# FINAL

## Report on Carcinogens Background Document for

# Diesel Exhaust Particulates

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Prepared for the:
**U.S. Department of Health and Human Services**
**Public Health Services**
**National Toxicology Program**
**Research Triangle Park, North Carolina 27709**

Prepared by:
**Technology Planning and Management Corporation**
**Canterbury Hall, Suite 310**
**4815 Emperor Boulevard**
**Durham, NC 27703**
**Contract Number NOI-ES-85421**

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# Summary Statements

## Result of NIEHS Report on Carcinogens Review Group (RG1) Review

### Carcinogenicity

Diesel exhaust particulates are *known to be a human carcinogen* based on findings of elevated lung cancer rates in occupational groups exposed to diesel exhausts (IARC 1989; Cohen and Higgins, 1995; Bhatia *et al.,* 1998) and supporting animal and mechanistic studies. Diesel exhaust exposure is associated with an increased relative risk for lung cancer in the majority of reported human studies. The association is found consistently in the more informative studies, which employed semiquantitative estimates of exposure (Steenland et al., 1998). The overall relative risk is about 1.3, and higher risks are found in more heavily exposed subgroups. The increased relative risk is not readily explained by confounding by smoking or asbestos exposure.

Studies of the carcinogenicity of diesel exhaust particulates in animals have shown a consistent lung tumor response in rats, but not in the mouse or hamster. The response in rats appears to be due to the particulate component of exhaust, as the filtered vapor phase of exhaust has been shown not to be tumorigenic. Solvent extracts of diesel exhaust particles are carcinogenic when applied to the skin, or administered by intratracheal instillation or intrapulmonary implantation to rats, mice or hamsters.

### Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Diesel exhaust is a complex mixture of combustion products of diesel fuel, with the exact composition depending on the type of engine, the speed at which it is run, and the composition of the fuel used. Diesel exhaust contains identified mutagens and carcinogens both in the vapor phase and associated with respirable particles. Diesel exhaust particles are considered likely to account for the human lung cancer findings because they are almost all of a size allowing penetration to the entire lung, and because mutagenic and carcinogenic chemicals including polyaromatic hydrocarbons and nitroarenes have been extracted from these particles with organic solvents, or with a lipid component of mammalian lung surfactant.

While diesel particulate exposures produce lung cancer in rats, the relevance of this result in predicting the human response has been questioned because diesel exhaust particulate exposure produces a characteristic spectrum of inflammatory and neoplastic pulmonary responses in the rat; responses also seen with other inhaled particles of varying toxicity, and apparently little influenced by the chemical constituents of the particles. Although the precise bioavailability of chemical mutagens and carcinogens from inhaled diesel particulates is not known, DNA adducts attributed to diesel exhaust particulate exposures have been demonstrated in the lung of exposed rats. Similarly, lymphocyte-DNA adducts were found higher in several studies of occupational groups exposed to diesel exhaust than in groups with lower or ambient exposures, although diesel exhaust exposure was not quantified in these studies and exposure to used motor oil likely contributed to the adducts observed in one study.

## Result of NTP Executive Committee Interagency Working Group for the Report on Carcinogens (RG2) Review

### Carcinogenicity

Exposure to diesel exhaust particulates is *reasonably anticipated to be a human carcinogen,* based on findings of elevated lung cancer rates in occupational groups exposed to diesel exhaust (IARC, 1989; Cohen and Higgens, 1995; Bhatia *et al.*, 1998) and supporting animal and mechanistic studies. An increased risk of lung cancer is found in the majority of human studies. The overall relative risk is about 1.3, and higher risks are found in more heavily exposed subgroups in some studies. The increased risk is not readily explained by confounding by either smoking or asbestos exposure. However, the increased risk cannot always be clearly ascribed to diesel exhaust exposure. Although some studies employed semiquantitative estimates of diesel exhaust exposure (*eg,* Steenland *et al.,* 1998), most studies used inadequate measures of exposure.

Studies of the carcinogenicity of diesel exhaust particulates in animals have shown a consistent lung tumor response in rats, but not in the mouse or hamster. The response in rats appears to be due to the particulate component of exhaust, as the filtered vapor phase of exhaust has been shown not to be tumorigenic. Solvent extracts of diesel exhaust particles are carcinogenic when applied to the skin, or administered by intratracheal instillation or intrapulmonary implantation to rats, mice or hamsters.

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# 1 Physical and Chemical Properties

## 1.1 Chemical Identification

Diesel engine exhaust is a complex mixture of numerous gases and fine particles. The engine emissions vary depending on engine type, operating conditions, fuel composition, lubricating oil, additives, and emission control system (Ullman 1989; Obert 1973). The emissions are separated using liquid chromatography and consist of a nonpolar fraction (57%) (Schuetzle 1983), a moderately polar fraction (9%), and a polar fraction (32%) (Schuetzle 1983; Schuetzle *et al.* 1985), with the remainder unrecoverable. The nonpolar fraction is composed of aliphatic hydrocarbons, with polycyclic aromatic hydrocarbons (PAH) and alkyl-substituted PAH as minor components. The moderately polar fraction contains oxygenated and nitrated PAH species. The polar fraction consists mainly of carboxylic and dicarboxylic acids of PAH and PAH derivatives. The nitro-PAH (nitroarenes) are present in diesel particulate extracts in much lower concentrations than the parent PAH (Schuetzle *et al*. 1981; Schuetzle *et al*. 1982; Paputa-Peck *et al*. 1983). In the moderately polar fraction oxy-PAHs were in higher concentration than nitro-PAHs (Schuetzle 1983).

Diesel engines operate with excess air (~25-30 parts air to 1 part fuel) (Lassiter and Milby 1978). As a result, the gas phase fraction is composed primarily of typical combustion gases such as nitrogen ($N_2$), oxygen ($O_2$), carbon dioxide ($CO_2$), and water vapor ($H_2O$). As a result of incomplete combustion, the gaseous fraction also contains pollutants such as carbon monoxide (CO), sulfur oxides ($SO_x$), nitrogen oxides ($NO_x$), volatile hydrocarbons, and low molecular weight PAH and their derivatives. Appendix A-3 summarizes a list of chemicals found in diesel particulates. Diesel particulate matter comprises mainly aggregates of spherical carbon particles coated with organic and inorganic substances. Table 1-1 presents the particulate matter emissions and distribution between Total Carbon (TC) and Organic Carbon (OC) for both the Heavy- (HDD) and the Light- (LDD) Duty Diesel engines.

**Table 1-1. Particulate matter emissions and distribution of Total Carbon (TC) and Organic Carbon (OC) for HDD and LDD engines**

| Emission | HDD | LDD | Reference |
|---|---|---|---|
| Particulate matter in g/mi (g/km) | 3.2 (2)<br>1.4 (0.87)<br>0.99 (0.62) | 0.6 (0.37) | Williams *et al*. (1989)<br>Pierson & Brachaczek (1983)<br>Westerholm *et al*. (1991) |
| TC (% wt/wt) | 78 | 80 | Williams *et al.* (1989) |
| OC (% of TC) | 58<br>30 | 36 | Williams *et al.* (1989)<br>Pierson and Brachaczek (1983) |

## 1.2 Identification of Compounds

The inorganic fraction of the particulate phase of diesel fuel combustion emissions primarily consists of small elemental carbon particles ranging from 0.01-0.08 microns in diameter. The organic and elemental carbon accounts for approximately 80% of the total particulate matter mass. The remaining 20% is composed of sulfate (mainly $H_2SO_4$) (Pierson and Brachaczek 1983) and some inorganic additives and components of fuel and motor oil. Table 1-2 summarizes the composition and emission of airborne particulate matter from on-road vehicles.

**Table 1-2. Airborne particulate composition and emission from on-road vehicles**

| Inorganic constituents | Diesel emissions (mg/km [% of total mass]) |
|---|---|
| H | 47 ± 11(5 ± 1) |
| B | 1.14 ± 0.16 (0.13 ± 0.02) |
| C | 725 ± 117 (84 ± 14) |
| N | 16 ± 2 (1.9 ± 0.3) |
| Na | 6.6 ± 1.0 (0.8 ± 0.1) |
| Mg | 8 ± 1 (0.9 ± 0.15) |
| Al | 8.5 ± 1 (1.0 ± 0.2) |
| Si | 14 ± 2 (1.6 ± 0.2) |
| P | 1.3 ± 0.2 (0.15 ± 0.02) |
| S [$SO_4^{-2}$] | 23[42 ± 5] (2.7[4.9 ± 0.9]) |
| K | 1.5 ± 0.2 (0.17 ± 0.03) |
| Ca | 5.8 ± 1.4 (0.7 ± 0.2) |
| Ti | 0.12 ± 0.03 (0.014 ± 0.004) |
| Mn | 0.34 ± 0.04 (0.04 ± 0.004) |
| Fe | 5.0 v 0.09 (0.6 ± 0.1) |
| Cu | 0.22 ± 0.09 (0.025 ± 0.01) |
| Zn | 1.4 ± 0.1 (0.16 ± 0.1) |
| Ba | 0.66 v 0.03 (0.08 ± 0.033) |
| Pb | 11.5 ± 3 (1.3 ± 0.3) |

Pierson and Brachaczek (1983)

In general, the organic compounds identified in diesel exhaust emissions contain hydrocarbons, hydrocarbon derivatives, PAH, PAH derivatives, multifunctional derivatives of PAH, heterocyclic compounds, heterocyclic derivatives, and multifunctional derivatives of heterocyclic compounds (Schuetzle 1988).

The organic fractions consist of soluble organic compounds such as aldehydes, alkanes, alkenes, and high molecular weight PAH and PAH-derivatives. Table 1-3 lists compounds identified in the PAH fraction of diesel exhaust particulates (DP). The particulate size in diesel exhaust is small and respirable. Carbonaceous, diesel emitted particles have high specific surface areas of 30-50 $m^2/g$ (Frey and Corn 1967). After removing extractable organic material, the surface area increases up to 90 $m^2/g$ (Pierson and Brachaczek 1976). Because of their high surface area, DP are capable of adsorbing relatively large amounts of organic material.

The adsorbed elements come from unburned fuel, lubricating oil, and pyrosynthesis during fuel combustion. A variety of mutagens and carcinogens such as PAH and nitro-PAH (see for example NRC 1982; Tokiwa and Ohnishi 1986; WHO 1996) are adsorbed by the particulates. There is sufficient evidence for the carcinogenecity for 15 PAHs (a number of these PAHs are found in diesel exhaust particulate emissions) in experimental animals (RoC 1997) (http://ehis.niehs.nih.gov/roc/). The nitroarenes (five listed) meet the established criteria for listing as "reasonably anticipated to be a human carcinogen" based on carcinogenicity experiments with experimental animals (http://ehis.niehs.nih.gov/roc/). The extractable fraction of DP is typically in the 20-30% range, but may be as high as 90% (Williams *et al.* 1989), depending upon vehicle type and operating conditions. In general, under low load, diesel engine incomplete combustion results in relatively low particle concentrations and a higher proportion of organic associated particles (Dutcher *et al.* 1984).

**Table 1-3. Identification of compounds in the polynuclear aromatic hydrocarbon fraction. Concentration range as found in particulate extracts of three VW passenger cars.**

| Compound | Molecular Weight | CAS number | Concentration (ng/mg extract) |
|---|---|---|---|
| Acenaphthalene | 152 | 208-96-8 | 30 |
| Trimethylnaphthalene | 170 | 28652-77-9 | 140-200 |
| Fluorene | 166 | 86-73-7 | 100-168 |
| Dimethylbiphenyl | 182 | 28013-11-8 | 30-91 |
| $C_4$Naphthalene | 184 | 2717-39-7 | 285-351 |
| 2,4,6-Trimethylbiphenyl | 196 | 3976-35-0 | 50 |
| Dibenzothiophene | 184 | 132-65-0 | 129-246 |
| Phenanthrene | 178 | 85-01-8 | 2186-4883 |
| Anthracene | 178 | 120-12-7 | 155-356 |
| Methyldibenzothiophene | 198 | 30995-64-3 | 520-772 |
| Methylphenanthrene | 192 | 31711-53-2, 28652-81-5 | 2028-2768 |
| Methylanthracene | 192 | 26914-18-1 | 517-1522 |
| Ethylphenanthrene | 206 | 30997-38-7 | 388-464 |
| 4H-cyclopenta[def]phenanthrene | 190 | 203-64-5 | 517-1033 |
| Ethyldibenzothiophene | 212 | 79313-22-7 | 151-179 |
| 2-Phenylnaphthalene | 204 | 612-94-2 | 650-1336 |
| Dimethylphenanthrene/anthracene | 206 | 29062-98-4 | 1298-2354 |

| Compound | Molecular Weight | CAS number | Concentration (ng/mg extract) |
|---|---|---|---|
| Fluoranthene | 202 | 206-44-0 | 3399-7321 |
| Benzo[def]dibenzothiophene | 208 | 30796-92-0 | 254-333 |
| Benzacenaphthylene | 202 | 76774-50-0 | 791-1643 |
| Pyrene | 202 | 129-00-0 | 3532-8002 |
| EthylMethylphenanthrene | 220 | 71607-66-4 | 590-717 |
| Methylfluoranthene/pyrene | 216 | 2381-21-7 | 1548-2412 |
| Benzo[a]fluorene<br>Benzo[b]fluorene | 216 | 238-84-6,30777-18-5<br>243-17-4,30777-19-6 | 541-990 |
| Benzo[b]naptho[1,2-d]thiophene | 234 | 205-43-6 | 30-53 |
| Cyclopentapyrene | 226 | 83381-96-8 | 869-1671 |
| Benzo[ghi]fluoranthene | 226 | 203-12-3 | 217-418 |
| Benz[a]anthracene | 228 | 56-55-3 | 463-1076 |
| Chrysene | 228 | 218-01-9 | 657-1529 |
| 1,2 –Binaphthyl | 254 | 4325-74-0 | 30-50 |
| Methyl[benz]anthracene | 242 (various isomers) | 2498-77-3, 2498-76-2, 316-49-4, 2381-31-9, 2381-16-0, 2381-15-9, 6111-78-0, 2422-79-9, 2541-69-7 | 30-50 |
| 3-Methylchrysene | 242 | 3351-31-3 | 50-192 |
| Phenyl(phenanthrene/anthracene) | 254 | 52856-52-7, 28802-91-7 | 210-559 |
| Benzo[j] Fluoranthene | 252 | 205-82-3 | 492-1367 |
| Benzo[b] Fluoranthene | 252 | 205-99-2 | 421-1090 |
| Benzo[k] Fluoranthene | 252 | 207-08-9 | 91-289 |
| Benzo[a]pyrene | 252 | 50-32-8 | 208-558 |
| Benzo[e]pyrene | 252 | 192-97-2 | 487-946 |
| Benz[a,h]anthracene | 278 | 53-70-3 | 50-96 |
| Indenol[1,2,3-cd]Pyrene | 276 | 193-39-5 | 30-93 |
| Benzo[ghi]perylene | 276 | 1105-45-7 | 443-1050 |
| Dibenzo[a,i]pyrene | 302 | 189-55-9 | 136-254 |

Tong and Karasek (1984)

1-Nitropyrene is the predominant component of diesel exhaust and concentrations range from about 7 to 165 μg/g of particles (Levsen 1988). A list of nitro-PAH identified in LDD and their concentrations are presented in Table 1-4. Nitro-PAH have, in recent years, caused great environmental concern because of their potent mutagenicity and potential carcinogenicity (Tokiwa and Ohnishi 1986; WHO 1996).

**Table 1-4. Concentration of Nitro-PAH in LDD particulate extract**

| Nitro-PAH[a] | Concentration (μg/g of particles) |
|---|---|
| 4-Nitrobiphenyl | 2.2 |
| 2-Nitrofluorene | 1.8 |
| 2-Nitroanthracene | 4.4 |
| 9-Nitroanthracene | 1.2 |
| 9-Nitrophenanthrene | 1.0 |
| 3-Nitrophenanthrene | 4.1 |
| 2-Methyl-1-nitroanthracene | 8.3 |
| 1-Nitrofluoranthene | 1.8 |
| 7-Nitrofluoranthene | 0.7 |
| 3-Nitrofluoranthene | 4.4 |
| 8-Nitrofluoranthene | 0.8 |
| 1-Nitropyrene | 18.9; 75[b] |
| 6-Nitrobenzo[a]pyrene | 2.5 |
| 1,3-Dinitropyrene[b] | 0.30 |
| 1,6-Dinitropyrene[b] | 0.40 |
| 1,8-Dinitropyrene[b] | 0.53 |
| 2,7-Dinitrofluorene[c] | 4.2; 6.0 |
| 2,7-Dinitro-9-fluorenone[c] | 8.6; 3.0 |

a: Campbell and Lee (1984), b: Paputa-Peck *et al*. (1983), c: Schuetzle (1983).

## 1.3 Physical—Chemical Properties

### *1.3.1 Diesel Engines*

Diesel engines are classified as either two or four stroke cycle, direct or indirect injected, and naturally aspirated or supercharged. Typically, they are further separated according to their service requirements, such as light- or heavy-duty, small or large industrial, and rail or marine. Most of the studies reviewed in the following sections considered light- and/or heavy-duty on road vehicles.

Light-duty and heavy-duty diesel engines' operating conditions differ in terms of engine speed, expected load, fuel composition, and engine emission controls. Typically, light-duty vehicles such as automobiles and light trucks operate at higher speeds than heavy-duty vehicles such as trucks. The total particulate emission concentration from light-duty diesel engines is much smaller than from heavy-duty diesel engines. In general, newer heavy-duty trucks emit about 20 times more particulates than catalyst equipped gasoline fueled vehicles (WHO 1996). Depending on operating conditions, however, light duty diesel engines can emit 50 to 80 times, and heavy duty diesel engines 100 to 200 times, more particulate mass than typically equipped gasoline engines (McClellan 1986).

### *1.3.2 Diesel Fuel*

EPA regulations from 1983-1993, and the Clean Air Act Amendments from 1994-1998, reduced federal emissions standards for nitrogen oxides and particulate matter in diesel exhaust emission, resulting in changes in diesel fuel quality. The contributing factors for diesel particulate emissions are the sulfur and PAH content in fuel. The higher the PAH and/or sulfur content, the higher the particulate emissions. Sulfur content in fuel ranged from 0.3 to 0.24 weight percent at the end of 1993.

A change in EPA regulations (which required a maximum sulfur content of 0.05 weight percent, effective October 1 1993, aimed to reduce sulfur oxides particulates in diesel exhaust) resulted in a sharp decline in the fuel sulfur content to 0.03 in 1994. The resulting sulfur oxide concentration in diesel exhaust would be proportional to 88% to 90% from the previous year (API 1998). The EPA regulation also included a requirement to equal or exceed 40.0 cetane index (35 volume percent aromatics content) (cetane index is a calculated value, derived from fuel density and volatility, giving a reasonably close approximation to cetane number. Cetane number is a measure of ignition quality of diesel fuel).

From 1994 the average aromatics in No. 2 regular diesel declined from 35 to 40 volume percent to 32 to 35 volume percent (API 1998). Premium grade diesel fuel differs from regular grade in having slightly lower sulfur and aromatics content, 90 percent lower distillation temperature, and a small increase in cetane number (API 1998).

### *1.3.3 Experimental Conditions*

In the studies reviewed in the following sections, experiments were conducted using conditions representative of the most frequent load and speed reached by light- and/or heavy-duty engines. In the course of these studies, exhaust was typically passed through a standard exhaust system into the experimental chamber duplicating the diesel exhaust from LDD and/or HDD engines.

# 2 Human Exposure

## 2.1 Environmental Exposure

Many studies have attempted to estimate levels of exposure to diesel exhaust particulates (DP). Based on numerous methods of analysis (various samplings, extraction, fractionation, and chemical analyses), carbon monoxide, nitrogen oxides, various hydrocarbons, and lead are known to be major constituents of diesel fuel combustion (IARC 1989). Various natural and artifical sources (such as tobacco smoke and combustion products), however, can generate particles that resemble DP, resulting in misleading exposure values. Because DP may undergo further atmospheric reactions, the data may be further confounded. Further, exposure sources may also be difficult to identify since populations at risk tend to be mobile (IARC 1989).

## 2.2 Biomarkers of Exposure

Various methods may be used to detect occupational exposures to respirable particles emitted by diesel and gasoline engines. Chemical tracers, unique to the combustion source, are used in apportionment studies. It is important to note that these promising tracers may be invalid. Owing to fuel source and combustion temperature variables, they may not be representative of the total sample. Biomarkers used in studies to determine exposure to DP include barium (IARC 1989), 1-hydroxyprene (Kanoh *et al.* 1993), elemental carbon (Zaebst *et al.* 1991), respirable-size particles, and dichloromethane (Hammond *et al.* 1988).

### *2.2.1 Barium*

Barium is used to determine levels of DP from various combustion sources because it is used exclusively as a diesel fuel additive. Comparison with lead emissions from diesel and gasoline vehicles can contribute to more accurate estimations of DP exposure. Barium has limited value as a characterization tool, however, because the diesel fuel used in certain industries (*e.g.*, mining, railroad, and heavy-equipment operations) do not use barium as an additive.

### *2.2.2 1-Hydroxypyrene*

1-Hydroxypyrene (1-POH) is a metabolite of pyrene that has shown promise as a marker for estimating polycyclic aromatic hydrocarbon concentrations, a byproduct of diesel fuel emissions. Studies have shown that 1-POH levels in rat urine are dependent upon pyrene doses. Human studies have also shown a linear relationship. Schoolchildren in areas of high traffic volume also had elevated urinary levels of 1-POH (Kanoh *et al.* 1993).

### *2.2.3 Elemental Carbon*

Field studies and laboratory results have shown how elemental carbon levels may help determine exposure to diesel fuel exhaust. A single-stage personal impactor with a quartz filter was used to collect submicrometer-sized diesel particles. Thermal-optical analysis can ascertain the concentration of elemental carbon. Background levels may be measured and an adjusted exposure level calculated. A controlled smoking chamber study was performed to observe the effect on elemental carbon levels. Elemental carbon was found to comprise 1.8% of the total carbon even in the presence of extremely high concentrations of tobacco smoke. Thus, elemental carbon is an excellent method to determine diesel fuel exhaust exposure independent of tobacco smoke levels (Zaebst *et al.* 1991).

### 2.2.4 Respirable-Size Particles and Dichloromethane

Because diesel particulates are much too complex to measure directly, respirable-sized particles (RSP) [< 10 μ] are used as a surrogate to estimate DP exposures. Measuring RSP is relatively simple and inexpensive. Concentrations of RSP, however, must be corrected for the presence of environmental tobacco smoke (ETS) and other inorganic particulate matter. Since nicotine is also a by-product of tobacco products, measurements of nicotine levels can help differentiate between DP and the fraction of the RSP from tobacco smoke. Inorganic sources of RSP can skew DP exposure data. Diesel fuel emissions have been found to contain high levels of dichloromethane, whereas inorganic particles have relatively low concentrations. Dichloromethane is, therefore, used to adjust RSP levels where non-diesel particles are present (Hammond *et al.* 1988).

## 2.3 Occupational Exposure

Various employee groups have been studied to determine their occupational exposures to DP. They include railroad workers, mine workers (use diesel-powered equipment), bus garage workers, trucking company workers, fork-lift truck operators, fire-fighters, lumberjacks, toll-booth and parking garage attendants, and many professions servicing or handling automobiles (car mechanics, professional drivers, *etc.*). The National Institute for Occupational Safety and Health (NIOSH) has estimated that approximately 1.35 million workers are occupationally exposed to DP in about 80,000 workplaces in the United States (NIOSH 1989).

### 2.3.1 Railroad Workers

Railroad workers' potential for exposure has increased since 1959, when almost all the U.S. railroad system (95%) was converted to diesel engines. Table 2-1 shows the varying degrees of exposure to DP (from 17 μg/m$^3$ for clerks to 134 μg/m$^3$ for locomotive shop workers) based upon job groups (Woskie *et al.* 1988a).

**Table 2-1. Personal exposures to Respirable Particulate Matter (RPM), and Adjusted Respirable Particulate Matter[1], among railroad workers by job group**

| Exposure Group | Job Group | Number | Arithmetic mean (SD) of RPM (μg/m$^3$) | Arithmetic mean (SD) of adjusted RPM (μg/m$^3$) |
|---|---|---|---|---|
| Clerks | Clerk/Station | 59 | 125 (75) | 42 (36) |
| Signal maintainers | Signal maintainer | 13 | 69 (39) | 58 (33) |
| Engineers/firers | Freight worker | 55 | 115 (67) | 94 (55) |
| | Yard worker | 50 | 108 (109) | 69 (70) |
| | Passenger | 23 | 75 (52) | 51 (35) |
| Brakers/conductors | Freight conductor | 62 | 126 (65) | 69 (52) |
| | Freight braker | 21 | 145 (80) | 102 (62) |
| | Passenger | 35 | 111 (62) | 104 (58) |
| | Yard worker | 21 | 180 (117) | 114 (76) |
| | Hostler | 8 | 231 (134) | 224 (130) |
| Shop workers | Electrician | 42 | 256 (332) | 192 (248) |
| | Machinist | 110 | 191 (146) | 147 (120) |
| | Supervisor, laborer, and | 24 | 244 (141) | 155 (83) |

| Exposure Group | Job Group | Number | Arithmetic mean (SD) of RPM (μg/m$^3$) | Arithmetic mean (SD) of adjusted RPM (μg/m$^3$) |
|---|---|---|---|---|
| | other shop workers | | | |

[1] Estimated cigarette smoke particulate matter (based on nicotine concentrations found in respirable particulate matter) was subtracted from the total respirable particulate matter.

Woskie *et al.* (1988a)

### 2.3.2 Miners

Diesel engines have been, and continue to be, commonly used in U.S. mines since their first introduction in the early 1950s. Exposure occurs from activities such as blastings, in which drillers and other heavy machinery using diesel are used. Holland (1978; cited by IARC 1989) conducted an exposure study in 24 U.S. coal mines (summarized in Table 2-2).

**Table 2-2. Levels of Diesel Exhaust Components (mg/m$^3$) in 24 U.S. mines**

| Contaminant | Diesel Exhaust Source | | | Personal and Area Samples | | |
|---|---|---|---|---|---|---|
| | # of samples | Mean | Range | # of samples | Mean | Range |
| Carbon monoxide | 6 | 140 | 11.5-344 | 21 | 14.2 | 0-26.3 |
| Nitric oxide | 3 | 6.3 | <0.1-16.5 | 10 | 12.7 | 0.5-70 |
| Nitrogen dioxide | 5 | 16.8 | 1-40 | 29 | 1.6 | 0-11 |
| Sulfur dioxide | 5 | 0.3 | 0-<1 | 6 | 2.1 | 0-13 |
| Sulfuric acid | 4 | 12.8 | <0.2-46 | 9 | 0.3 | <0.004-2 |
| Formaldehyde | 10 | 7 | 0-42 | 23 | 0.8 | 0-8 |
| Acrolein | 7 | 2.1 | <0.1-3.2 | 16 | <0.4 | <0.02-<5 |
| Total particulate matter | 8 | 50.2 | 0.5-236 | 13 | 4.6 | 0.2-14 |
| Anthracene | 8 | 0.05 | 0.02-0.2 | 13 | 0.001 | 0.00005-0.004 |
| Phenanthrene | 8 | 0.001 | 0-0.008 | 13 | 0.01 | 0-0.17 |

Holland (1978; cited by IARC 1989)

This study showed that, while certain PAH expected from DP were found (anthracene and phenanthrene), other PAH (benz[*a*]anthracene, benz[*a*]pyrene, benz[*e*]pyrene, chrysene, and pyrene) were not found "in measureable quantities" (Holland 1978; cited by IARC 1989). Cornwell (1982; cited by IARC 1989) found other PAH not found in Holland's study in diesel emissions from a molybdenum mine in Colorado. This mine was equipped with drills and various load haul-dumps.

### 2.3.3 Bus Garage and Other Bus Workers

Working areas around bus garages were studied to determine exposure levels to DP in garages. Table 2-3 presents results for American garages.

**Table 2-3. Levels of air contaminants in bus garages (mg/m$^3$, unless otherwise specified)**

| Contaminant | Concentration | Sampling | Location | Reference |
|---|---|---|---|---|

| Contaminant | Concentration | Sampling | Location | Reference |
|---|---|---|---|---|
| Carbon monoxide | 2-18 | Diesel areas | Denver, CO | Apol (1983)[1] |
| | 7-11 | Terminal (background) | Denver, CO | Pryor (1983)[1] |
| | 22-46 | Terminal (bus arrival/departure) | Denver, CO | Pryor (1983)[1] |
| | 6-8 | Package receiving area | Denver, CO | Pryor (1983)[1] |
| | 8-40 | Reservations room | Denver, CO | Pryor (1983)[1] |
| | 8-401 | Air inlet | Denver, CO | Pryor (1983)[1] |
| Nitric oxide | 1.4-6.9 | Peak morning period | Denver, CO | Apol (1983)[1] |
| | 0.5-2.8 | Later morning period | Denver, CO | Apol (1983)[1] |
| Nitrogen dioxide | 0.8-3.2 | Peak morning period | Denver, CO | Apol (1983)[1] |
| | 0.3-1.6 | Later morning period | Denver, CO | Apol (1983)[1] |
| | <0.07 | Package receiving area | Denver, CO | Pryor (1983)[1] |
| | 0.1-0.8 | Personal, mean TWA | USA | Gamble *et al*. (1987)[1] |
| Sulfur dioxide | ≤ 0.13 | Morning period | Denver, CO | Apol (1983)[1] |
| | 0.03-0.18 | Unspecified | Denver, CO | Pryor (1983)[1] |
| Aldehydes as formaldehyde | <0.3 ppm | Morning period | Denver, CO | Apol (1983)[1] |
| Total dust | 0.13-0.55 | TWA, including dust stirred up by buses | Denver, CO | Apol (1983)[1] |
| | 0.15-0.81 | Peak morning period | Denver, CO | Apol (1983)[1] |
| | 0.03-0.16 | Cyclohexane-soluble | Denver, CO | Apol (1983)[1] |
| | 0.01-0.09 | Unspecified | Denver, CO | Pryor (1983)[1] |
| Respirable dust | 0.12-0.61 | Personal, mean TWA | USA | Gamble *et al*. (1987)[1] |

[1] Cited by (IARC 1989)

Bus repair facilities were studied to determine diesel exhaust emissions (Apol 1983; cited by IARC 1989). The highest exposure levels were observed when buses were started at peak times of dispatch and return. Pryor (1983; cited by IARC 1989) studied area levels of DP at a bus garage. Elevated levels of diesel exhaust emissions were observed during peak hours of bus activity. Levels rapidly returned to normal 10 to 15 minutes after the passing of peak times. Gamble *et al*. (1987; cited by IARC 1989) observed 232 workers in four diesel bus garages to determine mean time weighted average (TWA) concentrations of certain diesel fuel emissions.

### *2.3.4 Truck Drivers*

Using elemental carbon, Zaebst *et al*. (1991) found an effective way of determining truck drivers' exposure to DP. Temperature played an influential role such that higher temperatures are related to higher levels of exposure. Table 2-4 presents these data.

**Table 2-4. Exposure to submicrometer-sized (< 1 μ) elemental carbon by job type and weather conditions**

| Exposure Group | N | Arithmetic (μg/m$^3$) | | Geometric (μg/m$^3$) | | 95% Confidence for Mean (μg/m$^3$) | |
|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SD | | |
| Road drivers (cold) | 34 | 2.4 | 0.3 | 2.0 | 1.94 | 1.5 | 2.6 |
| Road drivers (warm) | 38 | 7.6 | 0.5 | 7.0 | 1.51 | 5.3 | 9.2 |
| Highway background (cold) | 12 | 1.9 | 0.5 | 1.5 | 2.09 | 0.9 | 2.4 |
| Highway background (warm) | 9 | 5.3 | 0.7 | 4.9 | 1.52 | 2.8 | 8.7 |
| Residential background (cold) | 12 | 1.7 | 0.4 | 1.3 | 2.14 | 0.8 | 2.1 |
| Residential background (warm) | 11 | 1.1 | 0.2 | 0.9 | 1.85 | 0.5 | 1.5 |

Zaebest *et al*. (1991)

This study showed no discernible difference between the exposure levels of truckers (3.8 μg/m$^3$) and highway background concentrations (2.5 μg/m$^3$). These results would indicate that the truck is not the cause of the truck driver's exposure; rather, it is the highway environment (Zaebst et al. 1991).

### *2.3.5 Firefighters*

Firefighters in three major cities (New York, NY; Boston, MA; and Los Angeles, CA) were studied to determine exposure to DP. To measure total exposure to airborne particles, pumps with filters to trap a methylene chloride soluble fraction were used to evaluate personal exposure. Mostly nonsmokers were used, but the 14% who were smokers averaged higher concentrations of airborne particles (62.6 μg/m$^3$ more) than their nonsmoking counterparts. Sampling was performed only when firefighters were in the fire stations. Table 2-5 summarizes the study performed by Froines *et al*. (1987) to determine DP exposure in fire stations.

**Table 2-5. Firefighter exposure to total airborne particulates in fire stations in Los Angeles, New York, and Boston**

| Location and Fire Station | 1st shift (9 A.M.-5 P.M.) | # of FF sampled | Runs[1] | 2nd Shift (5 P.M.-1 A.M.) | # of FF sampled | Runs[1] | 3rd Shift (1 A.M.-9 P.M.) | # of FF sampled | Runs[1] |
|---|---|---|---|---|---|---|---|---|---|
| **Total Airborne Particulates (μg/m³) by Shift and Average Number of Runs** | | | | | | | | | |
| **Los Angeles** | | | | | | | | | |
| 1 | 161 ± 39 | 14 | 8 | 190 ± 38 | 12 | 7 | 182 ± 53 | 9 | 5 |
| 2 | 216 ± 15 | 10 | 10 | 111 ± 7 | 11 | 13 | 89 ± 12 | 6 | 3 |
| 3 | 237 ± 66 | 4 | 5 | 129 ± 18 | 4 | 6 | | | |
| 4 | 256 ± 77 | 3 | 7 | 207 ± 25 | 3 | 5 | 63 ± 3 | 3 | 4 |
| 5 | 748 ± 77 | 9 | 32 | | | | | | |
| **Boston** | | | | | | | | | |
| 1 | 91 ± 19 | 8 | 4 | 100 ± 29 | 7 | 2 | 35 ± 15 | 2 | 0 |
| 2 | 170 ± 28 | 8 | 7 | 207 ± 25 | 7 | 6 | 110 ± 60 | 2 | 1 |
| **New York** | | | | | | | | | |
| 1 | 289 ± 39 | 15 | 14 | 408 ± 52 | 14 | 20 | 60 ± 14 | 8 | 2 |
| 2 | 370 ± 36 | 10 | 7 | 260 ± 21 | 10 | 10 | 132 ± 8 | 9 | 2 |
| 3 | 370 ± 40 | 5 | 25 | 480 ± 59 | 5 | 15 | 154 ± 13 | 5 | 4 |
| 4 | 363 ± 43 | 10 | 11 | 253 ± 24 | 10 | 9 | 116 ± 9 | 5 | 4 |

[1] Each time a vehicle was started constitutes a run.

Froines *et al.* (1987)

For the three cities, total airborne particulate exposure had a TWA ranging from below 100 μg/m³ to 480 μg/m³. Froines *et al.* (1987) estimated an average particulate exposure of 300 μg/m³ in Boston and New York. With an adjustment for background levels and smoking (75 μg/m³), firefighters in these two cities were said to be exposed to a total DP level of 225 μg/m³. Los Angeles had the worst conditions of the three cities. Samplings allowed for a "worst-case" scenario in which the mean concentration levels were as high as 748 μg/m³. The authors noted that these were busy fire stations located in large metropolitan areas. Other factors such as smoking, building design, age and maintenance of vehicles, activities of the firefighters, and timing of runs also affect results (Froines *et al.* 1987).

### *2.3.6 Other Workers*

Three studies reviewed by International Agency for Research on Cancer (IARC) (1989) found that toll booth workers had elevated levels of exposure to carbon monoxide (CO) (although this was decreased with ventilation systems) and DP. CO exposure was also elevated among border-station and motor vehicle inspectors and parking garage attendants (IARC 1989). In many of these studies, however, it was difficult to differentiate between gasoline exhaust and diesel exhaust. Numerous studies have combined the two exhausts together making exact determinations of DP exposure difficult.

## 2.4 Regulations

There is no U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for diesel exhaust. OSHA is developing an action plan to reduce worker exposures to this hazard but currently is not initiating rulemaking.

In 1982, the U.S. Environmental Protection Agency (EPA) implemented emission standards for light-duty and heavy-duty diesel vehicles.

The American Conference of Governmental Industrial Hygienists (ACGIH) convened a committee to discuss the setting of a Threshold Limit Value (TLV) for diesel exhaust. As a result, diesel exhaust was placed on ACGIH's Notice of Intended Changes for 1995/1996 (0.15 $mg/m^3$) TWA with a designation as a suspected human carcinogen.

The U.S. Mine Safety and Health Administration (MSHA) is currently in the process of developing a proposed rule for limiting the exposure of mine workers to diesel particulate matter.

The National Institute for Occupational Safety and Health (NIOSH) issued a Current Intelligence Bulletin (1989) that recommended that "whole diesel exhaust be regarded as a potential occupational carcinogen." In its Bulletin, NIOSH concluded that "though the excess risk of cancer in diesel exhaust exposed workers has not been quantitatively estimated, it is logical to assume that reductions in exposure to diesel exhaust in the workplace would reduce the excess risk." NIOSH recommended that "all available preventive efforts (including available engineering controls and work practices) be vigorously implemented to minimize exposure of workers to diesel exhaust."

**Table 2-6. EPA Regulations**

| EPA | |
|---|---|
| **Regulatory Action** | **Effect of Regulation/Other Comments** |
| 40 CFR 58 SUBPART G—Federal Monitoring. Promulgated: 44 FR 27571, 05/10/79. | The Administrator may locate and operate ambient air monitors to determine emissions from motor vehicle diesel exhaust. |
| 40 CFR 60 SUBPART Cb—Emissions Guidelines and Compliance Schedules for Municipal Waste Combustors. Promulgated: 60 FR 65415, 12/19/95. | This subpart contains emission guidelines and compliance schedules for the control of certain designated pollutants, including emissions from diesel fuels, from certain municipal waste combustors in accordance with section 111(d) and section 129 of the Clean Air Act. |
| 40 CFR 75 SUBPART G—Reporting Requirements. Promulgated: 60 FR 26557, 05/17/95. | This subpart contains guidelines on sampling and analysis methods to determine levels of diesel particualtes. |
| 40 CFR 79 SUBPART D—Designation of Fuels and Additives. Promulgated: 59 FR 33093, 06/27/94. U.S. Codes: 40 U.S.C. 7545, 7601(a). | Fuels and additives designated and dates prescribed by the Administrator for the registration of such fuels and additives, pursuant to section 211 of the Act, are listed in this subpart. Manufacturers must identify and measure emissions from diesel fuel. |
| 40 CFR 85 SUBPART O—Urban Bus Rebuild | This subpart details diesel fuel emission testing and |

| EPA | |
|---|---|
| **Regulatory Action** | **Effect of Regulation/Other Comments** |
| Requirements. Promulgated: 58 FR 21386, 04/21/93. | reporting procdures for buses. |
| 40 CFR 86—Control of Air Pollution from New and In-use Motor Vehicles and New and In-use Motor Vehicle Engines: Certification and Test Procedures. Promulgated: 59 FR 16262, 04/06/94. U.S. Codes: 42 U.S.C. 7401-7671(q). | This part outlines general emission standards for various engines, including diesel engines in various light- and heavy-duty engines. |
| 40 CFR 86 SUBPART D—Emission Regulations for New Gasoline-Fueled and Diesel-Fueled Heavy-Duty Engines; Gaseous Exhaust Test Procedures. Promulgated: 42 FR 45154, 09/08/77. U.S. Codes: 42 U.S.C. 1857f-1, 1857f-5, 1857f-5a, 1857f-6, 1857g(a). | This subpart contains gaseous emission test procedures for gasoline-fueled and Diesel heavy-duty engines. It applies to 1979 and later model years. |
| 40 CFR 86 SUBPART I—Emission Regulations for New Diesel Heavy-Duty Engines; Smoke Exhaust Test Procedure. Promulgated: 59 FR 48521, 09/21/94. U.S. Codes: 42 U.S.C. 7521, 7524, 7541, 7542, and 7601. | The provisions of this subpart are applicable to new petroleum-fueled, diesel heavy-duty engines beginning with the 1984 model year; methanol-fueled, diesel heavy-duty engines beginning with the 1990 model year; and natural gas-fueled and liquefied petroleum gas-fueled, diesel heavy-duty engines beginning with the 1997 model year. |
| 40 CFR 89—Control of Emissions from New and In-use Nonroad Engines. Promulgated: 59 FR 31335, 06/17/94. U.S. Codes: 42 U.S.C. 7521, 7522, 7523, 7524, 7525, 7541, 7542, 7543, 7547, 7549, 7550, and 7601(a). | Contains emission standards and exhaust emission test procedures for nonroad engines, such as engines for locomotive, airplaines, water boats, and mining equipment. |

## Table 2-7. OSHA Regulations

| OSHA | |
|---|---|
| **Regulatory Action** | **Effect of Regulation/Other Comments** |
| 30 CFR 7.95 It is effective November 25 1996. This part sets out requirements for MSHA approval of certain equipment and materials for use in underground mines whose product testing and evaluation does not involve subjective analysis. | Part 7, subpart A general provisions apply to subpart F. Subpart F establishes the specific requirements for MSHA approval of diesel power packages intended for use in approved equipment in areas of underground coal mines where electric equipment is required to be permissible. |
| 30 CFR 75.1907. Information on Diesel Regulations, Updates to Diesel Regulations, Approved Diesel Engines for Permissible Equipment Mine Safety and Health Administration (MSHA) Diesel Regulation Information. | Permissible diesel-powered equipment manufactured on or after November 25 1999. Approved Diesel Power Packages for Permissible Equipment. Permissible diesel-powered equipment manufactured on or after November 25 1999, and used in an underground coal mine must be provided with a power package approved in accordance with the requirements of subpart F of part 7. |

# 3 Human Studies

Studies of diesel exhaust (DE) exposure and cancer published before 1989 are reviewed in International Agency for Research on Cancer (IARC) Volume 46 (IARC 1989). Thirteen studies of DE exposure and cancer have been published since the IARC report and are summarized in Tables 3-1 and 3-2. Also, several reviews of DE exposure and lung cancer have been published since the IARC analysis (Cohen and Higgins 1995; Muscat and Wynder 1995; Muscat 1996; Stober and Abel 1996; Morgan *et al.* 1997). These reviews include a formal meta-analysis by Bhatia *et al.* (1998) with a discussion by Silverman (1998).

## 3.1 IARC Report, 1989

The IARC Working Group (IARC, 1989) evaluated epidemiologic studies published through 1988. They cited ten surveys of mortality and morbidity statistics related to diesel exhaust exposure. Because of the well-known limitations of such studies, they were considered by the group to be hypothesis-generating and were not described in detail.

The Working Group reviewed 13 cohort studies of DE exposure, categorized by occupation as railroad workers, bus company workers, professional drivers, or miners. One of these (Raffle 1957) included data also presented in a later study (Waller 1981); one was a preliminary report (Steenland 1986); two did not present data on individual cancer sites (Stern *et al.* 1981; Edling *et al.* 1987); and exposure opportunities were limited in another (Waxweiler *et al.* 1973). Of the remaining eight studies, five showed associations of lung cancer with DE exposure (Ahlberg *et al.* 1981; Howe *et al.* 1983; Wong *et al.* 1985; Gustafsson *et al.* 1986; Garshick *et al.* 1988). Only three studies evaluated bladder cancer; DE exposure was associated with increased risk in one (Rushton *et al.* 1983).

Only two of the eight cohort studies provided semiquantitative estimates of DE exposure. Howe et al. (1983) studied 43,826 retired Canadian railroad workers. Occupation at the time of retirement collected from pension records was used to classify workers as unexposed, possibly exposed, or probably exposed. The respective relative risks (RRs) were 1.0 (referent), 1.2 [95% confidence interval (CI), 1.1-1.3], and 1.4 (95% CI, 1.2-1.5), with a significant trend ($p<0.001$). Similar results were obtained after excluding individuals with asbestos exposure. No adjustment for smoking was made. The risk of bladder cancer was not associated with DE exposure.

Garshick et al. (1988) studied 55,407 US railroad workers. In a separate study (Woskie et al., 1988a; 1988b), current DE exposures were measured at several railroad yards and combined with complete occupational histories from railroad company records to give estimates of historical exposure. For the workers with the longest exposure, the RR for lung cancer was 1.5 (95% CI, 1.1-1.9) in workers aged 40-44 in 1959, the year by which US railroads were mainly converted to use of diesel engines. Smaller risks were seen for older workers, who had lower prevalence and shorter duration of DE exposure. Similar results were obtained after excluding individuals with asbestos exposure. Although no adjustment for smoking was made, the use of unexposed members of the cohort as a comparison group may reduce the possibility of confounding by smoking. Bladder cancer was not evaluated.

These data were reanalyzed in an effort to conduct a quantitative risk assessment (Crump et al., 1991). The reanalysis replicated the major findings of Garshick et al. (1988), that lung cancer risk was related to exposure defined dichotomously, particularly in younger workers. The risk for several occupational subgroups was elevated, but the risk for shopworkers was not, although they were considered to be the most exposed subgroup. However, exposure among shopworkers may not have been uniform, since not all shops repaired diesel engines. Extensive efforts to use the data of Woskie et al. (1988a; 1988b) to relate risk to cumulative exposure in a more quantitative manner were unsuccessful; in fact, inverse relationships were found. Crump et al. (1991) pointed out that these negative findings might be due to weaknesses in the exposure data; they could also be due to unwarranted assumptions made in constructing the exposure metrics. Finally, the reanalysis exposed the fact that deaths in the cohort between 1976 and 1980 had been underascertained. While elevated cancer risk was found even in analyses limited to the years 1959 through 1976, this underascertainment could bias the reanalyses based on latency, which are heavily dependent on data from later years of follow-up.

The Working Group reviewed eight case-control studies which included data on lung cancer and DE exposure; all but one (Coggon *et al.* 1984) adjusted for smoking. Six of these found increased risk for lung cancer associated with DE exposure (Williams *et al.* 1977; Coggon *et al.* 1984; Buiatti *et al.* 1985; Garshick *et al.* 1987; Benhamou *et al.* 1988; Siemiatycki *et al.* 1988). Only two of the eight studies provided semi-quantitative estimates of DE. The study by Garshick *et al.* (1987) was a nested case-control study presented from statistics used in the later cohort study (Garshick *et al.* 1988). DE exposure was estimated from an industrial hygiene evaluation of jobs and work areas and a complete job history collected from railroad board records. Smoking histories were obtained by interviewing next-of-kin. After adjusting for smoking and asbestos exposure, lung cancer was associated with DE exposure (RR for each 20 years exposure=1.41; 95% CI=1.06-1.88). The study by Siemiatycki *et al.* (1988) was a population-based study of multiple cancer sites. Estimates of DE exposure were derived from an industrial hygiene evaluation of a complete job history obtained in an in-person interview. After adjusting for smoking and asbestos exposure, squamous cell lung cancer was associated with exposure to both DE (RR=1.2, 90% CI=0.9-1.6) and gasoline exhaust (RR=1.2; 90% CI=1.0-1.5).

The Working Group evaluated 11 studies of bladder cancer and DE exposure; all but one (Coggon *et al.* 1984) adjusted for smoking. Eight of these observed increased risk of bladder cancer associated with DE exposure (Howe *et al.* 1980; Coggon *et al.* 1984; Hoar and Hoover 1985; Silverman *et al.* 1983; Jensen *et al.* 1987; Iscovich *et al.* 1987; Steenland *et al.* 1987; Risch *et al.* 1988). Only the study by Siemiatycki *et al.* (1988) described above provided semi-quantitative estimates of DE exposure; no association of bladder cancer with exposure to DE or gasoline exhaust was found.

The IARC Working Group concluded that there is *limited evidence* for the carcinogenicity in humans of diesel engine exhaust.

## 3.2 Current Epidemiology Studies

Tables 3-1 and 3-2 give details of six cohort and eight case-control studies of cancer and DE exposure published in 1989 or later.

### *3.2.1 Cohort Studies*

Boffetta *et al.* (1988) studied 461,981 men aged 40-79 with known smoking habits. The men were followed for two years as part of the American Cancer Society prospective cancer mortality study, which involves 1.2 million men and women from the general U.S. population. Data on occupational history and smoking were collected with a self-administered questionnaire and analyzed with respect to self-reported exposure to DE and to employment in occupations presumed to involve DE. Unexposed members of the cohort were used as a comparison group. After adjusting for smoking and other occupational exposures, the overall risk (RR [95% CI]) associated with self-reported DE exposure was 1.18 (0.97-1.44), based on 174 deaths, and there was a dose-response for increasing duration of exposure ($p<0.01$). Twenty percent of the cohort did not report DE exposure status; the overall mortality rate was higher in this group than among those who did report DE exposure status. Elevated risks were observed for railroad workers, truck drivers, heavy equipment operators, and miners; the latter two were statistically significant. Truck drivers were the only group sufficiently large enough for further analysis. Risk was similarly elevated in truck drivers reporting DE exposure and those not reporting exposure. However, when duration of DE exposure was considered, a dose-response was found: 1-15 years, 0.87 (0.33-2.25); 16+ years, 1.33 (0.64-2.75). The strengths of this study are its large size, use of unexposed members of the cohort for comparison, and the availability of information on individual smoking habits. The limitations of the study are its reliance on self-administered questionnaires and the lack of actual DE exposure measurements.

Bender *et al.* (1989) conducted a mortality study of 4,849 men with at least one year experience as a highway maintenance worker for the Minnesota Department of Transportation; the cohort was followed from 1945 through 1984. DE exposure was defined as work as a highway maintenance worker, abstracted from personnel records. Workers were compared to the white male population of Minnesota. Risk (SMR, 95% CI) for all-cause mortality was significantly reduced 0.91 (0.86-0.96) as was mortality for all cancers (0.83, 0.73-0.94). This healthy worker effect suggests that the comparison population may have been inappropriate. Overall risk for respiratory cancers was significantly reduced (0.60, 0.52-0.90), based on 57 deaths; risk for urinary tract cancers was elevated after accounting for 40-49 years latency (2.92, 1.17-6.02), based on seven deaths; and risk of leukemia was elevated among those with 30-39 years exposure (4.25, 1.71-8.76), based on seven deaths. This study is limited by its small size, use of job titles to infer exposure, and lack of adjustment for smoking.

Gustavsson *et al.* (1990) conducted a mortality study of 695 male Stockholm bus garage workers; the cohort was followed from 1952 to 1986. Using occupation histories collected from company records, estimates were made by industrial hygienists for DE and asbestos exposures. Workers were compared to the occupationally active population of greater Stockholm. Overall risk (SMR, 95% CI) of lung cancer was increased (1.61, 0.94-2.57), based on 17 deaths, but there was no dose-response for increasing levels of exposure. Risk for bladder cancer was unchanged (0.52, 0.01-2.88), based on one death. The strength of this study is its use of industrial hygiene evaluation of complete job histories to assess DE exposure; it is limited by its small size and lack of adjustment for smoking or asbestos exposure.

Rafnsson and Gunnarsdottir (1991) conducted a mortality study of male Rejkjavik drivers; 868 truck drivers and 726 taxi drivers were followed from 1951 to 1988. Truck drivers were assumed

to be exposed to DE while taxi drivers were not. Comparisons were made with the male Icelandic population. Risk of cancer of the trachea, bronchus, and lung (SMR, 95% CI) was elevated for truck drivers (2.14, 1.37-3.18), based on 24 deaths, but there was no dose-response for increasing duration of employment. This may suggest confounding by smoking or may be due to small numbers. Risk of cancer of the trachea, bronchus, and lung was not elevated for taxi drivers (1.39, 0.65-3.83), based on 12 deaths. Risk of urinary tract cancer was not elevated in either group. Smoking history was available for a subset of the cohort. Current smoking was similar for both types of drivers and the general population, but more truck drivers than taxi drivers or members of the general population were ex-smokers. This study is limited by its small size, its lack of estimates of actual DE exposure, and the failure to adjust for either smoking or asbestos exposure.

Guberan *et al.* (1992) evaluated 6,630 male drivers in the Canton of Geneva for cancer incidence and mortality. Mortality follow-up was from 1949 to 1986, and incidence follow-up was from 1970 to 1986. The drivers were categorized in three groups as professional drivers (highest exposure) or non-professional drivers with more or less DE exposure. The male population of the Canton of Geneva was used as a comparison group. Professional drivers had an increased risk (SMR, 90% CI) of lung cancer mortality (1.50, 1.23-1.81), based on 77 deaths, and an increased risk (SIR, 90% CI) of lung cancer incidence (1.61, 1.29-1.98), based on 64 cases. The risk of lung cancer mortality increased with time since first exposure for the professional drivers ($p<0.02$). A significant trend for lung cancer incidence was seen across the three exposure groups ($p<0.05$). There was also an increasing trend for lung cancer mortality (SMRs = 1.21, 1.32, 1.50 for the three groups) which was not statistically significant. Professional drivers had elevated levels of bladder cancer mortality (1.43, 0.80-2.36), based on 11 deaths, and incidence (1.25, 0.74-1.99), which were not statistically significant. Professional drivers had significantly elevated levels of mortality from esophageal cancer (1.83, 1.08-2.91, 13 deaths), stomach cancer (1.79, 1.17-2.63, 19 deaths), and rectal cancer (2.58, 1.62-3.92, 16 deaths) and of incidence of stomach cancer (2.33 (1.56-3.36, 21 cases) and rectal cancer (2.00, 1.27-3.00, 17 cases). The strengths of this study are its large size and the use of incidence as well as mortality data. It is limited by the lack of estimates of actual DE exposure and the lack of adjustment for smoking or asbestos exposure.

Hansen (1993) conducted a mortality study of 14,225 Danish truck drivers; the cohort was followed from 1970 for ten years. DE exposure was defined as exposure associated with work as a truck driver, determined from self-administered census questionnaires; since World War II, all Danish trucks have used diesel engines. The comparison group consisted of 43,024 unskilled laborers selected on the basis of lack of occupational exposure to combustion products from fossil fuels, and similarity to truck drivers in terms of education, social class, lifestyle, and work requirement for physical fitness. Overall risk (SMR, 95% CI) of lung cancer was increased (1.60, 1.26-2.00), based on 76 deaths, but risk of bladder cancer was not (0.87, 0.32-1.89). Risk of multiple myeloma was elevated (4.39, 1.42-10.24). Although no adjustment was made for smoking, the use of a comparison group with similar lifestyle may reduce the possibility that the results are due to confounding. Although the control group included 36% rural workers, who may smoke more than others, the risk of lung cancer was still significant after adjusting for this difference (1.52, 1.19-1.90). The strengths of this study are its large size and the use of a similar

group of workers for comparison; its limitations are the lack of individual data on actual DE exposure and on smoking or asbestos exposure.

## Table 3-1. Cohort studies of diesel exhaust (DE) exposure and cancer*

| Reference | Exposed population | Comparison group | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| (Boffetta *et al.* (1988) | 461,981 men aged 40-79 with known smoking habits, followed for two years as part of the ACS prospective cancer mortality study.<br><br>Person years of observation among those exposed to diesel exhaust = 124,053. | Unexposed members of cohort. | Self-reported exposure to DE or employment in related occupations.<br>Self-administered questionnaire. | RR (95% CI) for lung cancer<br><br>Self reported exposure to DE (adjusted for age, smoking, and asbestos) (174 deaths):<br>Overall: 1.18 (0.97-1.44).<br>1–15 years exposure: 1.05 (0.80-1.39).<br>16+ years of exposure: 1.21 (0.94-1.56).<br><br>Work in DE related occupations (adjusted for age and smoking) (# of deaths):<br>Railroad workers (14): 1.59 (0.94-2.69).<br>Truck drivers (48): 1.24 (0.93-1.66).<br>Heavy equipment operators (5): 2.60 (1.12-6.06).<br>Miners (15): 2.67 (1.63-4.37).<br><br>No other site remarkable. | Risk observed in nonsmokers and ex-smokers as well as smokers.<br><br>Truck drivers may be exposed to asbestos. Miners may be exposed to radon, silica, or heavy metals. |
| Bender *et al.* (1989) | 4,849 men with at least one year experience as highway maintenance worker for Minnesota Dept. of Transportation, who had worked at least | White male population of Minnesota. | Work as a highway maintenance worker, abstracted from personnel records. | SMR (95% CI)<br><br>Respiratory cancers, 57 deaths: 0.69 (0.52-0.90). | No adjustment for smoking. |

| Reference | Exposed population | Comparison group | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | one day after Jan 1, 1945.<br><br>Person years of observation = 96,567. | | | Urinary tract cancers, 7 deaths, with 40-49 years latency: 2.92 (1.17-6.02).<br><br>Leukemia, 7 deaths, with 30-39 years work: 4.25 (1.71-8.76).<br><br>No other site remarkable. | |
| Gustavsson *et al.* (1990) | 695 male Stockholm bus garage workers who had worked at least six months between 1945 and 1970, identified from company records.<br><br>Person years of observation = 21,317.5. | Occupationally active population of greater Stockholm. | Personal exposure level calculated using estimated intensity of emission, ventilation, job type, and work practices.<br>Cumulative exposure calculated by multiplying exposure level by years of exposure for each job. | RR (95% CI)<br><br>Lung cancer, 17 deaths.<br>Overall: 1.61(0.94-2.57).<br>Low exposure: 0.97.<br>Moderate exposure: 1.52.<br>High exposure: 1.27.<br><br>Bladder cancer, 1 death:<br>Overall: 0.52 (0.01-2.88).<br><br>No other site remarkable. | No adjustment for smoking or asbestos exposure. |
| Rafnsson and Gunnarsdottir (1991) | Reykjavik drivers who were alive in 1951:<br>868 men who had worked only as truck drivers, identified from membership list of Truck Drivers' Union.<br>726 men who had worked only as taxi drivers, identified from | Icelandic men. | Work as truck or taxi driver. Truck drivers may have more DE exposure. | SMR (95% CI)<br><br>Cancer of trachea, bronchus, or lung:<br>For truck drivers, overall, 24 deaths: 2.14 (1.37-3.18).<br>For truck drivers, by years of employment, accounting for 30 year latency:<br><2 years: 2.70 (0.74-6.92) | Smoking history available for a subset: current smoking was similar for truck, taxi drivers, and general population; more truck drivers than taxi drivers or general population were ex-smokers. |

| Reference | Exposed population | Comparison group | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | membership list of cooperative taxi agency.<br>Person years of observation = 28,788. | | | 2-10 years: 2.46 (0.99-5.08)<br>11-30 years: 0.68 (0.01-3.76)<br>>30 years: 2.32 (0.85-5.04)<br>For taxi drivers, 12 deaths: 1.39 (0.65-3.83)<br><br>Urinary tract cancers:<br>For truck drivers, 3 deaths: 1.02 (0.21-0.21-2.97)<br>For taxi drivers, 1 death: 0.55 (0.01-3.08) | Truck drivers exposed to asbestos and welding fumes. |
| Guberan *et al.* (1992) | 6,630 men from Geneva with license to drive trucks, taxis, or buses in 1949-60.<br>Person years of observation = 198,667. | Male population of Geneva. | Professional drivers or non-professional drivers with more or less exposure, collected from license records. | For professional drivers:<br><br>Cancer mortality: SMR (90% CI):<br>Lung, 77 deaths: 1.50 (1.23-1.81).<br>Bladder, 11 deaths: 1.43 (0.80-2.36).<br>Esophagus, stomach, and rectum significantly increased.<br><br>Cancer incidence: SIR (90% CI):<br>Lung, 64 cases: 1.61 (1.29-1.98).<br>Bladder, 13 cases: 1.25 (0.74-1.99).<br>Stomach and rectum significantly increased; esophagus increased but not significantly. | No adjustment for smoking or asbestos exposure. |
| Hansen (1993) | 14,225 male Danish truck drivers, 15-74 years old, identified from 1970 census data and followed for ten years. | 43,024 unskilled laborers, 15-74 years old, identified from 1970 census | Work as a truck driver determined from self-administered census questionnaire. | SMR (95% CI)<br><br>Lung cancer, 76 deaths: 1.60 (1.26-2.00). | No adjustment for smoking or asbestos exposure. |

| Reference | Exposed population | Comparison group | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | Person-years of observation = 407,780. | data, with no exposure to fossil fuel combustion and similar to truck drivers in terms of education, social class, lifestyle, and work requirement for fitness. | | Bladder cancer , # of deaths not given: 0.87 (0.32-1.89).<br><br>Multiple myeloma, 5 deaths: 4.39 (1.42-10.24). | |

RR, relative risk; SMR, standardized mortality ratio; CI, confidence interval

### 3.2.2 *Case-Control Studies*

Hayes *et al.* (1989) pooled data from three studies of lung cancer conducted by the National Cancer Institute. Analyses were limited to 1,444 male lung cancer cases with in-person interviews, and 1,893 controls with in-person interviews matched to cases on the basis of age, sex, race, and area of residence. Information on employment in motor-exhaust related occupations was collected in a structured in-person interview. After adjusting for birth cohort, smoking, and study area, the risk (OR, 95% CI) associated with ten or more years employment in a motor-exhaust related occupation was 1.5 (1.2-1.9), based on 238 cases. Risks were observed in both smokers and nonsmokers. The strengths of this study were its large size, an exposure index based on a complete occupational history collected in person, and adjustment for smoking. It is limited by the lack of actual measurements of DE exposure.

Boffetta *et al.* (1990) studied 2,584 male lung cancer patients from 18 hospitals in six cities, as part of an ongoing multicenter study. They were compared to 5,099 controls with current diagnoses of nontobacco related diseases, recruited from the same hospitals and matched to the cases on the basis of age and sex. Information on self-reported DE exposure or employment in a job with possible or probable DE exposure was obtained from a structured in-person interview. After adjusting for age, race, smoking, education, and asbestos exposure, the risk (OR, 95% CI) associated with 31 or more years self-reported DE exposure was 2.39 (0.87-6.57), based on 12 cases; with 31 or more years in an occupation with probable DE exposure was 1.49 (0.72-3.11), based on 17 cases; and with 31 or more years as a truck driver was 1.17 (0.40-3.41), based on seven cases. The strengths of this study are an exposure index based on a complete occupation history collected in person, and adjustment for smoking and asbestos exposure. It is limited by the small number of exposed cases and the lack of actual measurements of DE exposure.

Gustavsson *et al.* (1990) conducted a nested case-control study as part of their cohort study of Stockholm bus garage workers described above, using 20 lung cancer cases, and 120 age-matched controls randomly selected from the cohort. A cumulative DE exposure index was calculated using information on estimated intensity of emission, ventilation, work practices, and years worked for each job type. There was a clear dose-response for this index, with a risk (OR, 95% CI) of 2.43 (1.32-4.47) associated with the highest level of exposure, based on ten cases. The strength of this study is its semi-quantitative estimate of DE exposure; it is limited by the small number of exposed cases and the lack of adjustment for smoking or asbestos exposure.

Steenland *et al.* (1990, 1998) conducted a case-control study of 994 lung cancer deaths among a cohort of men who had died in 1982-83 after having been members of the Teamsters Union for 20 or more years. The 1,085 controls were chosen by picking every sixth man from the same cohort, excluding lung and bladder cancer cases and motor vehicle accidents. A complete job history was obtained from self-administered or telephone interviews with next-of-kin. In the original analysis (Steenland *et al.* 1990), after adjusting for age, smoking, and asbestos exposure, the risk (OR, 95% CI) associated with 35 or more years work as a diesel truck driver was 1.89 (1.04-3.42), based on 56 deaths. Smaller risks were observed for gasoline truck drivers and mechanics. In a later analysis (Steenland *et al.* 1998), estimates of historical exposure to DE were based on current measurements of exposure to submicron elemental carbon and historical data on the prevalence of and emissions from diesel engines. After accounting for a five year latency and adjusting for age, race, smoking, diet, and asbestos exposure, the risk associated with

the highest quartile of exposure was 1.64 (1.09-2.49). The strengths of this study are its large size, its semiquantitative estimate of DE exposure, and adjustment for smoking, asbestos exposure, and other confounders.

Swanson *et al.* (1993) conducted a population-based case-control study of 3,792 male lung cancer cases from 1984-1987 in Detroit. They were compared to 1,966 male colorectal cancer cases. Occupational histories were collected in structured telephone interviews with cases or surrogates. In white men, after adjusting for age at diagnosis and smoking, the risk (OR, 95% CI) associated with driving heavy trucks for 20 or more years was 2.5 (1.40-4.4), based on 121 cases; the risk for driving light trucks for 10 or more years was 2.1 (0.9-4.6), based on 36 cases. The finding of significant linear trends for both heavy and light trucks, although the latter presumably have gasoline rather than diesel engines, suggests that some factor other than DE exposure may account for the risks. Risks were smaller and less precise in black men, possibly because the number of cases was smaller. The strengths of this study are its large size, an exposure index based on a complete occupational history collected in person, and adjustment for smoking; it is limited by lack of adjustment for asbestos exposure.

Emmelin *et al.* (1993) conducted a nested case-control study of lung cancer in the cohort of male Swedish dock workers described above (Gustafsson *et al.,* 1986). Fifty cases were compared to 154 controls, matched by age and port. Three semiquantitative estimates of DE exposure were made: “machine time” was years employed when diesel equipment was in use; “fuel” was cumulative diesel fuel used at the port while the worker was employed; and “exposed time” was years employed when fuel use at the port exceeded the lower quartile of use at all ports for all years. Risk (OR, 90% CI) for the highest level of machine time was 1.3 (0.3-5.6), based on 14 cases; for the highest level of fuel was 1.7 (0.5-5.9), based on 15 cases; and for the highest level of exposed time was 2.9 (0.8-10.7) based on 19 cases. After stratification by smoking, results suggested an interaction of smoking with DE exposure (measured as “exposed time”). The strengths of this study are its semi-quantitative estimate of DE exposure and adjustment for smoking; it is limited by the small number of exposed cases.

Brooks *et al.* (1992) conducted a case-case comparison of grade and stage of tumor in bladder cancer cases with or without potential DE exposure. The subjects were 1,415 white male incident bladder cancer cases from the Missouri Cancer Registry with recorded occupation status. Occupational and smoking histories were abstracted from medical records and reported to the Registry. Truck drivers and other motor vehicle operators had significantly more high grade cancers but there was no difference in stage. This study is limited by the small number of exposed cases and by its reliance on occupational information abstracted from medical records.

Muscat and Wynder (1995b) conducted a hospital-based case-control study of laryngeal cancer, including 235 white male cases from seven hospitals and 205 controls with conditions unrelated to smoking, frequency matched to cases by hospital, age, sex, race, and date of interview. Information on self-reported DE exposure of work in jobs related to DE exposure was collected in a structured in-person interview. After adjusting for current smoking, risk (OR, 95% CI) associated with self-reported DE exposure was 1.47 (0.5-4.1), based on 13 cases, and risk associated with jobs involved DE exposure was 0.96 (0.5-1.8), based on 36 cases. This study is limited by the small number of exposed cases and by the lack of information on duration of

exposure. Moreover, the large differences between cases and controls in smoking and alcohol use may not be adequately controlled in the analysis.

## Table 3-2. Case-control studies of diesel exhaust (DE) exposure and cancer*

| Reference | Study Design | Cases and Controls | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| Hayes *et al.* (1989) | Pooled data from three NCI case-control studies of lung cancer, two hospital- and one population-based. | Analyses limited to 1,444 male lung cancer cases with in-person interviews.<br><br>Analyses limited to 1,893 controls with in-person interviews, matched on age, sex, race, and area of residence. | Motor exhaust-related occupations.<br><br>Information collected by structured in-person interview; includes industry, occupation, and years employed for all jobs held six months or more. | OR (95% CI) adjusted for birth cohort, smoking, and study area.<br><br>By duration of employment in motor exhaust related occupations:<br>none, 948 cases: referent<br><10 years, 255 cases: 1.1 (0.9-1.3).<br>10+ years, 238 cases: 1.5 (1.2-1.9).<br><br>By type of employment, 10+ years:<br>Truck driver, 147 cases: 1.5 (1.1-1.9).<br>Heavy equipment operator, 14 cases: 1.3 (0.6-3.1).<br>Bus, 38 cases: 1.6 (0.9-2.8).<br>Taxi/chauffeur, 16 cases: 1.2 (0.5-2.6).<br>Other driver, 2 cases: 0.2 (0.0-1.6).<br>Mechanic, 26 cases: 1.7 (0.9-3.4).<br>All except truck, 136 cases: 1.4 (1.1-2.0). | Association observed in both smokers and nonsmokers.<br><br>No adjustment for asbestos exposure. |
| Boffetta *et al.* (1990) | Ongoing multicenter, hospital-based case-control study of lung cancer. | 2,584 male lung cancer cases from 18 hospitals in six cities.<br><br>5,099 controls, with current diagnoses of nontobacco related diseases from same hospitals, matched on age and sex. | Self-reported exposure to diesel exhaust (DE) or occupation with possible or probable exposure to diesel exhaust (DE).<br><br>Information collected by structured in-person interview in hospital. | OR (95% CI) for years of exposure, adjusted for age, race, smoking, education, and asbestos.<br><br>Self-reported exposure to DE:<br>1-15, 11 cases: 0.90 (0.40-1.99).<br>16-30, 12 cases: 1.04 (0.44-2.48).<br>31+, 12 cases: 2.39 (0.87-6.57). | Results adjusted for smoking and asbestos exposure. |

| Reference | Study Design | Cases and Controls | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | | | | Occupation with probable DE exposure:<br>1-15, 4 cases: 0.52 (0.15-1.86).<br>16-30, 15 cases: 0.70 (0.34-1.44).<br>31+, 17 cases: 1.49 (0.72-3.11).<br><br>Truck drivers:<br>1-15, 4 cases: 1.83 (0.31-10.73).<br>16-30, 12 cases: 0.94(0.41-2.15).<br>31+, 7 cases: 1.17 (0.40-3.41). | |
| Gustavsson *et al.* (1990) | Nested case-control study of lung cancer in cohort of male Stockholm bus garage workers (see Gustavsson *et al.* [1990] in Table 1) | 20 lung cancer cases.<br><br>120 age-matched controls randomly selected from cohort. | Personal DE exposure level calculated using estimated intensity of emission, ventilation, job type, and work practices.<br>Cumulative DE exposure index calculated by multiplying exposure level by years of exposure for each job. | OR (95% CI)<br><br>DE exposure index:<br>0-10, 5 cases: referent.<br>10-20, 2 cases: 1.34 (1.09-1.64).<br>20-30, 3 cases: 1.81 (1.20-2.71).<br>31+, 10 cases: 2.43 (1.32-4.47). | No adjustment for smoking or asbestos exposure. |
| Steenland *et al.* (1992) | Lung cancer study of men who had been members of Teamsters union for 20+ years and had died in 1982-83. | 994 lung cancer cases<br><br>1085 controls ascertained by identifying every sixth death from same group, excluding lung and bladder cancer and motor vehicle accidents. | Jobs involving exposure to DE, determined by self-administered (80%) or telephone (20%) interviews with next-of-kin. | OR (95% CI) for years of employment, adjusted for age, smoking and asbestos, compared to occupations with no exposure to DE.<br><br>Diesel truck driver:<br>1-24, 48 cases: 1.27 (0.70-2.27).<br>25-34, 72 cases: 1.26 (0.74-2.16).<br>35+, 56 cases: 1.89 (1.04-3.42).<br>Gasoline truck driver:<br>1-24, 72 cases: 1.24 (0.74-2.16).<br>25-34, 87 cases: 1.10 (0.67-1.80). | Results adjusted for smoking and asbestos exposure. |

| Reference | Study Design | Cases and Controls | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | | | | 35+, 86 cases: 1.34 (0.81-2.22).<br>Mechanic:<br>1-24, 11 cases: 1.69 (0.61-4.67).<br>25-34, 20 cases: 1.39 (0.63-3.07).<br>35+, 12 cases: 1.09 (0.44-2.66). | |
| Steenland *et al.* (1998) | Same as Steenland *et al.* (1990) | Same as Steenland *et al.* (1990) | Estimate of historical exposure to DE based on current measurements of exposure to submicron elemental carbon in different jobs and historical information on use of and emissions from diesel engines. | OR (95% CI) accounting for a five year latency and adjusted for age, race, smoking, diet, and asbestos exposure.<br><br>Quartiles of estimated cumulative exposure to DE, compared to those with no exposure above background:<br>0-169: 1.08 (0.72-1.63).<br>169-257: 1.10 (0.74-1.65).<br>257-331: 1.36 (0.90-2.04).<br>331+: 1.64 (1.09-2.49).<br><br>Cumulative exposure as a continuous variable: p=0.026.<br>Log cumulative exposure: p=0.011. | Results adjusted for both smoking and asbestos exposure. |
| Swanson et al. (1993) | Population-based case-control study of lung cancer, conducted between 1984 and 1987 in Detroit. | 3,792 male lung cancer cases, aged 40-84; 2,866 white, and 926 black.<br><br>1966 male colorectal cancer cases, aged 40-84; 1,596 white, and 370 black. | Driving heavy or light trucks.<br><br>Data collected by structured telephone interviews with the subject or his surrogate. | OR (95% CI) for years of exposure, stratified by race, and adjusted for age at diagnosis and smoking, compared to 0 years:<br><br>Heavy trucks, white men:<br>1-9, 78 cases: 1.4 (0.8-2.4).<br>10-19, 38 cases: 1.6 (0.8-3.5).<br>20+, 121 cases: 2.5 (1.4-4.4).<br>trend p<0.05. | Adjusted for smoking but not asbestos exposure. |

| Reference | Study Design | Cases and Controls | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | | | | Heavy trucks, black men:<br>1-9, 27 cases: 2.7 (0.8-9.2).<br>10-19, 16 cases: 1.9 (0.5-7.2).<br>20+, 16 cases: 2.1 (0.5-9.2).<br>trend p>0.05.<br><br>Light trucks, white men:<br>1-9, 46 cases: 1.7 (0.9-3.3).<br>10+, 36 cases: 2.1 (0.9-4.6).<br>trend p<0.05.<br>Light trucks, black men:<br>1-9, 11 cases: 1.7 (0.4-7.7).<br>10+, 8 cases: 1.4 (0.3-7.7).<br>trend p>0.05. | |
| Emmelin *et al*. (1993) | Nested case-control study of lung cancer in a cohort of male Swedish dock workers who had worked at least six months between 1950-74 at one of 15 ports; followed from 1960-82 (see Gustafsson *et al.* [1986] in Table 1). | 50 lung cancer cases.<br><br>154 controls matched by age and port. | Three semiquantitative estimates of exposure to DE, categorized as low, medium, and high exposure: (1) machine time = years employed when diesel equipment was in use; (2) fuel = cumulative diesel fuel used at port while person was employed; (3) exposed time = years employed when fuel use at port exceeded lower quartile of use at all ports over all years.<br><br>Smoking data from self-administered questionnaire to subjects or next-of-kin or | OR (90% CI), compared to lowest level of for each measure of DE exposure.<br>Machine time:<br>medium, 27 cases: 1.2 (0.4-4.2).<br>high, 14 cases: 1.3 (0.3-5.6).<br>Fuel:<br>medium, 25 cases: 1.1 (0.4-3.2).<br>high, 15 cases: 1.7 (0.5-5.9).<br>Exposed time:<br>medium, 19 cases: 1.6 (0.5-5.1).<br>high, 19 cases: 2.9 (0.8-10.7).<br><br>OR (90% CI) for exposed time in nonsmokers and smokers, compared to low exposure in nonsmokers: | Smoking histories may not be accurate.<br><br>Results suggest an interaction of smoking with exposure to DE. |

| Reference | Study Design | Cases and Controls | Exposure definition and measurement | Effects | Potential confounders |
|---|---|---|---|---|---|
| | | | interviews with foremen and other workers. | Nonsmokers:<br>medium 2 cases: 1.6 (0.2-12.5).<br>high, 2 cases: 2.9 (0.2-39.0).<br>Smokers:<br>low, 10 cases: 3.7 (0.9-14.6).<br>medium, 17 cases: 10.7 (1.5-78.4).<br>high, 17 cases: 28.9 (3.5-240). | |
| Brooks *et al.* (1992) | Data from Missouri Cancer Registry from 1984-1988 used to compare cases with high-grade invasive bladder cancer to those with low-grade, non-invasive cancer. | 1,415 white male incident bladder cancer cases with recorded occupational status. | Longest held job, abstracted from medical records and reported to Registry.<br><br>Smoking data from same source. | OR (p value) for high- *vs.* low-grade, adjusted for age and smoking:<br>Motor vehicle operators, 18 high-grade cases: 2.0 (0.10)<br>Truck drivers, 14 high-grade cases: 2.7 (0.05).<br>OR (p value) for late *Vs* early stage, adjusted for age and smoking:<br>Motor vehicle operators, 13 late stage cases: 0.9 (ns).<br>Truck drivers, 9 late stage cases: 0.8 (ns). | Adjusted for smoking but not asbestos exposure. |
| Muscat and Wynder (1995b) | Hospital-based case control study of laryngeal cancer. | 235 white male laryngeal cancer cases from seven hospitals.<br><br>205 controls hospitalized for conditions unrelated to tobacco use, frequency matched to cases by hospital, age, sex, race, and date of interview. | Self-reported exposure to DE or diesel fumes, or jobs related to DE or diesel fume exposure.<br><br>Collected in a structured in-person interview. | OR (95% CI).<br><br>Self-reported DE exposure, 13 cases: 1.47 (0.5-4.1).<br>Jobs involving DE exposure, 36 cases: 0.96 (0.5-1.8).<br>Self-reported diesel fume exposure, 17 cases: 6.4 (1.8-22.6).<br>Jobs involving diesel fume exposure, 1.30 (0.4-4.1). | Large differences between cases and controls; smoking and alcohol use may not be adequately adjusted for in analysis. |

OR = odds ratio; CI = confidence interval; ns = not specified

## 3.3 Discussion

The majority of studies reviewed found associations of DE exposure with lung cancer. Combining results for the studies reviewed by IARC (1989) and those published since then, 9 of 14 cohort studies and 12 of 17 case-control studies found that DE exposure was associated with increased risk of lung cancer. Of the more informative studies, *ie,* those with greater power and semi-quantitative estimates of DE exposure, 3 of 3 cohort studies and 4 of 5 case-control studies found that DE exposure was associated with increased risk of lung cancer. Overall risks in all studies ranged from 1.2 to 2.1, and were often higher in the more highly exposed subgroups. In a recent meta-analysis of 29 cohort and case-control studies, Bhatia *et al.* (1998), found a relative risk of 1.33 (1.18-1.51). Subanalyses of cohort *vs* case-control studies or studies that controlled for smoking *vs* those that did not produced similar results. Cohort studies that used internal comparisons, which would be likely to reduce the potential for confounding, found higher relative risks (1.43, 1.29-1.58) than those using external comparisons (1.22, 1.04-1.44). Moreover, a positive dose-response relationship was found for duration of exposure. Bhatia *et al.* (1998) concluded that "although the risk estimates for diesel exhaust exposure are small, they are consistently above one and are, in aggregate, unlikely to be due to chance. Confounding by smoking is also unlikely to explain all of the increased risks among diesel exhaust-exposed workers. Silverman (1998), in an accompanying editorial, pointed out that the small size of the effect could be due to low levels of exposure, exposure misclassification, or negative confounding but nevertheless concluded that the effect could not be considered causal because of its small size. Two recent qualitative reviews of DE exposure and lung cancer have come to opposite conclusions. Stöber and Abel (1996) judged that published studies were all biased by poor exposure assessment and/or confounding by smoking. Cohen and Higgins (1995), on the other hand, concluded that DE exposure "is associated with small to moderate relative increases in lung cancer occurrence and/or mortality. These elevations do not appear to be fully explicable by confounding due to cigarette smoking or other sources of bias. Therefore, at present, exposure to diesel exhaust provides the most reasonable explanation for these elevations. The association is most apparent in studies of occupational cohorts, in which assessment of exposure is better and more detailed analyses have been performed."

Studies of DE exposure and bladder cancer present a less consistent picture. Considering together studies reviewed by IARC (1989) and those published since then, 1 of 8 cohort studies and 9 of 12 case-control studies found an association of DE with bladder cancer. Of the more informative studies, 0 of 2 cohort and 0 of 1 case-control studies found an association. Most reviewers have concluded that the evidence does not clearly support a relationship of DE exposure and bladder cancer.

A major concern in interpreting the studies of DE exposure and cancer is the lack of quantitative estimates of DE exposure. In many studies, exposure was inferred from job title, sometimes based on a complete occupational history and translated into duration of exposure, but in other cases based on jobs ever held. Paucity of good exposure information will most likely result in misclassification among both the exposed and unexposed groups, especially in studies of the general population. However, there is in general no reason to believe that this misclassification will be differential for cancer cases and controls, and nondifferential misclassification will usually bias results in the direction of no effect. This may provide a partial explanation for the

small size of the risk. It is also noteworthy that most of the studies which included semiquantitative estimates of exposure found associations of lung cancer and DE exposure.

An additional concern is the possibility of confounding by smoking or other occupational exposures. With two exceptions, the cohort studies were unable to account for smoking. Garshick *et al.* (1988) used an internal comparison group whose smoking habits were presumably similar to those of the exposed workers, and Boffetta *et al.* (1988) adjusted for individual smoking habits; both of these studies found significant associations of DE exposure and lung cancer. In contrast, most case-controls studies did adjust for individual smoking habits: adjustment for smoking generally reduced but did not eliminate the risk associated with DE exposure. Truck drivers, railroad workers, and mechanics all may be exposed to asbestos, which is thus another potential confounder. Most studies did not adjust for asbestos exposure. Both Howe *et al.* (1983) and Garshick *et al.* (1988) analyzed data from their cohorts after excluding workers with asbestos exposure; in both cases, an association of DE exposure with lung cancer was still present. Three case-control studies were able to take asbestos exposure into account. Both Garshick *et al.* (1987) and Steenland *et al.* (1998) found evidence supporting a relationship of DE exposure with lung cancer after adjusting for asbestos exposure, although Boffetta *et al.* (1990) did not. Other potential confounders, such as other occupational exposure or lifestyle factors such as diet have rarely been taken into account.

In summary, DE exposure is associated with lung cancer in the majority of studies, with a overall relative risk of about 1.3. Some studies found dose-responses, with higher risks in the more heavily exposed groups. Although the risk is small, it is not readily explained by confounding by smoking or asbestos exposure. Most studies had little or no quantitative information on actual exposures, but the resulting misclassification would be more likely to disguise an effect than to produce a spurious one and may provide an explanation for the small size of the risk.

# 4 Experimental Carcinogenesis

As previously discussed, an International Agency for Research on Cancer (IARC) Working Group reviewed and evaluated studies of the carcinogenic potential for diesel and gasoline engine exhaust (IARC 1989). This report catalogued numerous animal experiments performed to test the carcinogenic potential of exposure to both diesel and gasoline-fueled internal combustion engine exhausts and components thereof. The studies were surveyed according to exposure subgroups: (i) whole diesel engine exhaust, DP; (ii) gas-phase diesel engine exhaust (with particles removed); (iii) diesel engine exhaust particles or extracts of diesel engine exhaust particles; (iv) whole gasoline engine exhaust; (v) condensates/extracts of gasoline engine exhaust; and (vi) engine exhausts in combination with known carcinogens.

## 4.1 Previously Reviewed Studies (IARC 1989)

### *4.1.1 Whole diesel engine exhaust (DP)*

Mice, rats, Syrian hamsters, and monkeys have been exposed by inhalation to whole diesel engine exhaust (DP), in some instances, over the life span of the test species. DP exposure resulted in dose-related increases in the incidences of benign and malignant lung neoplasms in rats in five studies. The Syrian hamster and monkey (Macaca fascicularis) studies were considered inadequate for carcinogenicity assessment. Interpretation of the mouse studies was questionable. In one experiment (Heinrich *et al* 1986a; cited in IARC 1989), exposure to DP was associated with an increased incidence of adenomas and adenocarcinomas of the lung. When adenocarcinomas were considered separately, there was also an increase in exposed groups. The Working Group noted that the incidence of lung tumors in the controls was low when compared to historical controls. In another study, Takemoto *et al* (1986; cited in IARC 1989) reported that exposure of mice to DP did not increase the incidence of tumors, although the Working Group determined that the Takemoto data did show a statistically significant increase in lung tumors in one of the two strains studied.

### *4.1.2 Diesel exhaust particles or organic extracts*

Organic extracts of diesel exhaust particles were applied to the skin or administered by intratracheal instillation or intrapulmonary implantation to rats, mice, or Syrian hamsters. An excess of pulmonary tumors developed in rats after the intrapulmonary implantation of beeswax pellets containing extracts of diesel particulates. Skin tumors were also caused in mice by skin application with diesel particulate extracts. Injection site tumors were observed following subcutaneous administration of diesel exhaust particles to mice. Again, carcinogenicity studies in Syrian hamsters were inconclusive.

### *4.1.3 Summary of carcinogenicity experiments reviewed by IARC (1989)*

Five inhalation studies of whole diesel engine exhaust using two different strains of rats showed an increased incidence of benign and malignant lung tumors. One study in rats did not show a tumorigenic effect. Of three studies in Syrian hamsters, two did not show induction of lung tumors, and the third was considered inadequate for evaluation of carcinogenicity. In two mouse studies, the incidences of lung tumors, including adenocarcinomas, were increased over that in concurrent controls. In one study, however, the total incidence of lung tumors was not elevated over the historical controls. Monkeys exposed to diesel exhaust for two years did not develop

lung tumors, but the short duration of the experiment rendered it inadequate for an evaluation of carcinogenicity.

## 4.2 Interpretations by Earlier Review Groups

The IARC Working Group (IARC 1989) concluded there is sufficient evidence that DP causes cancer in experimental animals. The group also reached this conclusion in the case of extracts of DP. The IARC Working found inadequate the evidence for carcinogenicity in experimental animals after gas phase diesel engine exhaust (*i.e.*, with particles removed) exposures (see Appendix A-1).

## 4.3 Recent Carcinogenicity Studies

Recent studies generally support findings of earlier ones by confirming that chronic DP exposure increases the incidence of pulmonary neoplasms in rats, whereas hamsters and mice are less susceptible. Soot appears to be a necessary potentiating element in respiratory carcinogenesis attributable to DP exposure. In hamsters, chronic DP exposure enhanced the carcinogenicity of diethylnitrosamine.

### *4.3.1 Inhalation Experiments*

#### *4.3.1.1 Studies conducted in rats*

Brightwell *et al*. (1989) and Nikula *et al*. (1995) exposed male and female rats to complete diesel exhaust and diesel exhaust passed through a particle filter. These workers generated exhaust in a Volkswagon 1.5-litre diesel engine.

In the Brightwell *et al.* study, rats were exposed for 16 hours per day, five days per week, for two years. Animals that survived the exposure phase were observed for an additional six months prior to sacrifice, necropsy, and histopathological examination. Engine exhaust was diluted with air and three levels of exposure were tested. Results of these experiments are presented in Tables 4-1 and 4-2.

**Table 4-1. Average concentrations of diesel exhaust emissions in exposure chamber air (v/v)**

| Dose level | Complete diesel exhaust | Mean particulate concentration | Filtered diesel exhaust |
|---|---|---|---|
| High | 8.2% | 6600 $\mu g/m^3$ | 8.2% |
| Medium | 2.7% | 2200 $\mu g/m^3$ | 2.7% |
| Low | 0.9% | 700 $\mu g/m^3$ | Not tested |
| Control | 0 | 0 | 0 |

Brightwell *et al.* (1989)

Rats exposed to filtered diesel exhaust (DE) had no excess tumors, but those exposed to complete diesel exhaust, DP, had dose-related, statistically significant increases in incidences of pulmonary tumors (Table 4-2).

**Table 4-2. Incidences of primary lung tumors in male and female rats exposed to complete diesel exhaust and filtered diesel exhaust for up to two years**

| Animals with lung tumors /animals examined | | | | |
|---|---|---|---|---|
| **Treatment group** | **Control** | **Low** | **Medium** | **High** |
| Complete Diesel exhaust | | | | |
| Males | 2/134 (1.5%) | 1/72 (1.4%) | 3/72 (4.2%) | 16/71 (22.5%) |
| Females | 1/126 (0.8%) | 0/71 (0%) | 11/72 (15.3%) | 39/72 (54.2%) |
| Filtered Diesel exhaust | | | | |
| Males | 2/134 (1.5%) | not tested | 0/72 (0%) | 0/72 (0%) |
| Females | 1/126 (0.8%) | not tested | 0/71 (0%) | 0/72 (0%) |

Brightwell *et al.* (1989)

Tumor response was dose-related in both genders, and females were more susceptible than males. Among animals surviving more than two years, 96% of females had tumors versus 44% of males. Diesel soot particles were clearly important causative factors and increased tumor incidences were observed only in rats exposed to either 2200 or 6600 $\mu g/m^3$. Animals exposed to 700 $\mu g/m^3$ (low dose DP) had no increase in tumor incidence. The absence of a carcinogenic response in the animals exposed to filtered diesel exhaust indicates the carcinogenic effect in rats is associated with the presence of high concentrations of diesel soot particles.

Many of the rats exposed to diesel emissions had more than one primary lung tumor. While only 39/72 high-dose females had lung tumors, a total of 75 primary tumors were identified in these animals. Multiple tumors, found in the same animal, were often of different histological types. In both male and female rats exposed to the high-level of diesel emissions, the following types and numbers of primary lung tumors were identified: adenoma (including scar tumors) (40), squamous cell carcinoma (35), adenocarcinoma (including scar tumors) (19), mixed adenoma/adenocarcinoma/squamous cell carcinoma (9), and mesothelioma (1).

Nikula *et al*. (1995) explored the importance of diesel exhaust soot associated organic compounds in the causation or rat lung tumors. Results of chronic exposures to (diluted) DP or aerosolized carbon black at identical particle concentrations for up to 24 months were compared. Male and female rats were exposed for 16 hours a day, five days a week, for up to 24 months, to either diesel exhaust or carbon black particles at target particle concentrations of 2.5 and 6.5 $mg/m^3$. Mean concentrations of particles to which rats were chronically exposed are shown in Table 4-3.

**Table 4-3. Mean concentrations of particles in exposure atmospheres to which male and female F 344 rats were exposed for up to 24 months**

| Exposure Atmosphere | Mean Particle concentration $\mu g/m^3$ |
|---|---|
| Control | 0.05 ± 0.02 |
| Low Carbon Black | 2.46 ± 0.03 |
| High Carbon Black | 6.55 ± 0.06 |
| Low Diesel Exhaust | 2.44 ± 0.02 |
| High Diesel Exhaust | 6.33 ± 0.04 |

(Nikula *et al.* 1995)

Both diesel exhaust soot and carbon black particles accumulated in the lungs of exposed rats, but the rate of accumulation was higher for diesel exhaust soot. After exposures of 23 months, mean lung particle burdens of DP soot were roughly double those for carbon black. Lung weights of diesel exhaust or carbon black-exposed rats increased in a time and dose-related fashion. Observed non-neoplastic changes included alveolar macrophage hyperplasia, alveolar epithelial hyperplasia, inflammation, fibrosis, alveolar proteinosis, bronchiolar-alveolar metaplasia, epithelial hyperplasia, and squamous metaplasia. Tumor incidence was generally dose-related and similar incidences of lesions occurred in animals exposed to each particulate.

Carbon black and diesel exhaust each caused concentration-related increases in the incidences of benign and malignant lung tumors in rats of both genders. The incidence of tumors, however, was consistently higher in females (Table 4-4).

**Table 4-4. Numbers of different types of lung neoplasms observed, and numbers of rats with each type of neoplasm**

| | C | | | LCB | | | HCB | | | LDE | | | HDE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | Male | Total | Female | Male | Total | Female | Male | Total | Female | Male | Total | Female | Male | Total |
| Number of susceptible rats[b] | 105 | 109 | 214 | 107 | 106 | 213 | 105 | 106 | 211 | 105 | 105 | 210 | 106 | 106 | 212 |
| Total rats with lung neoplasms | 0 | 3 | 3 | 8 | 2 | 10 | 28 | 4 | 32 | 8 | 5 | 13 | 29 | 9 | 38 |
| Tumor Type | | | | | | | | | | | | | | | |
| Adenoma | | | | | | | | | | | | | | | |
| Number of neoplasms | 0 | 1 | 1 | 2 | 1 | 3 | 17 | 0 | 17 | 6 | 2 | 8 | 22 | 5 | 27 |
| Rats with neoplasms | 0 | 1 | 1 | 2 | 1 | 3 | 13 | 0 | 13 | 5 | 2 | 7 | 19 | 4 | 23 |
| Adenocarcinoma | | | | | | | | | | | | | | | |
| Number of neoplasms | 0 | 1 | 1 | 6 | 1 | 7 | 23 | 1 | 24 | 3 | 1 | 4 | 32 | 3 | 35 |
| Rats with neoplasms | 0 | 1 | 1 | 6 | 1 | 7 | 20 | 1 | 21 | 3 | 1 | 4 | 19 | 3 | 22 |
| Squamous cell carcinoma | | | | | | | | | | | | | | | |
| Number of neoplasms | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Rats with neoplasms | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Adenosquamous carcinoma | | | | | | | | | | | | | | | |
| Number of neoplasms | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 1 |
| Rats with neoplasms | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 1 | 0 | 1 |
| Other | | | | | | | | | | | | | | | |
| Number of neoplasms | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rats with neoplasms | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Nikula *et al.* (1995)

[a] Several individual rats had multiple types of tumors and/or multiple tumors of a single type; thus, these rats are counted more than once in this table.

[b] Values include all rats examined by gross necropsy and microscopy except rats sacrificed at 3, 6, and 12 months. The first lung neoplasm was observed between 12 and 18 months of exposure; thus, all rats dying spontaneously, or euthanized in moribund condition, plus those sacrificed at 18 months or later were considered at risk for expression of lung neoplasia.

Tumor incidences were slightly higher among DP-exposed than carbon black-exposed rats (Table 4-5).

**Table 4-5. Incidences of diesel exhaust or carbon black exposed rats with lung tumors**

| Experimental group | Malignant tumors | Benign tumors |
|---|---|---|
| Control | | |
| F | 0/105 (0%) | 0/105 (0%) |
| M | 2/109 (1.8%) | 3.109 (2.8%) |
| Low carbon black | | |
| F | 7/107 (6.7%) | 8/107 (7.5%) |
| M | 1/106 (0.9%) | 2/106 (1.9%) |
| High carbon black | | |
| F | 21/105 (20%) | 28/105 (26.7%) |
| M | 4/106 (3.8%) | 4/106 (3.8%) |
| Low diesel exhaust | | |
| F | 4/104 (3.8%) | 8/105 (7.6%) |
| M | 3/105 (2.9%) | 5/105 (4.8%) |
| High diesel exhaust | | |
| F | 21/106 (19.8%) | 29/106 (27.4%) |
| M | 5/106 (4.7%) | 9/106 (17.9%) |

(Nikula *et al*. 1995)

The survival of carbon black-exposed males was shortened and this is likely to have reduced the expression of carcinogenicity. These results suggest that while the soot component of diesel exhaust is necessary for expression of diesel exhaust-induced carcinogenicity in rats, the tumor response is not necessarily unique to diesel exhaust soot.

Nikula *et al*. (1995) point out that carbon black was not less carcinogenic than diesel exhaust in their experiment. Based on that observation, the organic fraction of diesel exhaust did not appear to play an important role in the carcinogenicity of diesel exhaust in rats. This supports the hypothesis that the carcinogenic response of rats to diesel particulates is due more to particle exposure than diesel generated organics adsorbed to the particles. This line of reasoning approaches an interpretive dilemma as it could then be reasoned that, since there is inadequate evidence for human carcinogenicity of carbon black (IARC 1996), the pulmonary tumor response of rats to particulates is species specific with questionable predictive value for human health hazards. However, the genotoxic effect of particulate extracts (IARC 1989) should be considered as well as the production of skin tumors after dermal application of diesel particulate extract (Nesnow 1990).

Heinrich *et al.* (1995) conducted studies with female Wistar rats exposed to carbon black, $TiO_2$, four concentrations of diesel soot, and particle-free diesel exhaust. The rats were exposed by whole body inhalation for up to 24 months, 18 h/day, 5 d/week. After the exposure period, the rats were then kept in clean air conditions for an additional 6 mo. The dosage concentrations of diesel soot were 7.0 mg/m$^3$, 2.5 mg/m$^3$, and 0.8 mg/m$^3$ which coincide with 61.7 g/m$^3$ x h, 21.8 g/m$^3$ x h, and 7.4 g/m$^3$ x h. The concentration of carbon black and $TiO_2$ was 102.2 g/m$^3$ x h and 88.1 g/m$^3$ x h, respectively.

At 20 months of exposure, the incidences of bronchioloalveolar hyperplasia was 98/100 for the high dose of diesel, 96/100 for carbon black, and 96/100 for $TiO_2$. The intensity and frequency of interstitial fibrosis increased with particle exposure concentration. No differences could be detected among the high diesel exposure, the carbon black, and the $TiO_2$ groups. Particle-laden macrophages and particles in the alveolar region were observed in the lungs of all exposed rats. The resulting lung tumors, after a 24-month exposure period, followed with a 6-month exposure period to clean air, are shown in the following table (Table 4-6).

**Table 4-6. Lung tumors in rats after an experimental time of 30 month**

| Tumor type | Cumulative particle exposure ($g/m^3$ X h) | | | |
|---|---|---|---|---|
| | Clean air control | Diesel soot, 61.7[a] | Carbon black, 102.2[b] | $TiO_2$ 88.1[c] |
| Benign squamous-cell tumor[d] | 0/217 | 14/100 | 20/100 | 20/100 |
| Squamous-cell carcinoma | 0/217 | 2/100 | 4/100 | 3/100 |
| Adenoma | 0/217 | 4/100 | 13/100[g] | 4/100 |
| Adenocarcinoma | 1/217 | 5/100 | 13/100[g] | 13/100 |
| Hemangioma | 0/217 | 1/100 | 0/100 | 0/100 |
| Number of rats with tumors[e] | 1/217 | 22/100 (9/100)[f] | 39/100[h] (28/100[i])[f] | 32/100 (19/100)[f] |

Heinrich *et al.* (1995)

[a]7.0 $mg/m^3$ diesel soot for 24 mo.

[b]7.5 $mg/m^3$ carbon black for 4 mo. followed by 12 $mg/m^3$ for 20 mo.

[c]7.5 $mg/m^3$ $TiO_2$ for 4 mo. followed by 15 $mg/m^3$ for 4 mo. and 10 $mg/m^3$ for 16 mo.

[d]Benign keratinizing cystic squamous-cell tumor.

[e]Some animals had two lung tumors.

[f]Count without benign keratinizing cystic squamous-cell tumors given in parentheses.

[g]Significant a $p< 0.05$ compared to diesel soot group (Fisher's Exact Test).

[h]Significant at $p< 0.01$ compared to diesel soot group (Fisher's Exact Test).

[i]Significant at $p< 0.001$ compared to the diesel soot group (Fisher's Exact Test).

These values indicate a larger increase in lung tumor response in carbon black and $TiO_2$ exposure than in diesel soot. It is not possible to compare the carbon black and $TiO_2$ solely on exposure concentrations, due to the dosage increase in concentration throughout the study. The dosage concentrations of $TiO_2$ and carbon black varied throughout the study. Because of the high particle retention data in diesel soot-exposed rats, the aerosol concentrations of carbon black and $TiO_2$ had to be increased. The $TiO_2$ dosage for the first four months was 7.2 $mg/m^3$, followed by 14.8 $mg/m^3$ for four months and then 9.4 $mg/m^3$ for 16 months. The carbon black dosage was 7.4 $mg/m^3$ for four months, followed by 12.2 $mg/m^3$ for 20 months. The cumulative particle exposure concentration (particle exposure concentration multiplied by the actual total exposure time in hours) was determined for each exposure dosage. The comparison then showed that, regardless of the particle type used in this study, the lung tumor rate increased with increasing particle exposure concentration.

The 39% tumor incidence for carbon black was significantly different from the 22% tumor incidences of the high diesel group using the prevalence method (Hoel and Walburg 1972; cited by Heinrich *et al.* 1995). The TiO2 tumor incidence of 32% was not significantly different.

The IARC (1996) published a concise description of an hypothesized, mechanistic model for carcinogenic effects of particle exposure in the lung. This description is attached as *Appendix 2.—Studies conducted in hamsters*.

The potential carcinogenic effect of inhaled diesel exhaust was examined in hamsters exposed to DP or particle filtered diesel exhaust for 16 hours a day, five days a week, over a period of two years (Brightwell *et al*. 1989). Exposure atmospheres are described in Table 4-1. Half the animals in each exposure group were dosed with diethylnitrosamine, a known carcinogen, to evaluate possible co-carcinogenic interactions between diesel exhaust and the carcinogen. All surviving hamsters were sacrificed after two years of exposure. The respiratory tracts of all high-dose and control hamsters exposed to diesel exhausts (but not dosed with diethylnitrosamine) were examined microscopically. The incidence of tumors was always less than 1% (Table 4-7). Thus, under the conditions of this experiment, diesel exhaust was found not to cause cancer in hamsters.

The respiratory tracts of hamsters exposed to the high dose of diesel exhaust were compared to those of the controls. The only consistent changes seen in hamsters exposed to diesel exhaust was an increase in absolute and relative lung weights. The weight increase was far less than observed in similarly treated rats.

**Table 4-7. Respiratory tract tumors in male and female hamsters (sexes combined) exposed to diesel exhaust for up to 2 years**

| Exposure (Mean particulate concentration ($\mu g/m^3$) | Animals with Tumors of the Respiratory Tact | | | |
|---|---|---|---|---|
| | Nasal passages | Larynx | Trachea | Lungs |
| Control (0) | 0/411[a] (0/49) | 1/394 (0/49) | 0/403 (0/49) | 1/410 (0/49) |
| High dose (6,600 $\mu g/m^3$) | 1/207 (0/37) | 0/205 (0/36) | 0/205 (0/36) | 0/203 (0/36) |

Brightwell *et al.* (1989)

[a] Data presented as number of animals with tumors/number examined. Data in parentheses represents final sacrifice data only.

The respiratory tracts of all hamsters dosed with diethylnitrosamine prior to chronic exposure with diesel exhaust were examined microscopically. Tumor incidences in these groups are shown in Table 4-8.

**Table 4-8. Number of Diethylnitrosamine treated hamsters with primary tumors of the respiratory tract after exposure to Diesel Exhaust for up to 24 months**

| Treatment group | Animals with respiratory tract tumors (No. with tumor/No. examined) | | | |
|---|---|---|---|---|
| | Nasal passages | Larynx | Trachea | Lungs |
| **Males** | | | | |
| Control | 0/102 | 1/102 | 14/101 | 4/101 |
| Low diesel exhaust | 3/50 | 0/49 | 7/51 | 1/50 |
| Medium diesel exhaust | 1/52 | 1/51 | 12/52 | 3/52 |
| High diesel exhaust | 0/52 | 0/50 | 9/50 | 1/51 |
| Low filtered diesel | Not tested | Not tested | Not tested | Not tested |
| Medium filtered diesel | 0/51 | 0/51 | 4/50 | 3/52 |
| High filtered diesel | 0/52 | 0/52 | 6/51 | 5/52 |
| **Females** | | | | |
| Control | 1/104 | 0/102 | 18/103 | 3/101 |
| Low diesel exhaust | 2/50 | 0/50 | 8/50 | 1/51 |
| Medium diesel exhaust | 4/49 | 1/48 | 14/49 | 3/50 |
| High diesel exhaust | 0/47 | 0/45 | 13/48 | 3/50 |
| Low filtered diesel | Not tested | Not tested | Not tested | Not tested |
| Medium filtered diesel | 1/50 | 1/51 | 5/51 | 1/52 |
| High filtered diesel | 2/52 | 0/52 | 11/52 | 4/52 |

(Brightwell *et al.* 1989)

The incidence of tumors in female hamsters was generally higher than in males. While some of the diesel exhaust-exposed groups had a few more tumors than the control groups, there were no statistically significant increases in tumor incidences in exposed animals compared to controls.

Syrian golden hamsters were exposed 19 hours per day, five days per week, for 6, 10.5, 15, or 18 months to total diesel exhaust, diesel exhaust without particles, a mixture of nitrogen dioxide (5 ppm) and sulfur dioxide (10 ppm), or clean air. Two exposure groups from each test atmosphere were also treated by a single subcutaneous injection of either 3 mg or 6 mg of diethylnitrosamine/kg of body weight to evaluate an enhancing effect of diethylnitrosamine on exposure-related changes. Morphological changes were assessed by microscopic examination. Minor changes of the larynx and trachea were investigated by scanning electron microscopy, which showed a loss of ciliated cells in all exhaust-exposed groups. After exposure to diesel exhaust, with or without particles, focal metaplasia and dysplasia of the respiratory epithelium were seen. In the same specimens, attached mucous droplets indicated changes in mucous cells and mucous viscosity. Exposure to total diesel exhaust significantly increased the tumor rate in the upper respiratory tract of male hamsters that had been treated with 6 mg/kg of diethylnitrosamine. At the lower diethylnitrosamine dose, no exposure-related effects on the tumor rates were observed. The authors were undecided as to whether diesel-engine exhaust

should be classified as a co-carcinogen or as an enhancer for the test system used (Heinrich *et al*. 1989).

*4.3.1.2 Studies conducted in mice*

Earlier reports reviewed in IARC (1989) provided conflicting views of the response of mice to diesel exhaust. Mauderly *et al*. (1996) described carcinogenicity results from a bioassay conducted with CD-1 mice. Exposure to whole diesel exhaust (diluted to produce soot concentrations of 0.35, 3.5, or 7.1 mg/m$^3$) seven hours a day, five days a week, for 24 months, caused accumulations of soot in mouse lungs similar to those reported in lungs of rats (Nikula *et al.* 1995). Exposure to diesel exhaust did not affect survival or body weight. In contrast to dose-related neoplastic responses reported for rats exposed to diesel exhaust, exposure of mice did not increase the incidence of lung neoplasms (Table 4-9).

**Table 4-9. Summary of effect of chronic (up to 24 months) exposure to diesel exhaust on total lung neoplasms in CD-1 mice**

| Exposure group | Percent of mice with one or more malignant or benign pulmonary tumor | | |
|---|---|---|---|
| | Males | Females | Sexes Combined |
| High particle exposure (7.1 mg/m$^3$) | 6.1 | 8.7 | 7.5 |
| Medium particle exposure (3.5 mg/m$^3$) | 8.5 | 10.4 | 9.7 |
| Low particle exposure (0.35 mg/m$^3$) | 5.9 | 23.3 | 14.6 |
| Controls (0.013 mg/m$^3$) | 10.1 | 15.9 | 13.4 |

(Mauderly *et al.* 1996)

This finding is consistent with other data showing that mice, as well as Syrian hamsters, differ from rats in their lung neoplastic and non-neoplastic responses to heavy, chronic inhalation exposure to diesel exhaust soot.

Heinrich *et al*. (1995) conducted studies using two strains of female mice, NMRI and C57BL/6N. The NMRI mice were exposed by whole body inhalation to carbon black, $TiO_2$, and a high dose of 7.0 mg/m$^3$/h diesel soot for a period of 13.5 months. The dosage for carbon black was 7.0 mg/m$^3$ for 4 months, then 12 mg/m$^3$ for the remaining 9.5 months. $TiO_2$ dosage was 7.0 mg/m$^3$ for 4 months, then 15 mg/m$^3$ for 4 months, then, finally, 10 mg/m$^3$ for the remaing 5.5 months.

The NMRI mice were not exposed to low- and middle-doses of diesel soot. The total exposure time was 13.5 months, followed by 9.5 months of clean air.

The lung weight of NMRI mice showed substantial increase compared to controls as the study progressed. The particle lung burden of the NMRI mice after one year of exposure to diesel soot, carbon black, and TiO2 were 35, 37, and 26 mg, respectively.

A comparative study using NMRI mice and exposure to diesel exhaust, with 4.5 mg/m$^3$ soot and without soot particles, resulted in lower body weight after one year exposure, compared to the controls.

A 50% mortality rate was observed in the diesel soot group at 19 months and in all remaining groups at 20 months. Lung weight was 3.5-fold higher at 18 months in the soot-exposed mice compared to control animals (0.2 g). The retained particle mass in the lung was 29.5 mg/g lung wet weight of clean air control animals. The differences in the tumor rate between controls and diesel gas-phase-exposed mice was not significant at the 95% probability level (p=0.053). The percentages of adenomas/adenocarcinomas of the groups were 18.3/5% (total diesel exhaust), 31.7/15 (diesel gas phase), and 25/8.8 (clean air).

The C57BL/6N mice were exposed by whole body inhalation to dosages of 4.5 mg/m$^3$/h diesel soot, particle-free exhaust, and clean air. In these groups, the body weight development was similar and the mortality was 90% in exposed groups, compared to 80% in the control groups. The retained particle mass in the mouse lung was 12.6 mg/g lung wet weight. This shows comparable results with the rat 7.0 mg/m$^3$ diesel exposure in the particle lung load even though the dose values varied. This may be attributed to the higher deposition efficiency of particles and the higher volume, per gram, body weight for mice, as compared with rats.

The lung tumor rates at 30 months for diesel soot, particle-free exhaust, and clean air were 8.5, 3.5, and 5.1%, respectively. There was no significant increase in the lung tumor incidence of the exposure groups (Heinrich *et al.* 1995).

#### *4.3.2 Routes of Administration Other Than Inhalation*

Mouse skin tumorigenesis has often been used to evaluate complex mixtures, including human respiratory carcinogens. Nesnow (1990) examined the quantitative relationships between tumor induction in SENCAR mouse skin and the induction of respiratory cancer in humans using emissions from diesel engines. Unit risk (the lifetime probability of respiratory cancer death due to a constant lifetime exposure of 1 μg/m$^3$ of diesel exhaust in the inhaled air) was used to compare the potency of diesel exhaust emissions for mouse skin tumor induction and carcinogenic potential in the human respiratory tract. Human risk was based on rat inhalation data (Albert *et al.* 1983; cited in Nesnow 1990). The constant relative potency assumption compared the potencies of complex environmental mixtures and applied this to human respiratory cancer. The ratio derived was: Potency of lung cancer in man of X divided by the potency of lung cancer in man of Y equals K (constant) multiplied by the potency (mouse skin) of X divided by the potency (mouse skin) of Y. X and Y are human respiratory cancinogens while K is the proportionality constant. The proportionality constant can be measured experimentally from pairs of human carcinogens. When fitted to a linear regression, the mouse skin tumor data and human lung cancer risks were highly associated, with a correlation constant of 0.95 and a slope value of 0.89. The authors concluded that this comparative potency approach may be used in prospective analyses of human respiratory cancer risk to complex emissions.

# 5 Genotoxicity

## 5.1 Prokaryotic Systems

### *5.1.1 Induction of Reverse Mutation in Salmonella typhimurium*

In strain TA98 of *Salmonella typhimurium*, the mutagenic activity of dichloromethane extracts of diesel engine exhaust was largely dependent on the aromatic content of the fuel being burnt. Severe hydrogen treatment of the fuel (to reduce aromatic content) greatly reduced the mutagenicity of the resulting extract, whereas enrichment of the fuel with di- and tri-aromatics resulted in enhanced mutagenicity. Di- and tri-naphthenic compounds only had a borderline effect. The sulphur content of the fuel also had little influence (Crebelli *et al.* 1995).

Dichloromethane extracts of a standard sample of diesel particulate (SRM 1650) were fractionated by column chromatography and were tested for mutagenicity in *Salmonella* strain TA100 (in the absence of S9, dissolved in DMSO). The "primary mutagens" were found in three of the five fractions which the investigators described as "nitro-polycyclic aromatic hydrocarbons," "dinitro-polycyclic aromatic hydrocarbons," and "polar compounds." The two fractions largely bereft of mutagenic activity were those described as "aliphatic hydrocarbons" and "polycyclic aromatic hydocarbons" (Ostby *et al*. 1997).

Extraction of the mutagens from SRM 1650 using a range of solvents found that mutagenicity of the extracts (as assessed in Salmonella strains TA98 and TA100) decreased with increasing solvent polarity (from hexane, through 9:1 hexane/diethyl ether, 1:1 hexane/diethyl ether, diethyl ether, down to methanol). Dichloromethane was the most effective solvent examined. The direct mutagenic activity of a dichloromethane extract was over four-fold higher than that of a hexane extract. After initial dichloromethane extraction, only very low amounts of additional activity (<2%) were found in the extracts of subsequent solvents (Savard *et al*. 1992). A similar study, but comparing dichloromethane, with methanol, acetone and acetonitrile, also demonstrated the superiority of dichloromethane in extracting the mutagenic components from diesel particulates (Montreuil *et al*. 1992). These extraction studies, discussed in section 6.1.2, raise questions concerning the bioavailability and physiological disposition of mutagens associated with DP.

Another standard diesel particulate sample (SRM 1975) has been tested in a range of *Salmonella* strains (Hughes *et al*. 1997). The direct acting mutagenicity of dichloromethane extracts in the standard strains was much higher than that seen with the corresponding SRM 1650. The highest activity was found in strain YG 1021 which possesses a high nitroreductase activity (Hughes *et al*. 1997).

## 5.2 Mammalian Systems *In Vitro*

### *5.2.1 Diesel Exhaust-Induced DNA Adducts in Calf Thymus DNA and Human Lymphocytes*

Attempts to identify the range of adducts formed when calf thymus DNA was incubated with a diesel exhaust extract have come to no firm conclusion. Benzo[*a*]pyrene-derived reaction products were found at only low levels, and adducts derived from chrysene derivatives, benzo[*k*]pyrene and nitro-polyaromatic hydrocarbons were felt to be more important (Gallagher *et al*. 1993; King *et al*. 1994; Savela *et al*. 1995). A preliminary study had indicated that the incubation of a diesel exhaust extract with human lymphocytes produced five treatment-related

DNA adducts, one of which was probably the result of reaction with benzo[*a*]pyrene (Gallagher *et al*. 1993).

### *5.2.2 Induction of Chromosome Aberrations in Mammalian Cells in Culture*

Dichloromethane extracts of diesel exhaust particles from a light-duty engine produced chromosome damage in Chinese hamster lung (V79) cells, whereas the exhaust from a heavy duty engine did not. The exhausts from both types of engine induced sister chromatid exchange although the light duty engine was more active in this respect (Hasegawa *et al*. 1988). There was an increase in micronuclei in V79 cells and Chinese hamster ovarian fibroblasts exposed to DMSO extracts of diesel emission particles (Gu *et al*. 1992).

### *5.2.3 Induction of Cell Transformation by Diesel Exhaust Extract*

Dichloromethane extracts of the exhaust from a light-duty diesel engine induced a significant number of Type III foci in BALB/c 3T3 cells, and the transformed cells were shown to produce tumors when injected into mice. The corresponding extracts of exhaust from a heavy-duty engine was a much weaker inducer of cell transformation (Hasegawa *et al*. 1988).

## 5.3 Mammalian Systems *In Vivo*

### *5.3.1 Induction of DNA Adducts in Lymphocytes of Diesel Exhaust-Exposed Rodents*

Diesel particulate extract (dissolved in acetone) was applied to the skin of female C57 mice on four occasions over a 54-hour period at total doses of 20, 50, or 120 mg/mouse. Two mice were treated at each of the lower doses and a single mouse received the highest dose. There was a dose-related increase in the total yield of DNA adducts in the skin, lung, and liver. At the lowest dose the levels in the skin exceeded those in the lung, whereas in the mouse given 120 mg of the extract the total level of DNA adduct in the lung exceeded that seen in the skin. The chromatographic characteristics of the major adduct suggested it was partially (but not totally) derived from benzo[*a*]pyrene. This adduct made up from 46-67% of the total amount of adduct detected in the lung (Gallagher *et al.* 1990). The main DNA adduct detected in the lung and skin of the mice exposed to the two higher doses did not exhibit the chromatographic characteristics of either the benzo[*a*]pyrene derived 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene (BPDE-), benzo[b]fluoranthene-, benzo[*j*]fluoranthrene-, benzo[*k*]fluoranthene-, or the chrysene-DNA adduct standards (Savela *et al*. 1995). Two minor lung adducts migrated with a chrysene derivative- and benzo[b]fluoranthene-DNA adduct standards. Skin: DNA adducts showed chromatographic similarity to chrysene- and BPDE DNA-derived adducts (Savela *et al*. 1995).

In male F-344 rats exposed to diesel exhaust (7 h/d, 5 d/wk, for 12 weeks to 10 mg soot/$m^3$), the highest level of total DNA adducts in the respiratory tract was seen in the peripheral lung tissue (20 adducts/$10^5$ bases). Levels in the nasal tissues, which were about 1/4 to 1/5 those in the lung, were slightly higher than those found in the trachea, bronchi, and axial airway (Bond *et al*. 1988). The chromatographic pattern of the adducts was said to be unique to each of the regions, but at all sites one of the adducts exhibited chromatographic similarities to benzo[a]pyrene diol epoxide-DNA. This was one of the major adducts in the peripheral lung (Bond *et al.* 1988).

The overall level of DNA adduct in the lung was raised in F-344 rats exposed to diluted diesel exhaust for 7 h/d, 5 d/wk, for 12 weeks. The extent of DNA adduct formation, about twice that

seen in the controls, was similar throughout the tested dose range of 0.35 to 10 mg soot/m3 (Bond *et al*. 1990a). In a specific lung cell type, the alveolar type II cell, there was also a significant increase in total DNA adducts in male and female F-344 rats exposed 16 h/d, 5d/wk for 12 weeks to diluted diesel exhaust providing 6.2 mg soot/$m^3$. Eight adducts were detected in both the treatment group and in at least one control animal. None of the adducts were chemically characterized (although BPDE-DNA was said not to be present) (Bond *et al*. 1990b). By contrast, no increases in the total amounts of DNA adducts were found in the lungs of female Wistar rats exposed 18 h/d, 5 d/week to diesel emissions at 7.5 mg soot/$m^3$ for up to two years (Gallagher *et al*. 1994). There were, however, significant increases in the level of a specific adduct that was suspected to have arisen from the reaction of a nitro-polyaromatic hydrocarbon with DNA, as well as treatment-related decreases in the I-compounds (Gallagher *et al*. 1994).

### 5.3.2 Induction of Gene Mutations in Diesel Exhaust-Exposed Rodents

An analysis has been undertaken of the mutations present in the lung tumors that developed in diesel exhaust-exposed rats (16 h/d, 5 d/wk, for 24 months to 2.44 or 6.33 mg particles/$m^3$) (Swafford *et al*. 1995). In the 21 adenocarcinomas, no treatment-related effect was detected on the inactivation of the p-53 gene and there was only one from the activation of K-ras (a mutation on the second position of codon 12). This mutation was not present in the three adenocarcinomas that were found in rats exposed to filtered air. The p-53 protein exhibited an increased immunostaining (suggestive of gene dysfunction) in three of the six squamous cell carcinomas and the one adenosquamous carcinoma seen in the diesel exhaust–exposed rats, but was not present in either of the two tumors of these types occurring in the controls. The K-ras gene was unaffected in these tumor classes. Further analysis (by single strand conformational polymorphism and immunohistochemistry) of the p-53 protein failed to detect, however, any convincing evidence of treatment-related mutation.

### 5.3.3 Induction of Germ Line-Length Mutations in Diesel Exhaust-Exposed Rodents

There was some indication that diesel exhaust may be able to induce a mutation in male C57 Bl/6 mice (Hedenskog *et al*. 1997). The mice, exposed 8 h/d for 14 days to diesel exhaust diluted 10-fold by filtered air, were each mated 1 day and 18 days after the end of the exposure with two untreated females. Germ line-length mutations at one (PC-1) of the two measured minisatellites (PC-1 and 2) were found in the offspring of two of the 43 treated male mice (one from each of the two matings), but in none of the 43 controls. This increase was not statistically significant (presumably at $p< 0.05$). These same mutations were found in the offspring of six of the 35 mice treated with both PCB and diesel exhaust and in six of the 51 treated with PCB alone. The findings were described by the investigators as indicative of a treatment-related effect of the diesel exhaust.

### 5.3.4 Induction of DNA Adducts in Lymphocytes of Diesel Exhaust-Exposed Workers

$^{32}$P-Postlabeling was carried out on a subset of the study by Hou *et al*. (1995) to determine the DNA adduct levels. There was a statistically significantly increased level of total aromatic DNA adducts in the lymphocytes of 41 bus maintenance workers exposed to unquantified levels of diesel exhaust. The control group comprised 20 unexposed laboratory or hospital staff. The maintenance workers, all nonsmokers, were divided into three categories based on their diesel exhaust exposure. Significantly higher levels of adducts were found both in the 16 garage workers (highly exposed) and the 20 mechanics (intermediate exposure) (Hou *et al*. 1995). The

highest adduct levels were found in the workers with the combined genotype of negative glutathione-S-transferase (GSTMI) deficiency and slow acetylation (Lambert *et al*. 1995). Lambert further explains a significant correlation between the levels of DNA adducts (which were higher in the maintenance workers) and frequency of hprt mutant cells. In a similar study (Nielson *et al*. 1996), the adducts in the lymphocytes of diesel exhaust-exposed workers were chromatographically distinct from those seen in the control lymphocytes. Diesel exhaust exposures were not actually measured, diesel exhaust exposure from air pollution was not a contributing factor, although dermal exposure with the use of used lubricating oil was noted as a possible contributing source to the adducts seen in the workers lymphocytes.

#### *5.3.5 Genotoxicity of the Urine and Feces of Diesel Exhaust-Exposed Workers in the Salmonella Reverse-Mutation Assay*

There was no association between the diesel exhaust exposure and urinary mutagenicity of 87 U.S. railroad workers. Personal monitoring indicated that the median level of respirable particle concentration in the highest exposure group was 132 ug/m$^3$. Post-shift urine samples were tested in *Salmonella typhimurium* strain TA98 both in the presence and absence of S9 (Schenker *et al*. 1992).

Various extracts of the urine and feces of nine unexposed office workers and eight car mechanics exposed to "high concentrations" of diesel exhaust were tested for mutagenicity in strains TA98, TA98NR, and TA100 of *Salmonella typhimurium*. No convincing differences in the mutagenicity profiles were found (Willems *et al*. 1989).

#### *5.3.6 Induction of Genetic Damage in Diesel Exhaust-Exposed Humans*

##### *5.3.6.1 Induction of Gene Mutations at the hprt Locus in Lymphocytes of Diesel Exhaust-Exposed Workers*

A group of 47 non-smoking bus maintenance workers exposed to diesel exhaust and an unexposed control group of 22 laboratory and hospital fine-mechanic staff had a similar level of hprt mutation frequency in their lymphocytes (Hou *et al*. 1995). Lambert explains that in the group with the highest exposure, subjects with GSTMI deficiency, showed significantly higher hprt mutations than did GSTMI positive subjects. Coding errors were found in 80% of the mutations (Lambert *et al.* 1995).

##### *5.3.6.2 Induction of Micronuclei and Sister Chromatid Exchange in Human Lymphocytes exposed,* In Vitro*, to Diesel Exhaust*

Dichloromethane extracts of diesel exhaust particulates from a light-duty engine induced aneuploidy (kinetochore-positive micronucleus induction) in human lymphocytes (Odagiri *et al*. 1994). This effect was seen in the cells from the majority of the eight donors. A lower degree of extract-induced chromosome damage (kinetochore-negative micronuclei) was also reported. The corresponding extract of the particulates from a heavy-duty engine produced evidence of aneuploidy in the lymphocytes of only one of the volunteers (Odagiri *et al*. 1994).

### 5.4 Induction of Oxidative DNA Damage Diesel Exhaust–Exposed Mice

In male ICR mice given a suspension of diesel exhaust particles by intratracheal instillation, there was a dose-related increase in the levels of 8-hydroxy-deoxyguanosine, a marker of oxidative damage, in the DNA of the lungs. The mice were treated with 0.05, 0.1, or 0.2 mg

weekly for ten weeks and were killed one day later (the doses were sufficient to produce tumors at month 12) (Ichinose *et al*. 1997). The extent of DNA damage was greater in those animals on a high fat (16.34%) than a low fat (4.15%) diet. Dietary beta-carotene (0.02%) proved partially protective (Ichinose *et al*. 1997). Earlier work from the same group indicated that the maximum extent of oxidative DNA damage occurred two days after a single instillation of 0.3 mg. Higher doses produced less damage, probably because of cell death (Nagashima *et al*. 1995).

# 6 Mechanistic and Other Relevant Studies

## 6.1 Studies Reviewed Earlier by International Agency for Research on Cancer

The IARC Working Group (IARC 1989) survey revealed that inhalation exposure by unfiltered diesel exhaust produced a wide spectrum of toxicologic manifestations in a variety of mammalian species. The effects of unfiltered diesel exhaust were consistently more severe than those produced by filtered exhaust.

### *6.1.1 Disposition of Diesel Exhaust Particles in the Lungs of Experimental Animals*

The IARC Working Group reviewed factors affecting the deposition of diesel exhaust particles (DP) in the respiratory tracts of experimental animals as well as their dispositions (IARC 1989). Adsorption to DP of organic compounds generated during the burning of diesel fuel is an important element in the disposition of these compounds in lungs (Bond *et al*. 1986; cited in IARC 1989). The lung burden for an organic combustion product, after exposure to that compound coated on particles, may be many times that achieved by exposure to the compound alone. Further, prior exposure to DP could affect retention of subsequently inhaled materials. For example, one week after instillation, more benzo[a]pyrene was retained by the lungs of mice (exposed to diesel exhaust containing 6mg/m$^3$ for 9 months) than in controls (Cantrell *et al.* 1981; Tyrer *et al*. 1981; both cited in IARC 1989). The IARC Working Group (IARC 1989) suggested that this was most likely due to binding of the instilled compound to exhaust particles that had been retained in the lungs of the chronically exposed animals.

### *6.1.2 Diesel Exhaust Particulates and Metabolism*

Organic materials adsorbed to diesel particles may be extracted into biological fluids and metabolized to carcinogenic species of the parent compounds. This has been demonstrated, *ex vivo*, by treating with organic solvents, such as dichloromethane (DCM), or, more realistically, by incubating with simulated or actual bodily fluids and assaying for mutagenic potential using the Ames Test (Claxton 1983; Lewtas and Williams 1986; both cited in IARC 1989). Siak *et al.* (1981) and Brooks *et al*. (1981) evaluated the bioavailability of such materials under simulated *in vivo* physiological conditions. Attempts were made to extract mutagens from DP by incubating with saline, bovine serum albumin, and dipalmitoyl lecithin (DPL) with no significant increase in mutagenic effect in the Ames Test (Siak *et al*. 1981). Brooks *et al.* (1981) saw similar results in studies that also included extractions with serum and lung lavage fluid from canines. King *et al.* (1981) observed minor increases in mutagenic activity with extracts from human serum, but not from lung cytosol. They also reported a substantial (79-85%) decrease in organic solvent-extractable mutagenic activity from DP following incubation of the extracts with both serum (human) and lung cytosol (rabbit or rat). They concluded that DP mutagens may be inactivated when bound to serum or lung cytosol proteins. Further studies by King *et al.* (1983) showed that phagocytosis of DP by alveolar macrophages *in vitro* decreases mutagenic activity by 97-98% and, for 1-nitropyrene, 10-25%. Wallace *et al*. (1987), using DPL to simulate lung surfactant, observed mutagenic activity as high, or higher, than that seen using DCM extracts. In these studies, particles were constantly suspended whereas, in previous studies, finer particles must have been removed with extraction supernates.

Similar results have been observed in *in vivo* models. The metabolism of benzo[a]pyrene coated on DP was demonstrated by Sun *et al.* (1984; cited in IARC 1989). In those experiments, Fischer

344 rats were exposed (30 minutes) by inhalation to 3H-benzo[a]pyrene adsorbed to DP. Most of the radioactivity remaining in the lungs (65-76%) for up to 20 days was associated with benzo[a]pyrene, *per se,* but small amounts were also associated with known benzo[a]pyrene metabolites, benzo[a]pyrene-phenols (13-18%), and benzo[a]pyrene-quinones (5-18%). Other metabolites, such as the dihydrodiols, were not found.

Canine pulmonary macrophages, however, provided a wider variety of benzo[a]pyrene metabolites. In this model, macrophages were shown to metabolize 14-Carbon-benzo[a]pyrene, either in solution or adsorbed on diesel exhaust particulates, into benzo[a]pyrene-7,8-, -4,5- and -9,10-dihydrodiols as well as into dibenzo[a]pyrene-phenols and quinones. The *in vitro* efficiency of these metabolic transformations was the same whether benzo[a]pyrene was in solution or adsorbed onto diesel exhaust particles (Bond *et al.* 1984; cited in IARC 1989).

Inhalation exposure to diesel particles or to particle extracts has also been reported to have a moderately enhancing effect on aryl hydrocarbon hydroxylase activity in the lung and liver of mice and rats and in the lung of hamsters (Lee *et al*. 1980; Pepelko 1982; Dehnen *et al*. 1985; Chen 1986; cited in IARC 1989). This being the case, it is not surprising that reports exist showing that exposure to DP can increase the metabolism rate of organic compounds frequently associated with DP.

Exposure of Fischer rats by inhalation to diesel exhaust (7.4 mg/m$^3$ of particles) for four weeks resulted in a two-fold increase in the metabolism rate of 1-nitropyrene introduced via both nasal tissue contact and lung perfusion (Bond *et al.* 1985; cited in IARC 1989).

### *6.1.3 Genetic and Related Effects*

The soluble organic matter extracted from DP has been shown to induce DNA damage in bacteria in the absence of an exogenous metabolic system (Dukovich *et al*. 1981; cited in IARC 1989). Interactions between diesel exhaust and DNA have been demonstrated in *in vivo* mammalian models. Wong *et al*. (1986; cited in IARC 1989), reported that Fischer 344 rats chronically exposed to DP (containing 7.1 mg/m$^3$ particles) had more pulmonary DNA adducts than the rats of an unexposed group. Törnqvist *et al*. (1988; cited in IARC 1989), also reported dose-related increases in hemoglobin adducts of Fischer 344 rats and Syrian golden hamsters that had been exposed to diesel exhaust for six months to two years.

Exposure to diesel exhaust also promotes the synthesis of DNA in rodent lungs. After two days of continuous inhalation exposure to DP (containing 6 mg/m$^3$ of particles), the rate of DNA synthesis in the lungs of Fischer 344 rats was increased four-fold. The effect produced by the two day exposure was reversible, however, and DNA synthesis rate returned to normal one week after cessation of exposure (Wright 1986; cited in IARC 1989).

## 6.2 Studies Published Subsequent to the IARC Review

### *6.2.1 Disposition of Diesel Exhaust Particles in the Lungs of Experimental Animals*

Bevan and Ruggio (1991) studied the bioavailability of benzo[a]pyrene adsorbed to diesel exhaust particulate. Native DP (bearing a known concentration of benzo[a]pyrene) was labeled with $^3$H benzo[a]pyrene and instilled intratracheally into Sprague-Dawley rats. The distribution of radioactivity was analyzed at several time intervals over a three day post instillation period.

No attempt was made to identify metabolites. The results of this experiment are summarized in Table 6-1.

Distribution of radioactivity from the lung to the periphery is clear from the data shown in Table 6-1. One hour after instillation, radioactivity was found in all tissues sampled. Over 50% of the radioactivity, however, remained in the lungs for as long as three days and roughly 30% reached the feces/intestinal contents. The remaining radioactivity was distributed to other organs. These results confirm the bioavailability of benzo[a]pyrene (and/or its metabolites) when delivered in conjunction with DP. Also, the results suggest that, while the majority of a dose of the carcinogen inhaled is likely to remain for long periods in the lungs, systemic carcinogenesis is also a risk.

**Table 6-1. Distribution of radioactivity after intratracheal instillation of diesel particulate associated benzo[a]pyrene in rats**

| Organ/excreta | Percent of Recovered Dose at hours post instillation[1] | | | |
|---|---|---|---|---|
| | 1 | 6 | 24 | 72 |
| Lung | 77.4 ± 7.04 | 74.1 ± 3.1 | 68.5 ± 3.41 | 53.2 ± 2.25 |
| Liver | 2.19 ± 1.69 | 3.01 ± 0.71 | 3.13 ± 2.81 | 3.39 ± 0.98 |
| Intestine | 3.7 ± 3.03 | 3.23 ± 0.35 | 1.84 ± 1.59 | 1.98 ± 1.8 |
| Kidney | 1.32 ± 0.77 | 1.04 ± 0.086 | 0.7 ± 0.1 | 0.71 ± 0.25 |
| Stomach | 6.17 ± 3.32 | 2.61 ± 0.6 | 1.80 ± 1.20 | 2.59 ± 0.81 |
| Testes | 1.36 ± 1.16 | 0.32 ± 0.12 | 0.89 ± 0.41 | 0.69 ± 0.19 |
| Spleen | 1.46 ± 2.17 | 0.51 ± 0.17 | 0.42 ± 0.13 | 0.29 ± 0.072 |
| Heart | 0.96 ± 0.51 | 0.42 ± 0.13 | 0.50 ± 0.35 | 0.84 ± 0.28 |
| Blood | 0.65 ± 0.56 | 0.38 ± 0.06 | 0.99 ± 1.25 | 0.23 ± 0.074 |
| Carcass | 1.21 ± 1.88 | 1.32 ± 2.28 | 1.82 ± 1.67 | 1.68 ± 2.02 |
| Urine | 0.084 ± 0.111 | 0.19 ± 0.32 | 1.80 ± 0.95 | 3.29 ± 0.19 |
| Intestinal contents/feces | 3.50 ± 2.67 | 12.8 ± 1.86 | 17.6 ± 4.55 | 29.6 ± 3.48 |

[1]Cumulative values.

### *6.2.2 Diesel Exhaust Particulates and Metabolism*

As previously described, numerous organic products of combustion are adsorbed to diesel particles. Since they may be dissolved in biological fluids (both *in vitro* and *in vivo*), understanding the abilities of lung (and other) tissue to metabolize them to carcinogenic species is of practical interest. Moller *et al*. (1987) studied the metabolism of 2-nitrofluorene, which has been identified in urban air and diesel exhaust, in isolated, perfused rat lung and liver. The lung metabolized 2-nitrofluorene to hydroxylated derivatives, mainly 9-hydroxy-2-nitrofluorene which was shown to be mutagenic.

Tee *et al*. (1988) and Bevan and Ruggio (1991) conducted *in vitro* metabolism studies and reported that rabbit lung S9 fraction can metabolize 1,8-dinitropyrene by both reductive and oxidative pathways. Reductive metabolism is the major pathway for formation of stable

metabolites whereas oxidative metabolism results in the alkylation of cellular macromolecules. 1,8-dinitropyrene is commonly found in association with diesel exhaust particulates. The results of this experiment are summarized in Table 6-2.

**Table 6-2. Metabolism[1] of 1,8-Dinitropyrene by rabbit lung S9 under anaerobic and aerobic conditions**

| Metabolite | Anaerobic | Aerobic |
|---|---|---|
| 1,8-dinitropyrene (pmol) | 225.1 ± 27.0 | 542.7 ± 43.0 |
| Ether-extractable (pmol) | 588.6 ± 27.0 | 158.7 ± 41.8 |
| Percent of total metabolites | 98.2 ± 0.1 | 53.9 ± 7.8 |
| DNA bound (pmol/mg DNA) | 6.1 ± 0.3 | 14.8 ± 0.9 |
| Percent bound to DNA | 1.8 ± 0.1 | 9.9 ± 2.1 |

[1]Adding 815 nM of $^{3}$H1,8-dinitropyrene to the reaction mixture started the reaction.

Biotransformation of 1,8-dinitropyrene to stable metabolites (ether extractable) was more extensive under anaerobic conditions with more than twice as much substrate remaining unmetabolized under aerobic conditions. However, binding to calf thymus DNA was more extensive under aerobic conditions.

### *6.2.3 Genetic and Related Effects*

#### *6.2.3.1 Administration with known carcinogens*

Heinrich *et al*. (1986a) and Bevan and Ruggio (1991) exposed Wistar rats to diesel exhaust (with and without particles) and administered weekly subcutaneous doses of 250 or 500 mg/kg of dipentylnitrosamine (DPN) during the initial 25 weeks of the experiment. Exposures continued for up to 2.5 years after cessation of DPN administration. Diesel exhaust was diluted 1:17 and particle concentration in the unfiltered exhaust was 4.24 (1.42 mg/m$^3$ and the total doses of DPN were 6.25 and 500 g/kg. The results of these experiments are summarized in Table 6-3.

**Table 6-3. Percentage of rats with tumors in the lung and nasal cavity after exposure to diesel exhausts and Dipentylnitrosamine**

| Organ of origin | Tumor type | Control (%) | N | Filtered exhaust (%) | N | Total exhaust (%) | N |
|---|---|---|---|---|---|---|---|
| **Rats dosed with 6.25 g/kg DPN** | | | | | | | |
| Lung | All tumors | 84.8 | 46 | 67.4 | 46 | 83 | 47 |
| | Squamous cell carcinoma | 4.4 | 46 | 4.4 | 46 | 46.8 | 47 |
| Nasal | All tumors | 28.3 | 46 | 4.4 | 45 | 8.7 | 46 |

| Organ of origin | Tumor type | Control (%) | N | Filtered exhaust (%) | N | Total exhaust (%) | N |
|---|---|---|---|---|---|---|---|
| cavity | | | | | | | |
| **Rats dosed with 12.5 g DPN** | | | | | | | |
| Lung | All tumors | 93.8 | 48 | 89.6 | 48 | 89.6 | 48 |
| | Squamous cell carcinoma | 16.7 | 48 | 14.6 | 48 | 31.2 | 48 |
| Nasal cavity | All tumors | 52.1 | 48 | 31.3 | 48 | 22.9 | 48 |

The overall lung or nasal cavity tumor rates in animals dosed with dipentylnitrosamine were not influenced by exposure to diesel exhaust. However, the background incidence of tumors attributable to administration of dipentylnitrosamine was high (84.8% and 93.8% in low- and high-dose groups, respectively). This may have reduced the power of the test for detecting any co-carcinogenesis attributable to the inhalation of diesel exhaust. However, there was an unequivocal increase in the incidence of squamous cell carcinomas of the lung.

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# Appendix A-1…Species/ Derivatives Identified or Tentatively Identified in Diesel Exhaust

acenapthene (ARB 1993; Yergey 1982)
acenaphthenequinone (Volkswagen 1989)
acenaphthylene (ARB 1993; IARC 1989; Yergey 1982)
acetaldehyde (ARB 1993; Volkswagen 1989)
acetone (Volkswagen 1989)
acetylene (Volkswagen 1989)
acrolein (ARB 1993; IARC 1989; Volkswagen 1989)
aldehydes (Volkswagen 1989)
alkanes (DEC 1990)
alkylanthraquinone (Choudhury 1982)
alkylbenzenes (Schuetzle 1983)
alkyl-4H-cyclopenta[def]lphenanthren-4-one isomers (Newton 1982)
alkyl-9-fluorenones (Choudhury 1982; Newton 1982)
alkyl-9-fluorenone isomers (Newton 1982)
alkylnaphthaldehyde (Choudhury 1982)
alkylnaphthaldehyde isomers (Newton 1982)
alkylnaphthofuran carboxaldehyde (Choudhury 1982)
C -alkylnitroanthracene isomer (Newton 1982)
aluminum (ARB 1989)
ammonia (IARC 1989; ARB 1989; Volkswagen 1989)
aniline (IARC 1989)
anthanthrene (Volkswagen 1989)
anthracene (ARB 1993; IARC 1989; Jensen 1983; Newton 1982; Schuetzle 1983; Yergey 1982)
anthracene-x-aldehyde (Volkswagen 1989)
anthracene carboxaldehydes (Jensen 1983; Schuetzle 1983)
anthracene-9-carboxaldehyde (Choudhury 1982)
anthracene dicarboxylic acid anhydrides (Schuetzle 1983)
anthracene quinones (Jensen 1983; Schuetzle 1983)
9,10-anthracenedione (Yu 1981)
anthraldehyde isomer (Newton 1982)
anthraquinone (Choudhury 1982; Volkswagen 1989)
9, 10-anthraquinone (Newton 1982)
anthrones (Choudhury 1982; Jensen 1983; Schuetzle 1983)
antimony (ARB 1989)
arsenic (ARB 1993; ARB 1989)

barium (IARC 1989; ARB 1989)
benzacenaphthylene (IARC 1989)
benzacridines (IARC 1989)
benzaldehyde (Volkswagen 1989)
benz[a]anthracene (ARB 1993; IARC 1989; Schuetzle 1983; Volkswagen 1989; Yu 1981)
benz[a]anthracene carboxaldehydes (Schuetzle 1983)
benz[a]anthracenedione (Choudhury 1982)
benz[a]anthracene-7,12-dione (Volkswagen 1989)
7H-benz[de]anthracene-7-one (Choudhury 1982; Volkswagen 1989)
benz[de]anthracenone (Choudhury 1982)
benzanthrone isomers (Newton 1982; Schuetzle 1983; Yu 1981)
7H-benz[de]anthrone-7-one (Choudhury 1982; Volkswagen 1989; Yu 1981)
benzene (ARB 1993; Volkswagen 1989)
benzo[c]cinnoline (Yergey 1982)
benzo[def]dibenzothiophene (IARC 1989)
benzo,fluoranthene (Yergey 1982; Yu 1981)
benzo[b]fluoranthene (ARB 1993; IARC 1989; Volkswagen 1989)
benzo[ghi]fluoranthene (IARC 1989; Schuetzle 1983; Volkswagen 1989; Yergey 1982; Yu 1981)
benzo[j]fluoranthene (IARC 1989)
benzo[k]fluoranthene (ARB 1993; IARC 1989; Volkswagen 1989)
benzofluorene isomer (Newton 1982)
benzo[a]fluorene (IARC 1989)
benzo[b]fluorene (IARC 1989)
benzo[x]fluorene-y-one (Volkswagen 1989)
benzofluorenone (Choudhury 1982)
benzo[a]fluorenone (Choudhury 1982)
11 -benzo, [a]fluorenone (Yu 1981)
benzoic; acid (IARC 1989)
benzonaphthothiophene (IARC 1989)
benzo[b]naphtho[2,1-d]thiophene (IARC 1989; Yu 1981)
benzo[b]naphtho[2,1 -d]thiophene isomers (Yu 1981)
benzo[ghi]perylene (ARB 1993; IARC 1989; Volkswagen 1989; Yergey 1982)
benzo[c]phenanthrene (Volkswagen 1989)
1-benzopyran-2-one (Yergey 1982)
benzopyrenes (Yu 1981)
benzopyrene ketones (Choudhury 1982)
benzo[a]pyrene (ARB 1993; IARC 1989; Pitts 1982; Schuetzle 1983; Volkswagen 1989)

benzo[e]pyrene (IARC 1989; Volkswagen 1989)
benzo[xy]pyrene-z-one (Volkswagen 1989)
6H-benzo[cd]pyrenone isomers (Newton 1982; Volkswagen 1989)
benzo[cd]pyrenone (Choudhury 1982)
6-H-benzo[cd]pyrene-6-one (Volkswagen 1989)
beryllium (ARB 1993)
1,2-binaphthyl (IARC 1989)
2,2-binaphthyl (IARC 1989)
biphenyl (Yergey 1982)
biphenyl carboxaldehydes (Jensen 1983; Yu 1981)
biphenylene, (Yergey 1982)
bis[ethylhexyl]phthalate isomer (Newton 1982)
bromine (ARB 1989)
1,3-butadiene (ARB 1993)
cadmium (ARB 1993; ARB 1989)
calcium (ARB 1989)
carbon dioxide (Volkswagen 1989)
carbon monoxide (ARB 1993; Volkswagen 1989)
carbonate ion (ARB 1989)
chlorine (ARB 1993; ARB 1989)
chlorobenzene (ARB 1993)
chromium (ARB 1993; ARB 1989)
chrysene (ARB 1993; IARC 1989; Schuetzle 1983; Volkswagen 1989; Yergey 1982; Yu 1981)
cobalt (ARB 1989)
copper (ARB 1993; ARB 1989)
coronene (IARC 1989; Volkswagen 1989)
cresols (IARC 1989)
crotonaldehyde (Volkswagen 1989)
cyanides (Volkswagen 1989)
cyclopenta[cd]benzo[ghi]perylene (Rappaport 1980)
cyclopenta[jk]naphtho[1,8,7-efg]pyrene (Rappaport 1980)
cyclopentaphenanthrene-5 -one (Yergey 1982)
4H-cyclopenta[def]phenanthrene (IARC 1989)
4H-cyclopenta[def]phenanthren-4-one (Choudhury 1982; Jensen 1983; Newton 1982; Volkswagen 1989)
cyclopenta[cd]pyrene (IARC 1989; Rappaport 1980; Schuetzle 1983; Volkswagen 1989)
cyclopenteno[cd]pyrene (Yergey 1982)
dibenzacridines (IARC 1989)

dibenz[a,c]anthracene (Volkswagen 1989)
dibenz[a,h]anthracene (ARB 1993; IARC 1989; Volkswagen 1989)
dibenz[a,j]anthracene (Volkswagen 1989)
dibenzofurans (Yergey 1982)
dibenzofuran carboxaldehydes (Schuetzle 1983)
dibenzopyrene or -[def, p]chrysene (IARC 1989)
dibenzothiophene (IARC 1989; Jensen 1983; Yu 1981)
dibutyl phthalate (Choudhury 1982)
2,3-dihydro-inden-1-one (Yergey 1982)
dihydroxyfluorenes (Jensen 1983)
1,3-dihydroxynitropyrene (IARC 1989)
dihydroxyphenanthrenes (Jensen 1983)
dimethylanth.racenes (IARC 1989; Schuetzle 1983)
dimethylanthracene carboxaldydes (ARB 1993; Schuetzle 1983)
dimethylanthrones (Schuetzle 1983)
dimethylbiphenyl (IARC 1989)
1,9-dimethylfluorene (Jensen 1983)
dimethylfluorene quinones (Schuetzle 1983)
dimethylfluorenones (Schuetzle 1983)
dimethyhydroxyfluorene (Schuetzle 1983)
dimethylnaphthalene carboxaldehydes (Schuetzle 1983)
dimethylnaphthalene dicarboxylic acid anhydrides (Schuetzle 1983)
dimethylphenanthrene (IARC 1989)
dimethylphenanthrenes (Schuetzle 1983)
dimethylphenanthrene carboxaldehydes (Schuetzle 1983)
dimethylphenanthrones (Schuetzle 1983)
4,4-dinitrobiphenyl (Volkswagen 1989)
2,5-dinitrofluorene (IARC 1989)
2,7-dinitrofluorene (IARC 1989)
2,7-dinitro-9-fluorenone (IARC 1989)
dinitronaphthalene (IARC 1989)
1,3-dinitropyrene (IARC 1989)
1,6-dinitropyrene (IARC 1989)
1,8-dinitropyrene (IARC 1989)
dioxins (ARB 1993)
elemental carbon (IARC 1989; ARB 1989)
ethane (Volkswagen 1989)
ethylbenzene (ARB 1993)

ethyldibenzothiophene (IARC 1989)
ethylene, (Volkswagen 1989)
ethylmethylphenanthrene (IARC 1989)
2-or 9-ethylphenanthrene (IARC 1989)
fluoranthene (ARB 1993; IARC 1989; Jensen 1983; Schuetzle 1983; Volkswagen 1989; Yergey 1982; Yu 1981)
fluoranthene carboxaldehydes (Schuetzle 1983; Yu 1981)
fluoranthene quinones (Schuetzle 1983)
fluoranthones (Schuetzle 1983)
fluorene (ARB 1993; IARC 1989; Jensen 1983; Yergey 1982)
fluorene carboxaldehydes (Schuetzle 1983)
fluorene quiriones (Jensen 1983; Schuetzle 1983; Yergey 1982)
fluorenones (Schuetzle 1983; Yu 1981)
9-fluorenone (Choudhury 1982; Volkswagen 1989; Yergey 1982)
fluoren-9-one (Jensen 1983)
9-fluorenone isomers (Newton 1982)
formaldehyde (ARB 1993; Volkswagen 1989)
formic acid (IARC 1989)
furans (ARB 1993)
gallium (IARC 1989)
heptane (DEC 1990)
hexane(ARB 1993)
hexanaldehyde (Volkswagen 1989)
hydrogen (Volkswagen 1989)
hydrogen chloride (ARB 1993)
hydrogen cyanide (Volkswagen 1989)
hydrogen sulfide (Volkswagen 1989)
hydroxyanthracenes (Schuetzle 1983)
hydroxychrysene/triphenylene (Choudhury 1982)
hydroxydimethylanthracenes (Schuetzle 1983)
hydroxydimehtylphenanthrenes (Schuetzle 1983)
hydroxyfluoranthene (Choudhury 1982)
hydroxyfluorene (Schuetzle 1983)
hydroxyfluorenone (Schuetzle 1983)
hydroxymethylanthracenes (Schuetzle 1983)
hydroxymethylphenanthrenes (Schuetzle 1983)
hydroxyphenanthrenes (Jensen 1983; Schuetzle 1983)
hydroxypyrene (Choudhury 1982)

hydroxyxanthene (Schuetzle 1983)
hydroxyxanthone (Schuetzle 1983)
indeno[1,2,3-cd]pyrene (ARB 1993; IARC 1989; Volkswagen 1989)
indium (ARB 1989)
iron (IARC 1989; ARB 1989; Volkswagen 1989)
isobutyraldehyde (Volkswagen 1989)
lanthanurn (ARB 1989)
lead (ARB 1993; ARB 1989)
manganese (ARB 1993; ARB 1989; Volkswagen 1989)
mercury (ARB 1993; ARB 1989)
methane, (Volkswagen 1989)
methanol (IARC 1989; Volkswagen 1989)
methyl ethyl ketone (Volkswagen 1989)
methylanthracenes (Schuetzle 1983)
2-methylanthracene (IARC 1989)
methylanthracene carboxaldehydes (Choudhury 1982; Schuetzle 1983)
methylanthracene-9-carboxaldehyde (Choudhury 1982)
methylanthracene quinones (Schuetzle 1983)
methyl-9, 10-anthracenedione (Yu 1981)
methylanthraldehyde isomer (Newton 1982)
methylanthraquinone, (Choudhury 1982)
x-methylanthraquinone (Volkswagen 1989)
methylanthrones (Schuetzle 1983)
methylbenz[a]anthracene (IARC 1989)
7-methylbenzofuran (Yergey 1982)
methylbiphenyl carboxaldehydes (Yu 1981)
9-methylcarbazole (IARC 1989)
3-methylchrysene (IARC 1989)
methyl-4H-cyclopenta[def]phenanthren-4-one isomer (Newton 1982)
x-methyl-4-H-cyclopenta[def]phenanthrene-4-one (Volkswagen 1989)
methyldibenzothiophene (IARC 1989)
methylfluoranthenes (IARC 1989; Schuetzle 1983; Yu 1981)
methy1fluorenes (Yu 1981)
9-methylfluorene (Jensen 1983)
methylfluorene carboxaldehydes (Schuetzle 1983)
methylfluorene quinones (Schuetzle 1983)
methy1fluorenones (Schuetzle 1983; Yu 1981)
2-methylfluorenone (Yu 1981)

methyl-9-fluorenone (Choudhury 1982; Yergey 1982)
methyl-9-fluorenone isomers (Newton 1982; Volkswagen 1989)
methylhydroxyfluorene (Schuetzle 1983)
methylnaphthaldehyde (Choudhury 1982; Yu 1981)
methylnaphthaldehyde isomers (Newton 1982)
6-methyl-2-naphthaldehyde (Yu 1981)
methy1napthalene (Yergey 1982)
methylnaphthalene dicarboxylic acid anhydrides (Schuetzle 1983)
methy1nitroanthracene isomer (Newton 1982)
methy1nitrofluoranthenes (Schuetzle 1983)
x-methyl-9-nitroanthracene (Volkswagen 1989)
x-methyl-1-nitronaphthalene (IARC 1989)
methy1nitropyrenes (Newton 1982; Schuetzle 1983)
methylphenanthrenes (Schuetzle 1983)
1-methylphenanthrene (Jensen 1983; Yu 1981)
2-methylphenanthrene (IARC 1989; Jensen 1983; Yu 1981)
3-methylphenanthrene (IARC 1989; Yu 1981)
4-methylphenanthrene (Yu 1981)
9-methylphenanthrene (Jensen 1983; Yu 1981)
methylphenanthrene carboxaldehydes (Choudhury 1982; Schuetzle 1983; Yu 1981)
methylphenanthrene-9-carboxaldehyde (Choudhury 1982)
methylphenanthrene quinones (Choudhury 1982; Schuetzle 1983)
methylphenanthrones (Schuetzle 1983)
methylpheny1napthalenes (Yu 1981)
methylpyrenes (IARC 1989; Schuetzle 1983; Yergey 1982; Yu 1981)
1-methylpyrene (IARC 1989)
methylthioxanthones (Schuetzle 1983)
methylxanthones (Schuetzle 1983)
molybdenum (ARB 1989)
1-napthaldehyde (Yu 1981)
2-napthaldehyde (Yu 1981)
napthaldehyde isomers (Newton 1982)
naphthalene (ARB 1993; Jensen 1983; Yergey 1982)
naphthalene carboxaldehydes (Jensen 1983)
naphthalene dicarboxaldehydes (Schuetzle 1983)
naphthalene dicarboxylic acids (Jensen 1983)
1,8-naphthalene dicarboxylic acid (Volkswagen 1989)
naphthalene dicarboxylic acid anhydrides (Jensen 1983; Schuetzle 1983)

naphthoic acid (IARC 1989)
1-naphthol (IARC 1989)
2-naphthol (IARC 1989)
naphtho[1,8-cd]pyrene 1,3-dione (Schuetzle 1983; Yergey 1982)
nickel (ARB 1993; ARB 1989)
nitrates (ARB 1989)
nitric acid (IARC 1989)
nitric oxide (IARC 1989)
nitro-PAHs (Choudhury 1982)
1-nitro-3-acetoxypyrene (IARC 1989)
nitroanthracenes (Newton 1982; Schuetzle 1983)
nitroanthracene isomer (Newton 1982)
9-nitroanthracene (IARC 1989; Pitts 1982)
2-nitroanthracene (IARC 1989)
3-nitro-7H-benz[d,e]anthracen-7-one (Enya *et al.* 1998)
[1 or 3]-nitrobenzo[a]pyrene (Pitts 1982)
[3 or 1]-nitrobenzo[a]pyrene (Pitts 1982)
6-nitrobenzo[a]pyrene (IARC 1989; Pitts 1982; Volkswagen 1989)
x-nitrobenzoquinoline (IARC 1989)
2-nitrobiphenyl (IARC 1989)
3-nitrobiphenyl (IARC 1989)
4-nitrobiphenyl (IARC 1989)
1-nitrochrysene (IARC 1989)
nitrochrysene isomer (Newton 1982)
x-nitrodibenzothiophene isomers (IARC 1989)
x-nitro-y,z-dimethylanthracene (IARC 1989)
nitrofluoranthenes (Schuetzle 1983; Yu 1981)
nitrofluoranthene isomers (Newton 1982)
1-nitrofluoranthene (IARC 1989)
3-nitrofluoranthene (IARC 1989; Volkswagen 1989)
7-nitrofluoranthene (IARC 1989)
8-nitrofluoranthene (IARC 1989)
nitrofluorenes (Schuetzle 1983)
2-nitrofluorene (IARC 1989; Volkswagen 1989)
2-nitro-9-fluorene (IARC 1989)
3-nitro-9-fluorene (IARC 1989)
nitrogen (ARB 1993; Volkswagen 1989)
nitrogen oxides (ARB 1993; IARC 1989; Volkswagen 1989)

1-nitro-3-hydroxypyrene (IARC 1989)
9-nitro-1-methylanthracene (IARC 1989)
10-nitro-1-methylanthracene (IARC 1989)
10-nitro-9-methylanthracene (IARC 1989)
x-nitro-y-methylanthracene (IARC 1989)
1-nitro-2-methylnaphthalene (IARC 1989)
3-nitro-1-methylpyrene (IARC 1989)
6-nitro-1-methylpyrene (IARC 1989)
8-nitro-l-methylpyrene (IARC 1989)
1-nitronaphthalene (IARC 1989)
2-nitronaphthalene (IARC 1989)
3-nitro-1,8-naphthalic acid anhydride (IARC 1989)
2-nitrophenanthrene (IARC 1989)
nitrophenanthrenes (Newton 1982; Schuetzle 1983)
nitropyrenes (Jensen 1983; Newton 1982; Schuetzle 1983; Yergey 1982; Yu 1981)
1-nitropyrene (IARC 1989; Pitts 1982; Volkswagen 1989)
5-nitroquinoline, (IARC 1989)
8-nitroquinoline (IARC 1989)
x-nitroterphenyl (ARB 1989)
x-nitro-y,z,z'-trimethylanthracene [6 isomers] (ARB 1989)
x-trimethylnaphthalene [3 isomers] (ARB 1989)
nitrous acid (ARB 1989)
octane (DEC 1990)
organic Carbon (ARB 1989)
4-oxapyrene-5-one[l] (Pitts 1982)
oxygen (Volkswagen 1989)
oxy-PAHs (Choudhury 1982; Jensen 1983)
PAHs (ARB 1993; Choudhury 1982; ARB 1989; Jensen 1983; Rappaport 1980; Schuetzle 1983; Volkswagen 1989)
PAH derivatives (Choudhury 1982; ARB 1989; Jensen 1983; Volkswagen 1989)
PAH anhydrides (ARB 1989; Jensen 1983)
PAH carboxaldehydes (ARB 1989; Jensen 1983)
PAH coumarin (Volkswagen 1989)
PAH epoxides (Choudhury 1982)
PAH esters (ARB 1989)
PAH ketones (ARB 1989; Jensen 1983; Schuetzle 1983)
PAH quinones (ARB 1989)
PAH sulfonates (ARB 1989)

palladium (ARB 1989)

pentane (DEC 1990)

perylene (Volkswagen 1989; Yu 1981)

1-H-phenalene-1-one (Volkswagen 1989)

phenanthrenes (ARB 1993; ARB 1989; Jensen 1983; Newton 1982; ARB 1989; Yergey 1982; Yu 1981)

phenanthrene-x-aldehyde (Volkswagen 1989)

phenanthrene carboxaldehydes (Jensen 1983; Schuetzle 1983; Yu 1981)

phenanthrene-9-carboxaldehyde (Choudhury 1982)

2-phenanthrene carboxaldehyde (Yu 1981)

phenanthrene dicarboxylic acid anhydrides (Schuetzle 1983)

phenanthrene quinones (Jensen 1983; Schuetzle 1983; Yergey 1982)

phenanthrene-9, 10-quinone (Volkswagen 1989)

phenanthroic acid (ARB 1989)

phenanthrones (Choudhury 1982; Jensen 1983; Schuetzle 1983)

5H-phenanthro[4,5-bcd]pyran-5-one (Pitts 1982)

phenol (ARB 1989), (Volkswagen 1989)

phenylbenzaldehyde isomers (Newton 1982)

phenyinaphthalene (Jensen 1983; Yu 1981)

2-phenylnaphthalene (ARB 1989)

1-phenylphenanthrene (ARB 1989)

9-phenylphenanthrene (ARB 1989)

phenylphenanthrene or -anthracene (ARB 1989)

phosphorus (ARB 1993; ARB 1989)

phthalate (Volkswagen 1989)

phthalate Anhydride (Yergey 1982)

phthalic acid (ARB 1989)

platinum (ARB 1989; Volkswagen 1989)

POM [Polycyclic Organic Matter] (Pitts 1982)

potassium (ARB 1989)

propane (ARB 1989)

propionaldehyde (Volkswagen 1989)

propylene (DEC 1990)

pyrene (ARB 1993; ARB 1989; Jensen 1983; Newton 1982; Schuetzle 1983; Volkswagen 1989; Yergey 1982; Yu 1981)

pyrene carboxaldehydes (Schuetzle 1983; Yu 1981)

1, 12-pyrenedicarboxylic acid anhydride (Volkswagen 1989)

pyrene-3,4-dicarboxylic acid anhydride (Choudhury 1982; Rappaport 1980)

pyrene quinones (Schuetzle 1983)

pyridine (IARC 1989),
pyrones(Schuetzle 1983)
quercetin (Pitts 1982)
rhodium (Volkswagen 1989)
rubidium (ARB 1989)
selenium(ARB 1993)
silicon (ARB 1989)
siloxane (Newton 1982)
silver (ARB 1989)
sodium (ARB 1989)
strontium (ARB 1989)
styrene (DEC 1990)
sulfates (ARB 1989; Volkswagen 1989)
sulfur (ARB 1993; ARB 1989; Volkswagen 1989)
sulfur dioxide (Volkswagen 1989)
sulfuric acid (ARB 1989)
n-tetradecane (Yergey 1982)
tetramethylnaphthalene (ARB 1989)
thioxanthen-9-one (Jensen 1983)
9H-thioxanthen-9-one (Choudhury 1982)
thioxanthones (Schuetzle 1983)
tin (ARB 1989)
titanium (ARB 1989)
toluene (ARB 1993; Volkswagen 1989)
trimethylbiphenyl (ARB 1989)
2,2,4-trimethyl-1,3-diol diisobutyrate (Newton 1982)
trimethy1naphthalene (ARB 1989)
trimethy1naphthalene carboxaldehydes (Schuetzle 1983)
2,4,7-trinitro-9-fluorenone (ARB 1989)
triphenylene carboxaldehydes (Schuetzle 1983)
vanadium (ARB 1989)
water vapor (Volkswagen 1989)
xanthene carboxaldehydes (Schuetzle 1983)
xanthen-9-one (Jensen 1983)
9H-xanthen-9-one (Choudhury 1982)
xanthones (Schuetzle 1983)
xylenes (ARB 1993)
yttrium (ARB 1989)

zinc (ARB 1993; ARB 1989; ARB 1989)
zirconium (ARB 1989)

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**Appendix A-2…Epidemiology studies of carcinogenicity to humans.** International Agency for Research on Cancer. 1989. *Diesel and gasoline engine exhausts and some nitroarenes. IARC.* Vol. 46: 88-185.

exhaust (Janssen, 1976; Metz *et al.*, 1984) in which GC methods were compared with those of HPLC (Metz *et al.*, 1984).

Packed-column GC has been largely replaced by high-resolution glass capillary column GC using fused silica columns and careful sample injection procedures (on-column injection at low temperatures of 50–80°C or thermal desorption cold trap injection procedure). Detection limits down to the picogram level have been reached with GC when single-ion monitoring mass spectrometry is used as the detection system (Ramdahl & Urdal, 1982).

Various classes of organic compounds from vehicle exhausts have been analysed by GC and GC/MS, including paraffins, olefins, PAHs, thia-arenes, aza-arenes, oxo-arenes and aldehyde, phenol, quinone and nitro derivatives of PAHs and their acid anhydrides (Hites *et al.*, 1981; Schuetzle *et al.*, 1981; Lee & Schuetzle, 1983; Alsberg *et al.*, 1984; Ramdahl, 1984; White, 1985). More than 70 individual nitroarenes were found in diesel particulate extracts by means of fused-silica capillary column GC and a nitrogen-specific detector, with detection limits of 0.2–0.5 mg/kg (Paputa-Peck *et al.*, 1983). The emission of PAHs and nitroarenes by diesel engines from PAH-containing and other well defined fuels (hexadecane) were studied using GC/MS and tandem triple-quadrupole MS (Fulford *et al.*, 1982; Henderson *et al.*, 1983, 1984).

The rapid analysis of gaseous and other combustion-related compounds in hot gas streams by atmospheric-pressure chemical ionization/MS has been reported (Sakuma *et al.*, 1981).

(iv) *Other methods*

Photometric, infra-red, colorimetric, electrochemical and chemiluminescence techniques have been used for the analysis of gases such as sulfur dioxide, carbon monoxide, carbon dioxide, nitrogen oxides and formaldehyde (Hare & Baines, 1979; Deutsche Forschungsgemeinschaft, 1985). Test tubes in which various colour reactions are seen are available for the analysis of various constituents of vehicle exhaust such as carbon monoxide, carbon dioxide, sulfur dioxide and nitrogen dioxide (Leichnitz, 1986).

# 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/m$^3$ particles. Levels of 1.5 ± 0.3 ppm (3 ± 0.6 (SD) mg/m$^3$) nitrogen dioxide and 11.4 ± 2.1 ppm nitrogen oxides were found in whole exhaust and 1.2 ± 0.26 ppm (2.4 ± 0.5 mg/m$^3$) 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 cm$^3$ 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/m$^3$ particles; size, 0.32 $\mu$m; 2–4 ppm, 4–8 mg/m$^3$ nitrogen dioxide). Between months 13 and 28, lung tumours (adenomas and adeno-carcinomas) 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/m$^3$ soot from diesel exhaust; 8.3 ± 2.0 mg/m$^3$ soot from diesel exhaust plus 5.8 ± 3.5 mg/m$^3$ coal dust; 6.6 ± 1.9 mg/m$^3$ coal dust; or 14.9 ± 6.2 mg/m$^3$ 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/m$^3$ coal dust (<7 $\mu$m); 2 mg/m$^3$ diesel exhaust particles, with specific limits on gaseous/vapour constituents; or 1 mg/m$^3$ coal dust plus 1 mg/m$^3$ diesel exhaust particles (Lewis *et al.*,1986). The nitrogen dioxide concentration in the diesel exhaust was 1.5 ± 0.5 ppm (3 ± 1 mg/m$^3$); the exhaust was generated by a 7-1 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 cm$^3$ 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 $\mu$m) ranged from 2–4 mg/m$^3$, and those of nitrogen dioxide were 2–4 ppm (3–8 mg/m$^3$). 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/m$^3$ particles, 1.8 ± 1.8 ppm (3.6 ± 3.6 mg/m$^3$) 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

[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 1.8-1 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 11-1 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 1.5-1 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]

[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/m$^3$. 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/m$^3$), respectively. Sham-exposed controls received filtered air. The soot particles were approximately 0.25 $\mu$m 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 $\mu$m; mean particle concentration, 3.9 ± 0.5 mg/m$^3$; nitrogen dioxide, 1.2 ± 1.7 ppm (2.4 ± 3.4 mg/ m$^3$); nitrogen oxides, 18.6 ± 5.8 ppm) or filtered diesel exhaust (nitrogen dioxide, 1.0 ± 1.5 ppm (2 ± 3 mg/m$^3$); 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 $\mu$m) 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 C57B1 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. A11 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 $\mu$g 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 $\mu$g TPA three times per week. The concentration of TPA used in the initiation and promotion studies was changed after eight months to 1.5 $\mu$g. 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 $\mu$g 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 $\mu$g 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-L 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 $\mu$g 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-L 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-mine (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/m$^3$ gasoline exhaust (1:250 dilution of emission from a small gasoline engine; carbon monoxide, 300 ± 50 ppm (350 ± 60 mg/m$^3$); nitric oxide,0.21 ppm (0.3 mg/m$^3$); nitrogen dioxide, 0.08 ppm (0.16 mg/m$^3$) [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 adeno-carcinomas) in the urethane-treated group was 21/25. [The Workine 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 m$^3$ 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/m$^3$. 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/m$^3$). 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/m$^3$; nitric oxide, 28 ± 3 [13.7 ± 1.5 ] mg/m$^3$; nitrogen dioxide, 1.9 ± 0.4 [1.0 ± 0.2] mg/m$^3$; particles, 95.8 ± 16.5 [47.9 ± 20.2] mg/m$^3$. 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/m$^3$) carbon monoxide and 24–30 ppm hydrocarbon expressed as methane. The irradiated exhaust contained 0.5–1.0 ppm (1–2 mg/m$^3$) nitrogen dioxide, 0.1 ppm (0.12 mg/m$^3$) 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 $\mu$g 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 g/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 70), 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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/ 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 $\mu$g 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 $\mu$g/ 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 intratracheal instillations of 100 $\mu$g benzo[*a*]pyrene, 20 intratracheal instillations of 50 $\mu$g benzo[*a*]pyrene or ten intratracheal instillations of 50 $\mu$g 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 $\mu$g 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 $\mu$g 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 $\mu$g (females and males) or 10 $\mu$g (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 $\mu$g 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.]

## 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 $\mu$m (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 $\mu$m 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 $\mu$m; 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 | 10-7 | Dutcher *et al.* (1984) |
| Rat | 0.12 | 17[a] (calculated)<br>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 $\mu$m, total deposition in rats falls from 25 to 15%, tracheo-bronchial 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 $\mu$m 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] | |
| ND[b] | ND[b] | 77 | Chan *et al*. (1984) |
| 75 | 25 | ND[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]ND, Data not available

mucociliary clearance of exposures of six to 24 months to particulate concentrations of 0.4–7.1 mg/m$^3$ (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/m$^3$, 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/m$^3$, 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/m$^3$ (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

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

| Exposure | | | Pulmonary clearance | | Reference |
|---|---|---|---|---|---|
| Concentration ($mg/m^3$) | Duration (weeks) | h per day × days/week | Material studied | Half-time (days) | |
| 0 | 0 | 0 | Diesel | 77 | Chan *et al.* (1984) |
| 0.25 | 7 | 20 × 7 | exhaust | 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 | 87 | Griffis *et al*. (1983) |
| 0.94 | 18 | 7 × 5 | exhaust | 99 | |
| 4.10 | 18 | 7 × 5 | | 165 | |
| 6.00 | 1 | 20 × 7 | Diesel | 61 | Lee *et al.* (1987) |
| 6.00 | 3 | 20 × 7 | exhaust | 124 | |
| 6.00 | 6 | 20 × 7 | | 192 | |
| 0 | 0 | 0 | FAP[a] | 79 | Wolff *et al*. (9187) |
| 0.35 | 104 | 7 × 5 | | 81 | |
| 3.50 | 104 | 7 × 5 | | 264 | |
| 7.00 | 104 | 7 × 5 | | 240 | |

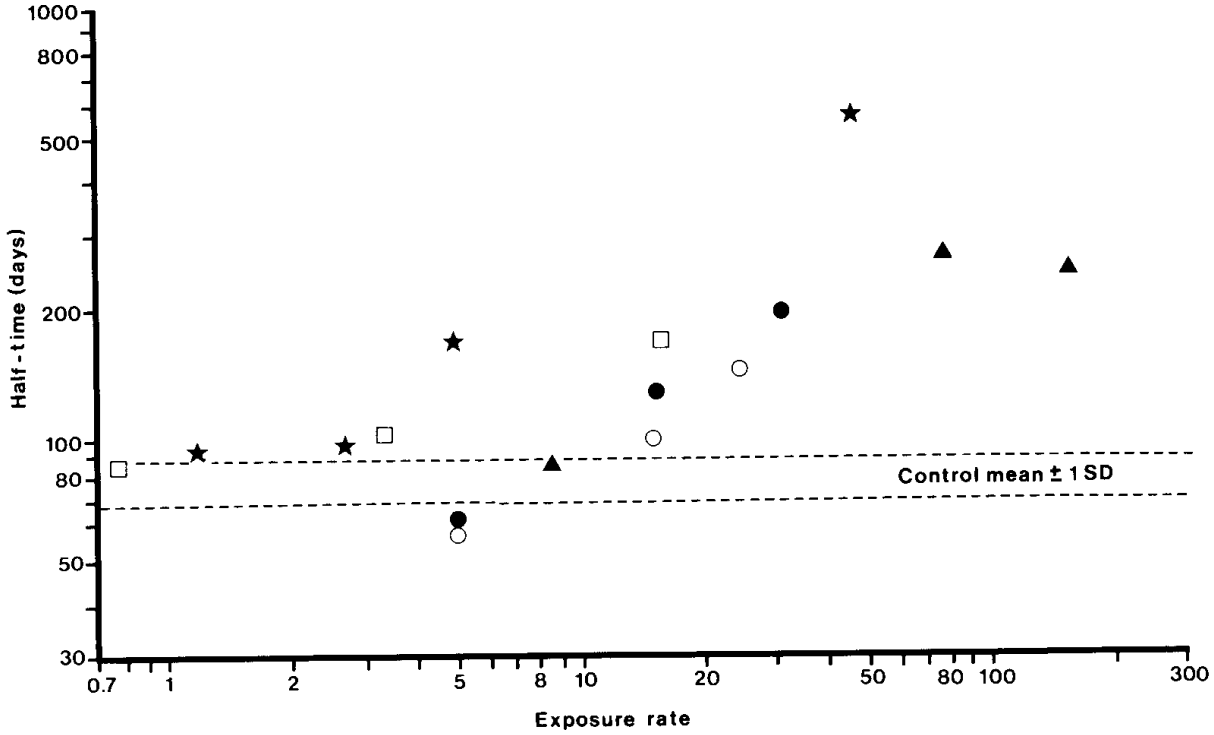
[a]FAP, radiolabelled ($^{134}Cs$) fused aluminosilicate particles

measurements, 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, $mg/m^3$ × 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 $mg/m^3$ × week (i.e., continuous exposure to 0.2 $mg/m^3$ or exposure for 40 h per week to 0.8 mg/ $m^3$ 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; ○, 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 ratet 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.]

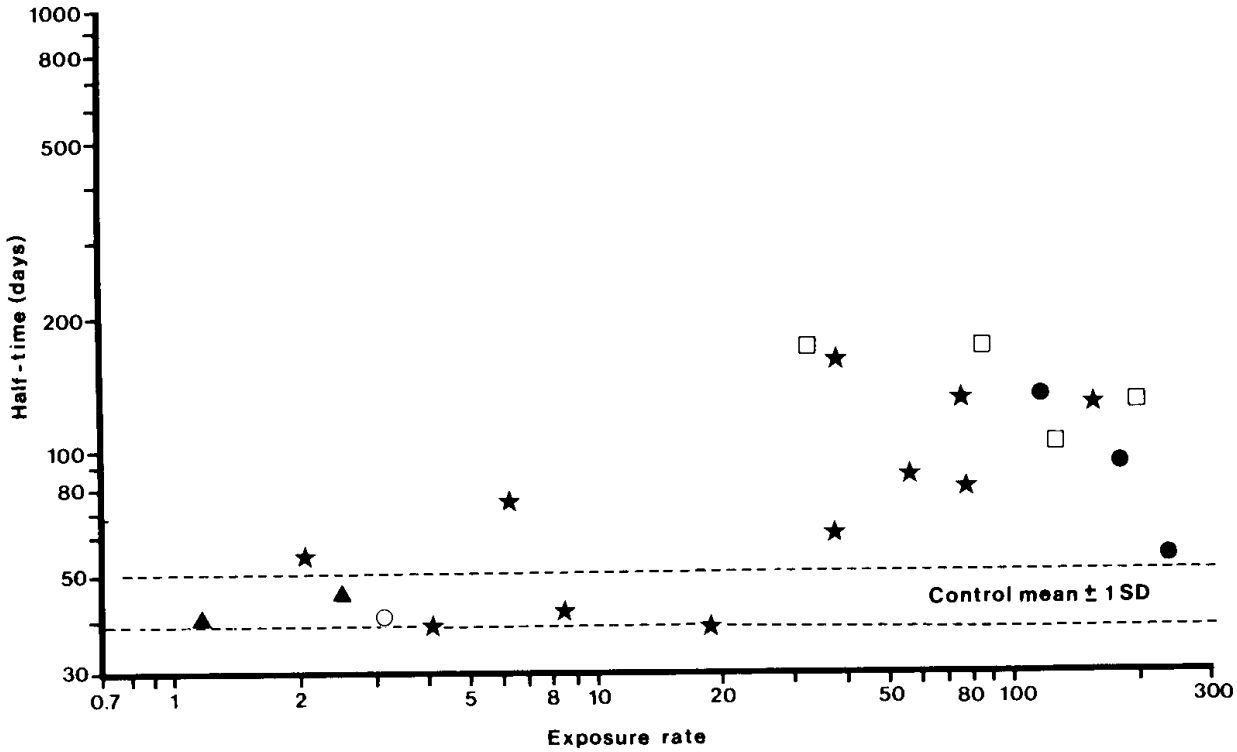
After only two months' exposure of rats to a diesel exhaust particulate concentration of 2 mg/m$^3$, clearance of metal oxide particles was significantly faster than in controls, sug-

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

| Exposure | | | Pulmonary clearance | | Reference |
|---|---|---|---|---|---|
| Concentration ($mg/m^3$) | 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 | $^{59}Fe_2O_3$ | 47 | Lewis *et al*. (1986) |
| 2 | 9 | 7 X 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 | $Fe_2O_3$ | 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; ○, Lewis *et al.* (1986), diesel exhaust; ▲, Bellmann *et al.* (1983), gasoline exhaust. Exposure rate = (mg × week/m$^3$) × (h/week)/168

gesting 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/m$^3$) 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/m$^3$), 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 lungclearance 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 $\mu$M $^{14}$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 $\mu$m) 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 | 36 |
| 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 (Tornqvist *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/m$^3$; 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/m$^3$; carbon monoxide, 14.3 ± 2.5 mg/m$^3$) 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/m$^3$) 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/m$^3$; 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/m$^3$), 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/m$^3$) 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/ m$^3$) 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/m$^3$, focal accumulation of soot was dose-dependent

and was paralleled by an active inflammation involving alveolar macro-phages 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 monthst 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 sinus 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 $\mu$g 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$^3$ nonirra-diated, 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). ln 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 $\mu$g/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 (Belisurio *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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/mL (Rudd, 1980; Mitchell *et al.*, 1981).

Particulate extracts (60 $\mu$g/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 $\mu$g/mL) induced mutation in Chinese hamster CHO cells, but no mutagenic activity was observed with samples from one light-duty (up to 300 $\mu$g/ mL) or one heavy-duty diesel engine (up to 750 $\mu$g/ mL; Casto *et al.*, 1981). In a third study, extracts from the exhaust of a light-duty diesel engine (25–75 $\mu$g/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 $\mu$g/ 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 $\mu$g/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 $\mu$g/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 $\mu$g/mL) of diesel exhaust induced mutation to trifluoro-thymidine 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 $\mu$g/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 $\mu$g/mL; Lockard *et al.*, 1982) and by diesel particulate extracts (10–200 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/mL; Zamora *et al.*, 1983, up to 40 $\mu$g/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 $\mu$g/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 $\mu$g/mL) from a heavy-duty engine (Casto *et al.*, 1981).

A particulate extract (5–10 $\mu$g/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 adminis-tration 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×C57B1)$F_1$, (C3H×C57B1)$F_1$ or T-stock female mice or when female (101×C3H)$F_1$ mice were similarly exposed for 7 weeks prior to matingwith 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×C57B1)$F_1$ 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; Lofroth, 1981 a,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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$g/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 $\mu$m (Chamberlain *et al.*, 1975) or 0.35 and 0.7 $\mu$m, 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 $\mu$m. 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 $\mu$m), 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 0.81 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 $\mu$m) 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 $\mu$m), although the air near roundabouts also contained a large proportion by mass of adventitious particles (2 $\mu$m) (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-$\mu$m 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, carboxy-haemoglobin (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 $FEF_{25\text{-}75\%}$ (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).

### 3.3 Epidemiological studies of carcinogenicity to humans

(*a*) *Introduction*

Although population-based studies to detect a possible association between exposure to engine exhausts and cancer in humans are the most direct methods for detecting human carcinogenesis, for low levels of risk the approach is complicated by several factors. These factors can be divided broadly into problems related to the documentation of levels of exposure and the potential for unidentified confounding factors to influence the results.

Nonoccupational exposure to engine exhaust is nearly ubiquitous in urban areas and in the vicinity of vehicles. Because emissions are diluted in the nonoccupational environment, it is unlikely that investigations of the general population would reveal risks when groups with heavy exposure show only a small risk.

'Unexposed' reference populations used in epidemiological studies are likely to contain a substantial number of subjects who are exposed nonoccupationally to engine exhausts. The 'exposed' group is often defined on the basis of job title, which may be an inadequate surrogate for exposure to exhaust emissions, and this may lead to an underestimation of risk. The situation is further complicated by the presence of possible confounding factors, such as smoking and other exposures (e.g., asbestos in railroad yards), which may influence results, especially when lung and bladder cancers are being studied. In addition, in many studies of the occupational setting, there is an inextricable link between exposure to exhaust emissions and to vapours from the fuels themselves. Some occupational groups, such as car-park attendants and toll-booth workers, which might be thought to be a source of more direct information due to their heavy exposure, are usually too small and/or too transient for a population-based study of cancer to be feasible.

Another important consideration is that occupational cohorts tend to have below-average mortality, both from all causes and from various major categories of specific causes. These deficits are, typically, manifestations of a selection process based on health status, referred to as the 'healthy worker effect'. In view of this overall deficit in cancer mortality in working cohorts, conventional statistical evaluation of site-specific standardized mortality ratios (SMRs) is usually conservative. That is, comparison of the SMR with an 'expected' value of 100 derived from the general population — rather than from some defined internal unexposed comparison group — may result in an underestimation of the true magnitude of any occupation-related increase in risk for specific cancers.

In the studies reviewed, retrospective assessment of an individual's exposure to engine exhausts is necessarily indirect, since there are generally no systematic or quantitative records of work-place or ambient exposures. In some studies, the title of a job or occupation with known or presumed exposure is used as a simple surrogate measure of exposure, and the cancer risk of groups of individuals in such jobs is compared with that of the general population or of persons in unrelated jobs. In some other studies, mainly of case-control design, each individual's past exposure is assessed by the use of a job-exposure matrix. In its simplest form, a job-exposure matrix is a two-way table in which each job or occupation is assigned a code indicating the presence (and sometimes the magnitude) of substances to which persons in that job would be exposed, on the basis of contemporary measurements and knowledge of working practices. The

job history obtained from the subject is then used to construct his or her record of past exposure from the matrix. Among the limitations of this approach is the fact that individual exposures may differ widely even within narrowly defined occupations, because of differences in working practices between individuals and work sites. from country to country and over time. It should be noted. however. that while such problems in exposure assessment reduce the precision with which any effect can be measured, they are not likely to give rise to a spurious association where none exists; consistency of results between different studies of this kind is therefore of particular importance in assessing the relationship between exposure and disease.

Several of the available case-control studies are hospital-based rather than population-based; i.e., the control group consists of subjects hospitalized for diseases different from those of the cases. Because little is known about the etiology of many diseases, some of which may be associated with exposure to engine exhaust, it is difficult to rule out bias resulting from the choice of specific sets of controls.

(*b*) *Mortality and morbidity statistics*

The Working Group noted that surveys of mortality or morbidity statistics suffer from many limitations, which reduce their usefulness in the evaluation of carcinogenic risks. Comparison of the results of different studies is complicated by the varying definitions and groupings of occupations and cancer sites. Generally, these studies have been designed to generate hypotheses about potentially exposed groups. For example, a striking difference in the male:female sex ratio for tumours unrelated to hormonal status within a specific geographical region might suggest an area that should be explored in either cohort or case-control studies, in which exposure can be assessed more readily.

Studies of this type that may relate to exposure to exhaust fumes include the following: Menck and Henderson (1976), Decouflé *et al.* (1977), Office of Population Censuses and Surveys (1978), Petersen and Milham (1980), Howe and Lindsay (1983), Milham (1983), Dubrow and Wegman (1984), Malker and Weiner (1984), Baxter and McDowall (1986) and Olsen and Jensen (1987).

(*c*) *Cohort studies*

(i) *Railroad workers*

Kaplan (1959) evaluated 6506 deaths among railroad workers from the medical records ofthe Baltimoreand Ohio Railroad relief department between 1953 and 1958, 818 of which were due to cancer and 154 of which were lung cancer. The cases were categorized into three groups by exposure to diesel exhaust. In comparison with national death rates, none of the groups had an excess risk for lung cancer. [The Working Group noted that, since changeover to diesel engines began in 1935 and was 95% complete by 1959 (Garshick *et al.*, 1988), few if any of the lung cancer deaths could have occurred in workers with more than ten years' exposure to diesel exhaust; in addition, smoking habits were not considered.]

Howe *et al.* (1983) studied a cohort of 43 826 male pensioners of the Canadian National Railway Company consisting of retired railroad workers who were known to be alive in 1965 plus those who retired between 1965 and 1977. Of the total of 17 838 deaths that

occurred in 1965–77, 16 812 (94.4%) were successfully linked to a record in the Canadian mortality data base. The expected number of cancer deaths was estimated from that of the total Canadian population, adjusted for age and calendar period. Available information included birth date, province of residence, date of retirement and occupation at time of retirement. Occupational exposures were classified into three types: 'diesel fumes', coal dust and other. The two statistically significant results for the whole cohort were deficits in deaths from all causes (SMR, 95 [95% confidence interval (CI), 93–96]) and from leukaemia (SMR, 80 [95% CI, 65–97]). For exposure to diesel engine exhaust, the risk for cancer of the trachea, bronchus and lung increased with likelihood of exposure: the relative risks were 1.0 for unexposed, 1.2 [1.1–1.3] for 'possibly exposed' and 1.4 [1.2–1.5] for 'probably exposed' ($p$ for trend < 0.001). The SMR for bladder cancer was 103 [88–119]. Similar results were found for the risk for cancer of the trachea, bronchus and lung from exposure to coal dust. Since there was considerable overlap in exposures to diesel fumes and coal dust, the risk was evaluated by calendar time during which one of these exposures predominated. The risk was largely accounted for by exposure to diesel exhaust. Since exposure to asbestos occurs during locomotive maintenance, workers thought to have had such exposure were removed from the analysis, with little effect on the risk associated with exposure to diesel engine exhaust. Exclusion of workers exposed to welding fumes did not alter the result. The authors noted that the data presented and the risks observed probably represent an underestimate of the true risk, for at least two reasons: exposure misclassification because of the use of job held last and failure to determine the cause of death for 5.6% of cases. [The Working Group noted that no data were available on duration of exposure, usual occupation or smoking habits and recognized the potential for competing biases in the way in which the cohort was composed.]

Garshick *et al.* (1988) studied a cohort of 55 407 white male railroad workers aged 40–64 in 1959 who had started railroad service ten to 20 years earlier. The cohort was traced from records of the pension scheme for US railway workers through to 1980; it was estimated that less than 2% left the industry during the period covered by the study. Death certificates were available for 88% of the 19 396 deaths, of which 1694 were from lung cancer; decedents for whom a death certificate was not obtained were classified as having died of unknown causes. Records of railroad jobs from 1959 through to death, retirement or 1980 were also available from the records of the pension scheme. Jobs were divided into regular exposure to diesel exhausts (train crews, workers in diesel repair shops) and no exposure (clerks, ticket and station agents, and signal maintenance workers). Job categories with recognized asbestos exposure, such as car repair and construction trades, were excluded from those selected for study. Information was available on duration of exposure. There was a significant excess risk for lung cancer in the groups exposed to diesel engine exhaust; this risk was highest in those who had the longest exposure: aged 40–44 (relative risk, 1.5; 95% CI, 1.1–1.9) and 45–49 (1.3; 1.0–1.7) and exposed to diesel exhaust in 1959. The groups aged 50–54 and 55–59 in 1959 also had excess risks, of 1.1 and 1.2, respectively, although these were not statistically significant. When workers with further potential asbestos exposure (shop workers) were excluded, similarly elevated lung cancer rates were observed. Although smoking habits were not considered directly, the authors pointed out that there was no difference in smoking habits

by job title in comparison studies of current workers or in a case-control study in which smoking was assessed. [The Working Group noted that exclusion of shop workers would also have excluded men exposed to welding fumes.]

As part of this study, exposure was assessed on the basis of several hundred time-weighted samples of respirable dust taken in the early 1980s both at stationary sites in parts of four existing, smaller railroad yards and with personal samplers carried by railroad workers in different job categories (Woskie *et al.*, 1988a). Samples were taken from workers in 39/155 Interstate Commerce Commission job codes, and the results were used to classify the jobs; these 39 categories were subsequently combined into 13 job groups, which could be further combined into five: clerks, signal maintenance, engineers/firers, brakers/-conductors and shop workers. The nicotine content was used to adjust the extractable respirable particulate content of each sample to account for the portion contributed by cigarette smoking. Mean exposure levels by national career groups in the five major categories of exposure suggested a five-fold range of exposure to respirable particles between clerks and shop workers (Woskie *et al.* 1988b). These values confirmed the a-priori assignment of the categories of diesel exposure used in the cohort study (Garshick *et al.*, 1988) and the assignment to appropriate exposure categories for the case-control study (Garshick *et al.*, 1987; see p. 140).

(ii) *Bus company employees*

Raffle (1957) determined deaths, retirements and transfers due to lung cancer in London Transport employees aged 45–64 years in jobs with presumably different exposures to exhaust fumes in 1950–54 and compared the figures with those for lung cancer mortality for men in England and Wales or in Greater London. No relationship between presumed exposure and lung cancer incidence was noted. In a subgroup of bus and trolley bus engineering staff aged 55–64, 30 deaths from lung cancer occurred while 21.2 were expected (observed:expected, 1.4) on the basis of the experience of other London Transport employees. [The Working Group noted that no information on smoking habits was available, and that all the deaths occurred in men over 55 years of age.] Waller (1981) compared lung cancer deaths and retirements or transfers to alternative jobs due to lung cancer in men aged 45–64 employed within five job categories of London Transport (bus drivers, bus conductors, engineers (garages), engineers (central works) and motor men and guards) to lung cancer mortality (age- and calendar time-adiusted) for men in Greater London. The study covered 25 years, ending in 1974, thus including some of the data described by Raffle (1957). A total of 667 cases of lung cancer were observed; although the risk was not elevated for any of the five job categories, the highest SMR occurred in the group that was presumably most heavily exposed to diesel exhaust (bus garage workers). [The Working Group noted that no data on smoking habits were available, and neither duration nor latency was examined.]

Rushton *et al.* (1983) examined a cohort of 8684 men employed as maintenance workers in 71 bus garages in London for at least one year in 1967-75. Follow-up until 31 December 1975 was completed for 8490 (97.8%) workers, and cause of death was known for 701 of 705 who had died. The SMRs were 84 [95% CI, 78–91] for all causes and 95 [83–109] for all

neoplasms, 101 [82–122] for lung and pleural cancer, 151 [60–307] for leukaemia, 121 [49–250] for central nervous system tumours and 139 [72–244] for bladder cancer. None of the rates for cancer at individual sites was statistically significantly increased. The authors noted the short follow-up period.

Edling *et al.* (1987) studied 694 men, five of whom (0.7%) were lost to follow-up, who had been employed as clerks, bus drivers or bus garage workers in five bus companies in south-eastern Sweden at any time between 1950 and 1959, and followed for 1951–83. The SMRs, based on age-, sex- and calendar time-adjusted national rates, were 80 (195 deaths observed; 95% CI, 70–90) for deaths from all causes and 70 (50–90) for deaths from malignancy. Dividing the data by exposure category, exposure time or latency did not appreciably change the risk ratios. The small sample size did not allow detailed examination of cancers at specific sites, although six lung cancer cases were observed compared to nine expected. [The Working Group noted that smoking habits were not addressed.]

(iii) *Professional drivers and some other groups exposed to vehicle exhausts*

Ahlberg *et al.* (1981) identified a cohort of Swedish drivers said by the authors to be exposed to diesel exhaust (1865 or 1856 [*sic*] fuel oil tanker drivers and 34 027 other truck drivers) from the national census of 1960. In this cohort, l 143 cancers were registered within the Swedish Cancer Registry in 1961–73. The reference population consisted of 686 708 blue-collar workers from the 1960 census who were thought to have had no exposure to petroleum products or chemicals. The data were adjusted for age and residence. The relative risk for lung cancer was elevated in the whole cohort (1.3; 95% CI, 1.1–1.6) and in Stockholm truck drivers in particular (1.6; 1.2–2.3). From a questionnaire study of 470 professional drivers in Stockholm, it was noted that 78% of fuel truck drivers and 31% of other truck drivers smoked. The authors cited an unpublished study indicating that the comparable smoking rate in Stockholm was 40% and concluded that the results could not be explained by smoking.

Wong *et al.* (1985) studied a cohort of 34 156 male members of a heavy construction equipment operators' union in the USA with potential exposure to diesel exhaust. Cohort members had to have been a union member for at least one year between 1 January 1964 and 31 December 1978, by which time 3345 had died and 1765 (5.2%) could not be traced. Death certificates were obtained for all but 102 (3.1%) decedents. No information was available for jobs held before 1967 and limited information was available on jobs held between 1967 and 1978. The SMRs, based on national figures, adjusted for age, sex, race and calendar time, were 81 (95% CI, 79–4) for all causes, 93 (87–99.6) for all cancers, 99 (88–110) for lung cancer (ICD7 162–163) and 118 (78–172) for bladder cancer. The data were also analysed by duration of union membership, latent period, retirement status, job category and exposure status. Significant upward trends in risk were detected for lung cancer with duration of union membership, used as a surrogate for duration of potential exposure to diesel exhaust, with SMRs for lung cancer of 45 [22–83], 75 [49–111], 108 [81–141], 102 [78–132] and 107 [91–125] for workers with <5 ,5–9, 10–14, 15–19 and ≥20 years of union membership, respectively. A significant upward trend was also noted for lung cancer with latent period. Mortality from cancers of the digestive system (SMR, 142; 116–173) and respiratory system

(SMR, 162; 138–190) and from lymphosarcoma and reticulosarcoma (SMR, 231; 111–425) was elevated in retirees. Exclusion of early retirees did not remove the risks for respiratory cancer or lymphatic cancer. In general, groups with jobs with presumed high exposure to diesel fumes did not show the excesses reported above. A random sample of union members was surveyed to determine smoking habits, and no significant difference between members and the general population was revealed.

In a review, Steenland (1986) presented data on a preliminary study of the mortality experience of about 10 000 teamsters (truck drivers, dock workers, mechanics and jobs outside the trucking industry) who had died in 1982-83 and had worked for at least ten years in a teamster job. Using occupational data on death certificates, proportionate mortality ratios were calculated for lung cancer for 255 mechanics (226; 95% CI, 162–309), 5834 truck drivers (154; 144–166),490 dock workers (132; 99–175) and 1064 others (116; 95–142). [The Working Group noted that this was an interim report and that judgement should be reserved until the final results are available.]

Gustafsson *et al.* (1986) studied 6071 Swedish 'dockers' assumed by the authors to have been exposed to diesel exhaust and first employed before 1974 for at least six months. The group had been followed for death from 1 January 1961 or from the date of first employment (if this date occurred later) through to 1 January 1981. Age-, calendar time- and region-specific rates were used to generate expected numbers of deaths. The SMRs were 89 (95% CI, 84–94) for all causes, 103 for all cancers, 132 for lung cancer (105–166) and 110 (85–142) for urogenital tract cancer. Cancer morbidity was determined among 6063 workers who had been alive and without cancer on 1 January 1961 and were followed through to 1 January 1980; a standard morbidity ratio of 110 (101–120; 452 cases) was seen for cancers at all sites and of 168 (136–207; 86 cases) for lung cancer. [The Working Group noted that there was no consideration of duration, intensity or latency of exposure or of smoking habits in this study.]

Stern *et al.* (1981) examined mortality patterns among 1558 white male vehicle exam-iners who had been employed in New Jersey, USA, for at least six months between 1944 and 1973. The vital status of all but eight (0.5%) of these was ascertained as of 31 August 1973; these eight were assumed to be alive. Approximately 63% of the cohort members had begun employment prior to 1957. A modified life-table analysis was used to generate the expected number of cause-specific deaths on the basis of national rates, adjusting for age and calendar time. There were 52 deaths from cancer (47.8 expected [SMR, 109; 95% CI, 81–143]). The SMRs for malignant disease increased significantly with latency: 0-9 years, 69 [25–151]; 10–19 years, 98 [56–159]; 20-29 years,107 [62–171]; >30 years, 189 [101–323]. Cancer at no specific organ site accounted for this excess. The exposure of interest was carbon monoxide, but the authors speculated that other components of automobile exhaust might have been responsible. No information on smoking habits was available for deceased workers, but COHb levels in currently nonsmoking workers increased during the work shift, indicating exposure to exhaust.

In a cohort study of white men enlisted in the US Navy (Garland *et al.*, 1988), 143 cases of testicular cancer were identified in the period 1974–79; age-specific incidence rates were similar to those for the US population, derived from the US National Cancer Institute

Surveillance, Epidemiology and End Results (SEER) programme for 1973–77. Of 110 occupational groups in the Navy, three involving maintenance of gasoline and diesel engines and daily exposure to their exhaust emissions (aviation support equipment technicians, enginemen and construction mechanics) had significantly high standardized incidence ratios for testicular cancer: 3.4 (95% CI, 1.9–5.6) in comparison to SEER rates, and 3.8 (2.1–6.3) in comparison to men in the US Navy as a whole, based on 15 cases. The authors noted that this was a hypothesis-generating study and that the men also had potential daily exposure to solvents and other chemicals.

(iv) *Miners*

Although diesel engines have been used in many mines for a number of years, the Working Group decided not to consider all groups of miners because they may be exposed concurrently to other potential lung carcinogens such as radon decay products, heavy metals and silica, and there was no way that the possible confounding effects of such factors could be determined from the data available in published reports.

Waxweiler *et al.* (1973) studied potash miners and millers, who are exposed to no known carcinogens in the ore, who had been employed for at least one year between January 1940 and July 1967 by eight companies. The vital status of the cohort was identified to July 1967. Of a total of 3886 men, 31 could not be traced and were assumed to be alive. Causes of death were compared with those of the general US population, standardized for age, race, sex and calendar time. Of the cohort, 2743 men had worked at least one year underground and less than one year on the surface and 1143 men had worked at least one year on the surface and less than one year underground. In only two of the eight mines were diesel engines used; one mine changed to diesel in 1949 and the other in 1957. Death certificates were available for 433 of the 438 workers who had died. The effect of smoking was taken into account. No excess mortality from lung cancer was seen in either surface or underground miners. Mortality rates did not differ between the mines with diesel vehicles and those without. The authors noted the short follow-up, the small expected numbers of deaths and the broad classification of causes of death.

(*d*) *Case-control studies*

(i) *Lung cancer*

Williams *et al.* (1977) examined cancer incidence and its relationship to occupation and industry in a study based on the US Third National Cancer Survey. In this study, detailed personal interviews were sought for 13 179 cancer patients (a random 10% sample of all incident invasive tumours occurring in three years in eight areas in the USA) and obtained for 7518 (57%). The numbers of cases of cancer at various anatomical sites were compared with that of cases at all other sites combined. The interview included occupational history (main employment and recent employment), other demographic data and information on smoking and drinking habits; the analysis also controlled for age, sex, race and geographical location. A statistically nonsignificant lung cancer excess (odds ratio, 1.5; [CI could not be calculated]) was observed for truck drivers, which could not be accounted for by smoking.

Intensity, duration of exposure and latency were not evaluated. [The Working Group noted the potential for bias due to the relatively low level of compliance with the questionnaire.]

In a population-based case-control study, Coggon *et al.* (1984) used the data on occupation on the death certificates of all men under the age of 40 years in England and Wales who had died of tracheobronchial carcinoma during the period 1975– 79; 598 cases were detected, 582 of which were matched with two and the rest with one control who had died from any other cause, for sex, year of death, local authority district of residence and year of birth. Occupations were coded using the Office of Population Census and Surveys 1970 classification of occupations, and a job-exposure matrix was constructed by an occupational hygienist, in which the occupations were grouped according to likely exposure to each of nine known or putative carcinogens. All occupations entailing exposure to diesel fumes were associated with an elevated odds ratio for bronchial carcinoma (1.3; 95% CI, 1.0–1.6); however, for occupations with presumed high exposure, the odds ratio was 1.1 (0.7–1.8). [The Working Group noted the limited information on occupation from death certificates, the young age of the subjects and the consequent short times of exposure and latency, and the lack of information on smoking habits and on the possible confounding effects of other carcinogenic exposures.]

In a hospital-based case-control study (Hall & Wynder, 1984) in 18 hospitals in six US cities, 502 men with histologically confirmed primary lung cancer (20–80 years old) and 502 control patients, matched for age, race and hospital were identified. Patients were interviewed between December 1980 and November 1982. Half of the controls had cancer; patients with tobacco-related diseases were excluded. The questionnaire included items on smoking habits, demographic variables and usual occupation. Occupations were grouped either dichotomously as exposed to diesel exhaust (warehousemen, bus drivers, truck drivers, railroad workers and heavy equipment repairmen and operators) or nonexposed, or. in a separate evaluation, in three presumed categories of frequency of exposure in the job (high, moderate, little). Using the dichotomous division, the exposed group had a significantly elevated odds ratio (2.0; 95% CI, 1.2–3.2), which, however, decreased to 1.4 (0.8–2.4; not significant) when adjusted for smoking. The crude odds ratios were 1.7 (0.6–4.6) for a high probability of exposure to diesel exhaust and 0.7 (0.4–1.3) for a moderate probability of exposure. [The Working Group questioned the possible consequences on risk estimates of excluding patients with tobacco-related diseases from the control group.]

In a hypothesis-generating case-control study, Buiatti *et al*. (1985) investigated the occupational histories of histologically confirmed cases of primary lung cancer among residents of metropolitan Florence, Italy, diagnosed during 1981-83 in the regional general hospital and referral centre for lung cancers in the Province of Florence. For the 376 cases (340 men, 36 women), 892 controls (817 men, 75 women), matched by sex, age, date of admission and smoking status in seven categories, were selected from the medical service of the same hospital, excluding patients with lung cancer, attempted suicides and patients not resident in metropolitan Florence. Each case and control completed a structured questionnaire on demographic variables and on all jobs held for more than one year. The jobs were classified into 21 major classes and 251 subclasses, using the International Labour Office

classification. Odds ratios for industries and occupations (ever *versus* never worked) were calculated using logistic regression, in which age and smoking status were included. Taxi drivers had an elevated relative risk for lung cancer after adjusting for tobacco smoking (l.8; 95% CI, 1.0–3.4). [The Working Group noted that multiple comparisons were made, increasing the probability that statistically significant results would be found.]

In a case-control study in northern Sweden, Damber and Larsson (1987) analysed the association between lung cancer and occupation. The cases were 604 male lung cancers reported to the Swedish Cancer Registry during 1972–77 and who had died before May 1979. For each case, a control was drawn from the National Registry for Causes of Deaths, and was matched for sex, year of death, age and municipality; cases of lung cancer and attempted suicide were excluded as controls. In addition, for each case, one living control (less than 80 years old) was drawn from the National Population Registry, matched for sex, year of birth and municipality. Information on residence, occupation, employment and smoking habits was collected by a questionnaire mailed to surviving relatives and to living controls; the response rates were 98% for cases and 96% and 97% for dead and living controls, respectively. Information was requested on all jobs held for at least one year and on lifetime smoking history. A linear logistic regression model, using three discrete levels of employment (<1 year, 1–20 years, and >20 years) and four levels of lifetime tobacco consumption, was used to calculate odds ratios. For professional drivers with more than 20 years' employment, the unmatched odds ratio was 1.5 (95% CI, 0.9–2.6) in comparison with dead controls; this was reduced to 1.2 (0.6–2.2) after adjustment for smoking. The figures obtained in comparison with living controls were 1.7 (0.9–3.2) and 1.1 (0.6–2.2), respectively.

Garshick *et al.* (1987) performed a case-control study on lung cancer deaths among employed and retired US male railroad workers with ten or more years of service, who had been born on 1 January 1900 or after and who had died between 1 March 1981 and February 1982. Cases of primary lung cancer (1256) were matched to two controls by age and date of death. Workers who had died from cancer, suicide, accident or unknown causes were not included among controls. Potential exposure to diesel exhaust was assigned on the basis of an industrial hygiene evaluation of the >150 railroad jobs and areas described by the US Interstate Commerce Commission. Job codes for each worker were available from the US Railroad Retirement Board starting in 1959 and ending with death or retirement. For workers who had retired between 1955 and 1959, the last railroad job held was available. Asbestos exposure prior to 1959 was categorized by job held in 1959 (end of steam locomotive era) or by the last job before retirement, if this was before 1959. Smoking history was obtained by questionnaire from the next-of-kin. Using multiple conditional logistic regression analysis to adjust for smoking and asbestos exposure, workers 64 years of age or younger at time of death who had worked in a diesel exhaust-exposed job for 20 years had a significantly elevated odds ratio for lung cancer (1.4; 95% CI, 1.1–1.9). No such effect was observed among older workers (0.91; 0.71–1.2), many of whom had retired shortly after the transition to diesel-powered locomotives and were therefore not exposed.

In a population-based case-control study (Lerchen *et al*., 1987), all white and Hispanic white residents of New Mexico, USA, aged 25–84 years, with primary lung cancer,

excluding bronchioalveolar carcinoma, diagnosed between 1 January 1980 and 31 December 1982, were identified from the New Mexico Tumor Registry. The cases (333 men and 173 women) were frequency matched with controls selected randomly from the telephone directory or, for persons 65 years or older, from the roster of participants in a health insurance scheme, for sex, ethnic group and ten-year age band at a ratio of approximately 1.5 controls per case (449 men and 272 women). Detailed occupational and smoking histories were obtained by personal interview, with response rates of 89% for cases and 83% for controls. Next-of-kin provided interviews for 50% of the male and 43% of the female cases and for 2% of the controls; the authors recognized the possible bias introduced by this practice. The odds ratio for exposure to diesel exhaust fumes, adjusted for age, ethnic group and smoking, was 0.6 (95% CI, 0.2–1.6). [The Working Group noted the possible bias in choosing controls from the telephone directory when cases are not required to have a telephone or to be listed.]

In a case-control study of lung cancer in France (Benhamou *et al.*, 1988), 1625 histologically confirmed cases and 3091 controls, matched for sex, age at diagnosis, hospital admission and interviewer, completed a questionnaire on residence, education, occupation, and smoking and drinking habits. All occupations held for more than one year were recorded and coded without knowledge of the case status of the patient, using the International Standard Classification of Occupations and according to chemical or physical exposures. The analysis was limited to men (1260 cases and 2084 controls); adjustment was made for age at starting smoking, amount smoked and duration of smoking. Several occupations were associated with increased odds ratios for lung cancer, including miners and quarry men (2.1; 95% CI, 1.1–4.3) and transport equipment operators (1.4; 1.1–1.8); the subcategory of motor vehicle drivers also had an increased risk (1.4; 1.1–1.9).

(ii) *Bladder cancer*

In a population-based case-control study in Canada (Howe *et al.*, 1980), all patients with bladder cancer newly diagnosed in three Canadian provinces between April 1974 and June 1976 were identified; 77% of the patients were interviewed, and for each patient one neighbourhood control, individually matched for age and sex, was interviewed. In the analysis, 632 case-control pairs (480 male and 152 female) were included. Lifetime smoking and employment histories were obtained, and exposure to dusts and fumes was elucidated. Elevated odds ratios were observed for railroad workers [not further defined] (9.0; 95% CI, 1.2–394.5; nine exposed cases) and for exposure to diesel and traffic exhaust (2.8; 0.8–11.8; 11 exposed cases).

In a death certificate-based case-control study (Coggon *et al.*, 1984; for details, see description on p.139), the occupations of 291 bladder cancer cases and 578 hospital controls were compared. The odds ratio for all diesel fume-exposed occupations was 1.0 (95% CI, 0.7–1.3) and that for occupations with high exposure was 1.7 (0.9–3.3). [The Working Group had the same reservations about this study as expressed on p. 139.]

In a population-based case-control study, the relationship between truck driving and bladder cancer was investigated (Hoar & Hoover, 1985). Cases consisted of all white residents of New Hampshire and Vermont, USA, who had died from bladder cancer in

1975-79. One control per case was selected randomly from all other deaths among residents, excluding suicides, and matched for state, sex, age, race and year of death. A second control per case was selected with the additional matching criterion of county of residencc. There were 230 and 210 eligible cases in the two states, respectively; the rate of response to interview was 87% for New Hampshire and 58% for Vermont, and the nonrespondents were similar to the respondents with respect to case-control status, sex, age and county of residence. The odds ratio for ever having been a truck driver was 1.5 (95% CI, 0.9–2.6), and there was a significant trend between bladder cancer risk and number of years of truck driving: odds ratios, 1.4 (0.6-3.3), 2.9 (1.2–6.7) and 1.8 (0.8–4.1) for those employed as truck drivers for 1–4, 5–9 and >10 years, respectively. Additional adjustment for age, county, coffee drinking or cigarette smoking (six categories) did not alter these crude odds ratios. [The Working Group noted the nonlinearity of the trend.]

In a hospital-based case-control study in Turin, Italy (Vineis & Magnani, 1985), 512 male cases and 596 male controls randomly selected from among other patients in the main hospital of the city of Turin between 1978 and 1983 were interviewed for lifetime occu-pational and smoking histories. Occupations were coded using the International Labour Office classification, and associations between specific chemicals and bladder cancer were studied using a job exposure matrix. Adjusting for age and smoking, the odds ratio for bladder cancer for truck drivers was 1.2 (95% CI, 0.6 - 2.5).

In a hospital-based case-control study, Wynder *et al.* (1985) examined the occupational histories and life style factors (smoking, alcohol and coffee consumption, demographic factors) of 194 male cases of histologically confirmed bladder cancer, 20–80 years of age, diagnosed during two-and-a-half years (January 1981 – May 1983) in 18 hospitals in six US cities, and of 582 controls, matched by age, race, year of interview and hospital of admission, hospitalized during the same period for diseases not related to tobacco use. The participation rate among eligible subjects was 75% among cases and 72% among controls. 'Usual' occupation was coded according to an abbreviated list of the US Bureau of Census codes. No significant association was detected between bladder cancer and occupations presumed to involve exposure to diesel exhaust: warehousemen and materials handlers, bus and truck drivers, railroad workers, heavy equipment operators and mechanics (odds ratio, 0.87; 95% CI, 0.47–1.6). [The Working Group questioned the possible consequences on risk estimates of excluding patients with tobacco-related diseases from the control group.]

Data from all ten areas of the US National Bladder Cancer Study were used to evaluate the association of motor exhausts with bladder cancer (Silverman *et al.*, 1986). The study group comprised 1909 white male cases with histologically confirmed bladder carcinoma or papilloma not specified as benign and 3569 frequency-matched controls. Significantly elevated age- and smoking-adjusted odds ratios for bladder cancer were observed for truck drivers or delivery men, and for taxi drivers or chauffeurs: 1.5 (95% CI, 1.1–2.0) and 6.3 (1.6–29.3) for 'usual' occupation, 1.3 (1.1–1.4) and 1.6 (1.2–2.2) for 'ever' occupation. For bus drivers, the odds ratios did not reach significance (1.3, 0.9–1.9 and 1.5, 0.6–3.9 for 'ever' and 'usual', respectively). When allowance was made for a 50-year latency, a significant trend with increasing duration of employment as a truck driver was observed: 1.2, 1.4, 2.1 and 2.2 for a duration of employment of <5, 5–9, 10–24 and >25 years, respectively

($p$ <0.0001). Information on subsets of this cohort has been published elsewhere (Silverman *et al.*, 1983; Schoenberg *et al.*, 1984; Smith *et al.*, 1985). In the Detroit subset (Silverman *et al.*, 1983), the adjusted odds ratio for bladder cancer for truck drivers who had never driven a vehicle with a diesel engine was 1.4 (0.7–2.9) and that for men who had ever driven a vehicle with a diesel engine was 11.9 (2.3–61.1).

Occupational risk factors were investigated as part of a population-based case-control study in Copenhagen, Denmark (Jensen *et al.*, 1987). Between May 1979 and April 1981, a total of 412 live patients with bladder cancer (invasive tumours and papillomas) were reported in the study, 389 of whom were interviewed. Live controls were selected at random from the municipalities where the cases lived, and the sample was stratified to match the cases with regard to sex and age in five-year groups. Among the 1052 controls approached, the overall participation rate was 75%. Cases and controls were interviewed for information on occupational history coded according to the Danish version of the International Standard Industrial Classification. Cigarette smoking was adjusted for in the analysis by using two dichotomous variables (ever/never smoked, current/noncurrent smoker) and a continuous variable (logarithm of pack-years smoked). The adjusted odds ratio for bladder cancer was elevated in land transport workers (1.6; 95% CI, 1.1–2.3). The adjusted odds ratios for bladder cancer for bus, taxi and truck drivers were 0.7 (0.4–1.5), 1.6 (0.8–3.4), 3.5 (1.1–11.6) and 2.4 (0.9–6.6) for durations of employment of 1–9, 10–19, 20–29 and >30 years, respectively, representing a significant trend with duration of employment. The trend was not significant for land transport workers.

In a hospital-based case-control study in Argentina (Iscovich *et al.*, 1987), 120 patients with histologically confirmed bladder carcinoma admitted to ten general hospitals in (Greater La Plata between March 1983 and December 1985 were identified. The 117 patients who could be interviewed represented approximately 60% of all incident cases. For each case, a hospital control from the same establishment was selected (patients with diseases associated with tobacco smoking constituted 12% of the control group); a neighbourhood control, matched for age and sex, was also selected. Information on smoking and past and present occupations was collected by questionnaire. An exposure index based on a job exposure matrix was generated. The adjusted odds ratio for truck and railway drivers was 4.3 [95% CI, 2.1–29.6].

Covering the period 1960–82, Steenland *et al.* (1987) identified 731 male bladder cancer (ICD-9 188) deaths in the Hamilton County, Ohio, region, where there is a known high bladder cancer rate. Six controls were matched to each case on sex and residence in the county at the time of death, year of death, age of death and race. Death certificates and city directories for all residents over 18 were used to identify job history. The first two controls that were listed in the directory within at least five years of the first listing of the cases were selected. Of the 648 cases (89%) listed in the directories, all but 21 had two controls; the remaining 21 had one control. A comparable analysis of all 731 cases and two controls per case was carried out using usual lifetime occupation from the death certificate. A significant increase in the frequency of bladder cancer was found for men with more than 20 years' duration of employment, identified through the city directories as truck drivers (odds ratio, 12.0 [95% CI, 2.3–62.9]; six cases, one control) and railroad workers (odds ratio, 2.2

[95% CI, 1.2–4.0]). Notably, those workers identified as 'drivers not otherwise specified' for ≥20 years had an odds ratio of 0.15 [95% CI, 0–0.8]. In contrast, on the basis of job ever held identified from either the death certificate or the city directory (without taking duration into account), none of the above findings was significant. [The Working Group noted that this study involved application of a new methodology for exposure ascertainment, which requires further validation.]

In a case-control study of bladder cancer incidence in Edmonton, Calgary and Toronto and Kingston, Canada (Risch *et al.*, 1988), 826 cases of histologically verified bladder cancer were compared with 792 population-based controls matched for age, sex and area of residence. Cases were aged 35–79 and had been ascertained between 1979 and 1982. Information was collected by questionnaire, administered by personal interview, covering family, medical, occupational, residential, smoking and dietary histories. Analysis of the occupational data included adjustment for lifetime smoking habits. Among other findings related to occupation and industry was that the 309 men who had had jobs with exposure to engine exhausts had an odds ratio of 1.5 (95% CI, 1.2–2.0) for 'ever' exposure and an odds ratio of 1.7 (1.2–2.3) for exposure during the period eight to 28 years prior to diagnosis. The authors also calculated that there was a significant increase in trend with duration of exposure for each ten years (1.2; 1.1–1.4). This relationship was not seen for women, but only 19 had been exposed. The relationship was also not seen when an analysis was undertaken by exposure to 18 categories of substances, including engine exhaust. [The Working Group found it difficult to interpret the differences in risk seen when exposure was defined in various ways.]

(iii) *Other and multiple sites*

In a hypothesis-generating, hospital-based case-control study in Sweden, Flodin *et al.* (1987) analysed the association between occupation and multiple myeloma. The cases were in persons diagnosed between 1973 and 1983 and still alive during 1981-83. From comparisons with cancer registry data, it was concluded that the cases represented one-third of all cases diagnosed in the area. Controls were drawn randomly from population registers. There were 131 cases and 431 controls for analysis. Information on occupational history, X-ray treatment and smoking habits were obtained by a mailed questionnaire. The crude odds ratio for occupational exposure to engine exhaust was 2.3 (95% CI, 1.4–3.7); this association remained significant after adjusting for confounding variables. In a study using the same set of controls (431) and source of cases, Flodin *et al.* (1988) investigated the association with occupational exposures for 111 cases of chronic lymphatic [lymphocytic] leukaemia. The crude odds ratio for occupational exposure to engine exhausts was 2.5 (95% CI, 1.5–4.0); the association remained significant after adjustment for confounding variables. [The Working Group noted that the study population and control of confounding were not clearly described, and that exposure to engine exhausts was self-reported and not further defined by the authors.]

In a large, hypothesis-generating, population-based case-control study in Canada (Siemiatycki *et al.*, 1988), the associations between ten types of engine exhaust and com-bustion products and cancers at 12 different sites were evaluated. The 3726 cancer patients

diagnosed in any of the 19 participating hospitals in Montreal were interviewed (rate of response, 82%). The patients were all men aged 35–70 years. For each cancer site, patients with cancers at other sites comprised the control group. The interview elicited a detailed job history, and a team of chemists and industrial hygienists translated each job into a list of potential exposures (Gérin *et al.*, 1985). The probability of exposure ('possible', 'probable', 'definite'), the frequency of exposure (<5, 5–30, >30% working time) and the level of exposure (low, medium, high) were estimated. Separate analyses were performed for oat-cell, squamous-cell, adenocarcinoma and other carcinomas of the lungs. After stratifying for age, socioeconomic status, ethnic group, cigarette smoking and blue-/ white-collar job history, an elevated odds ratio was observed for squamous-cell cancer of the lung and exposure to gasoline engine exhaust (OR, 1.2; 90% CI, 1.0–1.4). In a detailed analysis in which all covariables that changed the estimate of the disease-exposure odds ratio by more than 10% were included as confounders, further associations were revealed: long-term high-level exposure to gasoline engine exhaust (1.4; 1.1–1.8) and short-term high-level exposure to diesel engine exhaust (1.5; 0.9–2.7) were associated with squamous-cell cancer of the lung. The odds ratio for squamous-cell cancer of the lung (1.5; 0.9–2.5) was also elevated for bus, truck and taxi drivers (classified as exposed to gasoline engine exhaust) and for mining and quarrying (classified as exposed to diesel engine exhaust; 2.8, 1.4–5.8), but analyses by duration and intensity of exposure did not support a causal association. Marginally elevated odds ratios were also seen for colon cancer and exposure to diesel engine exhaust (1.3; 1.1–1.6); for cancer of the rectum (1.6; 1.1–2.3) and kidney (1.4; 1.0–2.0) with long-term high-level exposure to gasoline engine exhaust; for colon cancer (1.7; 1.2–2.5) with long-term high-level exposure to diesel engine exhaust; and for rectal cancer (1.5; 1.0–2.2) in bus, truck and taxi drivers. [The Working Group noted that 90% CI were used and that, at the 95% level, most of the intervals would have included unity.]

(*e*) *Childhood cancer*

Studies have been carried out to examine the hypothesis that exposure of adults to engine exhaust may result in mutations in germ cells, direct intrauterine exposure or early postnatal exposure.

In a case-control study in Québec, Canada (Fabia & Thuy, 1974), occupation of the father at time of birth was ascertained from the birth certificates of 386 children (out of 402 patients ascertained from death certificates, hospital insurance data and hospital records) who had died from malignant disease before the age of five years in 1965–70 and of 772 control children whose birth registration immediately preceded or followed that of the case in the official records. The occupation of the father was not known for 30 cases or for 56 controls. Father's occupation was recorded as motor vehicle mechanic or service station attendant for 29 (7.5%) cases and 29 (3.8%) controls [odds ratio, 2.1 (95% CI, 1.2–3.4)] and as driver for 19 (4.9%) cases and 49 (6.4%) controls [0.76 (0.4–1.3)].

In a case-control study in Finland (Hakulinen *et al.*, 1976), all 1409 incident cases of cancer in children under 15 years reported to the Cancer Registry in 1959–68 were ascertained. Paternal occupation was obtained from antenatal clinic records for the first trimester of pregnancy. After excluding twins and cases for which the father's occupation

was unobtainable, 852 cases were available for analysis. For each case, a child with date of birth immediately before that of the case and who had been born in the same maternity welfare district was chosen as a control. Leukaemias and lymphomas (339 pairs; 158 under five years of age), brain tumours (219 pairs; 77 under five years of age) and other tumours (294 pairs; 160 under five years of age) were analysed separately; analyses were carried out separately for the whole group (children under 15 years of age) and for children under five years of age at the time of diagnosis. Paternal occupation as a motor vehicle driver was not more frequent in any group of cases than in controls: the odds ratio for leukaemia in children under five (based on 14 cases) was 0.74 (95% CI, 0.34–1.6); that for leukaemia and lymphoma in the whole group (35 cases), 1.1 (0.63–1.8); that for brain tumours in children under five (four cases), 0.17 (0.00–1.4); and that for brain tumours in the whole group (16 cases), 0.67 (0.29–1.5). [The Working Group noted that only 60% of cases were available for analysis.]

In a case-control study in Connecticut, USA (Kantor *et al.*, 1979), paternal occupation was ascertained from birth certificates for all 149 cases of Wilms' tumour (aged 0-19 years) reported to the Connecticut Tumor Registry in 1935–73 and for 149 controls selected from State Health Department files and matched for sex, race and year of birth. The father's occupation was recorded as driver for eight cases and four controls [odds ratio, 2.1 (95% CI, 0.6–6.7)], as motor vehicle mechanic for six cases and one control [6.2 (0.8–49.8)] and as service station attendant for three cases and no control.

In a case-control study on the association between paternal occupation and childhood cancer (Kwa & Fine, 1980), 692 children born in 1947–57 or 1963–67 and who had died of cancer before the age of 15 in Massachusetts, USA, were identified from the National Center for Health Statistics. Two controls were selected from the registry of births for each case —one born immediately before the case and the other immediately after. Paternal occupation was taken from birth certificates and classified into one of nine categories on the basis of the type of chemical exposures involved. Mechanic/service station attendant was recorded as the father's occupation for 21 (4.9%) leukaemia/lymphoma cases [odds ratio, 1.1 (95% CI, 0.7–1.5)], six (4.5%) cases of neurological cancer [1.02 (0.4-2.4)], four (11.8%) cases of urinary tract cancer [2.9 (1.0—8.1); significant], four (4.2%) cases of all other cancers [0.93 (0.34–2.6)] and 61 (4.4%) controls. No excess of leukaemia/lymphoma, neurological cancer, urinary tract cancer or all other cancer was observed in the children of fathers who were motor vehicle drivers.

In a case-control study on associations between childhood cancer and parental occupation (Zack *et al.*, 1980), the parents of 296 children with cancer followed at a haematology clinic in Houston, TX, USA, from March 1976 to December 1977 and three sets of controls were interviewed for demographic information and job history in the year preceding the birth of the child until diagnosis of cancer. The first set of controls comprised 283 fathers and stepfathers and 283 mothers and stepmothers of children without cancer in the same clinic; the second set consisted of siblings of the parents of the case (413 uncles and 425 aunts), matched by age and number of children; and the third set was selected from among residents in the neighbourhood of the cases (228 fathers and 237 mothers). The proportion of

cases with paternal occupation as motor vehicle mechanic, service station attendant or driver did not differ from that in any control group [crude odds ratio in comparison with the first control group, 0.59 (95% CI, 0.28–1.2); that in comparison with the second control group, 0.79 (0.38–1.6); and that in comparison with neighbourhood controls, 0.92 (0.40–2.1)]. [The Working Group noted that the selection criteria were not given for either cases or controls, that it was unclear whether information on exposure was obtained from mothers or fathers or both, and that confounding factors were not taken into consideration.]

Hemminki *et al.* (1981) obtained data from the Finnish Cancer Registry on children less than 15 years old with cancer diagnosed in 1959–75 and on parental occupation, as in the study of Hakulinen *et al.* (1976; see pp. 145–146). The odds ratio for the father of a child with leukaemia in 1969–75 being a professional driver was 1.9 [95% CI, 1.1–3.7].

In a proportionate mortality study in England and Wales (Sanders *et al.*, 1981), paternal occupations recorded on the death certificates of children under 15 years of age during the years 1959–63 and 1970–72 (167 646 deaths; 6920 deaths from neoplasms) were investi-gated. Proportionate mortality ratios for neoplasms were not elevated for children of fathers employed as 'drivers of stationary engines, cranes, etc.', as transport workers or as warehousemen.

Associations between paternal occupation and childhood leukaemia and brain tumours were investigated in a case-control study in Maryland, USA (Gold *et al.*, 1982). Children under the age of 20 with leukaemia (diagnosed in 1969–74) or brain tumours (diagnosed in 1965–74) were ascertained in the Baltimore Standard Metropolitan Statistical Area from hospital records, death certificates, hospital tumour registries and from the pathology, radiotherapy and clinical oncology records of 21 of 23 Baltimore hospitals. There were two control groups: one consisted of children with no malignant disease, selected from birth certificates at the Maryland State Health Department and matched for sex, date of birth and race; the other group consisted of children with malignancies other than leukaemia or brain cancer, matched for sex, race, date of diagnosis and age at diagnosis. Information on occupational exposures of both parents before the birth of the child and between birth and diagnosis was collected by interviewing the mother. A total of 43 children had leukaemia and 70 had brain tumours. The paternal occupational category that included driver, motor vehicle mechanic, service station attendant or railroad worker was not more frequent for children with leukaemia or brain tumours than for the control children. [The Working Group noted the small numbers involved and found the results difficult to interpret.]

In a case-control study on childhood leukaemia and neuroblastoma (Vianna *et al.*, 1984), children born in 1949–78 who were diagnosed with acute leukaemia during the first year of life and reported to the Tumor Registry of the New York State Health Department or with neuroblastoma up to 12 years of age at diagnosis were identified. Using information from birth certificates, two sets of controls were selected: one was matched by year of birth, sex, race and county of residence; the other was additionally matched for age of the mother and birth order of the child. Information on parental age, race, education and occupation, and medical, obstetrical and therapeutic histories were obtained by telephone interview of the mothers. Of 65 eligible cases of leukaemia, 60, with two controls each, were finally included

in the analysis. The odds ratio for acute leukaemia for children with 'high' presumed paternal exposure to motor exhaust fumes (service station attendants, automobile or truck repairmen, aircraft maintenance personnel) was 2.5 [1.2–5.3] in comparison with the first control group and 2.4 [1.1–3.7] in comparison with the second. For 'lower' presumed exposure (taxi drivers, travelling salesmen, truck or bus drivers, railroad workers, toll-booth attendants, highwayworkers, police officers), the odds ratio was 3.4 [1.4–10.2] in comparison with the first control group and 1.3 [0.8–2.1] in comparison with the second. For the 103 cases of neuroblastoma, there was no significant difference from controls in the number of fathers who had had 'high' exposure. [The Working Group questioned the categorization of exposures as 'high' and 'lower' on the basis of the jobs listed.]

In a case-control study on paternal occupation and Wilms' tumour (Wilkins & Sinks, 1984), 105 patients were identified through the Columbus, OH, USA, Children's Hospital Tumor Registry during the period 1950–81. For each case, two controls were selected from Ohio birth certificate files; the first control series was individually matched for sex, race and year of birth, and the second series was additionally matched for mother's county of residence when the child was born. Due to changes in birth certification, the study included only the 62 cases and their matched controls for which father's occupation was recorded. The crude odds ratio for Wilms' tumour in children with paternal occupation as motor vehicle mechanic, service station attendant or driver/heavy equipment operator was 1.1 [95% CI, 0.36–3.5 compared to both controls taken together].

# 4. Summary of Data Reported and Evaluation

## 4.1 Exhaust composition and exposure data

Internal combustion engines have been used in cars, trucks, locomotives and other motorized machinery for about 100 years. Engine exhausts contain thousands of gaseous and particulate substances. The major gaseous products of both diesel- and gasoline-fuelled engines are carbon dioxide and water, but lower percentages of carbon monoxide, sulfur dioxide and nitrogen oxides as well as low molecular weight hydrocarbons and their derivatives are also formed. Submicron-size particles are present in the exhaust emissions of internal combustion engines. The particles present in diesel engine exhaust are composed mainly of elemental carbon, adsorbed organic material and traces of metallic compounds. The particles emitted from gasoline engines are composed primarily of metallic compounds (especially lead, if present in the fuel), elemental carbon and adsorbed organic material. Soluble organic fractions of the particles contain primarily polycyclic aromatic hydro-carbons, heterocyclic compounds, phenols, nitroarenes and other oxygen- and nitrogen-containing derivatives.

The composition and quantity of the emissions from an engine depend mainly on the type and condition of the engine, fuel composition and additives, operating conditions and emission control devices. Particles emitted from engines operating with gasoline are different from diesel engine exhaust particles in terms of their size distribution and surface properties. Emissions of organic compounds from gasoline (leaded and unleaded) and diesel

engines are qualitatively similar, but there are quantitative differences: diesel engines produce two to 40 times more particulate emissions and 20–30 times more nitroarenes than gasoline engines with a catalytic converter in the exhaust system when the engines have similar power output. Gasoline engines without catalytic converters and diesel engines of similar power output produce similar quantities of polycyclic aromatic hydrocarbons per kilometre; catalytic converters of the type used wlth gasoline vehicles reduce emissions of polycyclic aromatic hydrocarbons by more than ten times. Lead and halogenated compounds are also typically found in emissions from engines using leaded gasoline.

In urban areas, exposures to low levels and short-term peak levels of engine exhausts are ubiquitous. Higher exposures to engine exhausts may occur in some occupations, such as transportation and garage work, underground mining, vehicle maintenance and examination, traffic control, logging, firefighting and heavy equipment operation. The components of exhaust most often quantified in an occupational setting are particles, carbon monoxide and oxides of nitrogen; polycyclic aromatic compounds and aldehydes from engine exhausts have also been measured in work environments.

The exhausts of engines share similar physical and chemical characteristics with airborne materials from many sources. This makes it difficult to quantify the portion of an individual's exposure from the general environment that derives directly from engine exhausts and also complicates assessment of occupational exposures to engine exhausts.

### 4.2 Experimental data

Many studies have been carried out, using several animal species, to evaluate the potential carcinogenicity of exposure to whole exhaust and to components of exhaust from diesel- and gasoline-fuelled internal combustion engines. The studies are considered within six subgroupings: (i) whole diesel engine exhaust; (ii) gas-phase diesel engine exhaust (with particles removed); (iii) diesel engine exhaust particles or extracts of diesel engine exhaust particles; (iv) whole gasoline engine exhaust; (v) condensates/extracts of gasoline engine exhaust; and (vi) engine exhausts in combination with known carcinogens.

*Whole diesel engine exhaust*

Mice, rats, Syrian hamsters and monkeys (*Macaca fascicularis*) were exposed by inhalation to a range of concentrations of whole diesel engine exhaust, with observations in some studies extending to the lifespan of the animals. Five studies conducted using two different strains of rats showed an increased incidence of benign and malignant lung tumours that was related to the exposure concentration. Four of the studies involved exhaust from light-duty engines, and one the exhaust from a heavy-duty engine. One study of rats exposed to exhaust from a light-duty engine did not show a tumorigenic effect. Of three studies in Syrian hamsters, two did not show induction of lung tumours; the other was considered to be inadequate for an evaluation of carcinogenicity. In two studies in mice, the incidences of lung tumours, including adenocarcinomas, were increased over that in concurrent controls; however, in one study, the total incidence of lung tumours was not

elevated over that in historical controls. Monkeys exposed for two years to diesel exhaust did not develop lung tumours, but the short duration of the experiment rendered it inadequate for an evaluation of carcinogenicity.

*Gas-phase diesel engine exhaust (with particles removed)*

Three studies in which rats and Syrian hamsters were exposed to diesel engine exhaust from which soot particles had been removed by filtration did not show induction of lung tumours. In one study, mice exposed to filtered diesel engine exhaust had an increased incidence of lung tumours, including adenocarcinomas, compared to concurrent controls, a result similar to that seen with exposure to whole exhaust. However, the total incidence of lung tumours in this study was similar to that of historical controls.

*Diesel engine exhaust particles or extracts of diesel engine exhaust particles*

In other studies, organic extracts of diesel engine exhaust particles were used to evaluate the effects of concentrates of the organic compounds associated with carbonaceous soot particles. These extracts were applied to the skin or administered by intratracheal instillation or intrapulmonary implantation to mice, rats or Syrian hamsters. An excess of skin tumours was observed in mice in one study by skin painting and in one series of studies on tumour initiation using extracts of particles from several different diesel engines. An excess of lung tumours was observed in one study in rats following intrapulmonary implantation of beeswax pellets containing extracts of diesel engine exhaust particles.

In one study, an excess of tumours at the injection site was observed following subcutaneous administration of diesel engine exhaust particles to mice.

*Whole gasoline engine exhaust*

In one study in which rats were exposed by inhalation to whole leaded gasoline engine exhaust for up to two years and observed for up to an additional six months, the incidence of lung tumours was not different from that in controls. A similar study in Syrian hamsters also showed no induction of lung tumours. In a third study, dogs exposed to whole leaded gasoline exhaust for 68 months and held for an additional 32–36 months did not develop lung tumours.

*Condensates/extracts of gasoline engine exhaust*

Condensates/extracts of gasoline engine exhaust have been tested by skin painting, subcutaneous injection, intratracheal instillation or implantation into the lung. An excess of skin tumours was produced in five studies in mice by skin painting and in one series of tumour-initiation studies. An excess of lung tumours was observed in one study in rats that were given intrapulmonary implants of beeswax pellets containing condensates/extracts of gasoline engine exhaust. In one study, an excess of lung adenomas was observed in Syrian hamsters given intratracheal instillations of condensates/extracts of gasoline engine exhaust. Subcutaneous injections of condensates/extracts of gasoline engine exhaust also produced an excess of tumours at the injection site in one study in mice.

*Engine exhausts in combination with known carcinogens*

In studies in which known carcinogens were given to animals exposed either to diesel or gasoline engine exhausts or administered organic compounds from gasoline engine exhaust, inconclusive and inconsistent results were obtained.

### 4.3 Human data

*Studies of workers whose predominant engine exhaust exposure is that from diesel engines*

In the two most informative cohort studies (of railroad workers), one in the USA and one in Canada, the risk for lung cancer in those exposed to diesel engine exhaust increased significantly with duration of exposure in the first study and with increased likelihood of exposure in the second (in which smoking was not considered). Three further studies of cohorts with less certain exposure to diesel engine exhaust were also considered; two studies of London bus company employees showed elevated lung cancer rates that were not statistically significant, but a third, of Swedish dockers, showed a significantly increased risk for lung cancer.

In only two case-control studies of lung cancer (one of US railroad workers and one in Canada) could exposure to diesel engine exhaust be distinguished satisfactorily from exposures to other exhausts; modest increases in risk for lung cancer were seen in both, and in the first the increase was significant. In three further case-control studies, in which exposure to diesel engine exhaust in professional drivers and lung cancer risks were addressed, the Working Group considered that the possibility of mixed exposure to engine exhausts could not be excluded. None of these studies showed a significant increase in risk for lung cancer, although the risk was elevated in two.

In the three cohort studies (on railroad workers, bus company workers and ‘dockers’, respectively) in which bladder cancer rates were reported, the risk was elevated, although not significantly so. Four of the case-control studies of bladder cancer were designed to examine groups whose predominant engine exhaust exposure was assumed to be to that from diesel engines. Three showed a significantly increased risk for bladder cancer. In one of these, the large US study, a significant trend was also seen with duration of exposure; and in an analysis of one subset of self-reported diesel truck drivers, a substantial, significant relative risk was seen for bladder cancer.

*Studies of workers whose predominant engine exhaust exposure is that from gasoline engines*

Only one cohort study addressed workers exposed predominantly to gasoline engine exhaust (vehicle examiners). The risk for cancer increased with latency; no particular site accounted for this increase. In one case-control study, exposure to gasoline engine exhaust was isolated from that to diesel engine exhaust, but no consistent increase in risk was observed.

*Studies of workers whose predominant engine exhaust exposure cannot be defined*

In a cohort of Swedish drivers, a statistically significantly elevated risk for lung cancer was reported. A second cohort study of heavy construction equipment drivers showed significant increasing trends in lung cancer risk with duration of exposure, but the trend in risk for other smoking-related diseases was also increased. Increased risks for lung cancer were seen in three case-control studies of persons with mixed occupational exposures to engine exhausts in the USA, Italy and France; in two of these, the increase was significant.

In the one cohort study that addressed risk for bladder cancer, the risk was elevated, although not significantly so. In three case-control studies of bladder cancer in the USA, Italy and Denmark, modest increases in risk were seen; two showed significant trends with duration of exposure. In two further studies using the same set of controls, significant associations were also seen with multiple myeloma and chronic lymphocytic leukaemia. Three occupational groups in the US Navy with presumed exposure to engine exhausts were found to have a significantly high incidence of testicular cancer, although the influence of other exposures could not be assessed.

Possible associations between parental exposure to engine exhausts and cancer in children were considered in ten studies. No clear pattern of risk emerged.

### 4.4 Other relevant data

No relevant data were available on the toxic effects or metabolism of engine exhausts in humans, and there was no adequate study to evaluate whether diesel and gasoline engine exhausts induce chromosomal effects in humans.

Prolonged exposure of experimental animals to diesel engine exhaust leads to a number of effects related to the concentration to which they are exposed, including particle accumulation in macrophages, changes in the lung cell population, fibrotic effects and squamous metaplasia, which appear to be correlated with impaired pulmonary clearance. It has also caused exposure-related pathological changes in regional lymph nodes in mice and rats and an apparent increase in immunoglobulin M antibody response.

Prolonged exposure to diesel engine exhaust resulted in DNA adduct formation in rats and protein adduct formation in rats and hamsters.

Exposure of rodents to whole diesel engine exhaust induced sister chromatid exchange but not germ-cell mutations, micronuclei or dominant lethal mutations. Whole diesel engine exhaust induced sister chromatid exchange in cultured human cells. It did not induce sex-linked recessive lethal mutations in *Drosophila melanogaster* and gave inconclusive results in an assay for recombination in yeast. Particles or their extracts induced somatic gene mutations and sister chromatid exchange in rodents *in vivo* but did not induce micronuclei. They induced chromosomal aberrations, sister chromatid exchange and gene mutations in cultured human cells and cell transformation, sister chromatid exchange, gene mutations and DNA damage in rodent cells *in vitro* and inhibited intercellular communication. Particles or their extracts were weakly recombinogenic in yeast and induced mutations and DNA damage in bacteria. The gaseous phase was also mutagenic to bacteria.

Prolonged exposure to gasoline engine exhaust caused protein adduct formation in rats and hamsters.

Whole gasoline engine exhaust induced micronuclei in mice. Gasoline engine exhaust particle extracts induced cell transformation, aneuploidy, chromosomal aberrations, sister chromatid exchange, gene mutations and DNA damage in cultured animal cells but were not recombinogenic in yeast. Whole gasoline engine exhaust, particle extracts and the gaseous phase were mutagenic to bacteria.

### 4.5 Evaluation[1]

There is *sufficient evidence* for the carcinogenicity in experimental animals of whole diesel engine exhaust.

There is *inadequate evidence* for the carcinogenicity in experimental animals of gas-phase diesel engine exhaust (with particles removed).

There is *sufficient evidence* for the carcinogenicity in experimental animals of extracts of diesel engine exhaust particles.

There is *inadequate evidence* for the carcinogenicity in experimental animals of whole gasoline engine exhaust.

There is *sufficient evidence* for the carcinogenicity in experimental animals of condensates/extracts of gasoline engine exhaust.

There is *limited evidence* for the carcinogenicity in humans of diesel engine exhaust.

There is *inadequate evidence* for the carcinogenicity in humans of gasoline engine exhaust.

There is *limited evidence* for the carcinogenicity in humans of engine exhausts (unspecified as from diesel or gasoline engines).

#### Overall evaluation

Diesel engine exhaust *is probably carcinogenic to humans (Group 2A).*

Gasoline engine exhaust *is possibly carcinogenic to humans (Group 2B).*

[1]For definitions of the italicized terms, see Preamble.

**Summary table of genetic and related effects of diesel and gasoline engine exhausts**

| Nonmammalian systems | | | | | | | | | | | | | Mammalian systems | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proka-ryotes | | Lower eukaryotes | | | | Plants | | | Insects | | | | *In vitro* | | | | | | | | | | | | | | | | *In vivo* | | | | | | | | | | | |
| | | | | | | | | | | | | | Animal cells | | | | | | | | Human cells | | | | | | | | Animals | | | | | | | Humans | | | | |
| D | G | D | R | G | A | D | G | C | R | G | C | A | D | G | S | M | C | A | T | I | D | G | S | M | C | A | T | I | D | G | S | M | C | DL | A | D | S | M | C | A |
| **Diesel engine exhaust** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| $+^{1b}$ | $+^{ab}$ | | $?^{bc}$ | | | | | | | $-^{1c}$ | | | $+^{b}$ | $+^{b}$ | $+^{b}$ | | $?^{b}$ | | $+^{b}$ | $+^{1b}$ | | $+^{b}$ | $+^{b1c}$ | | $+^{1b}$ | | | | $+^{1c}$ | * | $+^{bc}$ | $-^{bc}$ | | $-^{1c}$ | | | $?^{c}$ | | $?^{c}$ | |
| **Gasoline engine exhaust** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | $+^{abc}$ | | $-^{1b}$ | | | | | | | | | | $+^{1b}$ | $+^{b}$ | $+^{b}$ | | $+^{1b}$ | $+^{1b}$ | $+^{b}$ | | | | | | | | | | | | | $+^{1c}$ | | | | | | $?^{c}$ | | $?^{c}$ | |

A, aneuploidy; C, chromosomal aberrations; D, DNA damage; DL, dominant lethal mutation; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei, R, mitotic recombination and gene conversion; S, sister chromatid exchange; T, cell transformation

*In completing the table, the following symbols indicate the consensus of the Working Group with regard to the results for each endpoint:*

+ considered to be positive for the specific endpoint and level of biological complexity

$+^{1}$ considered to be positive, but only one valid study was available to the Working Group

- considered to be negative

$-^{1}$ considered to be negative, but only one valid study was available to the Working Group

? considered to be equivocal or inconclusive (e.g., there were contradictory results from different laboratories; there were confounding exposures; the results were equivocal)

$^{a}$gas

$^{b}$particles or extracts thereof

$^{c}$whole exhaust

*positive in somatic cells$^{1b}$. negative in germ cells$^{1c}$

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**Appendix A-3…Carbon Black.** International Agency for Research on Cancer. 1996. *IARC Monographs*. Vol. 65:240-243.

the *hprt* clonal selection assay were used. Mutant frequencies ranged from 8.2 to 5.2 mutants/$10^6$ epithelial cells in the air control animals. Exposure to 52.8 mg/m$^3$ carbon black resulted in *hprt* mutant frequencies which were 4.3-, 3.2- and 2.7-fold greater than the air control group, immediately, three and eight months after exposure, respectively. A significant increase in the frequency of *hprt* mutants was detected immediately after 13 weeks of exposure to 7.1 mg/m$^3$ carbon black but not after three or eight months of recovery. No significant changes in the *hprt* mutant frequency were observed for alveolar epithelial cells from rats exposed to 1.1 mg/m$^3$ carbon black. This mutagenic response occurred at exposures that also resulted in significant pulmonary inflammation, epithelial hyperplasia and fibrosis.

## 4.5 Mechanistic considerations related to carcinogenicity

Figure 1 describes a hypothesized mechanistic model for effects of particle exposure in the lung. More specifically, the model, derived from inhalation studies in rats, pertains to exposure to low-toxicity low-solubility particles. This topic has also been discussed at a recent symposium (Mauderly & McCunney, 1996). Phagocytosis of such particles by alveolar macrophages leads to activation by alveolar macrophage and the subsequent release of inflammatory cytokines, growth factors, chemokines, enzymes and reactive oxygen species. This in turn recruits polymorphonucleocytes from the circulatory system into the alveolar space, thus amplifying the inflammatory response including the release of additional reactive oxygen species. This inflammatory response is dependent on the dose of deposited and phagocytized particles. Particularly, impairment of alveolar macrophage-mediated particle clearance due to lung particle overload results in further particle accumulation and amplifies this process, leading to chronic inflammation, including fibrotic changes. The continuous release of reactive oxygen species can result in increased mutation frequencies in specific target cells, which become manifest during increased cell proliferative responses. Subsequent responses include metaplastic changes, which finally result in tumour formation.

This specific mechanism involving reactive oxygen species is based on studies by Driscoll *et al.* (1996), which showed that carbon black in rats led in a dose-dependent manner to increased mutation frequency of the type II cells in those cases where significantly increased numbers of inflammatory cells were present. Earlier studies by Driscoll (1996) have shown that co-incubation of rat lung epithelial cells with inflammatory polymorphonucleocytes also resulted in increased mutation frequencies and that this response could be significantly decreased in the presence of antioxidants. Thus, oxidative damage to DNA due to released reactive oxygen species from inflammatory cells appears to be a plausible mechanism underlying the particle-induced rat tumour response. In further support of this hypothesis are the studies of Bond *et al.* (1990) who reported elevated levels of DNA damage in alveolar type II cells of rats exposed to concentrations of carbon black known to induce an inflammatory response.

An alternative mechanism relates to physical phenomena due to particles taken up by target cells. As pointed out in Figure 1, high pulmonary particle burdens result in

induced in mice at exposure concentrations and lung burden that exceeded the capacity of the lung to clear the particles and induced significant toxicity.

A central question is whether the toxic and defensive mechanisms suggested to operate in rats also operate in humans. Very little is known about the relationship between overload and lung cancer risk in humans, although it may be assumed that this overload-related mechanism could occur in humans exposed to sufficient levels (or doses). Limited indirect inferences regarding this issue may be derived from the available epidemiological studies of workers exposed to carbonaceous particles. The epidemiological studies of carbon black workers are not very informative in this regard. It is interesting to note that studies of coal miners have generally failed to detect an increased risk for lung cancer (Merchant *et al.*, 1986; Harrington & Levy, 1994). This evidence has been interpreted by some scientists as suggesting that a lung overload-related mechanism does not induce cancer in humans. However, there are other plausible interpretations for these observations that should be considered. It is difficult, as with nearly all epidemiological studies, to rule out that limitations in sample size, bias and other study design issues might explain these negative findings. The Working Group considered that the fact that coal miners are not permitted to smoke while working may have introduced a negative confounding bias for lung cancer in these studies. This bias is consistent with the observation of a deficit in lung cancer risk in these studies. Furthermore, it is important to consider the surface area characteristics of the inhaled low-toxicity, low-solubility particle (including coal dust) and its impact on dose.

Lung particle burden in the lungs of these workers by mass were on average ~15 mg/g lung. However, although pulmonary particle accumulation by mass is very high in coal miners, particle mass may not be the most relevant dose parameter for a correlation with specific long-term effects. It has been suggested, based on results of a number of studies, that surface area of retained particles may be a better parameter for correlation with pulmonary inflammation and neoplastic events. Thus, it may well be that coal miners, in spite of the high mass loading of particles in the lungs, did not reach a sufficient surface area of the retained particles. Also in rats, it has been found in some studies that lung particle mass burdens in this range, and even higher, did not result in increased tumour incidences. For example, chronic studies in rats with toner particles and pigment-grade titanium dioxide at exposure concentrations of 16 mg/m$^3$ and 50 mg/m$^3$, respectively, resulted in typical findings of particle overload (e.g. impaired particle clearance at lung burdens of ~12 and 60 mg/g lung, respectively) but with less inflammatory response and without induction of lung tumours. When these pulmonary mass particle burdens are expressed in terms of their surface area, the retained dose (by particle surface area) is lower than the dose (by surface area) observed to induce lung tumours in rats in other studies.

Epidemiological studies of diesel-exposed workers may also contribute something to this issue. A recent review of the epidemiological literature in this area concluded that these studies suggest a small-to-moderate increase in lung cancer risk, and that these findings do not appear to be fully explicable by confounding or other sources of bias (Cohen & Higgins, 1995). These studies are pertinent because it has been suggested, based on the recent experimental studies of rats exposed to high concentrations of diesel

exhaust and carbon black (Nikula *et al.*, 1994; Heinrich *et al.*, 1995), that the increased risk for lung cancer associated with diesel exhaust in rats might be explained by the carbonaceous core rather than the organic fraction of diesel soot. Workers in these studies were generally exposed to diesel exhaust levels below 200 $\mu g/m^3$, which is below the level at which overload of the lung in humans is believed to occur (Cohen & Higgins, 1995). These findings might be interpreted as suggesting either that lung overload occurs at lower levels than expected among humans, or that an overload-related mechanism may play the dominant role only at the high-exposure levels used in experimental studies.

In addition to these dose considerations, it is also of importance to consider differences in specific defence mechanisms between rats, mice and humans. The Working Group is not aware of studies which have evaluated specific pulmonary defences, including antioxidant levels, in humans under particle load conditions; such data, in contrast, are available for rats and mice. Thus, whether humans respond to chronic inhalation of particles, including carbon black, more like a rat or more like a mouse cannot be decided at present. It should be emphasized that the dose plays a most important role in the chain of mechanistic events outlined in Figure 1.

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure data

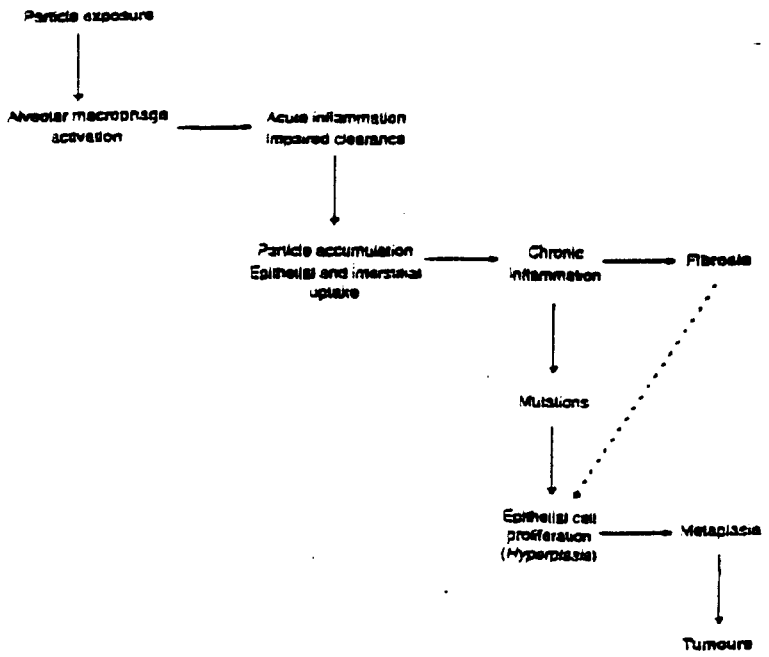
Carbon black is a powdered form of elemental carbon manufactured by the vapour-phase pyrolysis of hydrocarbon mixtures, such as heavy petroleum distillates and residual oils, coal-tar products, natural gas and acetylene. Worldwide production of carbon black in 1993 was approximately 6 million tonnes.

Carbon blacks are categorized as acetylene black, channel black, furnace black, lampblack or thermal black, according to the process by which they are manufactured. Lampblack is the oldest type of carbon black, having been used as a pigment for centuries. Channel black, produced from natural gas, was introduced in the late nineteenth century and was the major carbon black used worldwide in the early twentieth century for rubber and pigment applications; with the exception of a special product made in Germany, it is no longer produced. Acetylene, furnace and thermal blacks have been produced since the early twentieth century. Over 90% of all carbon black produced today is furnace black.

The primary use of carbon black is in rubber products, mainly tyres and other automotive products, but also in many other rubber products such as hoses, gaskets and coated fabrics. Much smaller amounts of carbon black are used in inks and paints, in plastics and in the manufacture of dry-cell batteries.

Types of carbon black are characterized by the size distribution of the primary particles, the degree of their aggregation and agglomeration and the various chemicals adsorbed onto the surfaces. Average primary particle diameters in several commercially produced carbon blacks range from 10 to 400 nm, while average aggregate diameters

**Figure 1. Mechanistic chain of events for pulmonary effects of low-toxicity, low-solubility particles assumed to be operative in rats**




Adapted from Oberdörster (1995)

increased uptake by epithelial cells and access of particles into the pulmonary interstitium. A study by Riebe-Imre *et al.* (1994) reported that a fetal Syrian hamster lung epithelial cell line, when incubated with different doses of carbon black, showed increased transformation, particularly when these cells were already differentiated. Carbon black also induced a dose-dependent enhancement of micronucleus formation in these cells, mostly due to clastogenic effects. Since these effects were observed in an epithelial cell line derived from hamsters after in-vitro exposures, the relevance to the in-vivo situation needs to be addressed. Particle-induced lung tumours have not been observed in hamsters, and target cells in the only species that shows particle-induced tumours (the rat) are most likely of alveolar origin, including the type II cells. This mechanistic hypothesis, based on physically induced DNA alterations, may be less plausible and relevant than the one of particle-induced oxidative damage.

The particle-associated rat lung tumours (e.g. diesel exhaust) cannot be extrapolated to mice or hamsters; these species do not, at comparable lung burdens of particles, develop lung tumours. For carbon black specifically, this species difference has been demonstrated between rats and mice. Several inhalation studies with low-solubility, low-toxicity particles, one of them with carbon black, have shown that no lung tumour was