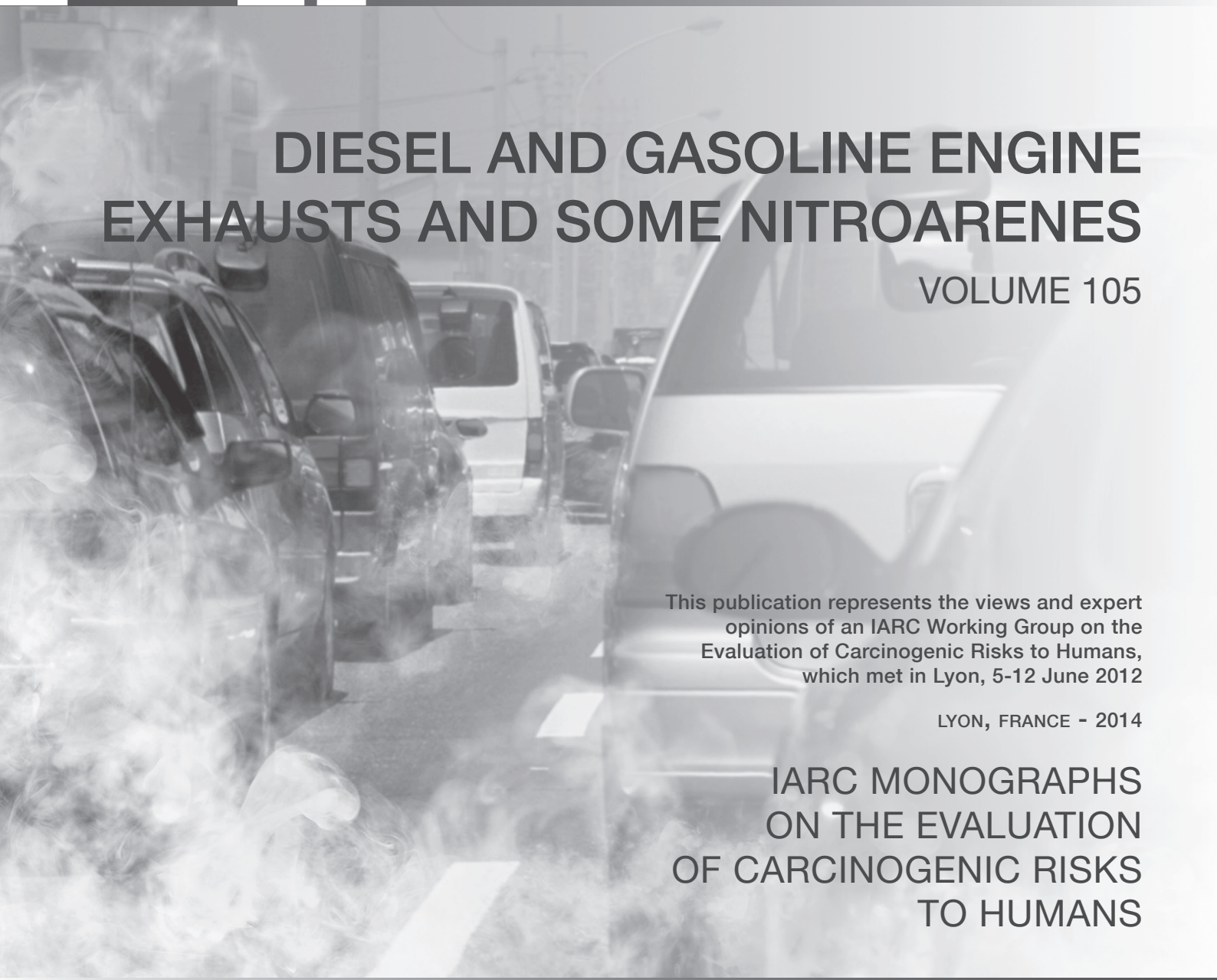
IARC MONOGRAPHS



DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES

VOLUME 105

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 5-12 June 2012

LYON, FRANCE - 2014

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

5. SUMMARY OF DATA REPORTED

5.1 Exposure data

Diesel and gasoline engines are the major sources of power used in motor vehicles. Both are internal combustion engines but differ fundamentally in terms of their fuel–air mixture preparation and ignition, and the fuels they use: diesel fuel is composed of petroleum fractions with a higher boiling range than those of gasoline.

Exhaust emissions from combustion engines comprise a complex and varied mixture of gases (e.g. carbon monoxide and nitrogen oxides), particles (including elemental and organic carbon, ash, sulfate and metals), various volatile (such as benzene) and semi-volatile organic compounds, and polycyclic aromatic hydrocarbons (PAHs), including oxygenated and nitrated PAH derivatives. The exact qualitative and quantitative composition of the exhaust depends on the fuel used, the type and age of the engine, the use of an emission control system, the tuning of the engine, its state of maintenance and its pattern of use (load and acceleration). Historically, diesel engine exhaust contained larger amounts of particulate matter, whereas gasoline engines contained higher levels of certain gases, such as carbon monoxide.

In addition to powering on-road vehicles, combustion engines are also used in a variety of off-road vehicles and equipment in different industrial sectors, such as mining, construction and transport. Diesel engines are generally used to power heavy-duty equipment (e.g. bulldozers and forklift trucks); both gasoline and diesel engines are used in lighter vehicles; and gasoline engines are used in hand-held equipment (e.g. chain saws, leaf blowers, hedge trimmers, brush cutters and clearing saws) and power generators.

Diesel-powered heavy-duty vehicles came on the market in the 1950s, and the predominant type has been sold between the 1960s and 1970s, resulting in their gradual infiltration into road traffic. Diesel engines were first used in underground mines in Germany in 1927, and their use increased considerably in the 1970s. Their use in railroad locomotives was first introduced around 1930 and they largely replaced steam engines between 1945 and the 1960s. In some countries, passenger cars may be almost exclusively powered by gasoline engines (e.g. Brazil, Switzerland and the USA), whereas cars in other countries may have either type of engine.

Increasing environmental concerns over the past two decades have resulted in regulatory actions to introduce successively stricter emission standards for both diesel and gasoline engines. Current emission standards for on-road vehicles vary across the world, and are generally introduced in North America and Europe initially, followed by other countries; however, many countries do not apply such regulations. Emission standards for off-road vehicles and industrial applications are mainly initiated after those for traffic vehicles; many off-road applications, such as for ships, trains and diesel generators, are still largely uncontrolled worldwide.

Standards and technology are strongly interlinked: standards drive improvements in technology and improved technology leads to more stringent standards. Diesel engines have progressed from 'traditional' models, for which particulate matter was not controlled, through 'transitional' models, with progressively advancing technology and lower emissions of particulates, nitrogen oxides and hydrocarbons, to 'new-technology' models that are characterized by the integration of wall-flow diesel particulate filters and diesel oxidation catalysts. Concurrently, the quality of diesel fuel has been improved in most parts of the world, especially with regard to the decrease in sulfur content (from 5000 ppm to 500 ppm, and subsequently to 15 ppm) to enable the use of advanced catalyst systems. New-technology diesel engines have only recently been introduced onto the roads in the USA and Europe. The rate of infiltration of these new technologies into on-road and off-road diesel vehicles/equipment is directly related to the level of sales of new-technology diesel engines to replace older vehicles that are currently in use.

Gasoline engine technologies have also evolved significantly. Tetraethyl lead had been banned as a fuel additive in most countries by 2000, although it is still used in a few geographical regions and in aircraft gasoline. Most gasoline automotive engines are now fitted with complex electronic feedback control systems, port fuel injection and three-way catalyst systems that have reduced emissions of particulate matter, nitrogen oxides, carbon monoxide and non-methane hydrocarbons, as well as unregulated emissions. To improve efficiency, the most recent gasoline engines have been fitted with a direct in-cylinder injection system, which may, however, increase emissions of particulate matter. Many industrial applications, especially small (below 10 kW) engines, still employ older gasoline engine technology.

The complex mixtures of gases and particulate matter in diesel and gasoline engine emissions require that suitable methods be used for their sampling and analysis. The gaseous species of interest are carbon monoxide, nitrogen oxides and various volatile and semi-volatile organic compounds. Particulate matter is a composite mixture of elemental and organic carbon, sulfate and various metals. The methods for sampling and analysing ambient air and engine exhausts have some similarities, although the concentrations tend to be much higher in exhaust. Both instruments for real-time measurement and sampling methods followed by off-line analyses are used.

Biomonitoring methods to estimate exposure to engine exhaust rely on the determination of biomarkers in body fluids and exhaled air. Analytes that have been used as biomarkers include the parent constituents of gasoline or diesel engine exhaust emissions, their metabolites and products of the covalent binding of activated biotransformation products to haemoglobin or DNA.

Exposure to diesel engine exhaust occurs in many different occupational settings, including the mining, railroad, construction and transport industries. The main determinants of exposure are the size, number and use of diesel engines indoors or outdoors, and the degree of ventilation. Several different markers of exposure have been used, such as elemental carbon, nitrogen oxides and PAHs. A generally accepted proxy for levels of exposure to diesel engine exhaust is elemental carbon, although this is not specific to diesel engine exhaust alone. Miners (in settings where diesel engines are used) and tunnel construction workers are the most highly exposed occupational groups, with average levels of exposure to elemental carbon above 100 μg/m^3. Dock workers, diesel mechanics and maintenance personnel are exposed on average to levels between 20 and 40 μg/m^3; train crews, construction workers and workers involved in loading and unloading ships are exposed to levels of elemental carbon of around 10 μg/m^3; and professional drivers

are exposed on average to lower levels of around 2 μg/m^3. Levels of exposure to elemental carbon vary largely within job titles, and these relative rankings can therefore vary in specific situations. Furthermore, the composition of diesel engine exhaust differs between occupational settings due to variations in use scenarios, operating conditions and engine technology.

Occupational exposure to gasoline engine exhaust occurs in a wide variety of professions. Exposure from on-road vehicles can occur in several occupations, with the following relative ranking: professional drivers > border inspectors > tollbooth workers > car mechanics > service station attendants > street workers > (traffic) policemen > car park attendants > shopkeepers. In addition to road traffic-related sources, exposure to gasoline engine exhaust may also occur during the use of gasoline engine-powered portable equipment, such as power chain saws. Several markers have been used to determine exposure to gasoline engine exhaust (lead, carbon monoxide, volatile organic compounds, nitrogen oxides, formaldehyde and particles), although none of these is highly specific for gasoline engine exhaust. The highest exposures, measured as carbon monoxide, are generally incurred by professional drivers, car mechanics, tollbooth workers and loggers.

Exposure of the general population to traffic emissions is dependent on proximity to such emissions, the volume and characteristics of the traffic and the presence of past traffic emissions in regional pollutants. The concentration of freshly emitted traffic-related pollutants decreases with distance from roads, reaching background levels at a distance of 100–600 m, depending on the pollutant. Source apportionment studies can help to determine the contributions of traffic to the complex mixture of air pollution. Studies conducted around the world indicated the contribution to particulate matter (< 2.5 μm in diameter) in general urban air pollution is 3–15% for diesel-powered vehicles and 8–30% for gasoline-powered vehicles. In urban areas, children may frequently be exposed when they walk or cycle near heavy traffic, or take a bus to neighbourhood schools. In addition, depending on the air exchange rate, gasoline and diesel engine exhaust emissions can represent a significant fraction of indoor air pollution.

5.2 Human carcinogenicity data

5.2.1 Diesel engine exhaust

(a) Cancer of the lung

The most informative studies were based on occupational cohorts of miners, railroad workers and workers in the transport industry with well characterized exposure, and supported a positive association between exposure to diesel engine exhaust and the risk for lung cancer.

The study of US miners included both a cohort analysis and a nested case–control study that adjusted for tobacco smoking. Both studies showed positive trends in the risk for lung cancer with increasing exposure, as estimated by elemental carbon. These trends were statistically significant in the nested case–control study, and risk increased in a near monotonic fashion. A two- to threefold increased risk was observed in the highest category for both cumulative and average exposure. The Working Group gave more weight to the nested case–control study, because it controlled for tobacco smoking, and to the combined analyses of surface and underground miners, in which the surface miners formed part of the low-exposure category. The studies of miner provided evidence of an association between exposure to diesel engine exhaust and the risk for lung cancer because few potential confounding exposures occurred in these mines, and high exposures to diesel engine exhaust had been well documented in concurrent surveys.

A large cohort study was carried out among US railroad workers. In the initial analyses, exposure was considered to have begun in 1959

after widespread conversion of the railroads to diesel engines. A significant 40% increased risk for lung cancer was found for exposed railroad workers compared with those who had low or no exposure, but no positive trends were found with duration of exposure. Indirect adjustment for tobacco smoking suggested that differences in this variate may have influenced the excess risk, but only slightly. In additional analyses, exposure assessment was refined by estimating exposure to diesel engine exhaust before 1959 on the basis of work history and the chronological conversion to diesel engines of different railroads. A significantly increased risk of 70–80% was observed for long-term and highly exposed workers, and positive trends were seen for duration of exposure, but not for an estimated index of cumulative exposure. The study of railroad workers supported an association between exposure to diesel engine exhaust and the risk for lung cancer.

A large cohort of workers in the US transport industry found an increased risk for lung cancer of 20–40% among workers with regular exposure to diesel engine exhaust (drivers and dock workers). Statistically significant positive trends of increasing risk with increasing duration of employment were found, with an approximate twofold risk after 20 years of employment. Indirect adjustment for tobacco smoking did not substantially alter these results. This study was extended using a refined exposure assessment that involved contemporary measurements and exposure reconstruction, based on elemental carbon. Exposure–response analyses showed positive trends for cumulative but not average exposure; these trends were more pronounced when an adjustment for duration of employment was included in the models in an effort to account for the healthy-worker survival effect. The studies in the transport industry supported an association between exposure to diesel engine exhaust and the risk for lung cancer.

Three further cohort studies were somewhat less informative because of limited sample size. These were carried out in Stockholm (Sweden) bus mechanics (20 cases of lung cancer), German potash miners (61 cases of lung cancer) and dock workers in Swedish ports (50 cases of lung cancer), and were supportive of an association between exposure to diesel engine exhaust and the risk for lung cancer through detailed exposure assessments. In a nested case–control study, mortality from lung cancer was assessed in persons who serviced buses in Stockholm; evidence of an increased risk with increasing exposure category was found, with borderline statistical significance. German potash miners were studied over a period of approximately 30 years. The results of the main and subgroup analyses of persons who had worked underground for 10 years or longer suggested exposure–response relationships. A nested case–control study was carried out in the cohort of dock workers in Swedish ports; tobacco smoking histories were obtained from next of kin and from retired workers. Three different indices of exposure gave similar results; the greatest odds ratio was found for workers with the highest exposure and evidence of an exposure–response relationship was observed after adjustment for tobacco smoking.

Several additional cohort studies that provided less accurate definitions of exposure (e.g. professional drivers and heavy-equipment operators) or used self-reported exposures were generally supportive of a positive association, although they showed less consistent results and were considered to be less informative.

Twelve independent case–control studies were available for evaluation after exclusion of multiple publications with overlapping study populations. A pooled analysis of studies from Europe and Canada included four of the 12 independent studies from Germany, Sweden, Turin and Montreal, but also included a new set of cases and controls that had not been reported earlier. Ten of the 12 independent studies were

population-based and two were nested within industrial populations of railroad and transport industry workers, respectively.

The two case–control studies of railroad and transport industry workers were large and provided more specific exposure assessments than general population studies. The study of railroad workers showed a tobacco smoking-adjusted increased risk of lung cancer that correlated positively with duration of employment in occupations that involved exposure to diesel engine exhaust in workers under the age of 65 years. The study of transport industry workers showed an elevated tobacco smoking-adjusted risk of lung cancer for long-term employment as a driver of heavy-duty diesel vehicles, and a positive association with cumulative exposure to diesel engine exhaust.

Seven of the 10 independent population-based case–control studies showed a positive association (statistically significant in six) between occupational exposure to diesel engine exhaust and the risk for lung cancer. All of these studies adjusted for tobacco smoking habits.

Two population-based studies showed no association with exposure to diesel engine exhaust. The study from the Turin area of Italy later showed a positive association after expansion and re-analysis with the job–exposure matrix used in a pooled European–Canadian study (see below). The other negative study from six cities in the USA was initially designed to investigate the effects of tobacco smoking and provided limited data on occupational history. A case–control study in the United Kingdom reported positive, non-statistically significant associations with some indicators of exposure to diesel engine exhaust, but did not adjust for tobacco smoking.

The European–Canadian pooled case–control study that applied a job–exposure matrix to classify exposure showed a positive association between exposure to diesel engine exhaust and the risk for lung cancer in a tobacco smoking-adjusted analysis, and a positive exposure–response relationship with duration of exposure.

Conclusions regarding cancer of the lung and diesel engine exhaust

In general, the more informative studies, many of which controlled for tobacco smoking, consistently showed a positive association between exposure to diesel engine exhaust and the risk for lung cancer. Most of the comparisons of exposed and unexposed groups indicated modest increases in risk, and analyses showed positive, statistically significant exposure–response trends.

Positive exposure–response trends were seen across different study designs and in several occupational settings. Therefore, it is improbable that the observed association between exposure to diesel engine exhaust and the risk for lung cancer was caused by chance, bias or confounding.

(b) Cancer of the urinary bladder

Eleven case–control studies reported risk estimates for exposure to diesel engine exhaust, one study reported a risk estimate for 'exhaust' and over 20 studies reported risk estimates for ever employment in an occupation associated with exposure to diesel or gasoline engine exhaust. None of the latter studies included a comprehensive exposure assessment that linked job/occupation specifically to quantitative measures of diesel or gasoline engine exhaust, which limited interpretation of the data because of potential exposure misclassification.

The most informative data resulted from a large pooled analysis of 11 European studies that observed a significantly increased risk among individuals in the highest category of exposure to diesel engine exhaust assessed from lifetime occupational histories and a job–exposure matrix. Four other studies – three (in Montreal, Canada, in Belgium and in Stockholm, Sweden) that used experts and one in British Columbia, Canada, that

used a job–exposure matrix to assess exposure to diesel engine exhaust – were also given greater weight in the evaluation. An excess risk of urinary bladder cancer was found among subjects with the highest exposure to diesel engine exhaust in the studies from British Columbia, Belgium and Stockholm. In the Montreal study, no excess risk for substantial exposure (the most comprehensive measure of exposure) was found, but elevated risks were observed among individuals in the expert-assessed categories for the highest confidence, frequency or duration of exposure to diesel engine exhaust. In all five studies, the odds ratios were generally greatest among individuals with the highest exposure, according to various metrics, but risk estimates in the different exposure categories were imprecise and most were not statistically significant. Of the two studies that reported trend tests for the level of exposure, only the study from British Columbia found a statistically significant trend in risk with increasing exposure. These studies adjusted for tobacco smoking in the analyses; however, the observed risks were small and subject to potential residual confounding from tobacco smoking or other occupational exposures.

The Working Group gave less weight to the evidence from six case–control studies that provided less accurate assessments of exposure to diesel engine exhaust and studies of occupations (heavy-duty vehicle, bus and taxi cab drivers, railroad workers and automobile mechanics) associated with potential exposure to diesel engine exhaust. Statistically non-significantly elevated risks were observed among subjects with the highest exposure to diesel engine exhaust in studies based only on job titles, but attribution of the association specifically to exposure to diesel engine exhaust was difficult.

Ten mortality risk estimates for urinary bladder cancer were based on nine occupational cohort studies. Standardized mortality ratios were 1.0 or less for six estimates and between 1.0 and 1.3 for four estimates. None of these studies included exposure–response analyses for urinary bladder cancer. The mortality studies had a limited ability to detect a positive association because they investigated mortality rather than incidence, which resulted in smaller numbers of cases, and lacked accurate exposure assessments.

Eight studies provided risk estimates for the incidence of urinary bladder cancer among workers potentially exposed to diesel engine exhaust. Four were record-linkage or population-based cohort studies and reported estimates based on a job–exposure matrix, self-reported exposure to diesel or unspecified engine exhaust or job title; only one risk estimate was significantly greater than 1.0. The studies were limited by low-quality exposure assessment, which was based on occupation at one point in time. In addition, three occupational cohort studies and a case–control study nested in one of the cohorts were available. Significantly increased standardized incidence rates for urinary bladder cancer were found in two cohorts of bus drivers. No association was found between occupation and the incidence of urinary bladder cancer in another cohort study. Most analyses in these studies did not adjust for tobacco smoking.

Overall, the epidemiological studies provide some evidence of a positive association between potential exposure to diesel engine exhaust and the risk for urinary bladder cancer.

(c) Cancer at other sites

Twenty-five case–control studies of adult cancers at sites other than the lung or urinary bladder were reviewed with regard to potential associations with exposure to diesel or gasoline engine exhausts. For most cancer sites in adults, only a small set of studies was available, the majority of which were limited with regard to exposure assessment, the number of exposed cases and other methodological problems. Occupational cohort studies showed no consistent patterns for other sites based on external comparisons and could not address potential confounders. Some

case–control studies of cancers of the larynx and colon suggested a positive association with exposure to engine exhaust (unspecified mixtures of diesel and gasoline) or proxies of exposure; however, these were not consistently supported by results from cohort studies. For pancreatic cancer, prostate cancer, multiple myeloma, leukaemia and lymphoma, the overall evidence did not support an effect of exposure to diesel and/or gasoline engine exhausts.

(d) Childhood cancer

Thirteen case–control studies assessed associations between exposure to unspecified mixtures of diesel and gasoline engine exhausts (or proxies of exposure) and childhood cancer, most of which focused on childhood leukaemia and brain tumours. The studies generally relied on the occupational titles of fathers and mothers to assess exposure, and most investigated parental occupation as a proxy for exposure to engine exhaust. Although several studies showed positive associations for acute leukaemias, the exposure assessment was generally not specific for diesel engine exhaust, which hampered their interpretation. Overall, no consistent evidence of associations between parental exposures to diesel and gasoline engine exhausts and the risk of childhood cancer was found.

5.2.2 Gasoline engine exhaust

(a) Cancer of the lung

Few studies attempted to disentangle the effects of diesel engine exhaust from those of gasoline engine exhaust. In some occupational environments, diesel engine exhaust is the only or primary source of exposure (e.g. those of railroad workers and non-metal miners), while many motor exhaust-related occupations involve exposure to a mixture of diesel and gasoline engine exhausts. The separate effects of gasoline and diesel engine exhausts have been investigated in US transport industry workers and in population-based studies in Sweden and Canada. The relative risks associated with exposure were consistently higher for diesel engine exhaust than for gasoline engine exhaust, and the modest excess risks associated with exposure to gasoline engine exhaust were possibly confounded by concomitant exposure to diesel engine exhaust. Little evidence was found for the carcinogenic effect of gasoline engine exhaust in these studies, but such an effect cannot be excluded.

(b) Cancer at other sites

The available data were too sparse and inconsistent to assess the carcinogenicity of gasoline engine exhaust at other sites.

5.3 Animal carcinogenicity data

5.3.1 Diesel engine exhaust

The whole diesel engine exhaust in these studies were generated from fuels and diesel engines produced before the year 2000, and included three basic components: elemental carbon particles in respirable clusters; organic matter adsorbed onto the surface of the carbon particles, which is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out using four animal species to evaluate the potential carcinogenicity of exposure to whole exhaust from diesel engines and its components. The studies were considered within four 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; and (iv) whole or gas-phase diesel engine exhaust in combination with known carcinogens.

(a) Whole diesel engine exhaust

Whole diesel engine exhaust was tested for carcinogenicity by inhalation exposure in four studies in mice, nineteen studies in rats, three studies in hamsters and one study in monkeys. In one study in mice, the incidence of lung adenocarcinoma in high-dose animals was significantly increased compared with that in concurrent controls. Significant increases in the incidence of lung tumours were not observed in the other studies in mice. In eleven studies in two different strains of rat, an increased incidence of benign and/or malignant lung tumours was related to exposure to whole diesel engine exhaust from light-duty engines (ten studies) and a heavy-duty engine (one study). One study in rats exposed to exhaust from a heavy-duty diesel engine did not show a significant increase in the incidence of lung tumours. Three studies in rats were inadequate for an evaluation of carcinogenicity and four gave negative results. None of the three studies in Syrian hamsters showed a significant increase in the incidence of respiratory tract tumours. Monkeys exposed to whole exhaust from a heavy-duty diesel engine for 2 years did not develop lung tumours, but the short duration of exposure was inadequate for an evaluation of carcinogenicity.

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

Gas-phase diesel engine exhaust (with particles removed) was tested for carcinogenicity by inhalation exposure in three studies in mice, seven studies in rats and three studies in hamsters. In one study in mice, the incidence of lung tumours was increased in treated animals compared with concurrent controls. However, the incidence of lung tumours in the control group in this study was significantly lower than that of historical controls in this laboratory. When this study was repeated in the same strain, and in a second strain, under the same conditions of exposure and duration, and with gas-phase diesel engine exhaust generated in the same way, there was no increase in the incidence of lung tumours in either strain tested, relative to controls. Therefore, the results of the first study reported were considered to be spurious. The seven studies in rats and three studies in hamsters did not show a significant increase in the incidence of respiratory tract tumours.

(c) Diesel engine exhaust particles or their organic extracts

Diesel engine exhaust particles were tested for carcinogenicity by intratracheal instillation in one study in mice, three studies in rats and one study in hamsters. The study in mice showed a non-significant increase in the incidence of lung tumours. One of the three studies in rats was inadequate for an evaluation of carcinogenicity. The other two studies showed a significant increase in the incidence of malignant and/or benign lung tumours. The study in hamsters gave negative results.

Organic extracts of diesel engine exhaust particles were tested for carcinogenicity by subcutaneous injection in three studies, by topical application in one study and by topical application in two initiation–promotion studies in mice, and by intrapulmonary implantation in one study in rats. An increased incidence of sarcomas at the injection site was observed following subcutaneous injection into mice in one study; the other studies in two strains of newborn mice were inadequate for an evaluation of carcinogenicity. An increased incidence of skin papilloma was observed in one initiation–promotion study using extracts of particles from heavy- and light-duty diesel engines; the other study was inadequate for an evaluation of carcinogenicity. The study of topical application in mice did not show an increase in the incidence of skin tumours. The study of intrapulmonary implantation in rats showed a significant increase

in the incidence of lung carcinoma following exposure to several of the isolated fractions.

(d) Whole or gas-phase diesel engine exhausts in combination with known carcinogens

Inconclusive and inconsistent results were obtained in two studies in mice and one study in rats in which a known carcinogen was administered to animals exposed to either whole or gas-phase diesel engine exhausts.

5.3.2 Gasoline engine exhaust

The gasoline engine exhausts evaluated in these studies were generated from fuels and engines produced before the year 2000, and included three basic components: particles composed primarily of elemental carbon and metallic compounds (especially lead, if present in the fuel); adsorbed organic material that is readily extractable with organic solvents; and a mixture of gas and vapour phases that include volatile organic compounds. Many studies have been carried out on four animal species to evaluate the carcinogenicity of whole gasoline engine exhaust and its components. The studies were considered within three subgroupings: (i) whole gasoline engine exhaust; (ii) condensates or extracts of gasoline engine exhaust; and (iii) whole gasoline engine exhaust in combination with known carcinogens.

(a) Whole gasoline engine exhaust

Whole gasoline engine exhaust was tested for carcinogenicity by inhalation exposure in three studies in mice, three studies in rats, three studies in hamsters and one study in dogs. The three studies in mice were inadequate for an evaluation of carcinogenicity. None of the studies of whole gasoline engine exhaust in rats, hamsters or dogs showed a significant increase in the incidence of respiratory tract tumours.

(b) Condensates or organic extracts of gasoline engine exhaust

Condensates or organic extracts of gasoline engine exhaust were studied to evaluate the effects of carbonaceous soot particles or concentrates of the organic compounds associated with these particles.

Condensates of organic extracts of gasoline engine exhaust particles were tested for carcinogenicity: in mice, by subcutaneous injection in one study which was inadequate for an evaluation of carcinogenicity, by topical application in five studies, two of which were inadequate for an evaluation of carcinogenicity and three of which demonstrated a significant increase in the incidence of carcinomas and papillomas of the skin, and by topical application in one initiation–promotion study which indicated that the extract of gasoline engine exhaust was a skin tumour initiator; in rats, by intrapulmonary implantation in one study, in which a significant increase in the incidence of lung carcinomas was observed; and in hamsters, by intratracheal installation in two studies, one of which showed an increase the incidence of pulmonary adenomas, while the other gave negative results.

(c) Whole gasoline engine exhaust in combination with known carcinogens

One of two studies of inhalation exposure to whole gasoline engine exhaust in mice that were also exposed to a known carcinogen was inadequate for an evaluation of carcinogenicity, while the other gave negative results. Of two studies of inhalation exposure to whole gasoline engine exhaust in rats that were also exposed to a known carcinogen, one indicated that whole gasoline engine exhaust was a lung tumour promoter, while the other gave negative results. One inhalation study with whole gasoline engine exhaust in hamsters in which a known carcinogen was also given gave negative results. One subcutaneous injection study with exhaust condensate in mice

also treated with a known carcinogen was inadequate for an evaluation of carcinogenicity.

5.4 Mechanistic and other relevant data

5.4.1 Diesel engine exhaust

(a) Deposition, clearance, retention and metabolism

The general principles regarding the inhalation, deposition, clearance and retention of poorly soluble particles, and the modelling of inhaled particle deposition in the human lung have been described previously. The number, concentration and size distribution of aerosol particles in the submicron range produced by combustion of diesel fuel and inhaled and exhaled by nonsmoking human volunteers have been determined. The average fraction from diesel exhaust retained in the human lung was 30 ± 9% (± standard deviation) and the count median diameter was 0.124 ± 0.025 μm.

Studies of the metabolism have been conducted on humans exposed to diesel engine exhaust, generally in the workplace, many of which focused on measurements of urinary concentrations of hydroxylated PAHs and amino-PAHs, mainly pyrenes. These studies demonstrated that humans exposed to diesel engine exhaust can adsorb, distribute, metabolize and excrete metabolites of PAHs. Other studies have reported the presence of urinary 1-hydroxypyrene and haemoglobin adducts of nitro-PAHs and low-molecular-weight alkenes (hydroxyethylvaline and hydroxypropylvaline) in populations exposed to diesel engine exhaust.

No adequate studies on the metabolism of diesel and gasoline engine exhaust mixtures in experimental animals were available to the Working Group. The deposition and clearance of diesel engine exhaust components, especially particulates, have been studied in some detail to improve the understanding of the potential mechanisms of species differences in the formation of lung tumours (e.g. exposure to diesel engine exhaust caused tumours in rats, but not in mice or hamsters). A particle overload mechanism for the induction of cancer in rats after high particle deposition entails an overloading of the process of particle clearance, which is mediated by macrophages through the phagocytosis of excessive quantities of particles in the deep lung, and results in the sequestration of particles within the lung. This engenders an influx of leukocytes that produces chronic pulmonary inflammatory effects, including the formation of reactive oxygen species, which increase oxidative DNA damage in proliferating epithelial lung cells that eventually results in lung cancer.

Inhalation studies of titanium dioxide and carbon black in rats revealed that, regardless of the particle type used, the lung tumour rate increased with increasing exposure concentrations of the particles. The conclusions from these and other inhalation studies in rats indicated that lower particle loads do not produce lung cancer, because they do not trigger compensatory inflammatory responses in the lung. Moreover, unlike other species, rats are uniquely sensitive to the inhalation of high particle loads, and mount significant lung physiological responses that eventually lead to cancer.

(b) Genetic and related effects

Exposures of humans to diesel engine exhaust increased the expression of genes associated with oxidative stress and inflammation in blood lymphocytes and those involved in inflammation in bronchoalveolar lavage cells. Exposure of humans to air that predominantly contained diesel engine exhaust induced bulky DNA adducts, DNA damage and micronucleus formation. Positive biomarkers of genotoxicity for exposure and effect were observed among humans exposed to diesel engine exhaust or

air with a predominant diesel engine exhaust content.

Diesel engine exhaust, diesel engine exhaust particulates and diesel particulate extracts induced DNA damage (e.g. oxidative lesions and bulky adducts), gene mutations, DNA strand breaks, chromosomal alterations (e.g. chromosome breaks, sister chromatid exchange and aneuploidy) and morphological cell transformation *in vivo* and *in vitro* in a wide range of experimental systems, including rats and mice, rodent and human cell lines, and rodent and human primary cells, as well as gene mutations in bacteria. The *in-vivo* effects have been documented after multiple routes of administration, including inhalation exposure, intratracheal instillation and oral administration of whole diesel engine exhaust and/or diesel engine exhaust particulates, and topical application and intraperitoneal injection of organic extracts of diesel engine exhaust particulates.

In rodent target tissues following inhalation exposure and in mammalian cells exposed to diesel engine exhaust, diesel particulate suspensions or diesel particulate extracts, gene expression profiles showed the upregulation of genes in pathways related to oxidative stress, inflammation, DNA damage, antioxidant responses, cell cycle, cell transformation and apoptosis.

Definitive statements regarding the effect of diesel engine exhaust aftertreatment or fuel formulation on the genetic and related effects of diesel engine exhaust have been hampered by variations in the types of device and/or fuel examined and the confounding effects of engine design, sample collection and processing, and engine test cycle (i.e. speed and load). Nevertheless, evidence has shown that oxidation catalysts can increase the activity of diesel engine exhaust *in vivo* and *in vitro* and that of extracts of diesel engine particulate matter or exhaust semi-volatile organic compounds (expressed per unit of extractable organic matter or per unit of mass particulate matter) *in vitro*. However, evidence has also been found that exhaust aftertreatment can contribute to substantial reductions in the activity of extracts of diesel engine particulate matter or exhaust semi-volatile organic compounds expressed per unit of engine work or volume of emitted exhaust. No comparative data were available to the Working Group to evaluate the genetic and related effects of new-technology diesel exhaust.

(c) Other effects

Numerous human clinical studies and experimental animal studies have been conducted to investigate the non-cancer health effects of diesel engine exhaust, including recent studies on new-technology diesel engine exhaust (e.g. US 2007 compliant technology or more recent). Biological responses to this type of exposure have been reported for a diverse range of health end-points, including lung function, lung inflammation, immunology and infection, systemic inflammation and cardiovascular effects, and brain inflammation. Prolonged exposure to high concentrations has been associated with the accumulation of particles in macrophages, changes in lung cell populations, fibrotic effects and squamous metaplasia, which appeared to be associated with impaired pulmonary clearance. Other responses such as systemic inflammation, susceptibility to infection, exacerbation of allergic response and cardiovascular responses have also been reported. These systemic responses were also observed with lower exposure concentrations at which pulmonary inflammation was mild or absent. Some of these responses appeared to be absent after exposure to new-technology diesel engine emissions. However, at the present time, new-technology diesel engine emissions have not been evaluated thoroughly.

(d) Susceptibility

Some studies have investigated the role of genetic polymorphisms on the modulation of biomarker responses following exposures to

air containing primarily diesel engine exhaust or mixed exhausts. However, the data were too limited to draw any conclusions. No studies were available to the Working Group on the influence of other susceptibility factors, such as vulnerable populations, underlying disease and the microbiome, in relation to exposure to diesel engine exhaust and the incidence of lung cancer.

(e) Mechanistic considerations

Diesel engine exhaust is a complex mixture that consists of both gaseous and particulate components. The gaseous phase of diesel engine exhaust is mutagenic to bacteria and contains a series of carcinogens including acetaldehyde, acrolein, benzene, 1,3-butadiene, formaldehyde, ethylene oxide, propylene oxide and naphthalene. The particulate phase contains carcinogenic PAHs, nitro-PAHs and metals.

Organic solvent extracts of particulates of diesel engine exhaust showed a broad spectrum of genotoxic activities *in vitro* and *in vivo*, inducing bulky DNA adducts, oxidative DNA damage, DNA strand breaks, unscheduled DNA synthesis, mutations, sister chromatid exchange, chromosomal aberrations and morphological cell transformation in mammalian cells, and mutations in bacteria. They increased the expression of genes involved in xenobiotic metabolism, oxidative damage, antioxidant response and the cell cycle in mammalian cells in culture, and induced skin papillomas and adenocarcinomas in mouse skin.

PAHs are bio-transformed by phase I metabolic enzymes to a series of dihydrodiols, phenols, quinones and polyhydroxylated metabolites. Dihydrodiols can be metabolized further to chemically reactive intermediates (diol epoxides), which bind covalently to DNA to form DNA adducts. PAHs can undergo one-electron reduction to form radical cations, which can bind to DNA to form depurinating PAH adducts. PAH quinones can redox cycle, generating reactive oxygen species that modify DNA. Many of these DNA modifications have been associated with the induction of mutation and, eventually, tumour formation. Further metabolism of PAH metabolites by phase II enzymes converts many of the primary metabolites to glucuronic acid, sulfate and glutathione conjugates that are excreted in the faeces and urine. Nitro-PAHs can be reduced by nitroreductases to hydroxylamino and amino metabolites, and the hydroxylamino intermediates have been shown to bind to DNA to form covalent DNA adducts. Some nitro-PAHs can undergo both oxidative and reductive metabolism, forming mixtures of metabolites and DNA adducts that containing nitro, dihydrodiol or amino functionalities.

The genotoxic organic compounds adsorbed onto the particles need to be bioavailable to manifest their genotoxic activities. Organic solvents are extremely efficient at removing organic compounds from diesel engine exhaust particulates, and some evidence has shown that biological fluids can facilitate bioavailability of genotoxic organic compounds bound to diesel engine exhaust particulates based on *in vitro* and *in vivo* assays.

The particulate phase of diesel engine exhausts was genotoxic *in vitro* and *in vivo*, inducing bulky DNA adducts, oxidative DNA damage, DNA strand breaks, germ-line mutations and transformed foci in selected organs and/or cells. Diesel engine exhaust particulates generated superoxide and hydroxyl radicals and increased the levels of 8-oxo-2'-deoxyguanosine in DNA *in vitro* and *in vivo*. Genes involved in phase I and II metabolism, oxidative stress, antioxidant response, immune/inflammatory response and cell cycle/apoptosis, and those that respond to cell damage were upregulated in cultured rat alveolar epithelial cells exposed to fractionated organic solvent extracts of diesel engine exhaust particles.

Exposure to whole diesel engine exhaust induced sister chromatid exchange in lung cells and lung tissues, increased the levels of bulky

DNA adducts and enhanced oxidative DNA damage in rodents, caused mutations in transgenic rats and induced angiogenesis and vasculogenesis in mice.

A particle overload mechanism has been proposed that includes overloading of the process of particle clearance carried out by macrophages (phagocytosis of excessive quantities of particles) in the deep lung, which results in the sequestration of particles within the lung. This engenders an influx of leukocytes that produces chronic pulmonary inflammatory effects, including the generation of reactive oxygen species, which increase oxidative DNA damage in proliferating epithelial lung cells, and eventually results in lung cancer. Inhalation studies of titanium dioxide and carbon black in rats revealed that, regardless of the particle type used, the lung tumour rate increased with increasing particle exposure concentrations. The conclusions from these and other inhalation studies in rats indicated that lower particle loads do not trigger compensatory lung inflammatory responses, which may account for the lack of tumour response observed at these exposure levels. Moreover, rats are more sensitive to the inhalation of high particle loads than hamsters and mice because they mount significant lung physiological responses that eventually lead to cancer. The rat lung response to particle overload is species specific and its occurrence after exposure to other particle types has been described. However, some aspects of the responses observed in rats are similar to those seen in humans exposed to diesel engine exhaust, which could help to elucidate the mechanism(s) of carcinogenic action in humans. The effect induced by high-loading particulates may be relevant for occupationally exposed humans. In addition, humans – in contrast to rodents – can mount an inflammatory response at levels encountered in occupational exposures.

Human studies indicated that some populations exposed to diesel engine exhaust excrete urinary 1-hydroxypyrene, an indicator of exposure to PAHs, and several amino-PAHs (e.g. 1-aminopyrene and 3-aminobenzanthrone), which are reduction products of 1-nitropyrene and 3-nitrobenzanthrone that are considered to be specific markers of exposure to diesel engine exhaust.

Populations exposed to diesel engine exhaust showed increased levels of bulky DNA adducts, DNA strand breaks, oxidative DNA damage and micronuclei in their blood lymphocytes, as well as upregulation of the genes related to oxidative stress and inflammation.

In controlled studies of chamber exposure to diesel engine exhaust, healthy subjects developed airway inflammation, with airway neutrophilia and lymphocytosis, increases in interleukin-8 protein in lavage fluid, increased interleukin-8 gene transcription in the bronchial mucosa and upregulation of the endothelial adhesion molecules. Moreover, diesel engine exhaust induced interleukin-6 and lymphocytes in airway lavage fluids, and increased growth-regulated oncogene-α protein expression in the bronchial epithelium in humans. Exposure of humans to diesel engine exhaust particles has been purported to induce oxidative stress leading to a cascade of downstream mitogen-activated protein kinase signalling pathways, the activation of which activates nuclear factor-κB and activator protein-1 transcription factors that increase the levels of pro-inflammatory mediators (e.g. interleukin-4, −6 and −8, and tumour necrosis factor-α), producing leukocyte infiltration and inflammation in the airways. Diesel engine exhaust particles increased interleukin-8 expression in airway epithelial cells isolated from normal adult human volunteers, and upregulated the expression of genes connected with key oxidative stress, protein degradation and coagulation pathways.

Diesel engine exhaust is complex in nature, and the mechanisms by which it induces cancer in humans are also complex; no single mechanism appears to predominate. Organic solvent and physiological fluid extracts of diesel engine

exhaust particles and several of their individual components are genotoxic, and some are carcinogenic, generally through a mechanism that involves DNA mutation. These modifications include the formation of bulky DNA adducts and oxidized DNA bases. Both the organic and particulate components of diesel engine exhaust emissions can generate oxidative stress through the formation of reactive oxygen species, which can be generated from washed particles, fresh particles, arene quinones formed by photochemical or enzymatic processes, metals and the phagocytosis process, and as a result of the inflammatory process. Reactive oxygen species can lead directly to the formation of oxidatively modified DNA and DNA adducts from the by-products of lipid peroxidation. They can also cause lipid peroxidation, which generates cytotoxic aldehydes, and initiate a signalling cascade that leads to inflammation, resulting in further induction of oxidative stress, which can then cause cell proliferation and cancer. In response to the inflammatory insult, cyclooxygenase-2 is upregulated and is a potent mediator of cell proliferation.

In conclusion, there is *strong mechanistic evidence* that diesel engine exhaust, as well as many of its components, can induce lung cancer in humans through genotoxic mechanisms that include DNA damage, gene and chromosomal mutation, changes in relevant gene expression, the production of reactive oxygen species and inflammatory responses. In addition, the co-carcinogenic, cell-proliferative and/or tumour-promoting effects of other known and suspected human carcinogens present in diesel engine exhaust probably contribute to its carcinogenicity in the human lung.

5.4.2 Gasoline engine exhaust

(a) Deposition, clearance, retention and metabolism

Human studies on the deposition of gasoline engine exhaust particulates have been reported previously. Total deposition was relatively constant at 30% over a wide range of breathing patterns for sizes of typical aerosols. As the size of primary particles decreased (below 0.1 μm), deposition increased sharply and the length of the respiratory cycle significantly affected deposition. In a separate analysis of the same data, deposition was shown to increase with the respiratory cycle in an approximately linear fashion, ranging from 10% at 3 seconds to 55% at 20 seconds; the slope of the gradient was somewhat dependent on tidal volume.

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

Exposure to gasoline engine exhaust has been associated with several biological responses that include lung inflammation, systemic inflammation and cardiovascular effects. The lower concentration (relative to diesel engine exhaust) of particulate matter in gasoline engine exhaust in most studies probably contributed to the reduced pulmonary responses compared with those elicited by diesel engine exhaust. However, some studies have suggested that the systemic responses to these two types of exhaust

in the cardiovascular system and the developing immune system are similar.

(b) Genetic and related effects

No studies on genetic and related effects in humans exposed to gasoline engine exhaust alone were available to the Working Group. Exposures of humans to air that contained a mixture of diesel and gasoline engine exhausts induced bulky DNA adducts, DNA damage, oxidative damage, chromosomal aberrations, sister chromatid exchange, micronuclei, mutagenic urine and altered expression of genes and proteins involved in oxidative stress. Thus, a wide array of positive genotoxicity biomarkers of exposure and effect were observed among humans exposed to mixtures of diesel and gasoline engine exhaust.

Gasoline engine exhaust induced chromosomal damage in mice, and changes in the expression of genes involved in pathways related to xenobiotic metabolism and inflammation in rat lung. Particulate matter from gasoline engine exhaust and its organic extracts induced DNA damage (e.g. strand breaks, oxidative lesions and bulky DNA adducts), chromosomal alterations (e.g. chromosome breaks and sister chromatid exchange) and morphological cell transformation in cultured rodent and human cells, and gene mutations in bacteria. The upregulation of genes involved in pathways related to inflammation, xenobiotic metabolism, tumour progression, metastasis and cell cycle has been noted in cultured human cells exposed to extracts of particulate matter from gasoline engine exhaust.

(c) Other effects

See Section 5.4.1

(d) Susceptibility

See Section 5.4.1

(e) Mechanistic considerations

The gaseous phase of gasoline engine exhaust was mutagenic to bacteria and contains several carcinogenic volatile carcinogens (benzene, 1,3-butadiene, formaldehyde and naphthalene); the particulate phase contains several carcinogenic PAHs.

Organic solvent extracts of gasoline engine exhaust particulates were mutagenic in bacterial and mammalian cells, induced DNA damage, sister chromatid exchange, micronuclei, chromosomal aberrations and morphological cell transformation in mammalian cells, and initiated tumours in mouse skin. These data provide *strong evidence* that a genotoxic mechanism is involved in the carcinogenicity of organic solvent extracts of gasoline engine exhaust particulates.

Whole gasoline engine exhaust was mutagenic to bacteria and induced micronuclei in mice. However, the mechanistic data from experimental and human studies of exposures to whole gasoline engine exhaust were insufficient to formulate a mechanism of action for this exposure.

6. EVALUATION

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of diesel engine exhaust. Diesel engine exhaust causes cancer of the lung. A positive association has been observed between exposure to diesel engine exhaust and cancer of the urinary bladder.

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

6.2 Cancer in experimental animals

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

There is *inadequate evidence* in experimental animals for the carcinogenicity of gas-phase diesel engine exhaust.

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

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

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

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

6.3 Overall evaluation

Diesel engine exhaust is *carcinogenic to humans* (Group 1).

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