposure to diesel exhaust did not alter the number of foci or induce 'remarkable' liver toxicity.

Lung function: Short-term exposure to diesel exhaust (28 days) led to a 35% increase in pulmonary air flow resistance in Hartley guinea-pigs (Wiester *et al.*, 1980) but increased vital capacity and total lung capacity in Sprague-Dawley rats (Pepelko, 1982a).

Prolonged exposure of rats to diluted diesel exhaust has led to impairment of lung function in some studies (Gross, 1981; Heinrich *et al.*, 1986a; McClellan, 1986) but not in others (Green *et al.*, 1983). No significant impairment of lung function was reported in hamsters (Heinrich *et al.*, 1986a).

A classic pattern of restrictive lung disease was observed in cats after 124 weeks of exposure to diesel exhaust (weeks 1–61: dilution factor, air:diesel, 18; particles, ~6 mg/m^3;

weeks 62–124: dilution factor, 9; particles, ~12 mg/m³; Moorman *et al.*, 1985). No such effect was observed during the first 61 weeks of the study (Pepelko *et al.*, 1980, 1981; Moorman *et al.*, 1985).

Lung morphology, biochemistry and cytology: After two years of exposure, the wet and dry weights of lungs from both mice and rats exposed to unfiltered, diluted (1:17) diesel exhaust (particles, 4 mg/m³; carbon monoxide, 14.3 ± 2.5 mg/m³) were two to three times higher than those of controls. The lung weights of Syrian golden hamsters exposed similarly had increased by 50 and 70% (Heinrich *et al.*, 1986a). An increased lung to body weight ratio was also observed in guinea-pigs following an eight-week exposure to a dilution of 1:13 (Wiester *et al.*, 1980).

Exposure of rats for 30 months to diesel exhaust (particles, 1–4 mg/m³) resulted in dose-dependent irregularity, shortening and loss of cilia in ciliated epithelia, particularly the trachea and the main bronchi (Ishinishi *et al.*, 1986a).

Increased numbers of alveolar macrophages containing diesel particles and of type II pneumocytes and accumulation of inflammatory cells within the alveoli and septal walls were observed after a 24-h exposure of Fischer 344 rats to high concentrations of diesel exhaust (particles, 6 mg/m³; White & Garg, 1981). Macrophage aggregates were still present six weeks after a two-week exposure (Garg, 1983).

Following prolonged exposure of rats to diesel exhausts (particles, 2–5 mg/m³), particle-containing alveolar macrophages and type II cell hyperplasia were observed (Heinrich *et al.*, 1986a; Iwai *et al.*, 1986; Vallyathan *et al.*, 1986). Increases in both the number and size of macrophages and in the number of polymorphonuclear leukocytes were also observed in rats and hamsters (Chen *et al.*, 1980; Vostal *et al.*, 1982; Strom, 1984; Heinrich *et al.*, 1986a). Elevated levels of lymphocytes have also been reported in rats and hamsters (Strom, 1984; Heinrich *et al.*, 1986a). Particle accumulation and cellular proliferation have been observed in guinea-pigs (Chen *et al.*, 1980; Wiester *et al.*, 1980; Barnhart *et al.*, 1981; Weller *et al.*, 1981), and granulocyte counts were increased dramatically (up to ten-fold) in hamsters (Heinrich *et al.*, 1986a).

In Fischer 344 rats exposed to diesel engine exhaust (particles, 2 mg/m³) for two years, depressed chemiluminescence and decreased surface ruffling of alveolar macrophage membranes were observed, indicating a depression of the phagocytic activity of the macrophages (Castranova *et al.*, 1985).

In specific-pathogen-free Wistar rats exposed to diesel exhaust (soot, 8.3 ± 2.0 mg/m³) continuously for up to 20 months, slight focal and diffuse macrophage accumulation and alveolar cell hypertrophy were observed after four months. After 20 months' exposure, focal macrophage accumulation was moderate and diffuse accumulation was slight to moderate. Alveolar cell hypertrophy was more marked (up to severe), and interstitial fibrosis and alveolar emphysema were more pronounced than after four months. Alveolar bronchiolization was seen in one group at four months, but was present in four of six groups up to a moderate degree after 20 months (Karagianes *et al.*, 1981). In a long-term inhalation study with pathogen-free Fischer 344 rats exposed for up to 30 months to whole exhaust diluted to contain soot concentrations of 0.35, 3.5 or 7.0 mg/m³, focal accumulation of soot was

dose-dependent and was paralleled by an active inflammation involving alveolar macrophages adjacent to terminal bronchioli. Progressive fibrosis was present in areas of soot accumulation. Epithelial hyperplasia and squamous metaplasia occurred adjacent to fibrotic foci (Mauderly *et al.*, 1987). However, although there was accumulation of particles, no histopathological sign of fibrotic change was observed after 12 or 24 months' exposure of Fischer 344 rats to diesel emissions (particles, 2 mg/m^3; Green *et al.*, 1983; Vallyathan *et al.*, 1986).

Fibrotic changes in the lungs of Hartley guinea-pigs exposed to diesel exhaust (particles, 0.25–6 mg/m^3) began after six months' exposure at a particulate concentration of about 0.75 mg/m^3; ultrastructural changes were concentration-dependent and started to appear after two weeks of exposure at this level. Alveolar septa were thickened following exposures above 0.25 mg/m^3 particles (Barnhart *et al.*, 1981, 1982).

After exposure of cats to diesel exhaust for 27 months (particles, 6 mg/m^3 for weeks 1–61; 12 mg/m^3 for weeks 62–124), bronchiolar epithelial metaplasia and peribronchial fibrosis were observed; the latter became more severe after an additional six months' exposure to clean air, but the bronchiolar epithelium returned to normal (Hyde *et al.*, 1985).

Biochemical changes in the lung associated with the changes described have been discussed by McClellan (1986). Lavage fluids from hamsters and rats after one and two years' exposure to unfiltered, diluted (1:17) diesel exhaust (particles, 4 mg/m^3; carbon monoxide, 14.3 ± 2.5 mg/m^3) contained increased levels of lactate dehydrogenase, alkaline and acid phosphatase, and glucose-6-phosphate dehydrogenase and of collagen and total protein (Heinrich *et al.*, 1986a). In contrast, acid phosphatase activity was reduced in rats and guinea-pigs exposed for one day to 12 months to diesel engine exhaust (particles, 0.25–6 mg/m^3); the effects were directly related to duration and levels of exposure (Weller *et al.*, 1981). Protein content and β-glucuronidase and acid phosphatase activities were elevated in lavage fluid cells from rats exposed to diesel exhaust for 48 weeks (particles, 1.5 mg/m^3) or 52 weeks (particles, 0.75 mg/m^3; Strom, 1984). Rats exposed to filtered diesel exhaust showed only small increases in glucose-6-phosphate dehydrogenase activity, collagen and protein content, while hamsters showed no increase (Heinrich *et al.*, 1986a). The total lung collagen level was elevated in the lungs of cats six months after exposure to diesel exhaust for 27 months. The cross-linked collagen content was more than doubled at the end of the exposure to air, and the collagen aldehydes:hydroxyproline ratio was elevated (Hyde *et al.*, 1985).

Sequestration (discussed above, p. 107) can be correlated with histopathological changes observed after prolonged exposure. Strom (1984) concluded that the apparent threshold of exposure of rats for increased influx of cells into the lung, beginning with alveolar macrophages, followed by polymorphonuclear leukocytes and lymphocytes, was 0.25–0.75 mg/m^3 for 28 weeks. [The Working Group noted that this would correspond to a calculated 'exposure rate' of 9 mg/m^3 × week, 110 h/week, which is not dissimilar to the point at which marked sequestration occurs (see Fig. 8).]

In Fischer 344 rats, DNA synthesis in lung tissue was increased four-fold after two days of continuous exposure by inhalation to diesel exhaust (particles, 6 mg/m^3). DNA synthesis returned to control levels one week after exposure. The labelling index of type II cells was

significantly greater than that in controls after two and three days of exposure to diesel exhaust. After one day of exposure, palmitic acid incorporation into phosphatidylcholine in lung tissue increased by three fold when tissue palmitic acid content decreased. Total lung fatty acid content decreased by 23% after one day of exposure (Wright, 1986).

Effects on metabolism: Exposure to diesel particles or diesel particulate extracts has been reported to have no effect (Chen & Vostal, 1981; Rabovsky *et al.*, 1984) or a moderate (<two-fold change) effect (Lee, I.P. *et al.*, 1980; Pepelko, 1982b; Dehnen *et al.*, 1985; Chen, 1986) on aryl hydrocarbon hydroxylase activity in the lung and liver of mice and rats and in the lung of hamsters.

Exposure of Fischer 344/Crl rats by inhalation to diesel engine exhaust (particles, 7.4 mg/m^3) for four weeks doubled the rate of 1-nitropyrene metabolism in both nasal tissue and perfused lung. In addition, the amount of ^{14}C covalently bound to lung macromolecules was increased four fold (Bond *et al.*, 1985). (See also the monograph on 1-nitropyrene.)

One week after instillation, there was significantly more residual benzo[*a*]pyrene in the lungs of A/Jax mice exposed to diesel engine exhaust (particles, 6 mg/m^3) for nine months, probably because benzo[*a*]pyrene had bound to exhaust particles. The amounts of free benzo[*a*]pyrene and of different unconjugated and conjugated metabolite fractions in lungs, liver and testis were similar to those in diesel exhaust-exposed and control mice (Cantrell *et al.*, 1981; Tyrer *et al.*, 1981).

Immunology and infection: In guinea-pigs exposed to diesel engine exhaust (particles, 1.5 mg/m^3) for up to eight weeks, B- and T-cell counts in lymph nodes were not altered (Dziedzic, 1981). No change was observed in the immunological function of splenic B- or T-cells from Fischer 344 rats exposed for up to 24 months to diesel engine exhaust (particles, 2 mg/m^3; Mentnech *et al.*, 1984).

CD-1 mice and Fischer 344 rats exposed to high (particles, 7 mg/m^3), medium (particles, 3.5 mg/m^3) or low (particles, 0.35 mg/m^3) levels of diesel engine exhaust for up to 24 months had exposure-related pathological changes in lung-associated lymph nodes, including enlargement, with histiocytes containing particles in the peripheral sinusoids and within the cortex. The total number of lymphoid cells in lung-associated lymph nodes was significantly increased after six months of exposure. In groups of mice and rats immunized at six-monthly intervals by intratracheal instillation of sheep red blood cells and analysed for IgM antibodies in lymphoid cells in rats and mice and for IgM, IgC and IgA antibodies in serum of rats, mice had an increased number of antibody-forming cells in lymph nodes from six months, but differences from controls were not statistically significant. In rats, the total number of IgM antibody-forming cells in lymph nodes was significantly elevated after six months of exposure to the high level of diesel exhaust and after 12 months of exposure to all levels. Antibody titres to sheep red cells in rat serum were not altered (Bice *et al.*, 1985).

The IgE antibody response of BDF_1 mice was increased after five intranasal inoculations at intervals of three weeks of varying doses of a suspension of diesel engine exhaust particles in ovalbumin solution. Antiovalbumin IgE antibody titres, assayed by passive cutaneous anaphylaxis, were enhanced by doses as low as 1 μg particles given at a three-week interval (Takafuji *et al.*, 1987).

Exposure to diesel engine exhaust may increase the susceptibility of mice to infection (Campbell *et al.*, 1981; Hahon *et al.*, 1985).

Gasoline engine exhaust

Lifetime exposure of specific-pathogen-free Sprague-Dawley rats to gasoline engine exhaust (carbon monoxide, 57 mg/m^3; nitrogen oxides, 23 ppm) reduced body weight (Stupfel *et al.*, 1973). Body growth rate was also reduced among Sprague-Dawley rats exposed for up to 88 days to exhaust (dilution, 1:11) from a gasoline engine operated with (carbon monoxide, 80 mg/m^3) or without (carbon monoxide, 240 mg/m^3) a catalytic converter (Cooper *et al.*, 1977).

Haematocrit and haemoglobin and erythrocyte counts were increased in Wistar rats exposed to gasoline engine exhaust (carbon monoxide, 583 mg/m^3) for five weeks (Massad *et al.*, 1986). Sprague-Dawley rats were exposed to diluted (~1:10) exhaust from a gasoline engine with and without a catalytic converter (particles, ~1.2 mg/m^3 irradiated, 1.1 mg/m^3 nonirradiated; carbon monoxide, 47 and 53 mg/m^3; and particles, 0.77 mg/m^3 nonirradiated, 3.59 mg/m^3 irradiated; carbon monoxide, 631 and 640 mg/m^3, respectively) for seven days. Haematocrit and serum lactate dehydrogenase activities were elevated in both groups exposed to emissions generated without a catalyst; no such change was observed in the groups exposed to emissions generated with a catalyst. No change was observed in serum glutamate oxaloacetate transaminase activity (Lee *et al.*, 1976).

Beagle dogs exposed for 61 months to gasoline engine exhaust (carbon monoxide, 114 mg/m^3; Malanchuk, 1980) developed arrhythmia and bradycardia (Lewis & Moorman, 1980).

Lung function: Long-lasting functional disturbances of the lung were observed in beagle dogs after exposure to raw or irradiated gasoline engine exhaust (carbon monoxide, 114–126 mg/m^3) for 68 months (Lewis *et al.*, 1974; Gillespie, 1980). In contrast, no impairment in lung function was detected in Crl:COBS CD(SD)BR rats exposed for 45 or 90 days to diluted (1:10) exhaust from a catalyst-equipped gasoline engine (particles, 11.32 ± 1.27 mg/m^3; carbon monoxide, 19.5 ± 3.5 mg/m^3; Pepelko *et al.*, 1979).

Lung morphology, biochemistry and cytology: In several reports of studies in beagle dogs, atypical epithelial hyperplasia was observed in animals exposed for 68 months to raw or irradiated gasoline engine exhaust (carbon monoxide, 114 mg/m^3). Increases in alveolar air space and cilia loss were observed after a long recovery period following exposure to irradiated exhaust (Hyde *et al.*, 1980). The collagen content of lung tissues following exposure to raw or irradiated exhaust, with and without a 2.5-3-year recovery period was not significantly different from that in unexposed animals; prolyl hydroxylase levels in the lung were highest in groups exposed to irradiated exhaust. Exposure to a mixture of sulfur oxides and irradiated exhaust also increased the level of this enzyme (Orthoefer *et al.*, 1976; Bhatnagar, 1980). Phosphatidyl ethanolamine content was lower in liver tissues of some dogs exposed for 68 months, and lung tissue phosphatidyl ethanolamine content was 90% of the mean control value. Lysobisphosphatidic acid and phosphatidyl glycerol levels in the lungs were increased (Rouser & Aloia, 1980).

Effects on metabolism: Extracts of gasoline engine particles instilled into hamster lungs increased aryl hydrocarbon hydroxylase activity of lung tissue by three to five fold (Dehnen *et al.*, 1985).

Immunology and infection : Increased sensitivity to infection has been demonstrated following exposure of mice to the exhaust of a gasoline engine with a catalytic converter, but the effect was less than that in mice following similar exposure to diesel engine exhaust (Campbell *et al.*, 1981).

(iii) *Effects on reproduction and prenatal toxicity*

Diesel engine exhaust

A three-fold increase in sperm abnormalities was observed in male Chinese hamsters exposed to diesel engine exhaust [dose unspecified] for six months, as compared to controls exposed to fresh air (Pereira *et al.*, 1981b). As reported in an abstract, a statistically significant dose-related increase in sperm abnormalities was observed in male (C57Bl/6 × C3H)F_1 mice receiving 50, 100 or 200 mg/kg bw diesel engine exhaust particles by intraperitoneal injection for five days. An eight-fold increase in sperm abnormalities over the spontaneous level was observed in mice receiving the highest dose. A significant decrease in the number of sperm was observed only at the highest dose; testicular weight was not affected (Quinto & De Marinis, 1984).

Gasoline engine exhaust

Fertilized white Leghorn eggs were incubated with diluted (1:11, exhaust:air) light-irradiated or unirradiated exhaust from a gasoline engine operated with and without a catalytic converter. Exposure was maintained for about 14 days at particulate levels of approximately 0.7 or 15 mg/m^3. Exposure to unirradiated exhaust resulted in decreased survival and embryonic weight; irradiated exhaust had a less pronounced effect. Similar effects were seen with the catalytic converter, but they were less pronounced (Hoffman & Campbell, 1977, 1978).

Two studies have shown decreased fertility in mice following exposure to irradiated automobile exhaust [unspecifed] (Hueter *et al.*, 1966; Lewis *et al.*, 1967).

(iv) *Genetic and related effects*

The genetic and related effects of diesel and gasoline engine exhausts have been reviewed (Lewtas, 1982; Claxton, 1983; Holmberg & Ahlborg, 1983; Ishinishi *et al.*, 1986b; Lewtas & Williams, 1986).

Since engine exhaust is difficult to administer in short-term tests, studies have been conducted on several components and fractions of exhausts. Early studies were conducted on exhaust condensates; recent dilution sampling methods have permitted the collection of soot particles. Biological studies have been conducted on collected particles and on various extracts of particles, primarily extractable or soluble organic matter. Several solvents are effective for extracting organic material from diesel and gasoline particles (Claxton, 1983);

dichloromethane is that used most commonly. More volatile organic compounds are collected on adsorbent resins and extracted for bioassay. Only limited studies have been conducted on direct exposure to gaseous and whole exhausts.

Studies of genotoxicity are thus conducted on particles, particulate extracts, volatile organic condensates or whole emissions, and the results are expressed as activity per unit mass. In order to compare different emissions, genotoxicity is often expressed as emission rate or genotoxicity per unit distance driven or per mass of fuel consumed. Thus, for example, the mutagenic activity in *Salmonella typhimurium* TA98 of several gasoline particulate extracts is greater than that of diesel particulate extracts per unit mass of organic extract, while the mutagenic emission factor per kilometre driven for gasoline automobiles is less than that for diesel engines (Lewtas & Williams, 1986). The data on gasoline engine exhausts are considered together, whether or not the engine used was equipped with a catalyst and regardless of the type of fuel used (e.g., leaded or unleaded). When this information was available to the Working Group, however, it is noted in the text.

The genotoxic activity of diesel particulate extracts is generally decreased by the addition of a metabolic activation system (e.g., Aroclor 1254-induced or uninduced liver 9000 × *g* supernatant (S9), lung S9, microsomal preparations). In contrast, the genotoxicity of gasoline particulate extracts is generally increased by the addition of metabolic activation (Claxton, 1983; Lewtas & Williams, 1986).

Diesel engine exhaust

The soluble organic matter extracted from diesel particles obtained from the exhaust of several types of diesel engines induced DNA damage in *Bacillus subtilis* in the absence of an exogenous metabolic system at doses of 60–500 μg/ml (Dukovich *et al.*, 1981).

The majority of studies on the mutagenicity of diesel exhaust have been conducted in *S. typhimurium* on soluble or extractable organic matter removed from soot particles. The dichloromethane extractable organic matter from soot particles collected from two diesel engines was mutagenic to *S. typhimurium* TA1537, TA1538, TA98 and TA100 in the presence and absence of an exogenous metabolic system from Aroclor 1254-induced rat liver. In the presence of activation, one soot extract was weakly mutagenic to TA1535 (Huisingh *et al.*, 1978). Other studies of particulate extracts from the exhausts of various diesel engines and vehicles also induced mutation in *S. typhimurium* TA1537, TA1538, TA98 and TA100 with and without an exogenous metabolic system, but not in TA1535 (Clark & Vigil, 1980; Clark *et al.*, 1981; Claxton, 1981; Claxton & Kohan, 1981; Dukovich *et al.*, 1981; Belisario *et al.*, 1984). Diesel engine exhaust particulate extracts were also mutagenic in *S. typhimurium* TM677 and TA100 in a forward mutation assay using 8-azaguanine resistance (Claxton & Kohan, 1981; Liber *et al.*, 1981) and in mutagenesis assays in *Escherichia coli* WP2 and K12 (Lewtas, 1983; Lewtas & Williams, 1986). In these assays, except in *E. coli* K12 where metabolic activation was required, the particulate extracts were mutagenic both in the absence and presence of an exogenous metabolic system.

Fractionation of diesel engine exhaust particulate extracts resulted in fractions (aliphatic hydrocarbons in a paraffin fraction) that were not mutagenic to *S. typhimurium*

TA1535, TA1537, TA1538, TA98 or TA100, as well as in fractions that were highly mutagenic and contained most of the activity (moderately polar and highly polar neutral fractions; Huisingh *et al.*, 1978). Similar studies in *S. typhimurium* TA98 using different fractionation procedures showed that most of the mutagenic activity of diesel engine exhaust particulate extracts was in neutral and acidic fractions (Petersen & Chuang, 1982; Pitts *et al.*, 1982; Handa *et al.*, 1983; Schuetzle, 1983; Austin *et al.*, 1985). Separation of the neutral fraction on the basis of polarity resulted in concentration of the mutagenic activity in the aromatic, moderately polar and highly polar oxygenated fractions (Huisingh *et al.*, 1978; Rappaport *et al.*, 1980; Pederson & Siak, 1981; Petersen & Chuang, 1982; Schuetzle, 1983; Austin *et al.*, 1985).

Chemical characterization by the use of bioassays has been reviewed (Schuetzle & Lewtas, 1986). Such studies have shown that nitrated PAHs contribute to the mutagenicity of diesel particulate extracts. The first evidence for the presence of nitroarenes in diesel particulate extracts was provided when a decrease in mutagenicity was observed in nitroreductase-deficient strains of *S. typhimurium* (Claxton & Kohan, 1981; Löfroth, 1981a; Pederson & Siak, 1981; Rosenkranz *et al.*, 1981; Pitts *et al.*, 1982). The contribution of mono- and dinitro-PAHs to the mutagenicity of these extracts (20–55%) was estimated by measuring both nitro-PAH and mutagenicity in *S. typhimurium* TA98 in the same diesel particulate extracts (Nishioka *et al.*, 1982; Salmeen *et al.*, 1982; Nakagawa *et al.*, 1983; Schuetzle, 1983; Tokiwa *et al.*, 1986). Other oxidized PAHs in diesel particulate extracts, such as PAH epoxides (Stauff *et al.*, 1980), pyrene-3,4-dicarboxylic acid anhydride (Rappaport *et al.*, 1980) and 5*H*-phenanthro[4,5-*bcd*]pyran-5-one (Pitts *et al.*, 1982), have been shown to be mutagenic to *S. typhimurium*. The formation of both nitro- and oxidized PAH has been reviewed (Pitts, 1983).

The use of the *S. typhimurium* mutagenesis assay to investigate the bioavailability of mutagens has also been reviewed (Claxton, 1983; Lewtas & Williams, 1986). Diesel particles dispersed in dipalmitoyl lecithin, a component of pulmonary surfactant, in saline were mutagenic to *S. typhimurium* TA98 (Wallace *et al.*, 1987). One diesel soot particulate sample collected by electrostatic precipitation from a diesel automobile was directly mutagenic to *S. typhimurium* TA98, TA100, TA1538 and TA1537 in the absence and presence of an exogenous metabolic system from Aroclor 1254-induced rat liver when particles were added directly to the top agar (1–20 mg/plate) without prior extraction or suspension in dimethyl sulfoxide. The sample was not mutagenic to *S. typhimurium* TA1535 when tested at up to 20 mg/plate (Belisario *et al.*, 1984). Diesel soot particles were either not mutagenic or weakly mutagenic to *S. typhimurium* when incubated with physiological fluids such as serum, saline, albumin, lung surfactant and lung lavage fluid (Brooks *et al.*, 1980; King *et al.*, 1981; Siak *et al.*, 1981). Serum and lung cytosol (proteinaceous fluids) inhibited mutagenicity of diesel particulate extracts in *S. typhimurium* (King *et al.*, 1981). Engulfment and incubation of diesel particles with lung macrophages decreased their mutagenic activity (King *et al.*, 1983).

Filtered diesel exhaust was mutagenic to *S. typhimurium* TA100 and to *E. coli* WP2*uvr*A/pkM101 in the absence but not in presence of an exogenous metabolic system; a marginal response was obtained in *S. typhimurium* TA104 in the presence of an Aroclor

1254-induced liver metabolic system (Matsushita *et al.*, 1986). Gaseous emissions from diesel exhaust collected by condensation after dilution and filtration of the particles were mutagenic to *S. typhimurium* TA98 and TA100 in the absence of an exogenous metabolic system; addition of an Aroclor-induced liver metabolic system reduced their mutagenic activity (Rannug, 1983; Rannug *et al.*, 1983). These two approaches to testing the gaseous emissions from diesel engine exhaust thus both show that they are mutagenic to *S. typhimurium* TA98 and TA100 in the absence of an exogenous metabolic system. The studies differ in the quantitative estimates of the contribution that the gaseous emissions make to the total mutagenicity of diesel exhaust: direct testing of gaseous emissions suggests that the gas phase contributes at least 30 times more to the total mutagenicity than the particles (Matsushita *et al.*, 1986); testing of the condensation extract indicated that the gaseous emissions contributed less (up to 30%) than the particles to the total mutagenicity (Rannug, 1983). [The Working Group noted that the latter procedure could result in loss of some volatile components during sampling, extraction or preparation for bioassay.]

The urine of female Swiss mice exposed for 8 h per day on five days per week to whole diesel exhaust (dilution, 1:18; particles, 6–7 mg/m^3) for seven weeks (Pereira *et al.*, 1981c) or of Fischer 344 rats exposed to diesel exhaust particles (1.9 mg/m^3) for three to 24 months (Green *et al.*, 1983; Ong *et al.*, 1985) was not mutagenic to *S. typhimurium*. However, positive responses were obtained with the urine of Sprague-Dawley rats given 1000–2000 mg/kg bw diesel exhaust particles by gastric intubation or by intraperitoneal or subcutaneous administration (Belisario *et al.*, 1984, 1985). [The Working Group noted that this result can be taken as evidence for the bioavailability of mutagens from diesel particles.]

Particulate extracts of diesel engine exhaust emissions increased the number of mitotic recombinants in *Saccharomyces cerevisiae* D3 (Lewtas & Williams, 1986). Mitchell *et al.* (1981) also found a slight elevation in the number of recombinants with concentrations of 100–2000 μg/ml diesel exhaust, but the authors concluded that the results overall were negative. An 8-h exposure to an approximately five-fold dilution of exhaust (particles, 2.2 mg/m^3) from a diesel engine did not increase the incidence of sex-linked recessive lethal mutations in *Drosophila melanogaster* (Schuler & Niemeier, 1981).

Extracts from the emissions of diesel engines (up to 250 μg/ml) did not induce DNA damage in cultured Syrian hamster embryo cells, as determined by alkaline sucrose gradient centrifugation (Casto *et al.*, 1981). However, diesel exhaust particles (1 and 2 mg/ml) induced unscheduled DNA synthesis in tracheal ring cultures prepared from female Fischer 344 rats (Kawabata *et al.*, 1986).

As reported in an abstract, diesel engine emission particles and particulate extracts were more cytotoxic for excision repair-deficient xeroderma pigmentosum fibroblasts than for normal human fibroblasts (McCormick *et al.*, 1980).

Particulate extracts (2.5–150 μg/ml) from the exhaust of one light-duty diesel engine induced mutation to ouabain resistance in mouse BALB/c 3T3 cells in the absence and presence of an exogenous metabolic system, while no significant increase in mutation frequency was found with particulate extracts from another light-duty or from a heavy-duty diesel engine (Curren *et al.*, 1981). Another diesel engine exhaust extract induced mutation in the absence of metabolic activation (Lewtas & Williams, 1986).

In two separate studies, particulate extracts of diesel engine emissions from several passenger cars and one heavy-duty engine all induced mutations in mouse lymphoma L5178Y $TK^{+/-}$ cells. Maximal increases in mutation frequency occurred at concentrations of 20–300 μg/ml (Rudd, 1980; Mitchell *et al.*, 1981).

Particulate extracts (60 μg/ml) from the exhaust emission of five light-duty diesel passenger cars induced mutations to 6-thioguanine resistance in Chinese hamster CHO cells both in the absence and presence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Li & Royer, 1982). In another study, similar particulate extracts from two light-duty diesel engines (tested at 25–100 and 100–400 μg/ml) induced mutation in Chinese hamster CHO cells, but no mutagenic activity was observed with samples from one light-duty (up to 300 μg/ml) or one heavy-duty diesel engine (up to 750 μg/ml; Casto *et al.*, 1981). In a third study, extracts from the exhaust of a light-duty diesel engine (25–75 μg/ml) induced mutation in Chinese hamster CHO cells in the presence, but not in the absence, of an exogenous metabolic system (Brooks *et al.*, 1984). In a study on whole particles from diesel engines (500–750 μg/ml), mutations were induced in Chinese hamster CHO cells in the absence of an exogenous metabolic system (Chescheir *et al.*, 1981).

Diesel particulate extracts (100–200 μg/ml) from emissions of light-duty and heavy-duty diesel engines induced 8-azaguanine and ouabain resistance in Chinese hamster V79 cells. The light-duty samples were more mutagenic than the heavy-duty samples (Morimoto *et al.*, 1986). In another study, particulate extracts (up to 100 μg/ml) generated by a light-duty diesel engine did not induce mutation to 6-thioguanine, 8-azaguanine or ouabain resistance in Chinese hamster V79 cells (Rudd, 1980). [The Working Group noted the small number of plates used.]

Particulate extracts (100 μg/ml) of diesel exhaust induced mutation to trifluorothymidine and 6-thioguanine resistance in human TK6 lymphoblasts in the presence, but not in the absence of an exogenous metabolic system (Liber *et al.*, 1980; Barfknecht *et al.*, 1981).

Particulate extracts of emissions from three light-duty and one heavy-duty diesel engines (100–400 μg/ml) induced sister chromatid exchange in Chinese hamster CHO cells (Mitchell *et al.*, 1981; Brooks *et al.*, 1984).

When whole diesel exhaust was bubbled through cultures of human peripheral lymphocytes from four healthy nonsmokers, sister chromatid exchange was induced in two of the samples (Tucker *et al.*, 1986). Sister chromatid exchange was also induced in cultured human lymphocytes by a light-duty diesel particulate extract (5–50 μg/ml; Lockard *et al.*, 1982) and by diesel particulate extracts (10–200 μg/ml) from emissions of light-duty and heavy-duty diesel engines (Morimoto *et al.*, 1986). In the last study, light-duty samples were more potent in inducing sister chromatid exchange than heavy-duty samples.

A particulate extract (20–80 μg/ml) from the exhaust emission of one light-duty diesel engine induced structural chromosomal abnormalities in Chinese hamster CHO cells (Lewtas, 1982), but an extract from a similar engine did not (Brooks *et al.*, 1984).

A particulate extract (0.1–100 μg/ml) from the exhaust of a light-duty diesel engine induced chromosomal aberrations in cultured human lymphocytes in the absence of an

exogenous metabolic system. In the presence of metabolic activation, no increase in the total percentage of cells with aberrations was observed, although an increase in the number of chromosomal fragments and dicentrics was observed (Lewtas, 1982, 1983).

Particulate extracts (2.5–100 μg/ml) from the exhaust of one light-duty diesel engine induced morphological transformation in BALB/c 3T3 cells in the absence, but not in the presence, of an exogenous metabolic system from Aroclor 1254-induced rat liver (Curren *et al.*, 1981). Similar extracts from two other light-duty diesel engines and a heavy-duty diesel engine did not induce morphological transformation in these cells in the absence or presence of a metabolic system (Curren *et al.*, 1981, up to 300 μg/ml; Zamora *et al.*, 1983, up to 40 μg/ml). An extract from a light-duty diesel engine (2–10 μg/ml) induced morphological transformation in BALB/c 3T3 cells initiated by treatment with 3-methylcholanthrene (Zamora *et al.*, 1983).

Particulate extracts (31–500 μg/ml) from the emissions of three light-duty diesel engines enhanced transformation of Syrian hamster embryo cells in the presence of SA7 virus. No significant enhancement of transformation was observed with the corresponding extract (up to 500 μg/ml) from a heavy-duty engine (Casto *et al.*, 1981).

A particulate extract (5–10 μg/ml) of exhaust from a light-duty engine inhibited intercellular communication, as measured by metabolic cooperation in Chinese hamster V79 lung cells (Zamora *et al.*, 1983).

Primary cultures of 12-day-old hamster embryos from pregnant Syrian hamsters that received intraperitoneal injections of the neutral fractions of light-duty or heavy-duty diesel particulate extracts (2000–4000 mg/kg bw) on day 11 of gestation had an increased number of 8-azaguanine-resistant mutations (Morimoto *et al.*, 1986).

Exposure of B6C3F1 mice to whole diesel engine exhaust emission (12 mg/m^3 particles) for one month did not induce sister chromatid exchange in bone-marrow cells, but injection [unspecified] of either diesel particles (300 mg/kg bw) or their extract (800 mg/kg bw) resulted in an increased incidence of sister chromatid exchange in the bone marrow of mice sacrificed two days after treatment (Pereira, 1982).

No increase in the frequency of sister chromatid exchange was observed in the peripheral lymphocytes of Fischer 344 rats exposed to whole diesel engine exhaust emission (1.9 mg/m^3 particles) for three months (Ong *et al.*, 1985), and no significant increase was observed in bone-marrow cells of rats exposed to 4 mg/kg whole emissions from light- or heavy-duty diesel engines for up to 30 months (Morimoto *et al.*, 1986). [The Working Group could not determine the accumulated dose.]

Intratracheal instillation of diesel engine exhaust particles (6–20 mg) in male Syrian hamsters increased the incidence of sister chromatid exchange in lung cells, as did exposure of Syrian hamsters for 3.5 months to whole diesel engine exhaust emissions (particles, 12 mg/m^3; Guerrero *et al.*, 1981). Exposure of pregnant Syrian hamsters to whole diesel engine exhaust emissions (particles, 12 mg/m^3) from day 1 of gestation, or intraperitoneal administration of diesel engine exhaust particles at the LD_{50} (300 mg/kg bw) on day 12 of gestation, did not result in increased frequencies of sister chromatid exchange in fetal liver,

as determined on day 13. However, an increase was seen after intraperitoneal administration on day 12 of a dichloromethane extract of the particles (Pereira, 1982; Pereira *et al.*, 1982).

No increase in the frequency of micronuclei in bone marrow was found in male ICR mice exposed to whole exhaust emission from a light-duty diesel engine at particulate concentrations of 0.4 and 2.0 mg/m^3 for up to 18 months (Morimoto *et al.*, 1986), or in Swiss-Webster CD-1 mice or Fischer 344 rats exposed to whole emission (particles, 1.9 mg/m^3) for six months and two years, respectively (Ong *et al.*, 1985), or in B6C3F1 and Swiss mice and Chinese hamsters exposed to exhaust emissions for one to six months (particles, 6 mg/m^3) or for one month (particles, 12 mg/m^3); however, an increase was observed in Chinese hamsters exposed to 6 mg/m^3 for six months. There was a slight increase in the number of micronucleated bone-marrow cells in B6C3F1 mice, but not in Chinese hamsters, administered an extract of diesel particles (800 and 1000 mg/kg bw) intraperitoneally (Pereira *et al.*, 1981b,c; Pereira, 1982; Pepelko & Peirano, 1983). As reported in an abstract, extracts of diesel engine exhaust particles given intraperitoneally at concentrations of up to 1000 mg/kg bw to Chinese hamsters did not increase the frequencies of chromosomal aberrations, micronuclei or sister chromatid exchange in bone-marrow cells (Heidemann & Miltenburger, 1983).

No increase in the incidence of dominant lethal mutations was found when male T-stock mice exposed for 7.5 weeks to diesel exhaust (particulates, 6 mg/m^3; 8 h/day, 7 days/week) were mated with (101×C3H)F_1, (SEC×C57Bl)F_1, (C3H×C57Bl)F_1 or T-stock female mice or when female (101×C3H)F_1 mice were similarly exposed for 7 weeks prior to mating with untreated males. No increase in the frequency of heritable point mutations was found after T-stock males were similarly exposed to diesel exhaust [length of exposure not given] prior to mating, and no oocyte killing was observed in (SEC×C57Bl)F_1 female mice after exposure for eight weeks prior to mating (Pepelko & Peirano, 1983).

Gasoline exhaust

Gasoline exhaust emissions from both catalyst and noncatalyst automobiles, collected using several standard methods, were mutagenic to *S. typhimurium* TA98 and TA100 (Claxton & Kohan, 1981; Löfroth, 1981a,b; Ohnishi *et al.*, 1982; Zweidinger, 1982; Clark *et al.*, 1983; Handa *et al.*, 1983; Rannug, 1983; Rannug *et al.*, 1983; Brooks *et al.*, 1984; Norpoth *et al.*, 1985; Westerholm *et al.*, 1988). Addition of a catalyst, however, significantly decreases the rate of emission from gasoline engine vehicles of material that is mutagenic to these strains (Ohnishi *et al.*, 1980; Zweidinger, 1982; Rannug, 1983; Rannug *et al.*, 1983; Lewtas, 1985).

Extracts of particles collected from the exhaust pipes of gasoline automobiles [assumed to be noncatalyst, using leaded fuel] were mutagenic to *S. typhimurium* TA1537, TA98 and TA100 both in the absence and presence of an exogenous metabolic system from Aroclor-induced rat liver (Wang *et al.*, 1978). Particulate and condensate extracts of the exhausts of a noncatalyst gasoline engine and a catalyst (oxidizing) gasoline vehicle were mutagenic to *S. typhimurium* TA1538, TA98 and TA100 in the presence of an exogenous metabolic system from Aroclor-induced rat liver. The samples were either not mutagenic or weakly

mutagenic to *S. typhimurium* TA1535 (Ohnishi *et al.*, 1980). Dichloromethane extracts of soot particles from a gasoline catalyst vehicle were mutagenic to *S. typhimurium* TA98 and TA100 in the absence and presence of an exogenous metabolic system but were not mutagenic to *S. typhimurium* TA1535 (Claxton, 1981). Particulate extracts of gasoline catalyst engine (unleaded fuel) emissions were mutagenic to *S. typhimurium* TA98 in the absence and presence of an exogenous metabolic system and in *S. typhimurium* TA100 only in the presence of an exogenous metabolic system (Westerholm *et al.*, 1988).

Gas-phase emissions collected from catalyst and noncatalyst engines by condensation after dilution and filtration were mutagenic to *S. typhimurium* TA98 and TA100 in the absence of an exogenous metabolic system, and the contribution of the gas phase to the total mutagenicity ranged from 50–90% in the absence of activation. In the presence of a metabolic system, the mutagenicity was decreased (Rannug, 1983; Rannug *et al.*, 1983; Westerholm *et al.*, 1988).

After fractionation of gasoline engine exhaust particulate and condensate extracts, the neutral aromatic fraction, which contains the PAHs, was found to be mutagenic to *S. typhimurium* TA98 in the presence of an exogenous metabolic system (Löfroth, 1981b; Handa *et al.*, 1983); the highest dose-dependent increase in mutagenicity was induced by the four- to seven-ring PAH fraction in *S.* typhimurium TA98 and TA100 (Norpoth *et al.*, 1985). Handa *et al.* (1983) found the acidic fraction to be significantly more mutagenic in *S. typhimurium* TA98 in the absence than in the presence of an exogenous metabolic system.

Nitro-PAH are either not detectable or present at much lower concentrations in particulate extracts from gasoline engine exhausts than in diesel particle extracts (Nishioka *et al.*, 1982; Handa *et al.*, 1983). In studies using strains of *S. typhimurium* that do not respond to nitro-PAH, gasoline engine exhaust particulate extracts (Brooks *et al.*, 1984) and whole catalyst gasoline engine emissions (Jones *et al.*, 1985) were less mutagenic than in TA98 (in the absence of activation), suggesting the presence of nitroaromatic compounds. Löfroth (1981a), however, using similar techniques, did not see a decrease in mutagenicity attributable to nitro-PAHs. [The Working Group noted that these results are not necessarily inconsistent, since different strains and sampling methods were used.]

Several studies of exhaust emissions from vehicles run on gasoline blended with alcohol (10–23% ethanol or methanol) have shown either no significant change or a decreased emission rate of material mutagenic to *S. typhimurium* TA98 and TA100 (Clark *et al.*, 1983; Rannug, 1983; Clark *et al.*, 1984).

Particulate extracts of one unleaded gasoline catalyst engine exhaust emission tested at up to 1500 μg/ml did not induce mitotic recombination in *S. cerevisiae* D3 (Mitchell *et al.*, 1981).

An extract of emissions from an unleaded gasoline catalyst engine (250 μg/ml) induced DNA damage in cultured Syrian hamster embryo cells, as measured by alkaline sucrose gradients, in the absence of an exogenous metabolic system (Casto *et al.*, 1981).

Particulate extracts from the exhaust of an unleaded gasoline catalyst engine (2.5–500 μg/l) induced mutation to ouabain resistance in mouse BALB/c 3T3 cells in the absence and presence of an exogenous metabolic system (Curren *et al.*, 1981). Particulate extracts from unleaded gasoline catalyst automobiles and leaded gasoline noncatalyst automobiles (20–350 μg/ml) were mutagenic to mouse lymphoma L5178Y $TK^{+/-}$ cells. Metabolic activation increased the mutagenic activity (Mitchell *et al.*, 1981; Lewtas, 1982). Particulate extracts from the exhaust emission from a gasoline engine with catalytic converter (50–400 μg/ml) induced mutations to 6-thioguanine resistance in CHO cells in the absence of an exogenous metabolic system (Casto *et al.*, 1981). In another study, extracts from an unleaded gasoline catalyst engine (25–75 μg/ml) induced mutations to 6-thioguanine resistance in the *hgpt* locus in Chinese hamster CHO cells only in the presence of an exogenous metabolic system (Brooks *et al.*, 1984).

Particulate extracts of unleaded gasoline catalyst engine emissions (10–200 μg/ml) induced sister chromatid exchange in Chinese hamster CHO cells in the absence of an exogenous metabolic system (Mitchell *et al.*, 1981). Extracts from another unleaded gasoline catalyst engine exhaust (10–50 μg/ml) also induced sister chromatid exchange in Chinese hamster CHO cells both in the absence and presence of an exogenous metabolic system (Brooks *et al.*, 1984). Leaded gasoline noncatalyst engine exhaust particulate extracts induced sister chromatid exchange in Chinese hamster CHO cells in the presence of an exogenous metabolic system (Lewtas & Williams, 1986). [The Working Group noted that no data were provided on responses in the absence of an exogenous metabolic system.]

Extracts from an unleaded gasoline catalyst engine exhaust (20–60 μg/ml) induced chromosomal aberrations in Chinese hamster CHO cells in the presence of an exogenous metabolic system (Brooks *et al.*, 1984). Particulate extract [type of fuel and presence of catalyst unspecified] (0.6–5 μg/ml) induced aneuploidy and polyploidy in Chinese hamster V79 cells in the absence of an exogenous metabolic system (Hadnagy & Seemayer, 1986) and induced disturbance of the spindle apparatus (Seemayer *et al.*, 1987).

Dichloromethane particulate extracts from the exhaust of an unleaded gasoline catalyst engine (2.5–500 μg/ml) increased the frequency of morphological transformation of BALB/c 3T3 cells in both the absence and presence of an exogenous metabolic system from Aroclor 1254-induced rat liver (Curren *et al.*, 1981). Dichloromethane extracts of the particulate emissions of an unleaded gasoline catalyst engine (31–500 μg/ml) enhanced morphological transformation of Syrian hamster embryo cells in the presence of SA7 virus (Casto *et al.*, 1981).

In male BALB/c mice exposed to whole gasoline engine exhaust [type of fuel and presence of catalyst unspecified] emissions for 8 h per day for ten days and killed 18 h after the last exposure period, an increased frequency of micronucleated bone-marrow cells was found (Massad *et al.*, 1986).

(b) Humans

(i) *Deposition, clearance, retention and metabolism*

The factors affecting the uptake of gases and vapours, including model calculations for their absorption in the different regions of the human respiratory tract, have been summarized (Davies, 1985).

Diesel engine exhaust

No data on the deposition, clearance, retention or metabolism of diesel engine exhaust were available to the Working Group. A model has been developed to predict the deposition of diesel exhaust in humans (Yu & Xu, 1986; Xu & Yu, 1987; Yu & Xu, 1987).

Gasoline engine exhaust

The results of two laboratory experiments in which human volunteers inhaled the exhaust from an engine run on gasoline containing ^{203}Pb-tetraethyllead are summarized in Table 29. In one of the experiments, the exhaust was contained in a 600-l chamber; the concentrations of carbon monoxide and carbon dioxide were reduced using chemical traps; median particulate size was about 0.4 μm (Chamberlain *et al.*, 1975) or 0.35 and 0.7 μm, resulting in an aerosol considered typical of urban environments (Chamberlain *et al.*, 1978). In the other experiment, the exhaust was rapidly diluted in a wind tunnel which prevented coagulation of the primary exhaust particles and resulted in aerosols with median particulate sizes of 0.02–0.09 μm. Both experiments were conducted with a variety of breathing patterns, which were monitored but not controlled. Total deposition was relatively constant at 30% over a wide range of breathing patterns for sizes typical of urban aerosols (Chamberlain, 1985). However, as the size of the primary particles decreased (below 0.1 μm), deposition increased sharply, and the length of the respiratory cycle (time between the start of successive breaths) significantly affected deposition. [The Working Group noted that these data are in broad agreement with those for other particulate materials of similar size (Heyder *et al.*, 1983; Schiller *et al.*, 1986.]

In a separate analysis of the same data, deposition was shown to increase with respiratory cycle in an approximately linear fashion — ranging from 10% at 3 sec to 55% at 20 sec; the slope of this line was somewhat dependent on tidal volume. A small, but significant effect of expiratory reserve volume on deposition was observed: total deposition dropped by a factor of 1.2 for an increase in expiratory reserve volume of 2.5 l (Wells *et al.*, 1977).

In a third study, measurements of total deposition were performed in the field by comparing inhaled and exhaled airborne lead concentrations; the method was found to give results comparable to experimental measurements involving ^{203}Pb. Total deposition was measured for inhalation at an average breathing pattern of 0.8 l and a respiratory cycle of 5.2 sec in persons seated by a motorway (61%), by a roundabout (64%), in an urban street (48%) and in a car park (48%). Median particulate sizes in the breath of persons near quickly moving traffic (0.04 μm) were found to be much smaller than those in persons in the urban

Table 29. Total deposition (%) of leaded gasoline particles as a function of size and breathing pattern[a]

Particulate diameter (μm)	Tidal volume in litres (respiratory cycle in seconds)										
	0.5 (2)	0.5 (4)	1.0 (4)	1.5 (4)	0.5 (6)	1.0 (6)	1.5 (6)	0.5 (8)	1.0 (8)	2.0 (8)	1.5 (12)
0.02	53	64		86		82		86			86
0.04			42		40	58	56		55	61	
0.09		35				32			27		
0.35										38	
~0.4[b]		32	26		42	46	36		37	62	62
0.70							40			50	

[a]Compiled by the Working Group from Chamberlain *et al.* (1978), except where noted

[b]From Chamberlain *et al.* (1975); individual data grouped by the Working Group according to breathing pattern and particle size

environment or in a car park (0.3 μm), although the air near roundabouts also contained a large proportion by mass of adventitious particles (2 μm) (Chamberlain *et al.*, 1978).

Lung clearance was best described by a four-component exponential clearance. The first two phases (half-times, 0.7 and 2.5 h) were similar for exhaust particles, lead nitrate (which is soluble) and lead oxide (which is insoluble), and therefore probably represent mucociliary clearance (Chamberlain *et al.*, 1975, 1978). On average, 40% of lung deposition of 0.35-μm aerosols was in the pulmonary region and 60% in the tracheobronchial region. The removal of lead compounds from the pulmonary region was described by a two-component exponential with half-times of 9 and 44 h; one exception was the removal of lead from highly carbonaceous particles, which exhibited half times of 24 and 220 h (Chamberlain *et al.*, 1978; Chamberlain, 1985).

No data on the metabolism in humans of gasoline engine exhaust were available to the Working Group.

(ii) *Toxic effects*

Early studies involving human volunteers showed that exposure to gasoline engine exhaust may cause headache, nausea and vomiting (Henderson *et al.*, 1921). Sayers *et al.* (1929) monitored the carbon monoxide content of gasoline engine exhaust gas-air mixtures and found a relationship between increasing carbon monoxide concentration, carboxyhaemoglobin (COHb) level and reports of headache in six men exposed to atmospheres containing 229–458 mg/m^3 carbon monoxide. In a more recent study of ten patients with angina (Aronow *et al.*, 1972), significant increases in COHb levels and significant reductions in exercise performance until onset of angina symptoms were observed in persons driving for 90 min in heavy traffic, as compared with tests both before the experiment and after breathing purified air for 90 min.

Among six volunteers exposed for 3.7 h to diesel engine exhaust gases containing about 4 mg/m^3 nitrogen dioxide, there was no increase in urinary thioether concentration (Ulfvarson *et al.*, 1987).

Effects of exposure to diesel engine exhaust on the lung have been reviewed (Calabrese *et al.*, 1981). Although bus garage and car ferry workers, exposed occupationally to mixtures of gasoline and diesel engine exhausts, had lower mean levels of respiratory function (forced respiratory volume in 1 sec (FEV_1) and forced vital capacity (FVC)) than expected, they showed no change in these measures over working shifts (for exposure measurements, see Tables 17 and 21, respectively). In contrast, workers on roll-on roll-off ships, exposed mainly to diesel engine fumes, showed statistically significant reductions in FEV_1 and FVC during working shifts (for exposure measurements, see Table 18). These reductions were reversible, however, the levels returning to normal after a few days with no exposure. The work-shift concentrations of nitrogen dioxide and carbon monoxide in these three groups averaged 0.54 mg/m^3 and 1.1 mg/m^3, respectively (Ulfvarson *et al.*, 1987). A small reduction in FEV_1/FVC and in $FEF_{25-75\%}$ (forced expiratory flow at 25–75% of forced vital capacity) was also observed at the end of a work shift among a group of chain-saw operators (Hagberg *et al.*, 1983; for exposure measurements, see Table 22). Concentrations of diesel engine emissions in coal mines, involving, on average, 0.6 mg/m^3 nitrogen dioxide and 13.7 mg/m^3 carbon monoxide, were not associated with decrements in the miners' ventilatory function (Ames *et al.*, 1982).

Studies in which changes in COHb levels were investigated over the course of a work shift are summarized in section 2 (pp. 69–73).

Possible effects on the lung of chronic occupational exposures to low levels of diesel engine exhaust emissions were studied cross-sectionally in railroad engine house workers (Battigelli *et al.*, 1964), in iron ore miners (Jörgensen & Svensson, 1970), in potash miners (Attfield *et al.*, 1982), in coal miners (Reger *et al.*, 1982; for exposure measurements, see Table 15), in salt miners (Gamble *et al.*, 1983), in coal miners exposed to oxides of nitrogen generated (in part) by diesel engine emissions underground (Robertson *et al.*, 1984) and in bus garage workers (Gamble *et al.*, 1987b). Effects of relatively high concentrations of automobile emissions have been described among bridge and road tunnel workers in two large cities (Speizer & Ferris, 1963; Ayres *et al.*, 1973; for exposure measurements, see Table 19). Changes in lung function over a five-year period have also been studied longitudinally among coal miners working underground in mines with and without diesel engines (Ames *et al.*, 1984). Some, but not all, of the results from these various studies showed decrements in lung function and increased prevalence of respiratory symptoms in subgroups exposed to engine emissions.

Exposure to engine exhaust has also been associated with irritation of the eyes (Waller *et al.*, 1961; Battigelli, 1965; Hamming & MacPhee, 1967; Hagberg *et al.*, 1983).

A 15-year follow-up of 34 156 members of a heavy construction equipment operators' union showed a highly significant overall excess of deaths certified as due to emphysema (116 observed, 70.2 expected), and this excess appeared to be higher among men with longer membership in the union (Wong *et al.*, 1985). No data on smoking habits were included in the mortality analyses, and the authors noted that they were unable to estimate the degree to

which exposure to diesel engine emissions (as distinct from other occupational factors, such as exposure to dust) might have contributed to the excess mortality from emphysema.

Another cohort study, of 1558 white motor vehicle examiners, yielded a slight excess of deaths from cardiovascular disease (124 observed, 118.4 expected) in a 29-year follow-up. The excess was more pronounced for deaths occurring during the first ten years of employment (28 oberved, 20.9 expected; Stern *et al.*, 1981). [The Working Group noted that the excesses observed are easily attributable to chance ($p > 0.1$).] A 32-year follow-up of 694 Swedish bus garage employees also showed a small, statistically nonsignificant, excess of deaths from cardiovascular disease (121 observed, 115.9 expected) which showed no pattern to indicate a relation to probable intensity or duration of exposure to diesel emissions (Edling *et al.*, 1987). Moreover, Rushton *et al.* (1983) found no excess of deaths from cerebrovascular or ischaemic heart disease among maintenance workers in London bus garages. A 27-year follow-up of 3886 potash miners and millers also showed no excess mortality that could be attributed to the presence of diesel engines in some of the mines that were studied; in only two of eight mines had diesel engines been used (Waxweiler *et al.*, 1973). None of these four analyses of mortality included adjustments for the men's smoking habits. However, the authors noted that the US potash workers whom they had studied included a greater proportion of cigarette smokers than among all US males.

(iii) *Effects on reproduction and prenatal toxicity*

No data were available to the Working Group.

(iv) *Genetic and related effects*

The frequency of chromosomal aberrations in cultured lymphocytes from 14 male miners exposed to diesel engine exhaust (five were smokers) was no greater than in 15 male office workers (five smokers; Nordenson *et al.*, 1981). The incidence of chromosomal changes was also investigated in four groups of 12 men: drivers of diesel-engine trucks, drivers of gasoline-engine trucks, automobile inspectors and a reference group, matched with respect to age, smoking habits and length in the jobs. The frequencies of gaps, breaks and sister chromatid exchange in lymphocyte preparations were not significantly different in the four groups (Fredga *et al.*, 1982). [The Working Group noted the small number of subjects in both of these studies.]

Among workers with relatively heavy exposure to diesel engine exhaust — in particular, crews of roll-on roll-off ships and car ferries and bus garage staff (the latter two groups also having exposure to gasoline engine exhausts) — no difference in mutagenicity to *S. typhimurium* TA98 or *E. coli* WP2 *uvrA* was observed between urine collected during exposed periods and that collected during unexposed periods. Similarly, no increase in urinary mutagenicity was found among six volunteers before and after an experimental exposure to diesel engine exhaust gases from an automobile run for 3.7 h at 60 km/h, 2580 revolutions/min (Ulfvarson *et al.*, 1987).